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The Abstracts

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KEYNOTE LECTURES

PL1

KL-01
DROSOPHILA AS A MODEL SYSTEM FOR INFLAMMATION

J. M. Reichhart[1,*]

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The adaptive immune system with its antibodies, B and T cells arose around 500 million years ago in the first vertebrates and in all invertebrates, the defence mechanisms are purely innate. We have been working since 1985 on the innate immune system, using *Drosophila* as a model system. In *Drosophila*, an infection provokes the rapid synthesis of powerful antibiotic peptides. As an example, the basal level of expression of the antifungal peptide DROSOMYCIN is increased a thousand fold within 30 min of septic injury. The control of this expression involves at least two pathways that, for sake of simplicity, I refer to as the TOLL and the IMD pathways. The paramount role of the TOLL and IMD pathways in the host defence of *Drosophila* is illustrated by experiments in which mutant flies are challenged with fungi or bacteria. In TOLL-deficient mutants, survival to fungal, but not to bacterial infection, is severely compromised. By contrast, IMD mutants are markedly affected by bacterial infections but resist fungi. In vertebrates, recognition of microbes by the innate immune system takes place at the cellular level by a family of transmembrane receptors homolog to *Drosophila* Toll, namely the Toll like receptors or TLRs. In *Drosophila* however, these recognition events take place in the open circulatory system via soluble excreted recognition proteins like the peptidoglycan recognition proteins (PGRPs). In turn, these recognition steps must be conveyed onto Toll by extracellular proteolytic signalling pathways. Upstream of the IMD pathway, other PGRPs are recognizing Gram-negative microbial determinants. We are now interested in how these two pathways are activated and regulated. Although anatomy of the Drosophila immune system differs widely from the vertebrate immune system, we will discuss the possibility that the fruit fly, with its powerful genetics and simple organization, could become a new paradigm to study inflammation.

PL2

KL-02
NEW THERAPEUTIC APPROACHES IN INFLAMMATION: THE EXAMPLE OF ENDOGENOUS GAS

J. L. Wallace[1,*]

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Over the past three decades, there has been a growing appreciation of the physiological and pathophysiological importance of endogenous gaseous mediators, such as nitric oxide, carbon monoxide and hydrogen sulfide. All three gases were initially considered poisons, but their physiological importance is now unquestionable. H_2S exerts a number of anti-inflammatory effects, including suppression of leukocyte adherence to the vascular endothelium, edema formation and NfKB activation. It also promotes resolution of inflammation and promotes wound healing. H_2S may therefore be exploited in the design of more effective and/or less toxic anti-inflammatory compounds. In recent years, we have attempted to achieve this using hydrogen sulfide-releasing moieties, covalently linked to a number of different anti-inflammatory drugs. For example, mesalamine is the first-line therapy for inflammatory bowel disease (Crohn's disease and ulcerative colitis), but it is a weak drug, with doses of up to 4 g per day required. An H2S-releasing derivative of mesalamine (ATB-429) was shown to be significantly more potent in two models of colitis. In addition to reducing inflammation, ATB-429 suppressed expression of a number of pro-inflammatory cytokines (IL-1, TNF, IL-12) and markedly reduced granulocyte infiltration into the colonic tissue. Another class of therapeutics we have focused on is nonsteroidal anti-inflammatory drugs (NSAIDs). While very effective at reducing pain and inflammation, these drugs continue to be limited in their utility by significant toxicity in the gastrointestinal tract (the advent of selective COX-2 inhibitors has been only a modest advance in this regard). Several H_2S-releasing NSAIDs have been developed and tested extensively. ATB-346 is an H_2S-releasing derivative of naproxen. It exhibits comparable anti-inflammatory and analgesic effects to naproxen, but does not produce any detectable GI injury in a variety of rodent models, including some in which gastrointestinal mucosal defence is severely impaired. Moreover, rather than impairing the healing of pre-existing ulcers, as is seen with naproxen and other NSAIDs, ATB-346 significantly enhanced healing. This approach could be taken to modify a wide range of drugs, as has been done with nitric oxide-releasing therapeutic agents.

PL3

KL-03
METAGENOMICS: IMPACT ON RESEARCH IN INFLAMMATION

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The incidence of chronic diseases is steadily rising over the past decades. Perturbations of gut microbial communities have been suggested in many of these. The main objective of the MetaHIT consortium is to explore associations of the bacterial genes, genomes or communities from the human gut with two chronic diseases, obesity and inflammatory bowel diseases. We developed a powerful new approach, Quantitative Metagenomics, to visualize the gut bacteria present in any individual. It is based on two main elements, the reference gene catalog of the gut bacterial genes and the high throughput sequencing of the total stool DNA. The reference gene catalog was established by massive Illumina sequencing of total fecal DNA from 124 individuals of European origin. It contains 3.3 million non-redundant genes, 150-fold more than encoded by our own genome. It captures over 85% of the genes from our cohort and includes about 80% of those found in previous studies of smaller scope, carried out in Japan and the US. This indicates that it represents well the human intestinal metagenome and has become known as our other genome (Qin et al. in Nature 464:59–65, 2010). Two case/control studies, one of obese and lean individuals and the other of the ulcerative colitis patients and family matched controls will be presented. They reveal links between bacterial diversity and inflammation, which may be relevant to numerous pathologies. Furthermore, they unveil association of bacterial species to chronic

disease. The species have a diagnostic/prognostic potential well above that of the alleles of our own genome, suggesting that personalized medicine should target, in preference, our other genome.

PL4

KL-04
SENESCENCE AND INFLAMMATION: NEW LINKS, NEW TARGETS

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Cellular senescence is a powerful anti-cancer mechanism by which cells undergo a permanent arrest of cell proliferation (growth). Cellular senescence is also thought to cause or contribute to aging. How can the same process have such apparently incongruous effects (tumor suppression and aging)?

The senescence growth arrest is accompanied by large-scale changes in gene expression. One outcome of these changes is a robust increase in the expression and secretion of numerous cytokines, growth factors and proteases, which we term the senescence-associated secretory phenotype (SASP). A striking feature of the SASP is that many SASP components are potent pro-inflammatory proteins. Inflammation, of course, drives virtually every major age-related disease, including, ironically, cancer. As an inflammatory response, cellular senescence also entails the expression of proteins that signal the immune system and targets senescent cells for clearance. Some senescent cells, however, escape immune clearance. Understanding how some senescent cells evade immune clearance provides novel targets for interventions aimed at facilitating the destruction of senescent cells by the immune system. Recent findings show that the SASP is primarily a DNA damage response (DDR) that may have evolved to allow damaged cells to communicate their compromised state to the surrounding tissue. The DDR engages a number of stress-responsive pathways that collaborate to stimulate the expression of SASP genes. These pathways include well-known transcriptional regulators of inflammation, such as NF-κB. They also include novel pathways that act at the level of translation, such as the mTOR pathway. These new data identify novel targets for suppressing the deleterious (pro-inflammatory) effects of cellular senescence, while preserving its beneficial (tumor suppressive) effects.

Disclosure of interest: None declared.

PL5

KL-05
DEVELOPMENT AND MODULATION OF IL-17 IMMUNE RESPONSES

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Interleukin 17 is a cytokine with central involvement in inflammatory immune responses. It is primarily produced by Th17 CD4 T cells, a subset of TCR$\gamma\delta$ T cells and some NKT cells. Over the past few years it has become clear that the production of IL-17 is regulated at multiple levels—both positively and negatively—to control its activity which when dysregulated can initiate pathogenic immunity and result in a variety of autoimmune syndromes. Cytokine production by Th17 effector cells is not a stable parameter, but instead appears to be modulated on several levels. As more states of CD4 T cell differentiation are uncovered, their flexibility is also beginning to be recognized. Components that control the plasticity of CD4 T cell populations include environmental influences at tissue sites, transcriptional circuitry and chromatin modifications. Understanding the rules that underlie adaptation and flexibility of T cell responses will be an essential part for future intervention strategies in human disease states.

EDUCATIONAL SESSIONS

EDUC1
IMAGING TECHNOLOGIES TO INVESTIGATE INFLAMMATORY IMMUNE RESPONSES

S. Shorte[1,]*

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Abstract not available at the time of printing.

EDUC2
MICRORNAS AS NEW PLAYERS OF INFLAMMATION

B. Mari, R. Rezzonicco, L. E. Zaragosi, N. Pottier, B. Marcet, B. Chevalier, T. Bertero, M. P. Puisségur, S. Grosso, K. Robbe-Sermesant, K. Lebrigand, G. Rios, S. Fourre, V. Magnone, G. Ponzio, R. Waldmann, P. Barbry

CNRS & University of Nice Sophia-Antipolis, Institut de Pharmacologie Moléculaire et Cellulaire, UMR6097, Sophia Antipolis, France

MicroRNAs (miRNAs) regulate gene expression at a post-transcriptional level by binding to complementary target mRNAs. They are involved into many aspects of immune system development, from hematopoiesis to activation of innate and adaptive immune response to infection. They also mediate complex multi-cellular regulations, for instance during regeneration of airway and skin *epithelia*. Epithelial–mesenchymal interactions, which are critical during vertebrate embryo development, and for maintenance of homeostasis in adult tissues, are modulated by miRNAs. Thus, miR-155 is induced in human fibroblasts by inflammatory cytokines TNF-α and IL-1β while it is down-regulated by TGF-β. Keratinocyte growth factor (KGF, FGF-7), a member of the fibroblast growth factor (FGF) family, was established as a target in human lung fibroblasts. In vivo experiments using a mouse model of lung fibrosis have shown that miR-155 expression level is indeed correlated with the degree of lung fibrosis. miRNA dysregulation is also central to the development and pathophysiology of many cancers. We have recently highlighted the importance of miR-210 and of its transcriptional regulation by the transcription factor Hypoxia-Inducible Factor-1 (HIF-1) in lung cancer. Over-expression of miR-210 at late stages of Non-Small Cell Lung Cancer causes an alteration of cell viability, a loss of mitochondrial membrane potential and the apparition of an aberrant mitochondrial phenotype. Expression profiling of cells

over-expressing miR-210 reveals a specific signature that includes several subunits of the Electron Transport Chain (ETC) complexes I and II. The transcript coding for one of these ETC components, SDHD, a subunit of Succinate Dehydrogenase (SDH) is a validated target for miR-210, and *SDHD* knockdown mimics miR-210-mediated mitochondrial alterations. Finally, miR-210-dependent targeting of SDHD was able to activate HIF-1, in line with previous studies linking loss-of-function SDH mutations to HIF-1 activation. miR-210 can thus regulate mitochondrial function by targeting key ETC components genes with important consequences on cell metabolism, survival and modulation of HIF-1 activity. We will discuss our current understanding of these general regulatory mechanisms, focusing on their involvement in inflammation.

Triboulet et al. Science. 2007;315:1579–82.
Pottier et al. PLoS One. 2009;4(8):e6718.
Le Brigand K, et al. Bioinformatics. 2010;26:3131–2.
Puisségur et al. Cell Death Differ. 2010.
Brest et al. Nat Genet. 2011.

SYMPOSIUM

News and goods to map and treat inflammatory bowel disease (SY01)

SY-01
MAPPING THE GENES FOR IBD

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[1]INSERM, [2]Université Paris Diderot, Hôpital Robert Debré, Paris, France

The genetic component of Inflammatory Bowel Diseases (IBD) is among the best known for complex genetic disorders. The role of genes in Crohn's Disease (CD) appears to be stronger than in Ulcerative Colitis (UC) as demonstrated by the percentages of familial cases [8–10% (CD) vs. 6–8% (UC)] and the concordance rates in monozygotic twins [20–50% (CD) vs. 2–6% (UC)]. Gene hunting was rarely fruitful in the past when using the functional candidate gene approach (e.g. HLA genes in UC), or linkage studies (e.g. NOD2 and SLC22A4/5 in CD). Conversely, the genome wide association studies (GWAS) have now provided more than 70 susceptibility genes for CD and more than 40 for UC in few years. These genes define some key biological functions like innate immunity and autophagy for CD and Th17 T-cell orientation for both diseases. However, despite of these important successes, GWAS failed to explain more than 25% of the heritability of IBD suggesting that the additional genetic variations, gene–gene or gene–environment interaction and stochastic events may play a role in the predisposition to IBD.

SY-02
NOD PROTEINS CONTROL EARLY INFLAMMATORY RESPONSES AT THE INTESTINAL MUCOSA IMPORTANT FOR PATHOGEN CLEARANCE

*Kaoru Geddes[1], Stephen J. Rubino[2], Joao G. Magalhaes[1], Catherine Streutker[3], Lionel Le Bourhis[1], Kyle Cho[1], Stephen E. Girardin[2], Dana J. Philpott[1,]**

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Nod1 and Nod2 are pattern recognition receptors of the innate immune system that detect peptidoglycan (PG) from the bacterial cell wall and trigger inflammation. Although both of these members of the Nod-like receptor (NLR) family respond to PG they respond to distinct substructures of this complex microbial-associated molecular pattern (MAMP). Indeed, Nod1 recognizes diaminopimelic acid-containing tripeptide fragments of PG, found mainly in Gram-negative bacteria, while Nod2 detects muramyl dipeptide, which is the minimal fragment of PG common to both Gram-negative and Gram-positive organisms. Detection of these muropeptides by Nod1 and Nod2 triggers a signal transduction cascade that culminates in the activation of NFκB and the production of pro-inflammatory mediators. Importantly, Nod1 and Nod2 have been implicated in inflammatory bowel disease (IBD), in particular Nod2 has been associated with Crohn's disease, yet a clear understanding of how dysfunctional Nod activation leads to aberrant inflammation is still lacking. While much of our previous work has focused on dissection of Nod signalling in in vitro and ex vivo models, our current interest is to understand how Nods function at the mucosal surface of the gastrointestinal tract and how they orchestrate inflammation and combat enteric infection. Specifically, we are interested in using bacterial colitis models, including the *Citrobacter rodentium* and streptomycin-treated *Salmonella typhimurium* models to probe Nod responses at the level of the intestinal mucosa. Our overall goal is to understand how Nod activation orchestrates mucosal responses and to delineate the functional importance of Nod triggering in order to gain a more complete understanding of their role in IBD.

SY-03
CAN WE PLAY WITH GUT MICROBIOTA TO TREAT IBD?

P. Langella[1,], L. Bermudez-Humaran[1], B. Pigneur[3], M. A. Maubert, F. Dumetz[2], F. Chain[1], C. Bridonneau[1], S. Hudault[1], J. Doré[2], H. Sokol[3], P. Seksik[3]*

A high decrease in the abundance and biodiversity of *Firmicutes* (one of two main phyla of the normal human gut microbiota) in Crohn's disease (CD) patients was previously described [1, 2]. *Faecalibacterium prausnitzii* is one of the dominant species of the *Firmicutes* phylum in the human gut. We first determined the composition of the mucosa associated microbiota of ileal CD patients at the time of surgical resection and 6 months later. We found that a proportion of *F.*

prausnitzii on resected ileal Crohn's mucosa was associated with a higher risk of endoscopic postoperative recurrence at 6 months. We thus hypothesized that *F. prausnitzii* could display anti-inflammatory effects. We thus analyzed the anti-inflammatory effects of *F. prausnitzii* in both in vitro and in vivo models. In vitro PBMCs stimulation by *F. prausnitzii* led to significantly lower IL-12 and IFNγ production levels and higher secretion of IL-10 [3]. Oral administration of either live *F. prausnitzii* or its supernatant markedly reduced the severity of TNBS colitis and tended to correct the dysbiosis associated with TNBS colitis. These anti-inflammatory effects are most probably partly due to secreted metabolites able to block NF-κB activation and IL-8 production. We are now evaluating whether these properties are or not a general trait of *F. prausnitzii* species by isolating and testing other strains. In order to decipher the interactions between *F. prausnitzii* and the host, animal models with simplified microbiota are actually developed. We are also trying to identify the anti-inflammatory molecules in the supernatant of *F. prausnitzii* and the bacterial genes encoding these molecules. Finally, this knowledge might be useful for the application of probiotics or pharmacological molecules in CD for which the current treatments are poorly effective and merely aimed at maintenance of the disease. Counterbalancing dysbiosis using *F. prausnitzii* as a probiotic is thus a promising strategy in CD treatment.

1. Sokol et al. Inflamm Bowel Dis. 2006;12:106–11.
2. Manichanh et al. Gut. 2006;55:205–11.
3. Sokol et al. Proc Natl Acad Sci USA. 2008;105(43):16731–6.

SY-04
NEW TARGETS TO TREAT IBD

Desreumaux[1,]*

[1]Lille, France

Abstract not available at the time of pricing

OC-001
PHOSPHOINOSITIDE 3-KINASE P110{GAMMA} IS REQUIRED FOR BACTERIAL CLEARANCE AND THE RESOLUTION OF EXPERIMENTAL COLITIS

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Adipokines new kids on the inflammation block (SY02)

SY-05
INFLAMMATION, ADIPOKINES AND OBESITY

K. Clement[1,]*

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The increase in circulating inflammatory factors found in obese subjects and the discovery of macrophage accumulation in human adipose tissue has opened a new field of research in obesity. This allowed reconsidering aspects of obesity physiopathology. The so called "low grade" inflammatory state is characterized by the moderate but chronic systemic rise of an increasing panel of molecules. A small or even modest reduction of weight, known to ameliorate obesity associated risks, significantly reduces circulating inflammatory markers. However knowledge regarding the molecules linking obesity to its complications is still lacking. White adipose tissue contributes significantly to low-grade inflammation in obesity. This tissue is composed of mature adipocytes, precursors (preadipocytes), endothelial cells, macrophages, mast cells, blood vessels, nerves and lymphatic and connective tissue. The phenotype, amount and biology of adipose tissue components are altered profoundly in human obesity. The evaluation of transcriptomic interactions characterizing the obese adipose tissue demonstrated the strong relationship linking inflammatory processes to extra cellular matrix remodelling components. Our group showed that interstitial fibrosis accumulates in obese adipose tissue as in many organs affected by low-grade inflammation in chronic diseases (i.e. liver, lung, kidney pathologies). In addition, to structural and biological alterations of adipose tissue, there are perturbations of its dialogue with central (i.e. brain) and peripheral organs (i.e. muscle, intestine, liver, bone, vessels) via multiple signals including inflammatory mediators. The aim of this lecture is to discuss up-to-date current knowledge regarding the importance of inflammation and adipose tissue remodelling especially in human obesity. A clinical angle will be taken.

SY-06
THE MULTIFACETED ROLE OF ADIPONECTIN

U. Müller-Ladner[1,], K. P. Frommer[1], C. Büchler[2], O. Distler[1], F. Meier, S. Gay[1], A. Schäffler[2], E. Neumann[1]*

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Adipose tissue is a structural component of many organs including skin, the intestine and the joints. The dominant cell type, the adipocyte but also other mesenchymal cells, produce highly bioactive substances, the so called adipokines or adipocytokines. Adipokines include a growing number of pluripotent factors such as adiponectin, resistin, leptin, and visfatin/PBEF. In addition, adipokines are able to actively modulate inflammation and the innate immune system. Besides stimulating IL-6 and MMP-3, **adiponectin** enhances RANKL expression in osteoblasts whereas OPG expression is inhibited. Recombinant human **resistin** increases the secretion of pro-inflammatory cytokines such as TNFα, IL-6 and IL-12 in murine and human macrophages. In addition, human endothelial cells are activated by recombinant human resistin leading to increased expression of endothelin-1 and several adhesion molecules as well as chemokines in these cells. **Visfatin** or pre-B cell colony enhancing factor (PBEF) activates human leukocytes and to induce co-stimulatory molecules on the cell surface and proinflammatory cytokines such as IL-1, TNF and IL-6 in monocytes. In addition, visfatin protects fibroblasts and neutrophils from apoptosis. Taken together, there is increasing evidence that adipokines produced by cells driving synovial pathophysiology actively take part in inflammatory, matrix destructive and fibrotic processes in rheumatic diseases.

SY-07
THE ROLE OF IMMUNE CELLS IN OBESITY

K. Lolmède[1], C. Duffaut[1], A. Bouloumié[1,]*

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Obesity is recognized as a low grade chronic inflammatory state. Several inflammatory factors are known to directly interact with insulin signaling pathway leading to insulin resistance. Such an effect is thought to contribute to the link between obesity and type 2 diabetes. Increasing evidences show that the adipose tissue (AT) itself is the site and the source of inflammation. Indeed, accumulation of innate and adaptive immune cells is observed in AT of various murine models of obesity (genetic and diet-induced obesity) as well as in human with increased adiposity index. Initial studies focused on adipose tissue macrophages (ATM). Characterization of human ATM (hATM) phenotypes and roles showed that hATM impact hAT remodeling, including adipocyte differentiation, angiogenesis and fibrosis. Through these local effects, ATMs may limit the AT capacity to buffer the toxic fatty acids into neutral triglycerides in adipocytes and thereby contribute to the ectopic lipid deposit into metabolic active tissues leading to insulin resistance. Kinetics studies of immune cells trafficking in AT clearly showed that T lymphocytes accumulate in mice AT before ATMs. The proportion of adipose tissue lymphocytes (ATL) subsets, including regulatory T lymphocytes, Th1 helper and cytotoxic T cells, are modulated with AT development. Analysis of the role of hATLs showed that hATL-derived secretions including interferon interfered with insulin-mediated up-regulation of the key lipogenic enzymes in adipocytes. Therefore, accumulation and activation of ATLs appears to be the primary event involved in obesity-associated inflammation and several recent evidences suggest that such an event might be related to cell-mediated immune response.
Disclosure of interest: None declared.

OC-002
SERUM ADIPONECTIN LEVELS PREDICT RADIOGRAPHIC JOINT DAMAGE IN EARLY RHEUMATOID ARTHRITIS: RESULTS FROM THE FRENCH ESPOIR COHORT

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OC-003
EVALUATION OF ANTI-TNF-ALPHA ANTIBODY EFFECTS UPON ADIPOKINE PRODUCTION AND INTESTINAL INFLAMMATION IN EXPERIMENTAL COLITIS

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OC-004
EXPERIMENTAL FOOD ALLERGY LEADS TO ADIPOSE TISSUE INFLAMMATION AND WEIGHT LOSS IN MICE

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New perspectives on T cells in inflammatory diseases (SY03)

SY-08
INTESTINAL MICROBIOTA AND THE DEVELOPMENT OF PRO-INFLAMMATORY IMMUNITY

G. Eberl[1,]*

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The mammalian intestine provides a unique niche for a large community of bacterial symbionts that complements the host in digestive and anabolic pathways, as well as in protection from pathogens. Only a few bacterial phyla have adapted to this predominantly anaerobic environment, but hundreds of different species create an ecosystem that affects many facets of the host's physiology. Recent data show how particular symbionts are involved in the maturation of the immune system, in the intestine and beyond, and how dysbiosis, or alteration of that community, can deregulate immunity and lead to immunopathology. The extensive and dynamic interactions between the symbionts and the immune system are key to homeostasis and health, and require all the blends of so-called regulatory and pro-inflammatory immune reactions. Unfortunately, pro-inflammatory immunity leading to the generation of Th17 cells has been mainly associated with its role in immunopathology. Here I discuss the view that the immune system in general, and type 17 immunity in particular, develop to maintain the equilibrium of the host with its symbionts.

SY-09
REGULATORY T CELLS AND TH17 MEDIATED INFLAMMATION

A. Y. Rudensky[1,]*

[1]Immunology Program and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Regulatory T lymphocytes (Treg cells) play a critical role in immune homeostasis by opposing immune-mediated inflammation in the context of autoimmunity, responses to infection, cancer, allergy, and interactions with commensal microbiota at the environmental interfaces. Transcription factor Foxp3 orchestrates differentiation and

function of these cells. Our studies suggest that Foxp3 cooperates with other regulators of gene expression and co-factors to ensure the maintenance of its own expression and Treg cell suppressor function under basal and inflammatory conditions. In this regard, the ability of Treg cells to suppress Th17 mediated inflammatory responses in the intestines is dependent upon IL-10 receptor mediated activation of transcription factor Stat3 and its cooperation with Foxp3.

SY-10
EFFECTOR AND REGULATORY T CELLS IN INFLAMMATORY DEMYELINATING DISEASES OF THE CENTRAL NERVOUS SYSTEM

R. Liblau[1,*]

[1]*INSERM U1043, CNRS UMR5282, Université Paul Sabatier, Toulouse, France*

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the brain and spinal cord leading to damage of nerve fibers resulting in neurological impairment and deficits.
The cause of MS is still mostly unknown. Demyelination in the central nervous system in MS is thought to be mainly mediated by T helper 1 cells, although this dogma is currently heavily discussed since recent evidence suggests an active pathogenic role of T helper 17 and CD8 T cells in MS, which recapitulates earlier suggestions that virus may have pathogenic role in MS. The contribution of regulatory CD4 Foxp3+ T cells in the control of the disease is also controversial. Currently the unraveling of immune-mediated pathogenesis in MS relies mostly on animal models. So far there is not one animal model that reflects all aspects seen in MS, but still the many different models are very suitable for targeting specific aspects of this disease. By using rodent models we pinpointed several cellular and molecular pathways of potential relevance for human inflammatory diseases of the central nervous system. The lessons learned from these models will be discussed.

OC-005
TREGITOPES AND TOLERANCE: HARNESSING REGULATORY T CELLS TO SUPPRESS INFLAMMATION

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OC-006
A CRITICAL ROLE FOR NF-KB1 IN THE PRODUCTION OF GM-CSF BY ACTIVATED CD4 T CELLS DURING INFLAMMATORY RESPONSES

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OC-007
ALTERNATIVELY SPLICED IL-4 PROTEIN IS NATURALLY PRODUCED IN HUMANS AND CAUSES IMMUNE INFLAMMATION IN VIVO

See Oral Communication Session section for abstract page 36

Cell death (SY04)

SY-11
APOPTOSIS, AUTOPHAGY AND INFLAMMATION

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Apoptosis is the most common form of physiological cell death and required for normal development and maintenance of tissue homeostasis, and its dysregulation is associated with various pathological conditions. In inflammation, for example, inhibition of apoptosis of inflammatory cells is a common phenomenon and essential to effectively fight against pathogens. On the other hand, specific tissue cells frequently undergo apoptosis in association with inflammatory responses that may cause or at least contribute to organ dysfunction. In contrast to apoptosis, autophagy is a cellular survival mechanism and usually induced when cells are exposed to stress. In infectious diseases, autophagy degrades intracellular pathogens and promotes cellular survival during pathogen invasion. Autophagy has also been shown to be involved in MHC class I and II antigen presentation, and may be required for the induction of self-tolerance. In addition, there is evidence that autophagy may provide negative feedback to dampen inflammatory signalling. Therefore, defects in autophagy may lead to autoimmunity. Moreover, in certain instances, induction of autophagy may promote cell death, including apoptosis, suggesting a cross-talk between apoptotic and autophagic pathways. Taken together, apoptosis and autophagy are critical to the overall fate of the cell and play important roles in immunity and inflammation.

SY-12
IMMUNOGENIC DEATH: CELL BIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

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Physiological cell death, which occurs as a continuous byproduct of cellular turnover, is non-immunogenic or even tolerogenic, thereby avoiding autoimmunity. However, cancer cell death elicited by radiotherapy and some chemotherapeutic agents such as anthracyclines and oxaliplatin can be immunogenic. Immunogenic death involves changes in the composition of the cell surface, as well as the release of soluble immunogenic signals that occur in a defined temporal sequence. This 'key' then operates on a series of receptors expressed by dendritic cells (DC, the 'lock') to allow for the presentation of tumor antigens to T cells and for the initiation of a productive immune response. Immunogenic cell death is characterized by the early cell surface exposure of calreticulin, which determines the uptake of tumor antigens by DC. The late release of the protein high mobility group box 1 (HMGB1), which acts on toll-like receptor 4 (TLR4), is required for the presentation of antigens from dying tumor cells. In addition, the release of ATP from dying cells causes the P2RX7 purinergic receptor-dependent activation of the NLRP3 inflammasome in DC, thereby allowing them to release interleukin-1ß and to polarize tumor antigen-

specific CD8 T cells towards a Tc1 cytokine pattern. We postulate that the immune system determines the long-term success of anti-cancer therapies, and that this immune response is dictated by immunogenic tumor cell death. Thus, therapeutic failure can result from failure to undergo immunogenic cell death (rather than cell death as such). Thus, agents that fail to induce immunogenic cell death cannot yield a long-term success in cancer therapy. Moreover, tumors that are intrinsically unable to undergo immunogenic cell death are incurable. Importantly, it appears that mitochondrial events determine whether cancer cells die or not in response to chemotherapy, while an endoplasmic reticulum stress (ER) response combined with autophagy determines whether this cell death is perceived as immunogenic. We suggest a series of strategies to restore the immunogenicity of cell death in the context of deficient ER stress and autophagy.

SY-13
LINEAR UBIQUITINATION PREVENTS INFLAMMATION AND REGULATES IMMUNE SIGNALLING

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Members of the Tumour Necrosis Factor (TNF) receptor superfamily have important functions in immunity and inflammation. Recently linear ubiquitin chains, assembled by a complex containing HOIL-1 and HOIP, were implicated in TNF signalling, yet their in vivo relevance remained uncertain. We identified Sharpin as a third component of the linear ubiquitin chain assembly complex, recruited to the CD40 and TNF receptor signalling complexes together with its other constituents, HOIL-1 and HOIP. Mass spectrometry of the native TNF receptor signalling complex revealed RIP1 and NEMO to be linearly ubiquitinated. Mutation of the Sharpin gene causes *chronic proliferative dermatitis* (*cpdm*) in mice characterised by inflammatory skin lesions and defective lymphoid organogenesis. Gene induction by TNF, CD40 ligand and interleukin-1β was attenuated in *cpdm*-derived cells which were rendered sensitive to TNF-induced death. Importantly, *Tnf* gene deficiency prevented skin lesions in *cpdm* mice. We conclude that by enabling linear ubiquitination in the TNF receptor signalling complex Sharpin interferes with TNF-induced cell death and, thereby, prevents inflammation. Our results provide in vivo evidence for the relevance of linear ubiquitination in preventing inflammation and regulating immune signalling.

OC-008
THE NUCLEAR PROTEIN MNDA MODULATES MCL-1 EXPRESSION AND NEUTROPHIL APOPTOSIS IN SEPSIS

See Oral Communication Session section for abstract page 37

OC-009
NUCLEOCYTOPLASMIC RELOCALIZATION OF THE PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) DURING DIFFERENTIATION IS ESSENTIAL FOR ITS ANTIAPOPTOTIC ACTIVITY IN MATURE NEUTROPHIL: IDENTIFICATION OF A NUCLEAR EXPORT SEQUENCE

See Oral Communication Session section for abstract page 37

OC-010
EFFECT OF NEUTROPHIL EXTRACELLULAR TRAPS ON DENDRITIC CELL MATURATION

See Oral Communication Session section for abstract page 37

Inflammatory cells: neutrophils and macrophages (SY05)

SY-14
NEUTROPHILS AS SHAPERS OF THE INNATE IMMUNE RESPONSE

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Historically, neutrophils have been considered as innate immune cells with an anti-infectious and pro-inflammatory function, due to their ability to phagocytose and to produce powerful antimicrobial peptides, proteolytic enzymes and reactive oxygen intermediates. Recently, however, unsuspected biological features of polymorphonuclear neutrophils have been uncovered. Indeed, it has been shown that in addition to their pivotal role in the defence against pathogens, neutrophils also display a high degree of plasticity as well as contribute to the control of adaptive immune responses. For instance, various experimental models have validated that, after migration to an infection site, neutrophils are stimulated by the causative agent to produce not only proinflammatory cytokines such as tumour necrosis factor-α (TNFα), but chemokines: initially, CXCL chemokines such as CXCL1, CXCL2, CXCL3 and CXCL8 which are chemo-attractive for neutrophils, followed by a second wave of selected CCL chemokines, for instance, CCL3, CCL4 and CCL20, that serve an instrumental role in

recruiting the required leukocyte subtypes necessary for the subsequent phases of response to infection. In addition, neutrophils were recently demonstrated to directly interact with immune and non-immune cell types, including dendritic cells (DC), NK cells, iNKT cells, polarized T cells and mesenchymal cells, a feature which greatly emphasizes their capacity to contribute as orchestrators of innate and adaptive immunity. All these novel aspects of the biology of neutrophils will be exhaustively covered, with particular emphasis on the more recent advances.
Disclosure of interest: None declared.

SY-15
HOW HUMAN NEUTROPHILS KILL INGESTED MICROBES

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Human polymorphonuclear neutrophils (PMN) represent an essential cellular component of innate host defense against infection. Upon engagement with a suitably opsonized microbe, PMN ingest and confine the organism in a specialized compartment, the phagosome, where cytotoxins can be delivered. Concomitant with phagocytosis, there is coordinated assembly and activation of the NADPH oxidase, the source of oxidants to fuel microbial killing, and fusion of PMN granules, a source of a host of proteolytic enzymes and antimicrobial proteins. Prominent among the granule proteins delivered to the phagosome is myeloperoxidase (MPO), a hemeprotein uniquely able to generate hypochlorous acid (bleach), a potent antimicrobial agent, in the presence of H_2O_2. Although optimal PMN microbicidal activity relies on MPO-mediated production of HOCl and its capacity to modify microbial targets, efficient killing and degradation of ingested organisms reflects the coordinated activities of many bioactive species. The mechanisms underlying the generation and antimicrobial action in phagosomes will be highlighted, as will specific synergies between soluble circulating proteins and PMN responses that collaborate to eradicate invading microbes. Lastly, it is critical to recognize that many ingested microbes immediately sense and respond transcriptionally to the cytotoxins present in the phagosome. Successful microbial reactions allow ingested microbes such as staphylococci to survive and cause persistent infection. Only by understanding the molecular basis for the dynamic interactions between host and microbe will novel advances in antimicrobial therapy be made.

SY-16
MACROPHAGE HETEROGENEITY DURING INFECTIOUS DISEASES

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Tissue macrophages can change their physiology in response to a variety of endogenous and exogenous signals generated in tissue. Many inflammatory signals can promote a classical macrophage activation state, characterized by the production of myriad inflammatory cytokines. These classically activated macrophages are an important component of host defense, but they are also responsible for host tissue damage during autoimmune diseases. We and others have identified signals that can 'reprogram' macrophages to assume physiologies that are quite distinct from classically activated macrophages. We have identified a so-called "regulatory macrophage" that produces high levels of the anti-inflammatory cytokine, IL-10. These cells are potent down-regulators of innate and adaptive immune responses and they can prevent or reverse autoimmune pathology. The 'reprogramming' step involves the activation of the Mitogen-Activated Protein Kinase, ERK, which leads to histone phosphorylation and chromatin remodeling at the IL-10 promoter. Regulatory macrophages secrete high levels of IL-10 but little to undetectable amounts of IL-12. Work is on-going to identify the various signals that can reprogram these cells to become regulatory macrophages and to discover biochemical markers that are specific to regulatory macrophages. The manipulation of the various macrophage subpopulations may lead to new approaches to diminish autoimmune pathologies, or conversely to develop more effective immune responses during vaccination.

OC-043
A NOVEL CROSS-TALK IN RESOLUTION: H2S ACTIVATES THE ANNEXIN A1 PATHWAY

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OC-044
CELLULAR MECHANISMS AND MOLECULAR DETERMINANTS OF HUMAN MACROPHAGE 3D MIGRATION

See Oral Communication Session section for abstract page 49

OC-045
M-CSF IS NOT ESSENTIAL FOR B-1 CELL DERIVED PHAGOCYTE DIFFERENTIATION IN VIVO

See Oral Communication Session section for abstract page 50

Neuronal control of inflammation and pain (SY06)

SY-17
MICROBIOME—NERVOUS SYSTEM INTERACTIONS: IMPLICATIONS FOR GI DISEASE

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Inflammatory and Functional Bowel Diseases are associated with intestinal dysbiosis and are also associated with abdominal pain and

behavioral illness including depression. We used oral antimicrobial therapy to induce dysbiosis in mice and assessed visceral pain responses and alterations in brain chemistry and behavior. Antibiotic treatment reduced lactobacilli and this was accompanied by a small increase in MPO activity but no structural damage in the gut. In addition, there was an increase in substance pain and in the visceromotor response to colorectal distension. These responses were attenuated in mice receiving either *lactobacillus paracaseii* or dexamethsone concomitantly with antibiotics. Oral antimicrobial therapy also increased Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus and an increase in anxiolytic behavior based on latency to step-down and light box-dark box preference testing. Antimicrobial-induced BDNF and behavioral changes were independent of vagus nerve integrity or changes in circulating pro-inflammatory cytokines and were not seen in germ free mice. The existence of a microbiome-brain communication was confirmed in adoptive transfer experiments where behavioral traits of one murine strain were induced in germ free recipients of another strain, following colonization with cecal bacteria. These results indicate that the intestinal microbiome communicates with the host enteric nervous system to alter visceral pain responses by an inflammation-dependent mechanism, and with the brain to alter behavior by an inflammation-independent mechanism. These findings may explain the frequent co-existence of pain and altered behavior in functional and inflammatory GI diseases.

SY-18
NEW TARGETS FOR INFLAMMATORY PAIN: TRPV1 AND TRPA1

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Approximately 30% of arthritic patients consider that their medicines could be improved, especially in terms of pain relief. The work in this laboratory has concentrated on providing evidence for role of members of the transient receptor potential (TRP) family, especially TRPV1 and TRPA1 in murine models of inflammatory hyperalgesia. Our first study revealed a role for the TRPV1 receptor in attenuating thermal hyperalgesia and inflammatory swelling in a model of CFA-induced inflammation, in a situation where TNFα levels remained high. Interestingly, we also showed that capsaicin induced TRPV1-dependent vasoconstriction in the knee and the consequences of this are at present unknown. Further studies enabled a link between TNFα levels and TRPV1-dependent hyperalgesia to be made, a mechanism that is dependent on COX-2 and other cytokines, as well as TRPV1. More recently we have gained evidence that TRPA1 is also involved in mediating pain. In this case TRPA1 is important in mediating mechanical-induced hyperalgesia, an anti-hyperalgesic effect that appears independent of an effect on inflammation.

Keeble et al. Arthritis Rheum. 2005;52:3248–56.
Russell et al. Pain. 2009;142:264–74.
Fernandes et al. Arthritis Rheum. 2010 (in press)
Supported by Arthritis Research UK

SY-19
MIGRAINE: AN INFLAMMATORY DISORDER?

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Migraine is a highly prevalent chronic condition characterized by disabling attacks of pulsating head pain associated with nausea and vomiting, as well as other symptoms. The pathogenesis of migraine is still unknown, however, recent evidence supports the role of calcitonin gene-related peptide (CGRP) in the pathway which results in headache and the associated symptoms of migraine attack. The role of CGRP released from the central terminals of trigeminal neurons and other dorsal root ganglion neurons is still uncertain, whereas CGRP released from peripheral and perivascular endings of these neurons causes a marked and prolonged arterial vasodilatation. CGRP receptor antagonists have been found to ameliorate the migraine attack. It is possible that blockade of the hitherto not well defined central actions, or of the well known vasodilator effect of CGRP of the peptide, are key mechanisms for migraine treatment. A series of agents identified as triggers of migraine attacks have been found to release CGRP, and through this mechanism to produce meningeal vasodilatation. These include, among many other agents, ethanol, which acts via the sensitization of the transient receptor potential vanilloi-1 (TRPV1) channel, or ingredients of cigarette smoke via the TRPA (ankyrin)-1 channel. TRPA1 activation and CGRP release are also produced by umbellulone, a compound contained in *Umbellularia californica*, known also as the headache tree because, beginning first with the Native Americans, all the way through present times, the scent of its leaves has been recognized as a cause of headache. Sir Thomas Lewis in 1936 proposed that a pro-inflammatory substance was released from nocifensive neurons to produce vasodilatation and sensitization of these neurons to painful stimuli. Recent acquisition strongly suggests that this substance is CGRP, which via these, and possibly additional mechanisms, plays a major role in migraine mechanism.

OC-046
PROTEINASE ACTIVATED RECEPTOR-2 CAUSES A TRPV1-DEPENDENT SENSITISATION OF KNEE JOINT AFFERENTS IN NAIVE RATS

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OC-047
METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 7 AND 8 IN THE CENTRAL NUCLEUS OF THE AMYGDALA PLAY OPPOSITE ROLE IN INFLAMMATORY PAIN CONDITIONS

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OC-048
ANTIGEN-DRIVEN T CELL RESPONSE INDUCES PERIPHERAL OPIOID-MEDIATED ANALGESIA IN MICE

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Rheumatoid arthritis: from pathophysiology to new targets (SY07)

SY-20
RHEUMATOID ARTHRITIS: A JOURNEY IN THE LIFE OF SYNOVIAL CELLS

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Synovial macrophages have been recognised in rheumatoid arthritis (RA) as major producers of proinflammatory cytokines, in particular of TNFa. On the other hand, the synovial fibroblasts have been considered for a long time as innocent bystanders or just as effector cells responding to the stimulation of immune cells. However, in recent years we and others have been showing that synovial fibroblasts (SF) are not only part of the innate immune system by the expression of Toll-like and NOD receptors, but also active and aggressively growing cells [1] participating in the spreading of the disease [2]. Since the RASF remain active after removing them from the inflammatory milieu we searched for endogenous factors and epigenetic mechanisms of regulation [3]. In this regard we could show that RASF are hypomethylated cells residing in a hyperacetylated synovial tissue and are characterized by a distinct pattern of miRNA expression [4].

1. Neumann E, Lefèvre S, Zimmermann B, Gay S, Müller-Ladner U. Trends Mol Med. 2010;16:458–68.
2. Lefèvre S, et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. Nat Med 2009;12:1414–20.
3. Karouzakis E, Gay RE, Gay S, Neidhart M. Epigenetic control in rheumatoid arthritis synovial fibroblasts. Nat Rev Rheumatol. 2009;5:266–72.
4. Stanczyk J, et al. Altered expression of miRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation. Arthr Rheum. 2010 (in press).

SY-21
TYROSINE PHOSPHORYLATION PATHWAYS IN AUTOIMMUNE INFLAMMATION

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Neutrophils are critical components of innate immunity but their improper activation may also lead to tissue damage during autoimmune inflammation. Integrins and immunoreceptors play critical roles in neutrophil activation, as well as in the neutrophil-mediated effector phase of various autoimmune inflammatory diseases. Our results indicate a surprising similarity between integrin and immunoreceptor signal transduction pathways in neutrophils and other cells. β_2 integrin-mediated neutrophil activation requires Src-family kinases, the ITAM-containing DAP12 and FcRγ adapter protein, the Syk tyrosine kinase and phospholipase Cγ2 (PLCγ2). The same molecules also appear to participate in β_2 integrin-mediated activation of macrophages. Furthermore, DAP12/FcRγ, Syk and PLCγ2 are also required for osteoclast development and osteoclast-mediated bone resorption. Taken together, a signal transduction pathway reminiscent of that utilized by classical immunoreceptors mediates non-immunoreceptor signaling processes such as integrin signaling or osteoclast development and function. To test whether the above pathway also participates in pathological processes, we tested the development of K/BxN serum transfer arthritis, an autoantibody-mediated arthritis model, in various genetically deficient mouse strains. Our results indicate that Src-family kinases, Syk and PLCγ2 are all required for the autoantibody-mediated effector phase of this disease. Components of this pathway may prove to be suitable targets of the pharmacological therapy of autoantibody-mediated diseases such as certain subsets of rheumatoid arthritis.

SY-22
THE EVOLUTION OF THE DISEASE-SPECIFIC IMMUNE RESPONSE IN (PRE)-ARTHRITIS: CAN WE IDENTIFY A MASTERSWITCH DETERMINING THE ONSET OF IMMUNE MEDIATED INFLAMMATORY DISEASES?

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Anti-citrullinated protein antibodies (ACPA) are a very distinctive feature of rheumatoid arthritis (RA) patients. The presence of these antibodies is highly predictive for both the development of RA and the extent of associated joint destruction. Recent evidence indicates that well-known genetic risk factors for RA: the HLA-DRB1 shared epitope (SE) alleles and the PTPN22 T-allele are predominantly associated with anti-CCP positive RA. These reports, together with the finding that ACPA can exacerbate arthritis in mice, suggest that anti-peptidylcitrulline immunity plays an important role in the pathogenesis of the disease. Studies investigating at which point in time ACPA first appear, have revealed that these antibodies can often be detected several years before disease onset. The mere presence of ACPA therefore does not appear to be sufficient to precipitate disease. An explanation for this observation could be that the anti-citrullinated protein immune response first needs to mature more fully, in the course of which ACPA could acquire distinct characteristics which are instrumental in mediating tissue damage. Taking into consideration that ACPA can be detected before the clinical diagnosis of RA, and that the presence of ACPA is strongly associated with disease progression, we hypothesized that epitope spreading, avidity maturation and/or isotype usage of the ACPA response may play a role in the evolution of the disease. Our data reveal that broadening of isotype-usage and Epitope spreading with recognition of more citrullinated antigens occurs before onset of RA and that immunological differences in these ACPA are associated with the future disease course.

OC-049
SYSTEMS APPROACH TO RHEUMATOID ARTHRITIS

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OC-050
SPECIFIC TARGETING OF IL-23 BY ACTIVE IMMUNIZATION IMPROVES INFLAMMATION AND DESTRUCTION IN A MURINE MODEL OF RHEUMATOID ARTHRITIS

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OC-051
GLPG0259, A SMALL MOLECULE INHIBITOR OF MAPKAPK5, DOSE-DEPENDENTLY REDUCES PRO-INFLAMMATORY CYTOKINES AND MMPS AND BLOCKS DISEASE PROGRESSION IN A MOUSE MODEL OF RHEUMATOID ARTHRITIS

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Cell signaling and communication (SY08)

SY-23
NOX FAMILY NADPH OXIDASES IN INFLAMMATION AND SIGNALING

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NOX enzymes are a gene family of reactive oxygen species (ROS)—generating NADPH oxidases. In mammals, 7 family members are known (NOX1-5, DUOX1,2). They transport electrons from cytosolic NADPH across the membrane and attach it to oxygen to form superoxide and down-stream ROS, including hydrogen peroxide. ROS are key players in inflammation, however in contrast with classical views, they have not only proinflammatory, but also antiinflammatory properties. I will discuss the two aspects. Concerning the proinflammatory properties of NOX-derived ROS and will discuss ROS-mediated neuroinflammation and -degeneration, commonly seen in a variety of diseases of the central nervous system, including Alzheimer's disease, ALS, stroke and epilepsy. Concerning the antiinflammatory properties of NOX-derived ROS, I will discuss hyperinflammation and increased autoimmune disease in patients with chronic granulomatous disease (CGD, a deficiency in NOX2 or subunits thereof), as well as in mouse models of CGD. I will then discuss redox-sensitive signaling pathways which might account for the complex role of NOX-derived ROS in inflammation.

SY-24
IKK/NF-KB SIGNALLING IN CHRONIC INFLAMMATION

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Intracellular signal transduction pathways activated downstream of pattern recognition receptors and cytokine receptors play a critical role for the regulation of immune and inflammatory responses. The NF-κB pathway is one of the most important signalling cascades activated by cytokine and innate immune receptors. NF-κB regulates cellular responses to infection, injury and other stressful conditions requiring rapid reprogramming of gene expression. NF-κB activation has been implicated in the pathogenesis of many diseases, particularly chronic inflammatory conditions and cancer. NF-κB activation is mediated by the IκB kinase (IKK), which consists of two catalytic subunits, IKK1 (IKKα) and IKK2 (IKKβ), and a regulatory subunit named NF-κB Essential Modulator (NEMO) or IKKγ. In vivo studies in genetic mouse models provided experimental evidence for the fundamental functions of NF-κB signalling in inflammation. Using tissue specific ablation of IKK subunits we have studied the role of NF-κB for the maintenance of immune homeostasis in epithelial surfaces. Epidermal keratinocyte specific blockade of NF-κB signalling caused strong inflammatory skin lesions resembling human psoriasis. Moreover, epithelial cell specific blockade of canonical NF-κB activation caused severe chronic inflammatory colitis. These results revealed that NF-κB signalling in epithelial cells plays a critical role for the maintenance of immune homeostasis in epithelial tissues. Our current experiments investigate the mechanisms by which NF-κB signalling acts in epithelial cells to regulate tissue homeostasis and prevent the pathogenesis of chronic inflammation. The results of our most recent studies will be discussed.

SY-25
NEW TARGETS IN INNATE IMMUNE SIGNALING FOR ANTI-INFLAMMATORY THERAPEUTICS

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In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple pathways involved in innate immunity. There are now 7 distinct receptor families that sense microbial products and in some cases products of inflamed tissues, and trigger the innate response, which includes induction of pro-inflammatory mediators as well as effector mechanisms in host defence. The best characterised are the Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). Genetic variation in several of these components has been linked to inflammatory diseases, notably in the TLR system and in the NLR protein Nalp3 and associated proteins. Work on knockout mice and the use of inhibitors continues to validate some of these proteins in disease. From work on Nalp3 there has also been a resurgence of interest in the IL1 system as a key driver of inflammation in diseases such as gout and diabetes (both Type I and Type II). IL1 family members such as IL1beta, IL1alpha, IL18 and IL33 are providing important information on the role of Th17, Th1 and Th2 cells in disease. For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes. We now have a good understanding of the

major components activated by TLRs, notably the TIR domain-containing adapters that initiate signalling following recruitment to TIR domains within the TLRs themselves, the IRAK family of protein kinases that are then recruited, and a series of ubiquitination and phosphorylation reactions that ultimately lead to the activation of transcription factors such as NF-kappaB and IRF family members. Furthermore inhibitory mechanisms are also being revealed that are likely to important for resolution of inflammation, notably miRNAs such as miR21. As we continue to unravel the molecular details of these processes, new therapeutic options will present themselves.

OC-052
ARACHIDONIC 5-LIPOXYGENASE ACTIVITY IS SUBJECT TO EPIGENETIC/ENVIRONMENTAL CONTROL IN INFLAMMATORY CELL POPULATIONS

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OC-053
INTERLEUKIN (IL)-31 ACTIVATES SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION FACTOR (STAT)-1/5, AND EXTRACELLULAR SIGNAL REGULATED KINASE (ERK) 1/2 AND MODULATES IL-12 PRODUCTION IN HUMAN MACROPHAGES FOLLOWING STIMULATION WITH STAPHYLOCOCCAL EXOTOXINS

See Oral Communication Session section for abstract page 53

OC-054
A TRIF-DEPENDENT NEGATIVE REGULATORY PATHWAY ORCHESTRATES SEPSIS ASSOCIATED ENDOTOXIN TOLERANCE IN MICE AND HUMANS

See Oral Communication Session section for abstract page 53

Resolution and inflammatory concert (SY09)

SY-26
THE LIPID MEDIATOR ORCHESTRA DURING INFLAMMATION-RESOLUTION

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Mechanisms controlling resolution of acute inflammation are of wide interest. The resolvins and other pro-resolving mediators are biosynthesized from polyunsaturated essential fatty acids in the resolution phase to promote resolution and homeostasis (Serhan in Am J Path 2010). In this presentation, we will review recent evidence for the role of microRNAs (miRNAs) in self-limited acute inflammatory exudates, their regulation by resolvin D1 (RvD1), and the resolvin D1 GPC receptors identified in murine and human systems. Using real-time PCR analysis, we report that in resolving exudates that produce RvD1 from DHA miR-21, miR-146b, miR-208a, miR-203, miR-142, miR-302, and miR-219 are selectively regulated ($P < 0.05$) in self-limited murine sterile peritonitis. RvD1 (300 ng/mouse) reduced zymosan-elicited neutrophil infiltration 25–50% into the peritoneum and shortened the resolution interval (R_i) by $\sim$4 h. In murine peritonitis at 12 h, RvD1 up-regulates miR-21, miR-146b, and miR-219 and down-regulates miR-208a in vivo. With human macrophages overexpressing recombinant RvD1 receptors that include both ALX/FPR2 and GPR32, these miRNAs were regulated ($P < 0.05$) by RvD1 at concentrations as low as 10 nM. Hence, isolated human macrophages exposed to RvD1 recapitulate the in vivo circuit obtained during the resolution phase of murine peritonitis. In addition, RvD1-miRNAs identified in these studies target cytokines and proteins involved in the immune system, e.g. miR-146b targeted NF-κB signaling, and miR-219 targeted 5-lipoxygenase reducing leukotriene production. These results indicate that resolvins regulate specific miRNAs target genes that are involved in resolution. Moreover, they establish a novel resolution circuit involving RvD1 receptor-dependent regulation of specific miRNAs in vitro and in vivo, which demonstrate the ability of pro-resolving GPCRs to regulate miR that impact inflammation-resolution.

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COI statement: CNS is inventor on patents assigned to BWH and Partners HealthCare on the composition of matter, uses, and clinical development of anti-inflammatory and pro-resolving lipid mediators and relates compounds. These are licensed for clinical development. Lipoxins to Bayer HealthCare and resolvins to Resolvyx Pharmaceutical. C.N.S. retains founder stock in Resolvyx.

SY-27
GLUCOCORTICOIDS CUT SHORT THE CONCERT OF INFLAMMATION THROUGH THE RELEASE OF ANNEXIN A1

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The process of acute inflammation relies on the active engagement of a series of pro-resolving mediators to assure its temporal and spatial completion. Within the ever-increasing group of players of the resolution of inflammation, a special place is occupied by cortisol. Appreciation of the role of cortisol in preventing the over-shooting of the host response to inflammation (Munck's theory) provides strong scientific bases to the widespread application of synthetic glucocorticoids in several pathologies. Thus, the striking efficacy of glucocorticoids suggests that mimicking a pathway operative in the resolution of inflammation is a viable approach for anti-inflammatory therapy in man. Can we harness this knowledge any further? Glucocorticoids modulate, among many others, the protein termed Annexin A1 (AnxA1) as well as its receptor, termed FPR2/ALX. In fact, rapid (non-genomic) mobilization of AnxA1 and gene induction of FPR2/ALX are observed in myeloid cells and become operational to affect

major processes of the resolution of inflammation, including inhibition of white blood cell recruitment and efferocytosis. In view of this 'duet' glucocorticoids and AnxA1/FPR2, we propose that detailed understanding of how the AnxA1/FPR2 pair impacts on experimental inflammation, and human cell biology, can provide novel indication for drug discovery programmes. Such a proposition is reinforced by the notion that potent anti-inflammatory lipids of the lipoxins and resolvins families can also signal via FPR2/ALX. Proof-of-concept studies in Fpr2 deficient mice reiterate the validity of this approach.

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SY-28
THE CHIMERIC NATURE OF CHEMERIN IN THE SYMPHONY OF INFLAMMATION: ANTI-INFLAMMATORY EFFECTS OF CHEMERIN-DERIVED PEPTIDES

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Acute inflammation is characterized by the activation and recruitment of innate immune cells. The recruitment of neutrophils and monocytes to sites of inflammation is orchestrated by a range of inflammatory mediators including chemoattractant cytokines. Failure to properly resolve acute inflammation leads to chronic inflammation, the pathological process that underlies many important diseases including angina, arthritis and inflammatory bowel disease. Work in the Greaves Lab has shown that the macrophage chemoattractant cytokine chemerin (TIG2) acting through G protein coupled receptors (GPCRs) including ChemR23 (CMKLR1) can mediate macrophage chemotaxis and adhesion to the cell adhesion molecules VCAM-1 and ICAM-1 and the extracellular matrix molecule Fibronectin. While full-length chemerin is a potent mediator of macrophage chemotaxis we have shown that proteolytic digestion fragments of chemerin can act as potent anti-inflammatory mediators that enhance macrophage uptake of microbial debris and apoptotic cells in vitro and in vivo. Importantly we have shown that polyclonal antibodies that specifically block the chemoattractant effects of full length chemerin and the anti-inflammatory effects of chemerin-derived C-terminal peptides lead to *increased* neutrophil and monocyte recruitment in a self resolving model of zymosan peritonitis.

Cash JL, Hart R, Russ A, Dixon JP, Colledge WH, Doran J, Hendrick AG, Carlton MB, Greaves DR. Synthetic chemerin-derived peptides suppress inflammation through ChemR23. J Exp Med. 2008;205:767–75.

Cash JL, Christian AR, Greaves DR. Chemerin peptides promote phagocytosis in a ChemR23- and Syk-dependent manner. J Immunol. 2010;184:5315–24

OC-087
ANTI-ALLERGIC CROMONES INHIBIT HISTAMINE AND EICOSANOID RELEASE FROM HUMAN MAST CELLS THROUGH AN ANNEXIN 1 DEPENDENT MECHANISM

See Oral Communication Session section for abstract page 65

OC-088
SATIATED-EFFEROCYTOSIS: A NOVEL FUNCTIONAL PROPERTY FOR RESOLUTION-PHASE MACROPHAGES REGULATED BY RESOLVINS, GLUCOCORTICOIDS, AND THE CHEMOKINE-SCAVENGING RECEPTOR D6

See Oral Communication Session section for abstract page 65

OC-089
EOSINOPHILS CONTRIBUTE TO THE RESOLUTION OF ACUTE PERITONITIS THROUGH THE 12/15-LIPOXYGENASE PATHWAY

See Oral Communication Session section for abstract page 66

Damps and pamps; critical endogenous and exogenous danger signals (SY10)

SY-29

HMGB1 IS A MULTI-FACETED DANGER SIGNAL

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HMGB1 is the prime example of a danger signal that originates from the damaged self rather than from invading pathogens. HMGB1 is passively released by cells that die traumatically, and actively secreted by severely distressed cells and by activated cells of the innate immunity system. HMGB1 is thus the archetypal Damage Associated Molecular Pattern molecule, or DAMP. Notably, HMGB1 signals via TLR4, the receptor for LPS, the archetypal Pathogen Associated Molecular Pattern molecule. In addition, HMGB1 forms highly inflammatory complexes with single-stranded DNA, LPS, IL-1β and nucleosomes, which interact with TLR9, TLR4, IL-1R and TLR2 receptors, respectively. The ability to act both alone and in combination with other inflammatory signals may be ideally designed to govern the choice between the sacrifice or reconstruction of tissues, as required by the presence or absence of pathogens.

SY-30
COUPLING OF DEAD CELL RECOGNITION TO ADAPTIVE IMMUNITY BY DENDRITIC CELLS

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Cell death can be sensed by dendritic cells (DC) and contribute to adaptive immunity against cell-associated antigens. We have been studying recognition pathways that allow DC to sense dead cells and present cell-associated antigens to T cells. One pathway involves the

C-type lectin DNGR-1, also known as CLEC9A, which signals via Syk kinase. DNGR-1 is selectively expressed by CD8α^+ DC in mouse and their equivalents in human, a sub-group of DC that possesses specialised properties including a unique propensity to phagocytose dead cell debris and to crosspresent exogenous antigens to $CD8^+$ T cells. DNGR-1 signalling via Syk in CD8α^+ mouse DC appears to regulate the retrieval and crosspresentation of dead cell-associated antigens and may also be involved in the activation of DC by dead cells. The study of DNGR-1 helps build a picture of the receptors and signalling pathways that regulate DC responses to self alterations and has applications in immunotherapy of cancer and infectious diseases.

SY-31
DANGER SIGNAL ACTIVATING THE INFLAMMASOME IN PULMONARY INFLAMMATION AND FIBROSIS

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The molecular mechanisms by which lung injury triggers inflammation and fibrosis remain poorly understood. We reported that Nlrp3 inflammasome activation and IL-1R1 signaling were required for bleomycin-induced inflammation and fibrosis in mice. Now investigating endogenous molecules released after lung injury, we show uric acid and extracellular adenosine tri-phosphate (eATP) are danger signals activating the Nlrp3 inflammasome in lung inflammation and fibrosis. Uric acid and ATP are locally released upon bleomycin-induced injury and involved in IL-1β production, lung inflammation and fibrosis. Fibrotic patients have elevated ATP content in BALF in comparison to control individuals. Since uric acid or silica crystals and soluble ATP are involved both in Nlrp3 activation and lung fibrotic diseases, we investigated whether ATP and purinergic signaling may be a corner stone of Nlrp3 inflammasome activation by particulate activators. We show that uric acid and silica crystals induce the active release of intracellular ATP by epithelial cells or macrophages in vitro. Moreover, IL-1β maturation and secretion by macrophages upon crystals depend on purinergic signaling through autocrine purinergic receptor loops. Our results suggest that uric acid and silica crystals are competent to induce maturation and secretion of IL-1β through a process that involves as a first event the extracellular release of endogenous ATP and as a second event the signaling through several purinergic receptors that triggers the activation of the Nlrp3 inflammasome. In conclusion, we propose that blocking purinergic signaling could represent a novel strategy to reduce lung inflammation and fibrosis.

Keywords: Uric acid crystals, ATP, Danger signal, Nlrp3 Inflammasome, Purinergic signaling.

OC-090
ENTEROHEMORRHAGIC ESCHERICHIA COLI, A NON-CYTOSOLIC PATHOGEN ACTIVATING CYTOSOLIC NLRP3 AND AIM2 INFLAMMASOMES THROUGH RELEASE OF BACTERIAL PRODUCTS INTO THE CYTOSOL

See Oral Communication Session section for abstract page 66

OC-091
RECTIVE OXYGEN SPECIES-MEDIATED INFLAMMASOME ACTIVATION AND IL-1B ARE ESSENTIAL FOR HOST RESISTANCE TO DENGUE VIRUS PRIMARY INFECTION

See Oral Communication Session section for abstract page 66

OC-092
MSU CRYSTAL-RECRUITED NON-INFLAMMATORY MONOCYTES DIFFERENTIATE INTO M1-LIKE MACROPHAGES CAPABLE OF DRIVING INFLAMMATION IN A MODEL OF GOUT

See Oral Communication Session section for abstract page 67

Vasculitis-autoimmunity (SY11)

SY-32

TH17 AND TH1 T-CELL RESPONSES IN GIANT CELL ARTERITIS

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Giant cell arteritis (GCA) is an inflammatory blood vessel disease that primarily affects the aorta and its branches. Clinical manifestations include blindness, stroke, aortic aneurysm and failure-to-thrive. At a molecular level, GCA is characterized by the apparently unopposed stimulation of T cell immunity ordinarily mobilized to fight infection. The typical lesion is a granulomatous infiltrate that injures the blood vessel wall and leads to intimal hyperplasia and luminal occlusion. Early stages of the disease involve activation of wall-embedded dendritic cells which express a broad spectrum of pattern recognition receptors and respond to PAMPs and DAMPs. In humanized mice engrafted with human aorta and human T cells GCA can be elicited though initial stimulation with PAMPs. T cells recruited into the granulomatous lesions include Th1 and Th17 cells. The contribution of Th1 and Th17 cells to the disease process has been examined in patients undergoing two successive artery biopsies. Th17 cells are rapidly responsive to immunosuppressive therapy with corticosteroids whereas Th1 cells persist in the inflamed arteries despite continuous treatment. In treated patients vessel-wall inflammation represents a "pure" Th1 response. In untreated patients, antigen-presenting cells in the affected temporal arteries produce IL-12, IL-1b, IL-6 and IL-23. IL-12 production is resistant to corticosteroid therapy and promotes chronic vasculitis in the absence of Th17 responses.

Conclusion: Multiple T cell lineages contribute to vasculitis with Th1 and Th17 cells being separable based on their susceptibility to steroid-induced immunosuppression. We conclude that at least two immune-activation cascades are functional in GCA and propose that the disease instigators eliciting Th1 and Th17 immunity are distinct.

SY-33
PATHOPHYSIOLOGICAL MECHANISMS IN ANCA-ASSOCIATED VASCULITIS

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ANCA-associated vasculitis (AAV) is a complex disease with a strong underlying autoimmune diathesis. Its precise aetiology is unknown but contributions from heritable factors acting through a polygenic contribution from relatively common variants, and environmental factors, including drugs, silica and infectious agents, seems certain. These factors may be linked through epigenetic modification of histone marks. The pathogenic mechanisms that are triggered involve diverse cell types, inflammatory mediators and signalling cascades. Autoantibodies to proteinase 3, myeloperoxidase and LAMP-2 are described that can engage with their autoantigenic targets when expressed at the neutrophil surface membrane after neutrophil priming or within neutrophil extracellular traps (NETS). Autoantibodies can activate neutrophils and promote neutrophil-endothelial interactions that initiate vascular damage. Components of the alternative complement pathway are also implemented as injury enhancers. The adaptive immune system is believed to be fundamental to development of autoimmune responses in AAV. Multiple changes in circulating T cell populations have been described. The Th17 subset may contribute since there are increased serum IL-17 and IL-23 levels during acute disease, increased autoantigen-specific IL-17-producing cells during disease remission and in animal models of anti-myeloperoxidase glomerulonephritis, mice deficient in IL-17A are protected. Interest in B cells increased markedly after efficacy in AAV of B cell depletion was demonstrated. The precise role of B cells in vasculitis still needs to be clarified, whether as precursors to antibody-producing plasma cells, antigen presenting cells, providers of cytokines and growth factors or other roles. That BLyS levels are increased in patients with active ANCA vasculitis may also perpetuate autoimmune B cells. Translating increasing knowledge of pathogenesis into better therapies for patients with AAV remains the challenge.

The author is now Vice-President, ImmunoInflammation CEDD, GlaxoSmithKline.

SY-34
BIOTHERAPIES IN SYSTEMIC VASCULITIS

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Biotherapies now hold a specific place in the therapeutic armamentarium for systemic vasculitides. These therapeutics include cytokines, like (pegylated) alpha interferon for HBV-related polyarteritis nodosa and HCV-related cryoglobulinemic vasculitis, and polyvalent immunoglobulins (IVIg), with now quite well defined indications and awaited results, but also now some more specifically targeted monoclonal antibodies, like anti-TNF-alpha or anti-CD20 for ANCA-associated vasculitides, or anti-IL5 and anti-IgE for Churg-Strauss syndrome. However, the exact indications of these latter new agents, as well as their optimal dosage and duration, are not yet precisely defined. Therefore, at present, they are mostly prescribed for patients with disease refractory to conventional therapy, with promising results. Results of international ongoing trials will tell us in the few next years whether they may also have a place as first line treatments. The first results show that rituximab is as effective as cyclophosphamide (RAVE study, not published) to induce remission of Wegener's granulomatosis. Rituximab is now under evaluation for maintenance therapy. Other biotherapies have been evaluated. If etanercept does not seem effective, to maintain remission, infliximab is effective in severe and refractory ANCA-associated vasculitis. However relapses occur as soon the drug is stopped. It seems now reasonable to expect dramatic changes in the treatment of ANCA-associated vasculitis with a larger place for biotherapies.

OC-093
THE LEADING ROLE OF THE TARGET: ENDOTHELIAL CELLS FROM DIFFERENT TISSUES DIFFERENTIALLY INFLUENCE IMMUNE ACTIVATION

See Oral Communication Session section for abstract page 67

OC-094
A LOSS-OF-FUNCTION VARIANT OF THE ANTIVIRAL MOLECULE MAVS IS ASSOCIATED WITH A SUBSET OF SYSTEMIC LUPUS PATIENTS

See Oral Communication Session section for abstract page 67

OC-095
IFN ALPHA CAUSES SLE AND ITS IMPLICATION TO SELF-ORGANIZED CRITICALITY THEORY OF AUTOIMMUNITY

See Oral Communication Session section for abstract page 68

Osteoarthritis (SY12)

SY-35

OSTEOARTHRITIS, ANGIOGENESIS AND INFLAMMATION

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Angiogenesis is a hallmark of chronic inflammation. Blood vessel growth facilitates synovitis, and angiogenesis inhibitors can reduce

synovial inflammation in animal models of OA. Neovascularisation leads to ossification of articular cartilage and of chondro-osteophytes following growth factor expression by inflammatory cells within the subchondral bone. Inhibiting vascular growth or inflammation can each reduce structural progression in OA models. Inflammation contributes to the pain of OA, and angiogenesis may increase pain by exacerbating inflammation. Furthermore, angiogenesis leads to specific structural changes within the joint that may contribute to pain, including sensory nerve growth and reduced integrity of the osteochondral junction. In OA, fine, unmyelinated sensory nerve fibres grow alongside new blood vessels into structures that normally receive no innervation, including the non-calcified articular cartilage and the meniscal fibrocartilage. Determining specific molecular pathways of angiogenesis and inflammation in the OA joint should lead to targeted interventions with potential to improve symptoms and joint damage.

SY-36
THERAPIES IN OSTEOARTHRITIS

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Osteoarthritis is often a progressive and disabling disease, which occurs in the setting of a variety of risk factors—such as advancing age, obesity, and trauma—that conspire to incite a cascade of pathophysiologic events within joint tissues. An important emerging theme in osteoarthritis is a broadening of focus from a disease of cartilage to one of the 'whole joint'. The synovium, bone, and cartilage are each involved in pathologic processes that lead to progressive joint degeneration. Additional themes that have emerged over the past decade are novel mechanisms of cartilage degradation and repair, the relationship between biomechanics and biochemical pathways, the importance of inflammation, and the role played by genetics. In this review we summarize current scientific understanding of osteoarthritis and examine the pathobiologic mechanisms that contribute to progressive disease.

SY-37
STEM CELLS FOR REPAIR IN OA: DREAM OR REALITY?

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Abstract not available at the time of printing.

OC-096
CARTILAGE-SPECIFIC PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARΓ) DEFICIENT MICE EXHIBIT ACCELERATED CARTILAGE DESTRUCTION IN A MICE MODEL OF OSTEOARTHRITIS (OA)

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OC-097
IMPACT OF MECHANICAL LOADING OF OSTEOBLASTS ON THE CHONDROCYTES PHENOTYPE: A NOVEL MURINE MODEL FOR ASSESSING BONE/CARTILAGE COMMUNICATION

See Oral Communication Session section for abstract page 68

OC-098
ANTIINFLAMMATORY EFFECTS OF CO-RELEASING MOLECULE-2 IN OSTEOARTHRITIC SYNOVIOCYTES

See Oral Communication Session section for abstract page 69

Yes, we have new opportunities for anti-inflammatory drug discovery (SY13)

SY-38

ARE CYCLIN-DEPENDENT KINASE INHIBITOR DRUGS ANTI-INFLAMMATORY AND PRO-RESOLUTION AGENTS?

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Neutrophilic and eosinophilic granulocytes are key effector cells in host defence against invading bacteria and parasites; however over-recruitment, dysregulated activation and defective removal of these cells play a prominent role in the initiation and propagation of chronic inflammatory conditions such as asthma and COPD. Apoptosis of granulocytes occurs during the normal resolution process, renders these cells unresponsive to further stimulation and marks them for removal by phagocytes (e.g., macrophages) by mechanisms that switch the phenotype of macrophages from pro- to anti-inflammatory. Although there is little doubt that apoptosis plays a critical role in many physiological and pathological processes, its precise role in inflammatory diseases is only recently being unravelled. The findings that modulation of granulocyte apoptosis with pharmacological agents such as cyclin-dependent kinase (CDK) inhibitor drugs can precipitated inflammatory resolution by inducing inflammatory cell apoptosis and augmenting phagocytic removal of apoptotic cells will be discussed. We propose that by defining novel mechanisms that selectively accelerate neutrophil and eosinophil apoptosis together with those that promote phagocyte clearance of apoptotic granulocytes will provide the scientific foundation for innovative approaches to the therapy of human inflammatory diseases.

SY-39
TARGETING PROSTAGLANDIN E PRODUCTION AND SIGNALING: WHERE ARE WE IN 2011?

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PGE_2 is the dominant PG induced during the early phases of inflammation due to the dramatic increase in levels of cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1) in response to pro-inflammatory cytokines and other stimuli to the inflammatory program. Pharmacologic inhibition of COX-2 enzyme activity either by non-specific or COX-2-selective non-steroidal anti-inflammatory drugs (NSAIDs) effectively blocks up-regulated production of PGE_2. Blocking PGE_2 using NSAIDs also inhibits cytokine-stimulated up-regulation of mPGES-1 expression, thereby enhancing their anti-inflammatory effects. This mechanism proceeds via the interaction of PGE_2 and its EP2 and EP4 receptors and can be compensated by direct application of PGE_2, EP2 or EP4 agonists, or by activating cAMP using forskolin. In addition to classical anti-inflammatory effects, PGE_2 acting via the EP4 receptor modulates the immune response promoting changes in the cytokine profile of antigen presenting cells to facilitate the IL-23/IL-17 axis important for autoimmune inflammatory diseases. In addition to inhibition of PGE_2, NSAIDs also inhibit production of other PG, such as PGI_2 and PGD_2. Since these PG, especially PGI_2, contribute to inflammation and pain, NSAID therapeutic effectiveness could relate in part to this lack of specificity. However, lack of specificity towards pro-inflammatory cytokines undoubtedly contributes to adverse effects. Inhibition of PGI_2, for example, may contribute to thrombophilia and atherogenesis as suggested by murine models. At present, it is unclear if specific inhibitors of mPGES-1 or EP4 receptors will be effective anti-inflammatory, analgesic, and antipyretic agents in humans. Development of drugs that inhibit mPGES-1 are hampered by species differences in activity leading to difficulties in implementation of murine models. However, experiments in PGES-1 null mice demonstrate that this strategy may yield promising results different from inhibition of COX-2. For example, mPGES-1 genetic deletion has important effects on neurons and microglia in a model of neuropathic pain that appear to be mediated via COX-1 rather than COX-2. Furthermore, mPGES-1 null mice appear protected from atherogenesis. Alternate strategies for inhibiting PGE_2 activity have now focused on EP4 receptor antagonists. In addition to showing promise in some animal models of arthritis and pain, EP4 receptor antagonists appear to inhibit platelet aggregation. Together, these properties suggest that EP4 receptor antagonism is another potential new therapeutic strategy for inflammation and pain.

SY-40
"INSIDE-OUT" SIGNALING OF SPHINGOSINE-1-PHOSPHATE: THERAPEUTIC TARGETS

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Sphingosine-1-phosphate (S1P) is a potent sphingolipid mediator formed inside cells by phosphorylation of sphingosine catalyzed by two sphingosine kinase isoenzymes, SphK1 and SphK2. SphK1 is activated by many pro-inflammatory cytokines leading to formation of S1P, which is exported by specific transporters to activate its own receptors in an autocrine and/or paracrine manner, a process, known as "inside out signaling" by S1P, is important for many of the immune cell functions known to be regulated by S1P and the diversity of these functions is explained by the repertoire of S1PR expression on various immune cells. We have recently also identified important roles for intracellular S1P during inflammation. We showed that activation of SphK1 by the pleiotropic cytokine TNF-alpha and production of S1P is necessary for Lys 63-linked polyubiquitination of RIP1, phosphorylation of IKK and IkBalpha, and IkBalpha degradation, leading to NF-kB activation, independently of S1P receptors. S1P specifically binds to TRAF2, a key component in NF-kB signaling triggered by TNF-alpha and stimulates its E3 ligase activity (Alvarez et al., Nature 465: 1084, 2010). Thus, S1P is the missing co-factor for TRAF2 E3 ubiquitin ligase activity, suggesting a novel paradigm for regulation of Lys 63-linked polyubiquitination. This highlights the key role of SphK1 and its product S1P in TNF-alpha signaling and the canonical NF-kB activation pathway and provides a mechanistic explanation for the numerous observations of the importance of SphK1 in inflammatory, anti-apoptotic, and immune processes. This work was supported by grants from the NIH R37GM043880, R01CA61774, RO1AI500941, and U19AI077435.

OC-131

S100A9, A NEW POTENTIAL THERAPEUTIC TARGET IN CHRONIC INFLAMMATORY DISEASES

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OC-132

THE FIRST DEMONSTRATION OF CLINICAL ACTIVITY BY A SMALL MOLECULE SIRT1 ACTIVATOR: SRT2104 REDUCES CYTOKINE RELEASE AND COAGULATION ACTIVATION IN A HUMAN ENDOTOXEMIA MODEL

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OC-133

NEXT GENERATION THERAPEUTICS FOR THE TREATMENT OF RESPIRATORY DISEASES - DISCOVERY AND CHARACTERIZATION OF AN INHALABLE HIGHLY POTENT AND SPECIFIC ANTI-IL-4RA SMALL PROTEIN ANTAGONIST

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SOCIETY SPONSORED SYMPOSIA

New pro- and antiinflammatory effects of microbes (SP01)

Symposium sponsored by the Inflammation Research Network

SP-01
MECHANISMS OF INNATE IMMUNE RESPONSES TO INFLUENZA VIRUS

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Influenza A virus (IAV) is the etiological agent of a highly contagious acute respiratory disease that causes epidemics and considerable mortality annually. The viral replicative intermediate double-stranded RNA (dsRNA) is considered a potent activator of the innate anti-viral immune response. Bronchial epithelial cells are the primary target and the principal host for IAV. We previously used an in vitro approach to establish a role for the pattern-recognition Toll-like receptor (TLR)3 in the immune response of lung epithelial cells to IAV-derived dsRNA and we demonstrated that in vivo, IAV-TLR3 interaction critically contributes to viral pathology. However, additional cellular dsRNA-recognition proteins have been implicated as key sensors of viral infection. These include two caspase recruiting domain-containing RNA helicases, i.e. the retinoic acid-inducible gene I (RIG-I) protein and the melanoma differentiation-associated gene (MDA-5) protein. In this context, we further demonstrated that the sensing of IAV by TLR3, RIG-I and MDA-5 clearly differs. Indeed, TLR3 activation appears critical for the induction of a pro-inflammatory response whereas RIG-I (but not MDA-5) activation is rather essential for the induction of a type I interferon-dependent antiviral response. Finally, we established that the innate immune response triggered by IAV is negatively regulated by the Suppressor of Cytokine Signaling (SOCS) 1 and SOCS3 proteins through a RIG-I/IFNAR-dependent pathway.

SP-02
MECHANISMS OF LUNG INFLAMMATORY DISEASE INDUCED BY PATHOGENS

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The activity of innate immunity is not simply dictated by the presence of an antigen but also by the balance between negative regulatory and immune potentiator pathways. Even in the absence of an antigen, innate immunity can "inflame" if negative regulators are absent. This resting state is adaptable and dictated by environmental influences, host genetics and past infection history. A return to homeostasis post inflammation may therefore not leave the tissue in an identical state to that prior to the inflammatory event. We show that respiratory pathogens cause long term modification of the lung microenvironment by a de-sensitisation to bacterial products and an increase in the myeloid negative regulator CD200R. These two events prevent subsequent inflammatory damage while the lung is healing but also may predispose to bacterial colonisation of the lower respiratory tract. This modulation of lung homeostasis also occurs with prior non-infectious inflammatory events. In murine models exposed to the common allergen house dust mite (HDM), *Streptococcus pneumoniae* persists, becomes invasive and systemic and leads to mortality, whereas healthy mice clear the infection without untoward consequences. We identify the molecular mechanisms associated with bacterial susceptibility by showing the disruption of bacterial recognition by pattern recognition receptors due to sequestration or diversion of key receptors or downstream signalling components in the HDM-remodelled lung. A deeper understanding of the consequences arising from innate immune cell alteration during acute and chronic inflammation and the subsequent development of bacterial complications has important implications for future drug development.

SP-03
IMMUNO-MODULATION BY THE NORMAL INTESTINAL FLORA

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We harbor an enormous load of commensal bacteria in our intestines, which are physically excluded from entering the body by a single layer of epithelial cells and overlying mucus. The mucosal immune system is strategically situated in very close proximity to this massive load of bacteria, which can provide a wealth of stimuli for maturation and shaping of the immune system. Comparing germ-free mice, which lack all intestinal bacteria, to mice that are specifically colonized with a defined bacterial population clearly demonstrates that the presence of bacteria has strong immunomodulatory effects on both the mucosal and systemic immune systems. It is known that small numbers of live bacteria can penetrate past the epithelial cell barrier. The majority of bacteria that do penetrate are efficiently phagocytosed and killed by intestinal macrophages. In addition, dendritic cells can carry bacteria to the Peyer's patches and mesenteric lymph nodes where they stimulate B cells to switch to IgA. The numbers of IgA plasma cells in the lamina propria and the concentration of secretory IgA in the lumen of the gut both increase dramatically following colonization and some of the IgA that is induced is highly specific for the bacteria present in the flora. We have also found that intestinal $CD4^+$ T cells also increase dramatically in response to the intestinal flora but overt inflammation, and the differentiation of T_H1 or T_H17 effector cells, is inhibited by commensal-dependent activation and expansion of colonic lamina propria regulatory T cells. Furthermore, we have shown that impaired bacterial killing due to innate immune deficiencies leads to induction of a systemic antibody response, which can compensate for decreased innate responsiveness and protects clean mice from death. Importantly, this continuum between the innate and adaptive immune systems may also underlie the observed over reactivity to the intestinal microflora that is observed in inflammatory bowel disease.

SP-04
HELMINTHS AND MACROPHAGES: NOT ALL MACROPHAGES ARE CREATED EQUAL

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Inflammatory bowel diseases (IBD) are chronic debilitating disorders, the incidences of which are increasing in westernized societies and for which there are no cures. Over the last decade, we, and others, have shown that infection with helminth parasites significantly modifies the outcome of concomitant disease in animal models, and models of IBD in particular. One facet of the T helper-2 environment generated in mice and humans following infection with helminths is the occurrence

of alternatively activated macrophages (AMMs), that arise as a consequence of exposure to interleukins (IL)-4 and -13. AMMs have been implicated in wound repair and may also promote fibrosis. Using the dinitrobenzene sulphonic acid (DNBS) murine model of colitis we have found that peritoneum-derived macrophages differentiated in vitro and return to mice in a prophylactic or a treatment strategy significantly reduced the chemically-induced colitis: an event that was in part dependent on IL-10. Subsequent analyses has shown marked differences between these cells and bone marrow-derived AAMs, and intriguingly that a hyper-polarized AAM phenotype can be induced in vitro by exposure to specific pro-inflammatory and anti-inflammatory cytokines. Ongoing investigations in which he function and anti-colitic properties of AAMs are being investigated will be discussed.

SP-05
SUPPRESSION OF EPITHELIAL PRO-INFLAMMATORY SIGNALING BY INTESTINAL PROTOZOA

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Numerous reports have described how acute infections may trigger inflammatory responses. Conversely, recent discoveries highlighted the remarkable ability of some parasites to downregulate host immunity and minimize pathology, particularly with parasitic Helminthes. Less is known on how Protozoa may tone down immunity. Research into these mechanisms, and into the causative products, will be key to the development of new anti-infectives, but, perhaps even more importantly, novel interventions against autoimmune and inflammatory diseases. Complement lysis by extracellular Protozoa as well as modulation of the phagosomal compartment by intracellular parasites have been well described. In addition, cysteine proteases of various Protozoa, including *Entamoeba histoloytica,* are able to inhibit cellular immune responses in the intestine of the host, at least in part by modulating host prostaglandin synthesis and cleaving chemotactic cytokines. A variety of Protozoa, including *Trypanosoma*, *Toxoplasma*, and *Leishmania*. reduce pro-inflammatory cytokines. *Trypanosoma* reduces TNF-α and IL-12 in activated dendritic cells, making them producers of anti-inflammatory IL-10 and TGF-β. Similarly, upon activation by TLR's, dendritic cells exposed to *Giardia* produce more IL-10 and less IL-12, perhaps by modulating the release of lipoxins, as shown with *Toxoplasma*. *Giardia* is the most common cause of waterborne parasitic diarrhea worldwide. For reasons that remain obscure, the parasite does not trigger any overt inflammatory cell infiltration in the intestine. In addition to the effects on dentritic cells, ongoing research points to novel epithelial immuno-modulatory events. Indeed, *Giardia* is able to inhibit IL-1-induced CXCL8 production by intestinal epithelial cells via post-translational mechanisms. Recent reports indicate that the production of diarrheal symptoms caused by *Giardia* infection may vary with between geographical areas of the World, and perhaps even protect against diarrhea. In view of the significance of community acquired diarrheagenic microbes, including *E. coli* pathotypes, as a cause of diarrhea in these endemic areas, interactions between *Giardia,* and pathogenic *E. coli* may represent an interesting avenue for future studies. Research needs to address whether and how enteric Protozoa such as *Giardia* may modulate host interactions with concurrent pathogens, as well as to assess its effects on the normal host microflora.

Disclosure of interest: None Declared.

Post-traumatic immune suppression (SP02)

Symposium sponsored by the SFAR (French Society of Anesthesiology and Critical Care Medicine)

SP-06
PATHOPHYSIOLOGY OF POST-TRAUMATIC IMMUNE RESPONSE

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Abstract not available at the time of printing.

SP-07
BIOMARKERS IN TRAUMA PATIENT

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Biomarkers, which can be described as disease-associated molecular changes in body tissues and fluids, are tools for improving diagnosis and tailoring the treatment in the patients. Trauma patients are characterized by an easy to determine starting point, by comparison with sepsis.

1. Inflammatory markers in trauma patients.

The immune response to trauma belongs to what has been described as the sterile inflammation. Trauma is responsible for an inflammatory response difficult to distinguish from inflammatory response to infection. Besides classical inflammatory markers such as CRP and procalcitonin, endogenous molecules termed damage associated molecular patterns (DAMPs) are involved, as these host derived nonmicrobial stimuli are released following tissue injury and have similar functions as pathogen associated molecular patterns (PAMPs) in terms of their ability to activate proinflammatory pathways. Among DAMPs, proteins such as S100 A8 and A9 have been recently studied in this context.

2. Other biomarkers utilization in trauma patients.

Biomarkers of organ lesion/failure. All the organ can be involved in trauma and the list of biomarkers is non specific of trauma. Biomarkers such as troponin (cardiac muscle), isoform of CPK (muscles), lipase (pancreas), hepatic enzymes (liver contusion), biomarkers of axonal injury in head trauma indicate organ injury. - Biomarkers of infection have been studied in the early phase of trauma to detect an organ injury associated with dissemination of bacteria (such as hollow viscus perforation). Parameters such as simple white blood cell count, CRP or procalcitonin could indicate the presence of an infection, but

the overlap with the trauma related inflammatory reaction is important. At a later phase, once the initial inflammation phase is resolved, the same biomarkers can be a help to diagnose a secondary infections. Recent studies have indicated that markers of the immune status, such as mHLA-DR, are able to predict the risk for secondary infections.

SP-08
SYSTEMIC IMMUNOSUPPRESSION VERSUS OVERWHELMING PULMONARY INFLAMMATION

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Abstract not available at the time of printing.

SP-09
TREATMENTS IN THE FUTURE

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Abstract not available at the time of printing.

Chemical toxicity and inflammation (SP03)

Symposium sponsored by the SPTC (French Society of in vitro *pharmacology and toxicology)*

SP-10
MECHANISMS OF CHEMICAL IMMUNE-MEDIATED SKIN INFLAMMATION

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Many protein-reactive chemicals can cause allergic contact dermatitis. This inflammatory skin disease is mediated by contact allergen-specific T cells. In order to trigger the adaptive immune response contact allergens must first activate the innate immune system to induce skin inflammation. Recent studies have revealed that contact allergen-mediated skin inflammation closely resembles the innate immune response to infection. Intriguingly, contact allergens activate similar innate immune mechanisms as microbial pathogens. Thus, metallic nickel ions can directly bind to human Toll-like receptor 4 (TLR4) and activate signal transduction. Some organic contact allergens indirectly activate TLR2 and TLR4 by causing the generation of endogenous ligands including breakdown products of the extracellular matrix component hyaluronic acid in the skin. Furthermore, by triggering the release of ATP from skin cells contact allergens activate the NLRP3 inflammasome via the ATP receptor $P2X_7$. In addition, oxidative stress responses result from contact allergen encounter. The tight collaborative interplay of these innate immune and stress responses has important implications for the development of causative treatment strategies for allergic contact dermatitis and for the development of animal-free in vitro methods for the identification of contact allergens.

SP-11
PULMONARY INFLAMMATION AND PARTICULATE MATTERS: ROLE OF IMMUNOSUPPRESSIVE RESPONSES

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The paradigm that pulmonary inflammation drives fibrogenesis has been challenged and it is proposed that, in some cases, the pathological process is mediated by alternative mechanisms in particle-related lung diseases. Our studies demonstrated that silica-induced lung fibrosis in mice results from the action of the immunosuppressive cytokines TGF-β and IL-10 produced to limit the development of chronic inflammation. TGF-β and IL-10 induce lung collagen deposition when overexpressed in silica-treated mice. It is generally accepted that TGF-β and IL-10 are essential for regulatory T lymphocyte (T reg) expansion and immunosuppressive activity. We demonstrated that T regs are recruited to control inflammatory responses induced by silica particles. The persistent accumulation of immunosuppressive T regs in the lungs also contributes to pulmonary fibrosis by stimulating fibroblasts through the secretion of growth factors. In conclusion, immunosuppressive responses may also contribute to particle-induced lung fibrosis and represent an additional etiopathogenic pathway of lung fibrosis.

SP-12
ROLE OF ENVIRONMENTAL POLLUTANTS IN ADIPOSE TISSUE INFLAMMATION

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Persistent Organic Pollutants such as dioxin accumulate in adipose tissue and may exert long term effects in humans and animals. We have carried cellular experiments in order to determine novel mechanisms of toxicity of these pollutants and in vivo studies in an attempt to correlate the kinetics of these pollutants with biological and clinical parameters. The cellular response to the influx of potentially toxic xenobiotics is initiated by xenobiotics receptors. We have focused on the dioxin receptor (Arylhydrocarbon Receptor, AhR) and on its ligands. Functional genomics studies allowed us to identify a variety of genes that could mediate part of the AhR ligands toxicity. In both human liver cell lines and adipocyte cell lines, inflammatory genes were among the most potently induced by dioxin and certain PCBs. The upregulation of inflammatory genes by dioxin in adipose tissue was confirmed in vivo in mice. We have also studied target genes involved in focal adhesion, signal transduction by integrins and those mediating epithelial mesenchymal transition in a liver cell line. In another set of experiments we have assessed in human the effect of drastic weight loss in obese individuals on persistent organic pollutant release in the bloodstream and correlated this release with the regulation of gene expression in blood

cells and in adipose tissue. We also correlated this release with a number of biological and clinical parameters and found significant effect on liver toxicity and metabolic parameters.

Bone inflammation and regeneration (SP04)

Symposium sponsored by the JSIR (Japanese Society of Inflammation and Regeneration)

SP-13
ANTI-OSTEOCLAST THERAPY

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Osteoclasts are bone-resorbing cells, which are involved in the pathogenesis of various bone and joint diseases including rheumatoid arthritis and osteoporosis. RANK lingad (RANKL) is a TNF family cytokine that is essential for the differentiation and function of osteoclasts. We have been studying the mechanism of osteoclast activation in the context of arthritis and found that aberrant expression of RANKL in synovium is responsible for the abnormal formation of osteoclasts. We identified IL-17-producing T helper cells (T_H17 cells) to be the exclusive T cell subset that has the ability to induce osteoclastogenesis and recently revealed IkBζ to be a crucial regulator of Th17 development. These mechanisms provide a molecular basis for developing novel therapeutic strategies against bone destruction in arthritis. RANKL signals the cell through numerous immunomodulatory molecules such as TRAF6, NF-κB, c-Fos and nuclear factor of activated T cells (NFAT) c1. NFATc1 activation requires calcium signaling, which is mediated in osteoclasts by immunoreceptor tyrosine-based activation motif (ITAM) in dual membrane adaptors, Fc receptor (FcR) common γ subunit (FcRγ) and DNAX activating protein (DAP12). FcRγ and DAP12 associate with multiple immunoreceptors such as OSCAR and TREM-2, which thus function as costimulatory receptors for RANK. Recent advances in the understanding of osteoclast regulation and osteoclast-targeted therapy will be discussed.

SP-14
NEURAL DIFFERENTIATION AND CELL THERAPY USING INDUCIBLE PLURIPOTENT STEM CELLS

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Abstract not available at the time of printing.

SP-15
EARLY DESTRUCTION OF OSTEOBLASTS AS A NICHE OF HEMATOPOIETIC STEM CELLS IN BONE MARROW-GVHD

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Disrupted bone marrow (BM) hematopoiesis and delayed immune reconstitution are life-threatening complications of allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, how GVHD impairs BM hematopoiesis has been largely unknown. Using several mouse models of MHC-mismatched allo-HSCT, we explored the effects of GVHD on the possible targets in BM, HSCs and hematopoietic niches. Lethally irradiated mice were transplanted with T cell depleted BM cells (TCD-BM) supplemented with T cells from allogeneic donor mice to induce GVHD. We first analyzed the hematopoietic reconstitution in MHC I- and MHC II-double mismatched GVHD model and found that the BM hematopoiesis, especially the B-lymphopoiesis, was severely impaired in the GVHD mice. The B-lymphopoiesis was also impaired in MHC II-single but not MHC I-single mismatched GVHD. This was further confirmed in a CD4 dominant minor mismatched model. Re-transplantation experiments revealed that GVHD-affected BM contains substantial number of HSCs that reconstitute all hematopoietic cell lineage. In contrast, hematopoietic niche in GVHD-affected BM failed to support the B-lymphopoiesis. Histological analysis of GVHD-affected BM revealed that osteoblasts were completely lost within 7 days after allo-HSCT. Accordingly, expression of mRNA for osteoblast markers, *Runx2, Twist, Msx2, Osterix, Dlx5* and *osteocalcin*, was severely decreased in GVHD-affected BM. Moreover, the bone formation determined by bone histomorphometry was completely impaired in the GVHD mice. Administration of anti-CD4 antibody in the early time largely prevented BM-GVHD, while preserving GVL/T effects. In conclusion, our study provides the first evidence for the destruction of osteoblastic niches mediated by allogeneic CD4 T cells during GVHD. The new concept of BM-GVHD would provide an explanation for disrupted hematopoiesis and delayed immune reconstitution after allo-HSCT and may bring novel preventive and therapeutic strategies for GVHD.

Monday June 27th, 2011

Cancer and inflammation (SP05)

Symposium sponsored by the IRA (Inflammation Research Society)

SP-16
BEYOND IMMUNE CHECKPOINT BLOCKADE: MANIPULATION OF T CELL REGULATORY CIRCUITS IN THE TREATMENT OF MELANOMA

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We conducted extensive pre-clinical studies in mouse models which showed that blockade of the inhibitory signals mediated by CTLA-4 in T cells, either alone or in combination with a variety of immunologic and conventional therapies, led to tumor rejection and long-lived immunity. Ipilimumab, an antibody to human CTLA-4 developed by Medarex (now Bristol Meyers-Squibb) has been given to over 4,000 patients in clinical trials and objective responses have been observed in melanoma, prostate, kidney, ovarian, and lung cancer. A recent report documented an increase in survival of patients with advanced

melanoma treated with Ipilimumab in a randomized placebo controlled trial, the first drug of any type to do so, with more than 20% of patients alive for over 4 years after treatment. While this trial documents that CTLA-4 blockade can lead to durable responses in patients, there is clearly a need to increase the response rate. We have that administration of anti-CTLA-4 results in an increase of the frequency of CD4 T cells that express ICOS in both the tumor tissue and blood of bladder cancer patients. The ICOS + population contained effector CD4 T cells that made IFNγ upon *v*stimulation with peptides from NY-ESO-1. We subsequently showed that survival of advanced melanoma patients treated with Ipilimumab had sustained elevation of ICOS + CD4 T cells (>twofold for 12 weeks) had significantly greater survival than those with no or transient increases. Together, these data suggested to us that ICOS might play an important role in the therapeutic effects of CTLA-4 blockade. To test this we used a mouse model of melanoma. We found that the efficacy of anti-CTLA-4 was markedly diminished in mice that were deficient in either ICOS or ICOS ligand (ICOSL), confirming that the ICOS/ICOSL pathway plays a critical role in anti-CTLA-4 therapy. These observations led us to test the possibility that engagement of ICOS could enhance the efficacy of anti-CTLA-4 therapy. To this end we transduced mouse B16F10 melanoma cells with a cDNA encoding ICOSL or a control construct. B16ICOSL + cells (Ivax) and control B16 cells were irradiated and used alone or in combination with anti-CTLA-4 to treat mice bearing established B16F10 tumors. We found that combination of the IVAX with anti-CTLA-4 was markedly more effective than the control vaccine plus anti-CTLA-4 or that of any single treatment alone. The increase in therapeutic efficacy was accompanied by a marked in increase in the density and functionality of CD4 and CD8 T cells within the tumor. These results suggest a novel strategy for manipulating the immune system to enhance anti-tumor responses: checkpoint blockade coupled with provision of agonist signals mediated by ICOS to enhance costimulation.

SP-17
CANCER IMMUNOEDITING: IMMUNOLOGIC CONTROL AND SHAPING OF CANCER

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Cancer Immunoediting is the process by which the immune system controls and shapes cancer. We originally envisaged that cancer immunoediting would occur in three phases: Elimination (also known as cancer immunosurveillance, the host protective phase of the process), Equilibrium (the phase in which tumor cells that survive immune elimination remain under immunologic growth control resulting in a state of functional tumor dormancy) and Escape (the phase where clinically apparent tumors emerge because immune sculpting of the tumor cells has produced variants that display either reduced immunogenicity or enhanced immunosuppressive activity). Strong experimental data has now been obtained using mouse models of cancer to demonstrate the existence of each phase of the cancer immunoediting process and compelling clinical data suggests that a similar process may also occur during the evolution of certain types of human cancer. Our efforts now focus on elucidating the molecular and cellular mechanisms that underlie each phase of cancer immunoediting and identifying the critical checkpoints that regulate progression from one phase of the process to the next. This approach has helped identify the nature of antigens seen by immunity in nascent developing cancers and has further shown that immunoselection is a major mechanism of immunoediting. Moreover, we have found that edited tumors can still be controlled by the immune system if natural mechanisms that prevent autoimmunity are suspended. As reported by others, we have confirmed that inhibition of CTLA-4 induces ejection of edited MCA sarcomas. However, we have also found that inhibition of PD-L1 does the same, although by perhaps different mechanisms. These differences will be discussed.

SP-18
INFLAMMATORY CYTOKINES AND AUTOCRINE TUMOR-PROMOTING NETWORKS

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A complex network of inflammatory cytokines and chemokines regulates communication between the malignant cells and supporting stroma in most experimental and human cancers. There is ample evidence from animal models that individual members of this cytokine network and their intracellular signalling pathways can make malignant cells resistant to apoptosis, can stimulate a tumor-promoting leukocyte infiltrate that may also suppress anti- cancer immune responses, and can regulate angiogenesis. Of all the individual cytokines and chemokines that have been implicated in tumor growth and spread, the most convincing and extensive animal model data relates to the cytokines TNF-α, IL-6 and the chemokine receptor CXCR4. These, and the CXCR4 ligand CXCL12, as well as the receptor Notch3 and its Jagged1 ligand, are co-expressed and co-regulated in ovarian cancer cell lines and human ovarian cancer biopsies. We have named this co-regulation the TNF network. High levels of expression of TNF network gene pathways in cell lines and ovarian cancer biopsies is significantly associated with pathways and process important to cancer growth and spread including angiogenesis, cell adhesion, stromal development and the immune cell infiltrate. In ovarian cancer biopsies high levels of malignant cell staining for TNF network members related to poor prognosis. To translate these observations to clinical practice we have focused on IL-6, conducting a phase II trial of the anti-human IL-6 antibody CNTO328 in women with relapsed ovarian cancer. Our results showed that IL-6 may be a therapeutic target in ovarian cancer with multiple actions within the tumor microenvironment.

Inflammation in severe sepsis: from bench to bedside (SP06)

Symposium sponsored by the SRLF (Société de Réanimation de Langue Française)

SP-19
INNATE RECOGNITION OF BACTERIA DURING SEPSIS

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Bacteria are recognized by human tissues via an array of receptors present mainly at the surface of cells of the innate immune system. These receptors have evolved with the capacity to recognize and bind conserved bacterial molecules, different from "self" molecules.

Among these receptors are the "Toll-like receptors" (TLRs), and associated molecules such as CD14 and MD-2, but also receptors important for the clearance of bacteria or bacterial products such as scavenger and mannose receptors. TLRs recognize bacterial endotoxin (TLR4/MD-2), bacterial lipopeptides and lipoteichoic acid (TLR2/1/6), viral RNA (TLR3), flagellin (TLR5), and CpG bacterial DNA (TLR9). Recognition of lipopolysaccharide (LPS, Gram-negative bacterial endotoxin). Ligation of microbial ligands by TLRs triggers intracellular signaling pathways. Upon ligand binding, many TLRs, in particular TLR2 and TLR4, activate inflammatory pathways via the so-called MyD88 pathway leading to NF-κB activation. TLR4, but also TLR3 activate also a non-MyD88 pathway, leading to the production of β-interferon. The step of recognition of (bacterial) PAMPs by TLRs is therefore essential to initiate an inflammatory response. TLRs have been also identified as key receptors in antigen presenting cells, boosting the adaptive immune system. Some TLRs are also expressed in non-professional immune cells such as endothelial and epithelial cells, and ligation of those TLRs can also initiate inflammatory responses in tissues. Most studies addressing TLR specificity have been performed using purified bacterial ligands and transfected cells. Only a few studies have investigated PAMPs and their receptors in mediating human cell activation using whole bacteria. Bacteria are essentially sensed by human immune cells as whole microorganisms. PAMPs can also stimulate immune cells in the form of microparticles and soluble mediators released from bacteria. The predominant PAMPs stimulating TLRs are largely unknown in the context of a whole bacterium. Using either TLR-transfected cells or human leukocytes, endothelial cells, or epithelial cells treated with specific anti-TLRs, –MD-2, and -CD14 monoclonal antibodies (mAbs), we have determined the contribution of cell-surface TLRs and their accessory proteins toward cellular responses to prototypic Gram-negative and Gram-positive bacterial strains responsible for human septic shock. We have shown that whole Gram-negative and Gram-positive bacteria activate human leukocytes, endothelial, and epithelial cells through TLRs and CD14. The blockade of these receptors with specific mAbs efficiently abrogated cell activation in response to their exposure to whole bacteria. TLR4 is the main TLR involved in cell activation by Gram-negative bacteria. In the absence of TLR4 or at high concentration of Gram-negative bacteria, TLR2 was also activated. Higher concentrations of Grampositive bacteria were needed to activate cells in a TLR2-dependent fashion. This activation appeared to be completely TLR4/MD-2 independent. These results largely confirm studies performed in genetically modified mice and demonstrate that antibody blockade of CD14 and TLRs can potentially decrease excessive and detrimental inflammation in response to bacteria, making it an attractive therapeutic avenue to explore in patients with severe sepsis and septic shock. Indeed, during the initial phase of shock, the blockade of TLR-dependent hyperactivation of cells without interfering with CD14-dependent internalization of bacteria and bacterial products might be beneficial. Interfering with the initial inflammatory response with TLR antagonists carries also the theoretical risk of bacterial overgrowth, a problem that should be minimized by an appropriate antibiotic therapy, particularly in patients with severe bacterial infection and septic shock. Work is underway to determine how leukocytes from premature babies recognize and get activated by bacteria, as well as immature neutrophils in adults, a prominent circulating cell type in patients with septic shock. These results will be presented. Elson G, Dunn-Siegrist I, Daubeuf B, Pugin J. Contribution of Toll-like receptors to the innate immune response to Gram-negative and Gram-positive bacteria. Blood. 2007;109:1574–83.

SP-20
NEUROIMMUNOMODULATION IN SEPSIS

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The brain mediates via the autonomic nervous system and neurohormones the growth and proliferation of most if not all tissues involved in immunity, and all immune cells have membrane or cytosolic receptors for a number of neuromediators. The systemic inflammatory response to infection results in brain activation, which subsequently generates appropriate anti-inflammatory response. However, excess in pro-inflammatory mediators entering the brain can cause cerebral damage. In turn, dysfunction of the autonomic nervous and neuro-endocrine systems may alter immunity in a vicious circle resulting in metabolic derangements and organ failure. Two major pathways allow neuro-immune communications: the circumventricular organs (CVOs) located in the midline ventricular system, and the *vagus nerve*. CVOs are lacking a blood–brain barrier. Thus, they permit a direct communication between brain and blood stream. Some CVOs are located in the vicinity of neuroendocrine structures as and others are located close to brainstem autonomic centres. Thus, blood borne cytokines enter the brain through these areas, which express receptors for cytokines and bacterial fragments. In addition, specific carriers allow circulating cytokines to reach hypothalamic nuclei. Circulating IL-1 activates afferent vagal fibres terminating in the nucleus tractus solitarius, with subsequent stimulation of the hypothalamic–pituitary–adrenal axis. Efferent activity in the vagus nerve, termed 'cholinergic anti-inflammatory pathway', releases acetylcholine in the vicinity of macrophages within the reticuloendothelial system and leads to cellular deactivation and inhibition of cytokine release. The sympathetic and parasympathetic systems are thought to work hand-in-hand to modulate inflammatory responses. Diffuse endothelial activation induced by sepsis may result in blood–brain barrier breakdown allowing circulating mediators to enter massively into the brain. They induce expression of CD40 and adhesion molecules on brain endothelial cells. NF-kappa-B induced over-expression of iNOS further increases blood–brain barrier permeability. Components of innate and adaptive immune systems are expressed in the brain during LPS challenge. They are first expressed within the CVOs, and then spread to deeper areas of the brain controlling neuroendocrine and autonomic functions. Toll-like receptors (TLR)4, TLR2 and TLR9 are expressed by glial cells, such as microglia, astrocytes and oligodendrocytes at rest and after exposure to LPS. Whether TLRs are expressed in neurones remains controversial. Endotoxin also up-regulates the iNOS in the brain, causing accumulation of NO which behaves as a neurotoxic effector. Prostaglandins and purines, such as ATP and adenosine, are further key mediators in brain response to sepsis, particularly inducing fever. A number of other mediators may be involved in the cerebral immune response, such as macrophage migrating inhibitory factor, macrophage inflammatory protein, endothelin-1, angiotensin II, platelet-activating factor, superoxide radicals and carbon monoxide. Exposure to LPS may cause apoptosis of brain cells via dysfunction of mitochondrial respiration, activation of mitogen-activated protein kinase and NF-kappa-B pathways, and release or accumulation of calcium and reactive oxygen species. In septic shock, post-mortem studies showed iNOS induced neurone and glial apoptosis, mainly within

cardiovascular autonomic centres and the hippocampus. Cell culture experiments indicate an important role for microglial cells in immune surveillance, host defense and tissue repair. Indeed, they clear apoptotic cells and downregulate cytokines and encephalitogenic T-lymphocytes. During systemic inflammation, NO, cytokines and prostaglandins modulate brain neurotransmission, especially R-adrenergic system, GABAergic synapses, central muscarinic cholinergic regulation, corticotrophin releasing factor, ACTH, and vasopressin synthesis, and medullary autonomic centre output. Neurotoxic substances such as ammonium, or amino acids may also be involved. Prolonged LPS-exposure impaired synaptic transmission and neuronal excitability of pyramid neurons in the hippocampus, involved in behavioural and emotional systems. Finally, neurotransmitters and neurohormones modulate cerebral expression of inflammatory mediators. The final behavioural, neuroendocrine and autonomic responses are therefore variable because they depend on a complex and spatio-temporally organized process that involves both stimulatory and inhibitory pathways.

SP-21
GENETIC, SEPSIS AND INFLAMMATION

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Severe sepsis continues to be a major and increasing heath care burden worldwide. Despite aggressive organ support and optimal microbial therapy, sepsis remains the commonest cause of death in ICU with an overall mortality rate of 25–40%. The relative failure of conventional therapeutic strategies has stimulated a major interest in the development of new research axes, such as identification of genetic factors that influence sepsis susceptibility, therapeutic response, or outcome. Genetically-determined differences in host immune responses against pathogens might explain why some people get sick and die when they encounter a pathogen whereas others stay perfectly healthy. Explosion of knowledge both in human genomics and in host inflammatory response explains the increasing interest in immunogenetics over the last 15 years. Indeed, twin and adoptee studies have suggested more than 20 years ago, that host genetic factors are major determinants of susceptibility to infectious diseases in humans. Recently, candidate gene studies and human genome wide analysis have been used to identify infectious diseases susceptibility and resistance genes. Rarely, a single gene defect has been directly related to devastating consequences such as interferon-gamma receptor mutations leading to fatal infections with ubiquitous mycobacteria. For clinical practice, gene polymorphisms of specific immunological or physiological mediators appear to be of major importance. These genetic variants, which modify the regulation or function of either Pathogen Recognition Receptors or inflammatory mediators, have been associated with susceptibility and/or outcome of severe sepsis and septic shock. All steps of the host response to bacteria clearance have been shown to be potentially affected by genetic factors. However genetic studies in sepsis have produced contradictory results related in part to methodological faults. Improved adherence to published guidelines of good study design will help to ensure that genetic epidemiology contributes to a better classification of the heterogeneous septic population. The impact of these findings on the understanding of infectious disease pathogenesis and on the design of future preventive and therapeutic strategies should also be considerable.

New trends in the control of lung inflammation (SP07)

Symposium sponsored by the BIS (Brasilian Inflammation Society)

SP-22
MODULATION OF THE CHEMOKINE SYSTEM IN PULMONARY FIBROSIS

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Pulmonary fibrosis is a chronic respiratory disease with lethal consequences. Fibrosis may be triggered by a series of inflammatory conditions that usually precedes deposition of collagen in the lung parenchyma. Bleomycin-induced lung fibrosis in mice is the most commonly used model to study the pathogenesis of Pulmonary Fibrosis. Bleomycin instillation results in oxidative damage to the DNA of the alveolar epithelium and promotes cytokine release, which subsequently drives recruitment and activation of leukocytes in the airways. Leukocyte transmigration into the airways contributes to the chronification of the inflammatory process by inducing the production of cytokines, triggering fibroblast proliferation and excessive collagen deposition. Studies conducted by our group has shown that blockade of the chemokine receptor CXCR2 prevents development of fibrosis induced by bleomycin in mice. Importantly, blockade of CXCR2 was also protective even when started many days after induction of disease, suggesting it may be useful therapeutically in huams. The reduction of pulmonary fibrosis in those animals treated with CXCR2 inhibitors was associate with inhibition of angiogenesis. Similarly, preventive or therapeutic blockade of CCL3 with a *chemokine binding protein-CCL3 (Evasin-1)* decreased pulmonary fibrosis, an effect associate with decreased migration of leukocytes into the airways and reduced production of pro-fibrogenic cytokines. Chemokine receptors may signal via PI3 Kγ. PI3 Kγ deficiency led to attenuation of lung angiogenesis, reduction in leukocyte influx into the airways, decreased lung fibrosis and increased animal survival. Pharmacological inhibition of PI3 Kγ caused functional changes in leukocytes, endothelial cells and fibroblasts in vitro, suggesting that PI3 Kγ plays a role in multiple levels in the context of pulmonary fibrosis. More recently, we evaluated the role of Bradykinin receptors, B1 and B2, during the development of pulmonary fibrosis. B1R deficiency caused an increase in lung leukocyte influx, inflammatory cytokines and increased loss weight, and accelerated and increased mortality (100%) induced by bleomycin. However, B2R deficiency was associated with decreased production of inflammatory cytokines and chemokines and protected mice from lethality, weight loss, inflammation and fibrosis. These results unravel a novel role of B2 receptor in controlling inflammation and fibrosis after bleomycin challenge and suggest that B2 receptors may be interesting targets in the control of pulmonary fibrosis in humans.

SP-23
ROLE OF NLPR3 INFLAMMASOME ACTIVATION IN ALLERGIC ASTHMA

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Inflammasome activation with the production of IL-1β received substantial attention recently in inflammatory disease, but the role of inflammasome in the pathogenesis of asthma is not clear. Using an adjuvant-free model of allergic lung inflammation induced by ovalbumin, we investigated the role of NLRP3 inflammasome. We find that allergic airway inflammation depends on NLRP3-ASC complex producing IL-1β. Dendritic cell recruitment into lymph nodes, activation of Th2 lymphocytes in the lung and secretion of Th2 cytokines and chemokines including IL-33 and TSLP are reduced in the absence of NLRP3 and contribute to a dramatic reduction of allergic inflammation. Absence of NLRP3 and IL-1β is associated with reduced expression of other proinflammatory cytokines such as IL-13, IL-33, TSLP and IL-6. Mechanistically, we find that ATP which activates the inflammasome is released upon ovalbumin challenge. Furthermore, the critical role of IL-1R1 signaling in allergic inflammation is confirmed in IL-1R1, IL-1β and IL-1α deficient mice suggesting an additional role of IL-1α. By contrast t using the potent adjuvant alum during antigen immunization causes NLPR3 and IL-1R1 independent allergic asthma. In conclusion, NLRP3 inflammasome activation leading to IL-1 production is critical for the induction of a Th2 inflammatory allergic response, and the NLPR3 dependence of an allergic response may be determined by the adjuvant.
Disclosure of interest: None declared.

SP-24
A NEW MODEL OF ALLERGIC LUNG INFLAMMATION SUITABLE FOR INVESTIGATING GLUCOCORTICOID-RESISTANT ASTHMA IN THE MOUSE

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The importance of developing new animal models to assess the pathogenesis of glucocorticoid (GC)-resistant asthma has been stressed. Since following repeated allergen provocations, several features of asthma appeared less marked in many mice strains (Balb/c, C57blk6 and others) as compared to A/J mice, we have investigated the sensitiveness of mice of strain A/J to allergic provocations in the presence or absence of dexamethasone (DEX) treatment. Animals were sensitized on days 0 and 14 by a mixture of Al(OH)3 and ovalbumin (OVA), and challenged for 2 or 4 consecutive days starting at day 19 post-sensitization. The animals were subjected to treatment with DEX (3 mg/kg, oral), or vehicle, 1 h before each provocation. Invasive methodology was employed for measurement of AHR. Peribronchial leukocyte infiltration and subepithelial airway fibrosis were analyzed by histomorphometry. Cytokines and chemokines were assessed by ELISA. All changes were done 24 h after the last challenge. Our findings show that A/J mice developed asthma-like pathological changes that become resistant to DEX treatment as they are exacerbated by successive provocations. Since, the expression of GATA-3 also appeared increased and less sensitive to DEX, it is not unlikely that an overexpression of GATA-3 may contribute to the state of GC refractoriness seen in this model. This study suggests the existence of a causative relationship between steroid resistance and continued allergic inflammation.
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SP-25
MODULATION BY ESTRADIOL OF THE ACUTE LUNG INFLAMMATION AFTER INTESTINAL ISCHAEMIAREPERFUSION (I/R) IN FEMALE AND MALE RATS

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Sexual dimorphism modulates Th1 and Th2 lymphocyte functions, and accordingly estradiol (E2) interferes with acute lung injury (ALI) due to intestinal I/R. Female rats are relatively resistant to end-organ injury caused by hemorrhagic shock and gut from female animals is more resistant than the males's to the deleterious effects of ischemic injury. Nitric oxide (NO) is a dual mediator of gut trauma-induced lung injury, but interaction of estradiol and NO is still not clear. Here we assessed the effect of E2 on the lung and intestinal inflammation after intestinal I/R and its interaction with the inducible NOS (iNOS) pathway. Anesthetized rats were subjected to occlusion of the superior mesenteric artery (SMA) for 45 min, followed by 2 h of reperfusion. Groups of rats were treated with E2 (17β estradiol, 280 µl/kg, s.c. 24 h before ischemia, or i.v. 30 min after induction of ischemia) and with the selective NOS inhibitor, aminoguanidine (50 mg/kg iv) or the NO-donor L-arginine (300 mg/kg, i.p.) 1 h before occlusion. Lung and intestinal vascular permeability (LVP and IVP) were assessed by Evans blue dye extravasation and neutrophil recruitment to the tissues by the myeloperoxidase (MPO) method. IL-10 was quantified by ELISA in cultured lung tissue. Female ovariectomized (OVX) rats had LVP, IVP and MPO increased after I/R whereas IL-10 levels were reduced. E2 reverted the increased LVP, but not IVP nor MPO. Aminoguanidine alone reduced LVP in OVX rats, but failed to do so when associated to E2. Aminoguanidine used in non-OVX rats markedly exacerbated LVP and IVP. Thus endogenous NO probably reduces the vascular effects of intestinal I/R. In addition, the NO-donor L-arginine imitated E2 since it reduced ALI after intestinal I/R. In confirmation, male rats treated with E2 were also protected. In conclusion, estradiol reduces lung inflammation after intestinal I/R, but the concomitant blockade of NOS suppresses protection.
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SP-26
SENSING ALLERGIC INFLAMMATION

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Allergy is on the rise worldwide. Asthma, food allergy, dermatitis, and systemic anaphylaxis are amongst the most common allergic diseases. The association between allergy and altered behavior patterns has long been recognized. The molecular and cellular pathways in the bi-directional interactions of nervous and immune systems are now starting to be elucidated. In my talk I will outline the consequences of allergic diseases, especially asthma and food allergy, on behavior and neural activity; and, secondly, on the neural modulation of allergic responses.

Auto-inflammatory diseases (SP08)

Symposium sponsored by the SNFMI (National Society of Internal Medicine)

SP-27
THE CLINICAL SPECTRUM OF AUTO-INFLAMMATORY DISEASES

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The auto-inflammatory diseases encompass a large group of inflammatory disorders defined as Mendelian genetic diseases involving the innate immune system, and especially interfering with the regulation of Interleukin 1 pathway. The core group of these diseases is represented by hereditary recurrent fever syndromes: Familial Mediterranean fever; Tumor necrosis factor receptor-associated periodic fever syndrome; the cryopyrinopathies (Cryopyrin-associated periodic syndrome or CAPS) which include familial cold autoinflammatory syndrome, Muckle–Wells syndrome, and Chronic infantile neurological cutaneous and articular/Neonatal onset multisystemic inflammatory disease; the group of mevalonate kinase deficiency; nucleotide-binding oligomerisation domain, Leucine rich Repeat and Pyrin domain (NLRP12)-associated syndrome, and the deficiency of the interleukin 1-receptor antagonist. All these disorders are characterized by recurrent episodes of inflammatory attacks manifested by fever, local inflammatory symptoms due to serositis involving mainly the abdomen, the musculo-skeletal system, the thorax, and the skin, and an elevation of acute-phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA). Specificities characterize each of these entities. Other diseases sharing the Mendelian pattern of inheritance but not the intermittent character can be also considered as auto-inflammatory diseases such as pyogenic arthritis pyoderma gangrenosum and acne syndrome, Majeed syndrome, Ghosal syndrome. The concept of auto-inflammation is expanded to diseases in which innate immunity dysregulation is involved but without a Mendelian genetic basis such as atherosclerosis or gout; or disorders which share the implication of genes involved in Mendelian recurrent fever syndromes such as Crohn disease; or diseases which mimick the intermittent inflammatory attack feature such as Still's disease.

Key words: Auto-inflammatory diseases, clinical aspects.
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SP-28
INFLAMMASOMES

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Inflammasomes are multimolecular platforms controlling caspase-1 activation and proinflammatory cytokine maturation like Interleukin-1beta (IL-1β). These platforms play major roles in the sensing of pathogens and stress signals. The best-characterized inflammasome is the NLRP3-inflammasome, which plays a critical role in protection against infections but also in auto-inflammatory disorders including Familial Periodic Fevers and gout disease. How the NLRP3-inflammasome activity is regulated is still not completely understood. Intracellular potassium levels as well as ROS production have been proposed to signal to NLRP3. New findings indicate that the NLRP3-inflammasome activity is controlled by autophagy.

SP-29
WHICH GENETIC APPROACH FOR AUTO-INFLAMMATORY DISORDERS?

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A growing number of illnesses, including Mendelian as well as multifactorial disorders, are recognized as autoinflammatory syndromes (AIS). So far, few studies have investigated susceptibility loci for complex AIS. However, major advances have been made in the characterization of monogenic AIS. Five genes have been involved in the first-recognized and most common AIS called hereditary recurrent fevers (HRF) and, depending on the definition of so-called AIS, 5–10 additional genes have been shown to be responsible for these disorders. Genetic testing allows confirmation of the diagnosis, avoids years of unsuccessful investigations, and permits targeted therapy, as well as genetic counselling. However, molecular analyses also show some limitations. (1) Most variations correspond to missense mutations whose deleterious effect remains often difficult to ascertain in sporadic cases due to the lack of appropriate functional assays. In addition, for several variations identified in HRF genes, it is not clear whether they are simple polymorphisms, confer a milder phenotype, or act as modifiers for other inflammatory loci. (2) Many patients with familial Mediterranean fever—FMF—(the most frequent monogenic AIS) carry only one mutation in the disease-causing gene (*MEFV*); this questions the recessive model of inheritance of FMF, although this observation depends on the clinical definition of the disease. (3) Notably, there is no molecular explanation in more than 70% of patients.

Several approaches can be used to identify new AIS genes: classical linkage studies, homozygosity mapping, as well as candidate gene approaches. The few recent novelties in the field include the involvement of *NLRP12* in HRF, *IL1RN* in the DIRA syndrome, as well as *IL10RA* and *IL10RB* in early-onset enterocolitis.

Tuesday June 28th, 2011

Translational research strategies in inflammation (SP09)

Symposium sponsored by the SCIL (Society for Cytokines, Inflammation and Leukocytes)

SP-30
ANTI TNF THERAPY IN RHEUMATOID ARTHRITIS

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The discovery of anti TNF therapy, now routinely used in the treatment of rheumatoid arthritis and multiple diseases, was founded on several premises. First a hypothesis is that cytokines were important autoimmunity. Secondly the most accessible tissue to test was rheumatoid synovium. Thirdly by 1987/88 the molecular biology revolution had led to the cloning of multiple cytokine cDNAs, enabling the analysis of cytokine expression. Fourth, improved culture systems enabled the upregulated cytokine expression to be analysed and TNF emerged as a therapeutic target, with tested reproducibility. But the success is limited: there are no cures. Further work is need to address the reasons why this is so, and potential synergistic approaches may improve efficacy but at unknown risk. Importantly the cytokine analysis in diseased tissue approach has been successfully used by Claudia Monaco to show that upregulated cytokines are important in atherosclerosis, especially in active plaques.

SP-31
TREATING ALLERGY—WHERE TO FROM HERE?

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Allergic diseases constitute major and growing health burdens worldwide. Th2-polarised immune responses to allergens drive the adverse reactions, notably IL-4 which induces IgE class switching and mast cell inflammatory mediator release and IL-5 which promotes local eosinophil activation. Allergen specific immunotherapy (SIT) can modify the natural course of allergic diseases. Conventionally this treatment is given subcutaneously but recently sublingual-swallow allergen-specific immunotherapy (SLIT) is gaining popularity, with meta-analyses confirming long lasting clinical efficacy with decreased adverse events. Data on the immunological mechanisms underlying effective SLIT are emerging. The most convincing studies to date indicate that SLIT induces decreased allergen-stimulated T-cell proliferation, immune deviation (decreased IL-4 and IL-5 and increased IFN-γ), T cell anergy (allergen-specific non-responsiveness with enhanced IL-10 secretion), blocking antibodies (allergen-specific IgG_4), and induction of regulatory T cells, as have been established for subcutaneous injection immunotherapy. Peripheral T-cell tolerance to allergens is an important mechanism for maintaining homeostasis in healthy individuals and in clinically efficacious allergen-specific immunotherapy. It is likely that functional naturally-occurring $CD4^+CD25^+Foxp3^+CD127^{lo}$ T reg and peripherally-induced IL-10- and/or TGF-β-secreting Treg are important in effective SLIT. Additionally, there is evidence that the oral mucosa presents distinct regulatory features with oral Langerhans-like DC playing a major role. The precise molecular delineation of the allergen-DC receptor interaction which favours tolerance induction and whether particular adjuvants specifically tailored for the sublingual route will allow improved allergen formulations and delivery strategies for greater efficacy of treatment remain to be determined.

SP-32
THE ROLE OF AIRWAYS INFLAMMATION IN DEVELOPMENT OF PERSISTENT ASTHMA DURING CHILDHOOD: EMERGING TARGETS FOR ASTHMA PREVENTION

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Epidemiological evidence from several independent groups suggests that the development of what eventually becomes chronic asthma is commonly initiated in early childhood, driven by a range of environmental factors which confer varying degrees of risk. Two factors stand out from the perspective of potential intervention targets. Firstly, the association between allergic sensitization to aeroallergens (as marked by IgE production) and asthma development has been recognised for many years, and in this context atopy can function as an independent risk factor. Secondly, early lower respiratory tract infection, particularly severe infection marked by inflammatory symptoms (wheeze and/or fever), also operates as an independent risk factor, and risk increases as a function of infection frequency. Moreover, maximum risk is encountered when these inflammatory pathways intersect, i.e., in children experiencing both sensitization and severe lower respiratory tract infections. This suggests direct interaction between these risk factors which results in inflammation-mediated disturbance in the maturation of normal respiratory functions. These interactions also appear to operate when levels of IgE are relatively low, within the range commonly encountered transiently in up to 50% of the normal infant population. Ongoing studies in our lab on viral-induced severe asthma exacerbations provide a plausible explanation for these interactions, notably "recruitment" of underlying IgE-associated allergen-specific Th2 immunity into the inflammatory milieu of the infected airway via the innate immune response against the virus, which is mediated by upregulation of high affinity IgE-receptor expression on local airway mucosal Dendritic Cells. Additionally, it is becoming evident that mucosal dwelling bacteria also play a role in early asthma pathogenesis, and recent evidence on the nature of their participation will also be presented.

Genomics and epigenetics of inflammation (SP10)

Symposium sponsored by the BIRAS (British Inflammation Research Association)

SP-33
EXPRESSION PROFILING IN INFLAMMATION

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SP-34
THE ROLE OF SMALL NON-CODING RNAS IN INFLAMMATION

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Abstract not available at the time of printing.

SP-35
EPIGENETICS AND LUNG INFLAMMATION

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Epigenetics, as the name implies, is used to describe the events that alter cell, organ whole animal phenotype that does not require changes in DNA sequence. It has been proposed to account for much of the missing heritability in many diseases including asthma, a chronic inflammatory disease of the airway. In addition to asthma, other airways diseases such as COPD and pulmonary fibrosis have been proposed to have epigenetic components. Changes in cell function involve co-ordinated post-translational modifications of histones and DNA including changes in DNA methylation and histone acetylation/methylation. These changes are under the control of several enzymes and enzyme complexes that write, read and erase these epigenetic marks. Epigenetic changes occur through life in response to environmental, physiological and pathological signals and may affect asthma susceptibility. For example, increasing the dietary content of methyl donors can increase asthma susceptibility and inflammatory components associated with airway hyperresponsiveness in animal models of asthma. Furthermore, changes in lung function and airway inflammation in response to environmental pollutants such as traffic/diesel pollution and cigarette smoking have been linked to changes in DNA methylation and histone acetylation. It is hoped that the development of novel drugs that target these writers, erasers and readers may produce novel therapeutic agents for chronic inflammatory diseases and at least cancer.

SP-36
NEW TOOLS FOR EPIGENETICS-FUTURE THERAPEUTICS

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Abstract not available at the time of printing.

Atherosclerosis: the role of inflammation (SP11)

Symposium sponsored by the NSFA (Nouvelle Société Française d'Athérosclérose)

SP-37
CHEMOKINES AS KEY REGULATORS ORCHESTRATING VASCULAR INFLAMMATION

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Atherosclerosis is a chronic inflammatory disease of the arterial wall that is characterized by a disturbed equilibrium of immune responses and lipid accumulation, leading to the development of plaques. The atherogenic influx of mononuclear cells, namely monocytes and T-cells, and the interaction of antigen-presenting cells, T cells and monocytes is orchestrated by chemokines and their receptors. Studies using gene-deficient mice and antagonists based on peptides and small molecules have generated insight into targeting chemokine-receptor axes for treating atherosclerosis, which might complement lipid-lowering strategies and risk factor modulation. Combined inhibition of multiple chemokine axes could interfere with the contributions of chemokines to disease progression at specific cells, stages or sites. In addition, the recently characterized heterophilic interactions of chemokines might present a novel target for the treatment and prevention of inflammatory diseases such as atherosclerosis.

SP-38
THE MACROPHAGE IN METABOLIC CONTROL AND VASCULAR INFLAMMATION: MODULATION BY NUCLEAR RECEPTORS

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Macrophages play a pivotal role in the development of atherosclerosis. After recruitment in the sub-endothelial space, monocytes differentiate into macrophages, accumulate lipids thus forming foam cells and secrete pro-inflammatory and matrix-degrading factors, thus contributing to plaque development, inflammation and instability. Recently, a

role for macrophages in adipose tissue dysfunction related to obesity and diabetes has also been demonstrated. Therefore, pharmacological modulation of macrophage functions represents an attractive strategy for the prevention and treatment of metabolic and cardiovascular diseases related to atherosclerosis. Macrophages are heterogeneous cells, which adapt their response to micro-environmental signals. While Th1 cytokines promote pro-inflammatory M1 macrophages, Th2 cytokines promote an "alternative" anti-inflammatory M2 macrophage phenotype. In recent years, the role of ligand-activated nuclear receptors, such as the peroxisome proliferator-activated receptors (PPAR) and Liver X receptors (LXR), in the modulation of macrophage polarization, lipid homeostasis and immune-inflammatory responses has been studied extensively. It has been shown that PPARγ promotes the differentiation of monocytes into anti-inflammatory M2 macrophages in humans and mice, while a role for PPARβ/δ in this process has been reported only in mice. Our findings show that, in contrast to PPARγ, expression of PPARα and PPAR$\beta\delta$ overall do not correlate with the expression of M2 markers in human atherosclerotic lesions, whereas a positive correlation with genes of lipid metabolism exists. Moreover, unlike PPARγ, PPARα or PPAR$\beta\delta$ activation does not influence human monocyte differentiation into M2 macrophages in vitro. Thus, PPARα and PPARβ/δ do not appear to modulate the alternative differentiation of human macrophages, whereas PPARγ exerts anti-inflammatory actions by stimulating M2 polarization.

SP-39
LRP1: CONTROL OF VASCULAR REMODELING AND PROTECTION FROM ATHEROSCLEROSIS

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The low-density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional protein that binds numerous ligands and controls multiple signaling pathways. LRP1 integrates TGFβ and PDGF signaling pathways in vascular smooth muscle cells (vSMCs) and disruption of this signal integration results in increased TGFβ signaling, Marfan-like changes of the vessel wall architecture, decreased cellular cholesterol export, massive foam cell accumulation and accelerated atherosclerosis. Recently, we found that the nuclear hormone receptor PPARγ and the LDL receptor-related protein LRP1 functionally interact to control the metaplastic cartilaginous transformation of vSMCs. Loss of PPARγ in vSMCs promotes cartilage formation in the medial muscular layer, but surprisingly this is prevented in the absence of LRP1. Wnt5a expression, and recapture and nuclear translocation of SPARC, both regulators of the cartilage inducers Sox9 and Cart1, require LRP1. By contrast, PPARγ controls the expression of the Wnt5a antagonist sFRP2, and LRP1 and PPARγ both suppress fibrosis- and calcification-promoting TGFβ signals. Thus, LRP1 and PPARγ jointly control the vascular wall architecture, by regulating cellular lipid and extracellular matrix homeostasis, as well as the differentiation state of vSMCs.

Inflammation and repair in lung diseases (SP12)

Symposium sponsored by the SPLF (Société de Pneumologie de Langue Française)

SP-40
PULMONARY INFLAMMATION AND REPAIR IN FIBROSIS

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SP-41
VASCULAR INFLAMMATION IN PULMONARY ARTERIAL HYPERTENSION

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Inflammatory processes are prominent in various types of human and experimental pulmonary hypertension (PH) and are increasingly recognized as major pathogenic components of pulmonary vascular remodeling. Macrophages, T and B lymphocytes, mast cells, and dendritic cells are present in the vascular lesions of PH, whether in idiopathic pulmonary arterial hypertension (PAH), or PAH related to more classical forms of inflammatory syndromes such as connective tissue diseases or human immunodeficiency virus. Similarly, the presence of circulating chemokines and cytokines and increased expression of growth (such as vascular endothelial growth factor and platelet-derived growth factor) and transcriptional (e.g., Nuclear Factor of Activated T cells or NFAT) factors in these patients are thought to contribute directly to further recruitment of inflammatory cells, and proliferation of smooth muscle and endothelial cells. Thus, the recognition of complex inflammatory disturbances in the vascular remodeling process offers potential specific targets for therapy and has recently lead to clinical trials investigating, e.g., the use of tyrosine kinase inhibitors. This presentation will provide an overview of specific inflammatory pathways involving cells, chemokines and cytokines, cellular dysfunctions, growth factors, highlighting their potential role in pulmonary vascular remodeling and the possibility of future targeted therapy.

SP-42
BRONCHIAL INFLAMMATION

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Abstract not available at the time of printing.

ORAL FREE COMMUNICATIONS

News and goods to map and treat inflammatory bowel disease (SY01)

OC-001
PHOSPHOINOSITIDE 3-KINASE P110{GAMMA} IS REQUIRED FOR BACTERIAL CLEARANCE AND THE RESOLUTION OF EXPERIMENTAL COLITIS

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Introduction: PI3kγ has recently been identified as a potential target for the treatment of inflammation, primarily due to its role in leukocyte chemotaxis. Moreover, this signalling molecule is vital for defence against invading microbes, and its loss could potentially result in persistent infection. We chose to examine the effects of genetic ablation of PI3K p110γ on the development and resolution of acute murine colitis induced by the hapten trinitrobenzene sulfonic acid (TNBS).
Methods: Colitis was induced in wild-type C57/Bl6 or PI3k$\gamma^{-/-}$ mice by intra-rectal administration of 4 mg TNBS in 30% ethanol. Colon tissue was collected on day 3 to examine severity of acute colitis, and on days 7 and 14 to observe the resolution of colitis. Indices of colitis assessed include: weight loss, gross inflammatory and histological score, and myeloperoxidase (MPO) activity.
Results: While PI3k$\gamma^{-/-}$ mice exhibited significant protection from TNBS colitis by all measures during the early stages of colitis (i.e., 3 days post-TNBS), they also failed to properly resolve the inflammation by day 7 or 14. This was marked by a significant inability to gain weight following colitis (98.7 $\pm$ 5.2 vs. 107.7 $\pm$ 3.4% initial body weight on day 14), persistence of elevated MPO levels (day 7: 78.6 $\pm$ 1.3 vs. 4.2 $\pm$ 1.3 Units/mg tissue; day 14: 2.6 $\pm$ 0.5 vs. 0.2 Units/mg tissue for PI3k$\gamma^{-/-}$ and wild-type, respectively), and noticeably more serve remaining histopathology in colonic segments of PI3k$\gamma^{-/-}$ mice.
Conclusions: While inhibition of PI3kγ p110 is effective in blocking bouts of acute inflammation, its activity is required for the proper resolution of inflammation. This may be of particular importance in areas of high microbial load, such as the gut. Thus the pharmacokinetics of inhibitors of PI3Kg activity would be of paramount importance when considering PI3kγ inhibition as a therapy for colitic conditions, such as inflammatory bowel disease.
Disclosure of interest: None declared.

Adipokines new kids on the inflammation block (SY02)

OC-002
SERUM ADIPONECTIN LEVELS PREDICT RADIOGRAPHIC JOINT DAMAGE IN EARLY RHEUMATOID ARTHRITIS: RESULTS FROM THE FRENCH ESPOIR COHORT

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Background: Adipokines are products of adipose tissue that may be involved in the pathophysiology of rheumatoid arthritis (RA).
Objectives: To investigate whether serum adipokines are associated at baseline with radiographic joint alterations and may predict structural damage at 1 year in early RA.
Methods: We assessed 791 patients in the French ESPOIR early arthritis cohort fulfilling the 2010 EULAR-ACR criteria for RA (n = 632) or had undifferentiated arthritis (UA, n = 159). We measured serum levels of adiponectin, leptin and visfatin at enrolment. We investigated in RA patients the association between adipokine levels and X-ray joint damage as assessed by Sharp/van der Heijde score (SHS) at baseline and 1 year in a multivariate analysis including pertinent confounders: age, sex, BMI, insulin resistance as assessed by HOMA-IR index (model 1). In model 2, we included in addition factors of severity of RA (DAS28, CRP, HAQ, rheumatoid factor or anti-CCP status and radiographic destruction at baseline for the 1 year analysis) and the steroid use.
Results: The levels of adipokines were not different between UA and RA ($p > 0.47$). Within the RA population, adiponectin level at inclusion was associated with structural joint damage at baseline [OR = 1.11 (1.03–1.19), $p = 0.005$] and 1 year [OR = 1.12 (1.05–1.20), $p = 0.0009$] independently of confounding factors modulating adiponectin concentration (model 1). This association was also independent of the factors of RA severity and of the steroid use [OR = 1.12 (1.03–1.21), $p = 0.007$ at baseline and OR = 1.13 (1.05–1.23), $p = 0.002$ at 1 year] (model 2). Conversely, neither leptin nor visfatin levels were associated with SHS at baseline or 1 year.
Conclusion: In early RA, serum adiponectin level is an independent factor associated with radiographic joint damage and predicts

structural damage at 1 year, independently of metabolic confounders and intrinsic features of RA severity.
Disclosure of interest: None declared.

OC-003
EVALUATION OF ANTI-TNF-ALPHA ANTIBODY EFFECTS UPON ADIPOKINE PRODUCTION AND INTESTINAL INFLAMMATION IN EXPERIMENTAL COLITIS

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Abnormalities of fat in the mesentery have been long recognized as characteristic features of Crohn's Disease (CD). The therapy with antibodies anti-TNF-α, i.e., infliximab, has been employed to treat CD patients. Although adipose tissue from CD patients can secretes a large number of hormones that act modulating immune responses and inflammation, there is no relate about infliximab action upon adipokine production by mesenteric adipose tissue (MAT) during intestinal inflammation. Thus, the aim of this work was to study infliximab effects upon adipokine production by MAT and intestinal inflammation in trinitrobenzenosulphonic acid (TNBS)-induced colitis in rats. Wistar rats with colitis induced by TNBS received infliximab (1 or 5 mg/kg/week). Colitis was characterized by histopathological analysis, MPO content and inflammatory mediators production in colon. Morphology, adipokines release and inflammatory markers were assessed in MAT. Infliximab treatment reduces MPO activity and improves all inflammatory parameters evaluated in colon. The TNF-αand IL-10 level in colon was not modified while iNOS expression was strongly inhibited in both infliximab groups. Leptin and adiponectin release by MAT was also inhibited in both infliximab groups, while resistin released was increased after infliximab treatment at 5 mg/kg dose. MAT also expressed level of PPAR-γ and adipocyte size after infliximab (5 mg/kg) as healthy animals. TNF-α blockade in experimental colitis was able to reduce the inflammatory response in colon. In MAT, we could observe an inhibition in leptin production, an important pro-inflammatory adipokine. Interestingly, infliximab treatment restores adipocyte morphology and PPAR-γ expression, which could act as an additional anti-inflammatory mediator, providing evidences that therapeutic intervention modifies not only intestinal inflammation but also MAT alterations induced by inflammation.
Financial support: CNPq.
Disclosure of interest: None declared.

OC-004
EXPERIMENTAL FOOD ALLERGY LEADS TO ADIPOSE TISSUE INFLAMMATION AND WEIGHT LOSS IN MICE

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Food allergy affects approximately 5% of children and 3% of the adult population in the western world. This disease is the manifestation of an abnormal immune response to antigens introduced into the organism orally and it is often mediated by IgE. Our group developed a mouse model for the study of food allergy induced to ovalbumin. One of the most remarkable alterations observed in the allergic mice is a loss of weight. However, the disturbances that trigger this loss of body weight are not clear. Thus, the aim of this study was to investigate the mechanisms involved in weight loss of mice with ovalbumin-induced food allergy. With this purpose, BALB/c mice were subcutaneously sensitized with ovalbumin in aluminum hydroxide and challenged with the antigen containing diet for 7 days. The allergic mice showed significant weight loss with loss of epididymal adipose tissue, although it was not observed a reduction in food intake. These mice demonstrated adipose tissue inflammation characterized by increased leukocyte recruitment and infiltration of mast cells, macrophages and regulatory T cells in the stroma. Moreover, we demonstrated high concentrations of TNF-α, IL-6, IL-10 and the chemokine MCP-1/CCL-2 in this tissue. The metabolic changes in adipose tissue of allergic animals were represented by increased glucose uptake and lipolysis in adipocytes, resulting in atrophy of these cells. Changes were also seen in systemic metabolism characterized by decreased serum concentrations of glucose, triglycerides, total cholesterol and free fatty acids in allergic mice. Based on our results, we conclude that food allergy induce adipose tissue inflammation by producing mediators that lead to atrophy of this tissue. The decrease in adipose tissue mass has systemic consequences and results in loss of body weight.
Disclosure of interest: None declared.

New perspectives on T cells in inflammatory diseases (SY03)

OC-005
TREGITOPES AND TOLERANCE: HARNESSING REGULATORY T CELLS TO SUPPRESS INFLAMMATION

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Activation of immune regulatory pathways is critically important for treatment of inflammation in autoimmunity, allergy, and transplantation. We discovered regulatory T cell epitopes (Tregitopes) located in IgG Fc and framework regions that induce regulatory T cells (Treg) in vitro and in vivo. We report efforts directed at using Tregitopes to suppress antigen (Ag)-specific inflammatory immune responses by inducing immunological tolerance. Studies in allergy (OVA model), transplantation (MLR, cardiac transplant model), protein therapeutics (OVA), gene therapy (AAV), and autoimmunity (EAE, NOD) have demonstrated that tolerance to a range of Ags can be induced by co-administration of Tregitopes with target Ags in vitro and in vivo. Tregitopes activate CD4 + CD25 + FoxP3 + natural Treg (nTregs). In vitro, co-incubation of Ag with Tregitopes leads to suppression of Teff responses (i.e. cytokine secretion, proliferation) and expansion of Ag-specific adaptive

Tregs (aTregs). In vivo, co-administration of Tregitopes with a range of proteins (i.e. ovalbumin, and autoantigens) results in Treg expansion and functions, with suppression of Ag-specific T cell and antibody responses. The in vivo Tregitope effect is long-lasting; 100 days in transplant, and up to 30 weeks in the NOD mouse model. Tregitope treatment appears to be pleiotropic; in different model systems induction of IL-2, IL-10, suppression of gamma-interferon, induction of TGF-Beta and IL-9 are observed, perhaps due to the complex interplay of APC, nTregs, Teff, and aTregs. Stimulation of regulatory T cells to control a range of inflammation-mediated pathologies is promising. Moreover, the potential to stimulate Tregs in an Ag-specific manner may reduce side effects (i.e. infections) associated with more widely immunosuppressive treatments. Adaptation or incorporation of Tregitopes into established protein therapeutics also may have broad-reaching implications for the fields of allergy, autoimmunity and transplantation.
Disclosure of interest: A. De Groot Employee of: EpiVax, Inc.; Founder, CEO & CSO, N. Najafian: None Declared, D. Hui: None Declared, F. Mingozzi: None Declared, L. Cousens Employee of: EpiVax, Inc., L. Moise Employee of: EpiVax, Inc., S. Khoury: None Declared, W. Elyaman: None Declared, W. Martin Employee of: EpiVax, Inc.; Founder, COO & CIO, D. Scott Consultant/Speaker's bureau/Advisory activities with: EpiVax, Inc.

OC-006
A CRITICAL ROLE FOR NF-KB1 IN THE PRODUCTION OF GM-CSF BY ACTIVATED CD4 T CELLS DURING INFLAMMATORY RESPONSES

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We previously reported that NF-kB1-deficient ($Nfkb1^{-/-}$) mice had reduced CD4 T cell-dependent acute inflammatory arthritis (1). We also previously reported that MoDC differentiated from monocyte precursors in the spleen during acute inflammatory peritonitis (2). Here, we show that $Nfkb1^{-/-}$ mice are protected from acute inflammatory peritonitis and that protection in both of these models of acute inflammation is due to a requirement for the NF-kB1 subunit in CD4 T cells. Protection was not due to enhanced apoptosis or failure to upregulate cytokine receptors, but to a selective defect in the production of the cytokine GM-CSF by activated CD4 T cells. We also show that the in vivo differentiation of $CD11c^{int}CD11b^{hi}Ly6C^{+/hi}MHCII^{hi}$ MoDC, which could be identified in inflamed synovial tissue and draining lymphoid tissues, was dependent on the expression of NF-kB1 in CD4 T cells. These data provide in vivo evidence that NF-kB1-mediated production of GM-CSF by activated CD4 T cells is required for MoDC differentiation under inflammatory conditions.
(1) Campbell IK, Gerondakis S, O'Donnell K, Wicks IP. Distinct roles for the NF-κB1 (p50) and c-Rel transcription factors in inflammatory arthritis. J. Clin. Invest. 2000;105:1799–1806.
(2) Naik SH, Metcalf D, van Nieuwenhuijze A, Wicks I,Wu L, O'Keeffe M, Shortman K. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. Nat. Immunol.2006;7:663–671.
Disclosure of interest: I. Wicks: None Declared, A. Nieuwenhuijze: None Declared, I. Campbell Patent licensing of: GM-CSF antagonism, K. O'Donnell: None Declared, M. Hommel: None Declared, S. Gerondakis: None Declared, J. Villadangos: None Declared, E. Coghill: None Declared

OC-007
ALTERNATIVELY SPLICED IL-4 PROTEIN IS NATURALLY PRODUCED IN HUMANS AND CAUSES IMMUNE INFLAMMATION IN VIVO

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Abstract: Interleukin-4 mRNA exists naturally in two isoforms, one encoded by exons 1–4 (IL-4), and the other encoded by exons 1, 3, and 4 (IL-4δ2). We have previously identified and named IL-4δ2 mRNA. We and others studied IL-4δ2 mRNA expression in pathology, and discovered changes in patients with scleroderma, asthma, tuberculosis, and other diseases. However, it is not known whether a corresponding IL-4δ2 protein is also naturally produced and under what conditions. The in vivo effects of IL-4δ2 protein have never been studied. We have developed an mAb against IL-4δ2 with no cross-reactivity to IL-4. Freshly purified T cells from 10 patients with mild or moderate asthma and from 5 healthy controls were tested after up to 96 h activation with PMA/ionomycin. Levels of IL-4 and IL-4δ2 mRNAs were measured by RT-Q-PCR, and secretion of IL-4 and IL-4δ2 proteins was measured by ELISA. Basal expression of IL-4 and IL-4δ2 mRNAs was detected in all samples. Stimulation caused a 5–12-fold increase in IL-4 mRNA, and a 20–70-fold increase in IL-4δ2 mRNA, peaking at 12–24 h of activation. T cells from controls secreted IL-4 but not IL-4δ2 protein, whereas T cells from asthmatics produced both IL-4 and IL-4δ2 proteins in comparable amounts. Levels of IL-4 protein peaked at 24–48 h of activation, whereas IL-4δ2 protein peaked at 72 h. Adenoviral gene delivery of mouse IL-4 or mouse IL-4δ2 to mouse lungs in vivo induced immune inflammation with accumulation of T and B cells in both cases. However, delivery of IL-4 but not IL-4δ2 induced pulmonary eosinophilia, suggesting that IL-4 does and IL-4δ2 does not engage STAT6 signaling. Western blotting experiments in primary human T cell cultures tested phosphorylation of STAT6 and STAT3. Only IL-4 but not IL-4δ2 induced phosphorylation of STAT6 or STAT3 in a time- and dose-dependent fashion. Thus, IL-4δ2 is naturally secreted as a protein; it is biologically active in vivo without engaging STAT6 or STAT3 signaling.
Disclosure of interest: None declared.

Cell death (SY04)

OC-008
THE NUCLEAR PROTEIN MNDA MODULATES MCL-1 EXPRESSION AND NEUTROPHIL APOPTOSIS IN SEPSIS

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Sepsis and septic shock are major medical challenges that result from a harmful host response to infection. Suppressed neutrophil apoptosis, a hallmark of sepsis, perpetuates inflammation and delays resolution. We found that the myeloid nuclear differentiation antigen (MNDA) protein is necessary and fundamental to execution of the neutrophil death program. During constitutive apoptosis of human neutrophils, MNDA is initially involved in suppression of *Mcl-1* transcription. Subsequently, MNDA is cleaved by caspases and relocated from the nucleus to the cytoplasm where it shortened the half-life of the anti-apoptotic protein Mcl-1 by promoting its proteasomal degradation, thereby accelerating collapse of mitochondrial transmembrane potential. Conversely, culture of neutrophils with mediators of sepsis prevented MNDA cleavage and translocation. MNDA knockdown with short hairpin RNA attenuated Mcl-1 turnover and conferred resistance to stress-induced apoptosis in myeloid HL-60 cells. We also show that neutrophils from septic patients exhibited impaired MNDA relocation/cleavage parallel with Mcl-1 increased half-life, preservation of mitochondrial function and suppression of apoptosis. Thus, the nuclear protein MNDA functions as a central mediator of a novel nucleus-mitochondrion circuit that promotes progression of apoptosis. Disruption of this circuit contributes to neutrophil longevity in septic patients, thereby identifying MNDA as a potential target for treatment of sepsis and other inflammatory pathologies.
Disclosure of interest: None declared.

OC-009
NUCLEOCYTOPLASMIC RELOCALIZATION OF THE PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) DURING DIFFERENTIATION IS ESSENTIAL FOR ITS ANTIAPOPTOTIC ACTIVITY IN MATURE NEUTROPHIL: IDENTIFICATION OF A NUCLEAR EXPORT SEQUENCE

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We have recently described in mature neutrophils, a new survival pathway regulated by the proliferating cell nuclear antigen (PCNA), a nuclear factor involved in DNA replication and repair of proliferating cells. Notably, in neutrophils, which are non-proliferating cells, PCNA localized exclusively in the cytoplasm due to its nuclear-to-cytoplasmic relocalization occurring during myeloid differentiation. The first aim of the study was to elucidate the molecular basis of this PCNA cytoplasmic relocalization. We identified a leucine-rich nuclear export signal (NES) that can mediate an active CRM1-dependent nuclear export. However, because this NES sequence was located at the inner face of the PCNA trimer, this NES was fully functional in PCNAY114A monomeric mutant, which accumulated within the cytoplasm. Using the DMF-differentiated PLB985 cell line, we next tested whether PCNA nuclear-to-cytoplasmic relocalization during differentiation would impact its anti-apoptotic activity in mature neutrophils. PLB985 cells expressing the monomeric and cytoplasmic PCNAY114A showed the same anti-apoptotic activity assessed by mitochondria depolarization as those expressing PCNA as compared to control PLB985. In contrast, no antiapoptotic activity was observed in PLB985 cells expressing the nuclear SV40NLS-fused PCNA, thus strongly suggesting that nuclear-to-cytoplasmic relocalization but not trimerization was pivotal for PCNA survival activity in mature neutrophils. We concluded that (1) PCNA nuclear-to-cytoplasmic relocalization is part of the specific features of the neutrophil differentiation programme since it is essential for its pro-survival activity and (2) this phenomena is dependent on the newly identified monomerization-dependent PCNA NES.
Disclosure of interest: None declared.

OC-010
EFFECT OF NEUTROPHIL EXTRACELLULAR TRAPS ON DENDRITIC CELL MATURATION

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Polymorphonuclear neutrophils (PMN) are key components of the innate response via several mechanisms, in particular the production of Neutrophil Extracellular Traps (NETs). NETs are composed of decondensed chromatin fibers associated with granule and cytoplasmic proteins and are released upon activation. NETs play an important role in antimicrobial defenses but seem also to contribute to some auto-immune and inflammatory diseases. Our aim was thus to investigate whether NETs could interact with dendritic cells and thus modulate key immune functions. We first developed a reproducible model to induce, isolate and quantify NETs from blood PMN. Calcium ionophore A23187 was chosen to induce NETs and the restriction enzyme Alu I allowed the recovery of heterogeneous-sized fragments of NETs, as observed by DNA electrophoresis. Their composition was assessed by Western blotting and the use of various antibodies against some proteins potentially involved in DC interactions; proteomic analysis is under investigation. The incubation of isolated NETs with moDC did not directly induce DC maturation as measured by phenotypic modifications (CD80, CD86, CD83, CD40, HLA-DR…). In contrast, NETs were able to modulate the effect of lipopolysaccharide (LPS) on moDC maturation. In particular, we could observe the decrease of LPS-induced HLA-DR up-regulation. Our preliminary results thus suggest that NET formation, that takes place during inflammation, could also constitute a regulatory mechanism by modulating some DC functions. The next steps of this study will be to better understand the mechanisms of NETs-DC interactions, in particular the signaling pathways involved and the consequences on T cell activation. These results add new arguments to document

the involvement of PMN in the adaptive immune response, via netosis, a very peculiar PMN cell death mechanism.
Disclosure of interest: None declared.

Infection and inflammation (OC01)

OC-011
ROLE OF PYRIN INFLAMMASOME IN IL-1B PROCESSING IN RESPONSE TO BURKHOLDERIA CEPACIA

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Aims: Bacterial recognition by mononuclear cells often initiates assembly of an inflammasome resulting in activation of caspase-1 and processing of IL-1β, a key pro-inflammatory cytokine. *B. cepacia* is one of the major opportunistic pathogens colonizing airways and inducing progressive respiratory inflammation in cystic fibrosis patients. However, the *Burkholderia*-sensing inflammasome remains uncharacterized. The purpose of this study was to investigate pyrin as a potential sensor of *B. cepacia* and its effect on caspase-1 activity.
Methods: Human monocytes (Mo) and THP-1 cells, stably expressing either siRNA against pyrin or YFP-pyrin and universal inflammasome adaptor molecule ASC (YFP-ASC), were infected with *B. cepacia* and analyzed for the inflammasome activation.
Results: *B. cepacia* efficiently activates the inflammasome and IL-1β release in Mo and THP-1 cells. Suppression of pyrin levels in Mo and THP-1 cells reduced caspase-1 activation and ability to process and release IL-1β by *B. cepacia* challenge. In contrast, over-expression of pyrin or ASC induced the robust IL-1β response to *B. cepacia*, which correlated with the enhanced host cell death. In initial efforts to identify the pathogen-associated molecular pattern (PAMP) responsible for *B. cepacia* recognition by pyrin, Mo were infected with *Burkholderia* mutants, defective in several types of secretion systems.Inflammasome activation and IL-1β release was abolished in cells infected with T4SS mutants of *B. cepacia.*
Conclusions: For the first time we showed an association between pyrin levels and release of the major pro-inflammatory cytokine IL-1β by mononuclear cells infected with *B. cepacia*. The inflammatory reaction is likely induced by a yet uncharacterized PAMP of the T4SS. Pyrin in association with caspase-1 and ASC forms pyrin inflammasome and thus up-regulates mononuclear cell IL-1β processing and release in response to *B. cepacia*.
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Disclosure of interest: None declared.

OC-012
NRF2-MEDIATED CD36 OVER EXPRESSION INDEPENDENTLY OF PPAR GAMMA IN INFLAMMATORY MACROPHAGES IMPROVES THE OUTCOME OF SEVERE MALARIA

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CD36 receptor expressed on mononuclear phagocytes is a sensor of a broad range of microbial products that mediates phagocytosis and inflammation. CD36 is the main receptor mediating nonopsonic phagocytosis of *P. falciparum* parasitized erythrocytes (*Pf*PEs). Accordingly, CD36$^{-/-}$ mice display a combined defect in parasite clearance and cytokine response leading to higher mortality rates. Interestingly, CD36 macrophage overexpression promotes *Pf*PEs clearance and modulates parasite-induced TNFα secretion. CD36 is upregulated on macrophages following exposure to rosiglitazone and IL13, a Th2 cytokine, via the PPARγ pathway. Nrf2 transcription factor has also been described to control CD36 expression on macrophages.

Severe malaria is associated with an excessive production of pro-inflammatory cytokines such as TNFα. Here, we demonstrated that inflammatory processes generated through TLR2 engagement and TNFα negatively regulate CD36 expression both on human and murine macrophages, and hence decrease CD36-mediated *Pf*PEs clearance, directly favoring the worsening of malaria infection in vivo. Our main objective was to determine whether PPARγ or Nrf2 activators could restore CD36 expression and function on macrophages during inflammatory processes. In these conditions, we showed that PPARγ ligands were ineffective to promote CD36 expression. This phenomenon was directly associated with a failure in the expression and activation of PPARγ. Interestingly, pharmacological and molecular approaches using specific Nrf2 activators on PPARγ- and Nrf2-deficient macrophages showed that CD36 expression and function were restored both on human and murine inflammatory macrophages via Nrf2, independently of PPARγ. These results were validated in vivo where only Nrf2 and not PPARγ activators improved the outcome of severe malaria highlighting that Nrf2 activators represent a novel class of host immunomodulatory drugs that might be useful for treatment of severe malaria.
Disclosure of interest: None declared.

OC-013
RESISTANCE OF ALVEOLAR MACROPHAGES TO ENDOTOXIN TOLERANCE: INVOLVEMENT OF CYTOKINE MICROENVIRONMENT

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Monocytes and macrophages endotoxin tolerance, and more widely Toll-like receptors (TLR) agonists tolerance is a phenomenon corresponding to a modification of functional response of these cells (particularly an alteration of proinflammatory cytokines) during iterative stimulation by theses agonists, notably the lipopolysaccharide (LPS). Tolerance is observed with monocytes and macrophages from many tissue (peritoneal cavity, spleen, bone marrow), but is not detected with alveolar macrophages (AM). We investigated the cytokine and cell specificities of lung microenvironment possibly involved in that singular behavior of AM. We confirmed the absence of tolerization of AM ex vivo in three different mice strains: BALB/c, C57BL/6 and 129SV. In vivo systemic inhibition of granulocyte–macrophage colony-stimulating factor (GM-CSF) (produce in large amounts by type II pneumocytes, IIPC) led to a modification of the second response of AM to LPS. Similarly, AM obtained from interferon-γ (IFN-γ) receptor KO mice, or after in vivo inhibition of this cytokine, allowed the observation of tolerance. The responses of AM isolated from different

KO mice (*rag2−/−*, *rag2/γc−/−*, *cd3ε−/−*, *μ−/−*, *il-15−/−*, *Jα18−/−*) lacking various leukocytes lineages led us to demonstrate the involvement of NK cells and B cells in the IFN-γ production within the lung microenvironment. In summary, AM are resistant to tolerance to endotoxin. This functional singularity is underlined by the presence of GM-CSF (produced by IIPC) and of IFN-γ of which production is depending of NK and B cells) within lung microenvironment.
Disclosure of interest: None declared.

OC-014
AIDS PROGRESSION IS ASSOCIATED WITH THE EMERGENCE OF IL-17 PRODUCING INKT CELLS EARLY AFTER SIV INFECTION

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Invariant NKT (iNKT) cells have a role in antitumor immune responses and antiviral immunity. Recently, a new subset of iNKT cell has been reported to produce Interleukin-17 (IL-17) and implicated to inflammatory diseases. In the context of pathogenic Simian Immunodeficiency Virus (SIV) infection of rhesus macaques, we report the emergence of IL-17 expressing cells during the acute phase. This subpopulation expresses phenotypic markers of iNKT cells and appears at day 14 post-infection concomitantly with an increase in TGF-b and IL-18 expression. This IL-17 producing iNKT population (iNKT-17), rather than IL-17 producing CD4 T cells (Th17), persist during the chronic phase in peripheral blood and in a large number of tissues, except colon. In addition, the levels of these cells were associated with disease progression to AIDS. Consistent with the role of TGF-b and IL-18 production, we demonstrated that, in vitro, TGF-b and IL-18 induce the differentiation and expansion of iNKT-17 cells. The activity of IL-17 is classically defined by its ability to induce the expression of a variety of pro-inflammatory mediators, which ultimately leads to the recruitment and activation of phagocytes (polymorphonuclear neutrophils and monocytes). The emergence of iNKT-17 cells during SIV/HIV infection could therefore contribute to the chronic activation of immune cells and inflammation. These abnormalities may be involved in the increased risk of serious non-AIDS conditions, such as cardiovascular, kidney, liver diseases, and non-AIDS-defining malignancies, in individuals with HIV infection, compared to the general population.
Disclosure of interest: None declared.

OC-015
RECEPTOR-MEDIATED OVER-REGULATION OF THE ALVEOLAR MACROPHAGES FOLLOWING VIRAL INFECTION

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Background: Recent studies suggest that prolonged alterations in innate immunity following a primary viral infection are orchestrated by up-regulation of negative regulators in innate immune effectors, primarily alveolar macrophages. The majority of negative regulators investigated in this study have not been explored following viral infection before and are therefore novel.
Methods: Using a murine model, we tentatively assessed the involvement of various negative regulators [Glucocorticoid Receptor (GR), TAM receptors, IL-10R, Ron-β, ST2L, EP2, LXR-α/β and PPAR-γ] on alveolar macrophages following influenza. We also studied the antagonism of GR (by RU486 administration) following primary influenza infection and its effect on subsequent secondary bacterial challenge because of its therapeutic potential.
Results: Following primary influenza, the expression profiles of the regulators altered dramatically. There was a biphasic expression pattern: early phase regulators, which peaked at day 7 (e.g. Axl, GR, PPAR-γ, and GRK2) and late resolution phase regulators (e.g. MerTK, EP2, IL-10R, EP2 and LXR) which peaked around days 19–26. GR antagonism following primary influenza infection reduced the severity of subsequent secondary bacterial pneumonia.
Discussion: The up-regulation of negative regulators in a biphasic manner is interesting and may represent any or all of the following: (a) apoptosis of alveolar macrophages leaving behind those that preferentially express a particular receptor, (b) maturation of macrophages at different times so that receptor expression is seen in waves, (c) recruitment of macrophages leaving a receptor at particular time points (d) alteration of macrophages following engulfment of apoptotic cells.
Conclusions: These findings would help to develop alternative interventions against secondary bacterial pneumonia and we have shown that antagonism of these negative regulators, as we have shown in GR antagonism, may lead to effective clinical application.
Disclosure of interest: None declared.

OC-016
PLATELET-ACTIVATING FACTOR RECEPTOR (PAFR) DEFICIENCY DECREASES SUSCEPTIBILITY TO EXPERIMENTAL CEREBRAL MALARIA

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Introduction: The mechanisms underlying the pathogenesis of cerebral malaria (CM) remain unclear. The inflammatory infiltrate in the brain, with the recruitment of CD8+ T cells, and endothelium damage seems to be relevant in this process. Platelet-activating factor (PAF) is an inflammatory mediator involved in leukocyte recruitment and vascular permeability.
Objective: To investigate the role of the PAFR in the outcome of *Plasmodium berghei* ANKA (PbA) infection and the relevance of this molecule for the neuroinflammatory process involved in CM.
Method: C57Bl/6 wild-type (WT) and PAFR$^{-/-}$ mice were infected with PbA (10^6 parasitized erythrocytes) by intraperitoneal injection. Parasitemia and survival parameters were monitored daily. Leukocyte rolling and adhesion were evaluated by intravital microscopy in the

pial vessels. Recruitment and activation of CD8+ T cells of brain-sequestered leukocytes were analyzed by FACS. The expression of cleaved caspase-3 in total brain extracts was analyzed through Western blot. Histopathological analyses were also performed. To further investigate the role of PAFR, WT mice were treated with PAFR specific antagonist (UK-74,505).
Results: Infected $PAFR^{-/-}$ mice resisted to the infection for a longer period (21 days) than WT (7 days). Perivascular inflammatory infiltrates and parenchymal hemorrhage were more intense in WT mice than $PAFR^{-/-}$ mice. However, intravital microscopy showed similar levels of rolling and adhering leukocytes in WT and $PAFR^{-/-}$ mice. The recruitment and activation of CD8+ T cells were reduced in PAFR-deficient mice, as the activation of caspase-3. In WT mice infected with PbA, treatment with UK74,505 was also associated with the reduction of infection severity.
Conclusions: PAFR signaling seems to be relevant for the development of experimental CM and its blockade is a promising pharmacological strategy in CM.
Disclosure of interest: None declared.

OC-017
LUNG SENSITIZATION BY CANDIDA ALBICANS PROTECTS FROM PSEUDOMONAS AERUGINOSA-INDUCED LUNG INJURY THROUGH MACROPHAGE ACTIVATION NOT POLYMORPHONUCLEAR RECRUITMENT

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Objectives: *Pseudomonas aeruginosa* is often isolated with *Candida albicans* in critically ill patient airways. Lung sensitization with *C. albicans* in mice attenuated subsequent *P. aeruginosa*-induced lung injury and decreased bacterial burden. Interestingly, this protection persisted when direct interactions between the two pathogens were blocked. Our objective was therefore to determine the cellular mechanisms that may account for the *Candida*-triggered protective effect against *P. aeruginosa* infection.
Methods: Strains were *C. albicans* CA SC5314 and *P. aeruginosa* PaO1. Balb/c Mice were colonized by endotracheal instillation of 10^5 CFU of *C. albicans*. *P. aeruginosa* pneumonia was induced 48 h later by endotracheal instillation of 10^7 CFU of *P. aeruginosa*. Lung immune response was assessed 24 h later in bronchoalveolar lavage (BAL) and lung homogenates from mice infected with PaO1 with or without *C. albicans* sensitization. BAL and lung cellularity were determined through flow cytometry (FACS).
Results: Lung sensitization by a colonizing and persistent inoculum of *C. albicans* alone, led to significant alveolar macrophage cell recruitment and activation with a higher expression of MHC II in BAL ($p < 0.05$ vs. control). In contrast, *P. aeruginosa* pneumonia alone (Pa) significantly increased both PMN and macrophages but decreased alveolar macrophage activation ($p < 0.05$ vs. control). Importantly, lung sensitization by *C. albicans* significantly enhanced alveolar macrophage recruitment and activation with an overexpression of both CD86 and MHC II ($p < 0.05$ vs. Pa). Consistently, lung total lymphoid cell populations were significantly decreased by *P. aeruginosa* pneumonia alone, but were significantly increased by *C. albicans* airway colonization alone and by *Candida* sensitization prior to *P. aeruginosa* pneumonia.
Conclusion: *C. albicans* airway sensitization attenuates *P. aeruginosa*-induced lung injury through alveolar macrophage and possibly related lymphoid recruitment and activation.
Disclosure of interest: None declared.

OC-018
BACTERIAL COLONIZATION OF TISSUES OF CHRONICALLY ISCHEMIC LOWER LIMB

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Objectives: Reaction of arterial wall to bacterial infection as etiological factor in pathogenesis of atherosclerosis remains a contentious issue. The majority of available data on identification of bacterial antigen originates from studies of coronary arteries but not of lower limbs arteries.
Aim: To investigate the presence of bacterial cells and microbial DNA with use of broad-range PCR, targeting conserved region (16sRNA), *Chlamydia pneumoniae* (CP) and *Helicobacter pylori* (HP) in fragments of femoral and popliteal arteries of patients undergoing reconstructive surgery or amputation.
Methods: Fragments of arteries were harvested and cultured, from the remaining fragment DNA was extracted. PCR amplification was performed with primers for gene fragment coding bacterial 16sRNA, major outer membrane protein (ompA) of CP and urease gene of HP DNA with positive and negative controls. Routine bacteriological cultures of specimens were carried out.
Results: Using routine microbiological methods popliteal and femoral arteries contained isolates in 51% of cases (Staph. epidermidis, aureus, Enterococcus, Pseudomonas), carotid arteries 4.1% and aorta 0.7%. Microbial DNA (16sRNA) was detected in 64% of examined femoral and popliteal specimens. CP could be demonstrated in 69% of positively-tested patients while HP was detected in 3.8%. In carotid arteries 29% of cases contained bacterial DNA, 29% CP and 0% HP specific genes. Thirty one aortic specimens contained bacterial DNA, 65% CP and 18% HP.
Conclusions: Bacterial isolates and DNA were found in lower limb arteries. Aorta and carotid arteries only sporadically contained isolates. The microbes colonizing limb vascular bundles may be responsible for complications after arterial surgery as anastomosis dehiscence and wound suppuration.
Disclosure of interest: None declared.

New therapeutic targets in inflammation (OC02)

OC-019
MECHANISMS AND ANTAGONISTIC APPROACHES OF MIF/CXCR-MEDIATED INFLAMMATORY LEUKOCYTE RECRUITMENT

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MIF is a structurally unique inflammatory mediator that plays a critical role in several inflammatory diseases, including atherosclerosis. We recently discovered that MIF, contrary to its eponymous name, functions as a chemokine, promoting the recruitment of leukocytes to inflamed and atherogenic endothelium (Bernhagen et al., Nat. Med. 2007). This function is mainly based on non-cognate interactions with the chemokine receptors CXCR2 and CXCR4, but MIF also binds to the MHC class II chaperone CD74. Blockade of Mif but not of the canonical ligands of Cxcr2 or Cxcr4, Cxl8 and Cxcl12, respectively, in mice with advanced atherosclerosis led to plaque regression. In an attempt to devise novel MIF/CXCR-based therapeutic approaches, we comprehensively mapped the MIF/CXCR interface and identified a *pseudo*-(E)LR and an N-like-loop motif in MIF as a critical structural basis for MIF's promiscuous interaction with CXCR2 (Weber et al., *PNAS* 2008; Kraemer et al., *FASEB J*. 2010). Applying peptide spot array technology, here we fully characterize the MIF/CXCR4 interface and identify the motifs and residues on the receptor side of CXCR2 and CXCR4 responsible for MIF binding. Importantly, we will report on the identity, formation, and function of novel hetero-oligomeric MIF receptor complexes, which are involved in inflammatory monocyte and lymphocyte recruitment by MIF. Based on this previously unavailable structural information, we report on peptide-based anti-MIF strategies that may have future applicability in inflammatory disease and atherosclerosis.
Disclosure of interest: None declared.

OC-020
GLUTAMINYL CYCLASES ARE NOVEL TARGETS FOR THE TREATMENT OF CCL2-RELATED DISORDERS

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Monocyte chemoattractant protein 1 (MCP-1, CCL2) plays a major role in inflammatory conditions, such as atherosclerosis, and Alzheimer's disease. CCL2 is post-translationally modified by a pyroglutamyl (pGlu-, also pE-) residue formed by intramolecular cyclization of N-terminal glutaminyl residues. Here, we show that the Glutaminyl Cyclases (QCs) facilitate this reaction. The pGlu-residue confers resistance to proteolytic degradation and ensures proper receptor activation. In this context, upregulation of pGlu-cytokines in disease may exert protective but also deleterious effects for surrounding tissues. Chronic or overshooting cytokine response can drive toxicity and tissue damage. Accordingly, we have characterized the role of QCs in inflammatory responses in vitro, in situ and in vivo. QC activity originates from two distinct genes termed *QPCT* (QC) and *QPCTL* (isoQC). Especially QC is up-regulated by appropriate inflammatory stimuli; however, inhibition of isoQC activity or genetic deficiency of *QPCTL* prevents the N-terminal pGlu-formation of Gln-cytokines. Consequently, the secretion of such immature peptides attenuates their stability and receptor activation, which alleviates pathology in animal models. Our findings open up alternative pathways for counteracting the excessively overproduced pGlu-cytokines by QC-inhibitors. Hence, this novel small-molecule approach has the potential to therapeutically modulate inflammatory responses in CNS and peripheral tissues. Disclosure of interest: H. Cynis Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG, R. Eichentopf Employee of: Probiodrug AG, A. Kehlen Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG, S. Graubner Employee of: Ingenium Pharmaceuticals, Stock ownership or royalties of: Ingenium Pharmaceuticals, D. Friedrich Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG, T. Hoffmann Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG, S. Schilling Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG, H.-U. Demuth Board member of: Probiodrug AG, Employee of: Probiodrug AG, Stock ownership or royalties of: Probiodrug AG.

OC-021
A PROTEIN EPITOPE MIMETIC (PEM) CCR10 ANTAGONIST, POL7085, INHIBITS OVALBUMIN-INDUCED AIRWAY EOSINOPHILIA IN MICE

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A potential involvement of the CCR10/CCL28 axis was recently reported in a murine model of allergic asthma[#]. Blockade of the CCR10 receptor might therefore represent a novel alternative to the current treatment of asthma. We have evaluated the effect of the CCR10 antagonist POL7085, a Protein Epitope Mimetic (PEM) in two different hypereosinophilic asthma models in mice. Nine week-old male Balb/c mice were sensitized to ovalbumin (OVA) administered intraperitoneally in the presence of alum, and challenged to OVA administered intranasally first in a 8-day (D5-D7) and then in a confirmative 21-day (D18-D21) model. POL7085 was administered once daily, 1 h before each OVA challenge at increasing doses up to 18 μmol/kg intra-nasally (I.N.) versus dexamethasone (DEX; 2.3 μmol/kg) versus saline. Bronchoalveolar lavage (BAL) was performed 24 h after the last challenge, and BAL cells differentially counted. In both models, in saline treated animals, OVA induced recruitment of eosinophils, neutrophils, macrophages and lymphocytes into the BAL fluid. POL7085 dose-dependently and significantly decreased eosinophil numbers in BAL by 85 ± 7.1 and 55 ± 4.3% in the 8-day and the 21-day models, respectively. In addition, the higher doses of POL7085 also reduced the number of BAL neutrophils and lymphocytes in both models, although not significantly. In conclusion, these preliminary data show that the PEM CCR10 antagonist, POL7085, significantly and dose-dependently decreases eosinophilia, and reduces the influx of other cell types in two murine allergic model of asthma after once daily local administration. These data suggest that CCR10 antagonists may represent a promising novel approach for treating asthma.
English et al., Immunol Lett. 2006.
Disclosure of interest: None declared.

OC-022
INHIBITION OF PIM KINASE ATTENUATES ESTABLISHED INFLAMMATION IN A MURINE MODEL OF COLITIS

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PIM kinases consist of three oncogenic serine/threonine kinases, whose activity is regulated at all levels of expression. PIM-1 and PIM-3 have been shown to be involved in T cell proliferation and survival following T cell receptor engagement and have reported roles in cytokine induced survival. These activities suggest that a PIM1/3 kinase inhibitor may be useful in targeting T cell mediated inflammatory conditions, such as Crohn's disease. Herein we report the ability of a small molecule PIM 1/3 kinase inhibitor to modulate disease progression and severity of the CD45RB adoptive transfer murine colitis model, which is a model where progression involves Th1 and Th17 cytokines. AR00472159 is a selective and potent inhibitor of PIM1 and 3 kinases in both the enzyme (160 pM PIM 1 and 300 pM PIM 3) and cellular setting (88 nM PIM 1 cell assay). When administered orally once daily at 10 and 100 mg/kg to CB17 SCID mice beginning 28 days after adoptive transfer of CD4 + CD45RBhi T cells (from Balb/c donors) and dosed for 21 days, AR00472159 significantly inhibited colon size (g/cm) by 68 and 69%, respectively. Colon pro-inflammatory cytokines IL-1b, IFNg and TNFa were also reduced with AR00472159 treatment. Furthermore, beneficial effects were seen by histopathology with up to 85% inhibition of mucosal thickening, 64% inhibition of colon inflammation and 65% inhibition of colon gland loss. These data support an important role of PIM kinases in a Th1/Th17 driven model of colitis.
Disclosure of interest: None declared.

OC-023
SUPPRESSIVE EFFECT OF C-JUN NH2-TERMINAL KINASE (JNK) INHIBITOR SP600125 ON EXPERIMENTAL SILICOSIS IN MICE.

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Environmental and occupational lung disorders are important aspects of clinical medicine and the worldwide economic costs are staggering. Silicosis is a dysfunction caused by long-term inhalation of silica particles, characterized by intense inflammation and fibrosis, including the presence of granulomas in the lung tissue. In spite of the therapeutic arsenal currently available, there is no specific treatment for this disease. In this study, we investigated the potential effect of the JNK inhibitor, SP 600125, on the fibrotic component of the experimental model of silicosis in mice. Swiss-Webster mice were injected intranasally with silica particles, and further, daily treated with the JNK inhibitor SP 600125, from day 21 to 27 after silica provocation. The analyzes were performed 24 h after the last administration. We showed that treatment of silicotic mice with the JNK inhibitor SP 600125 markedly reduced granuloma formation, collagen deposition as well as the increased labeling for macrophages and myofibroblasts in the lung tissue. Generation of the chemokine KC and the fibrogenic factors TNF-α and TGF-b were inhibited by the drug. In line with the previous data, SP 600125 suppressed the increase in airways resistance (central and peripheral) and static elastance in silicotic animals. The high levels of phosphor-JNK noted in silicotic lungs, as compared to the controls, were clearly reduced by SP 600125. Additionally, incubation of lung fibroblasts with SP 600125 in vitro abolished the proliferative response to IL-13 stimulation. In conclusion, our findings indicate that JNK inhibitor SP 600125 effectively suppressed the fibrotic phase in silicosis in mice, which is clearly reflected by the impairment of lung function alterations, strongly supporting the therapeutic potential of orally available JNK inhibitor for the treatment of pulmonary fibrosis.
Support: PAPES5/FIOCRUZ, CNPq, FAPERJ.
Disclosure of interest: None declared.

OC-024
LASSBIO-1135: AN ANTI-TNF-A AND TRPV-1 ANTAGONIST COMPOUND ORALLY EFFECTIVE IN MODELS OF ACUTE AND CHRONIC PAIN

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Treating chronic pain is one of the hardest challenges for scientists. Multi-target therapies focusing in neuro-immune interaction has been arisen as a successful treatment approach. Male and female Wistar rats and BALBc mice, weighing 120–180 and 25–35 g, respectively, were used for in vivo pain tests ($n = 8$; $^*p < 0.05$ ANOVA One Way). All the studies have been approved by the UFRJ ethic committee. In vitro studies for TNF-α and p38 MAPK were carried out in murine macrophages. TRPV-1 antagonism was evaluated by electrophysiological studies in *Xenopus laevis* oocytes. LASSBio-1135 (100 μmol/kg, p.o.) was able to inhibit the thermal carrageenan-induced hypernociception (Lavich TR et al., Braz J Med Biol Res. 2005;38: 445–451) in 71%*, reducing cell infiltration into the injured paw in 58%* evaluated by the myeloperoxidase (MPO) activity of plantar tissue. LASSBio-1135 inhibited the TNF-α production of LPS-stimulated peritoneal macrophages in a concentration-dependent manner, presenting an $IC_{50} = 9.2$ μM, this blockade depended on inhibition of the activation of p38 MAPK, as seen in Westernblot assays in this culture. Moreover, LASSBio-1135 orally administered (100 μmol/kg) inhibited the capsaicin-induced hypernociception by 91%* (Mizushima T et al. Pain 2005, 113:51–60; Lacerda, R.B. et al. Bioorg. Med. Chem., 2009, 17: 74), while when administered directly into the paw it inhibits by 47.4%* the hypernociception. Electrophysiological studies showed that LASSBio-1135 at 50 mM inhibited the capsaicin-induced current by $44.8 \pm 13.5\%$ ($^*p < 0.001$; $n = 6$) and in a concentration dependent manner. In a neuropathic pain model, LASSBio-1135 also inhibited the hypernociception induced by partial sciatic ligation after 3 days of treatment, remaining the effect until the 10th day of treatment. LASSBio-1135 arises as a multi-target compound which is able to control chronic pain by mechanisms that involve modulation of neuro-immune interaction. Acknowledges: INCT-INOFAR, FAPERJ, CNPQ.
Disclosure of interest: None declared.

OC-025
MOLECULAR ENGINEERING OF SHORT HALF LIFE SMALL PEPTIDES (VIP, αMSH AND γ3MSH) INTO LATENT ASSOCIATED PEPTIDE (LAP) RESULTS IN TARGETED ANTI-INFLAMMATORY AND ANTI-RHEUMATIC THERAPEUTICS

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The engineering of a latent cytokine using the latency associated peptide (LAP) of TGF-β1 fused via an MMP cleavage site to interferon (IFN)-b prolongs in vivo half–life and the MMP cleavage site permits IFN-b release specifically at a site of inflammation (1). We aimed to assess whether small peptides, such as VIP, αMSH and γ_3MSH can be similarly delivered and modulate inflammation and arthritis. Latent forms of VIP, αMSH and γ_3MSH were generated by fusion to the LAP of TGF-β through an MMP cleavage site, purified, and ability to be hydrolyzed by MMP1 confirmed. The biological activity of LAP-γ_3MSH was compared to protein alone in vivo in murine monosodium urate crystal (MSU) peritonitis (16 h Gr1 + cell influx) and LAP-MMP (control), LAP-MMP-VIP or LAP-MMP-αMSH were delivered by gene delivery via encoding plasmid DNA i.m. in collagen-induced arthritis (CIA). The recruitment of MSU induced PMN (GR1+) infiltration was reduced by 35% with γ_3MSH at 1 nmol whereas administration of purified latent LAP-MMP-γ_3MSH at 0.03 nmol attenuated leucocyte influx by 50%. Gene delivery of plasmids coding LAP-MMP-VIP and LAP-MMP-αMSH at disease onset reduced the development of CIA compared to empty LAP-MMP. A significantly lower degree of paw inflammation, inflammatory cell influx, and the erosion of cartilage and bone was seen with LAP-MMP-VIP and LAP-MMP-αMSH. Collagen type II autoantibody and serum inflammatory cytokine production were also reduced in these two groups when compared to LAP-MMP. The short half lives of VIP and αMSH limit their therapeutic use. Incorporation of these small anti-inflammatory peptides into the LAP shell and delivered either locally or through gene therapy can control inflammatory and rheumatic disease. This concept can be developed to control human arthritides and other autoimmune diseases. Adams G, Vessillier S, Dreja H, Chernajovsky Y. Targeting cytokines to inflammation sites. Nat Biotechnol. 2003;21(11):1314–20.
Disclosure of interest: None declared.

OC-026
THE "MULTI-EPITOPE-TARGETING" APPROACH TO ANTIGEN-BASED IMMUNOTHERAPY OF MULTIPLE SCLEROSIS-LIKE DISEASE IS MORE EFFICACIOUS THROUGH A SPECIFICALLY ENGINEERED MULTI-EPITOPE PROTEIN THAN THROUGH PEPTIDES

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Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS, associated with complex anti-myelin autoimmunity. Among all approaches proposed for MS therapy, an approach that neutralizes only the pathogenic T cells reacting against myelin, while leaving the innocent immune cells intact, is the ultimate goal in the immune-specific therapy for MS. The multiplicity of primary target antigens, along side the dynamic nature of autoimmunity in MS, whereby the specificity of anti-myelin pathogenic autoreactivities may shift or expand in the same patient with disease progression, impose major difficulties in devising immune-specific therapy to MS. To overcome this multiplicity and the potential complexity of pathogenic autoreactivities in MS, we have put forward the concept of concomitant multi-antigen/multi-epitope targeting as, a conceivably more effective approach to immunotherapy of MS. We constructed an EAE/MS-related synthetic human Target Autoantigen Gene (MS-shMultiTAG) designed to encode in tandem only EAE/MS related epitopes of all known encephalitogenic proteins. The MS-related protein product (designated Y-MSPc) was immunofunctional and upon tolerogenic administration, it effectively suppressed and reversed EAE induced by a single encephalitogenic protein. Furthermore, Y-MSPc also fully abrogated the development of "complex EAE" induced by a mixture of five encephalitogenic T-cell lines, each specific for a different encephalitogenic epitope of MBP, MOG, PLP, MOBP and OSP. Strikingly, Y-MSPc was consistently more effective than treatment with the single disease-specific peptide or with the peptide cocktail, both in suppressing the development of "classical" or "complex" EAE and in ameliorating ongoing disease. Overall, the modulation of EAE by Y-MSPc was associated with energizing the pathogenic autoreactive T-cells, downregulation of Th1/Th17 cytokine secretion and upregulation of TGF-b secretion together with induction of regulatory $CD4^+FoxP3^+$ cells.
Disclosure of interest: None declared.

Pain, neuroinflammation (OC03)

OC-027
THE CIA31 LOCUS ON MOUSE CHROMOSOME 15 IS INVOLVED IN DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS—AN ANIMAL MODEL FOR MULTIPLE SCLEROSIS

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In the search for genes involved in the development of autoimmune diseases, the animal model for Multiple Sclerosis, Experimental Autoimmune encephalomyelitis (EAE), are used. By crossing the EAE-resistant mouse strain RIIIS/J and the EAE-susceptible mouse strain B10.RIII, the *Eae2* locus on mouse chromosome 15, have been found. The *Eae2* is also linked to the expression of the co-stimulatory molecule CD86 on stimulated macrophages. Within *Eae2*, a sub-locus linked to Collagen Induced Arthritis (CIA), *Cia31*, has been identified. The aim of this project is to investigate whether genes within *Cia31*, in addition, are linked to EAE and to investigate putative candidate genes. The

congenic B10.RIIIRIIIS/J_*Cia31* mice have eight genes (2 Mbp) from the RIIIS/J mouse strain. Induction of EAE by immunization with the myelin basic protein peptide 89−101 resulted in later onset and lower severity of disease in the congenic B10.RIII RIIIS/J_*Cia31* mice compared to littermate controls. By gene expression analyses and bioinformatics studies, we have suggested the *Ctnnd2* and *March6* genes as two potential EAE candidate genes in *Cia31*. *Ctnnd2* codes for δ-catenin, a brain protein involved in the transduction of signals between neurons. Deletions or mutation in this gene results in severe retardation in humans. *March6* codes for an E3 ubiquitin ligase. The March family of proteins regulates membrane molecules, among those, CD86. Sequence analyses of the *Ctnnd2* and *March6* genes show variations between the RIIIS/J and B10.RIII strains in both coding and non-coding regions. Furthermore, we have found a differential expression of the CD86 protein on stimulated peritoneal macrophages from congenic B10.RIII$^{RIIIS/J_Cia31}$ mice compared to littermate controls. We hypothesize that this might be linked to an amino acid change in the March6 protein.
Conclusion: A gene(s) in the congenic *Cia31* fragment in mouse chromosome 15, are involved in development of EAE, the animal model for multiple sclerosis.
Disclosure of interest: None declared.

OC-028
DYNAMIC OF CXCL12 EXPRESSION IN THE CNS OF EAE MICE AND ITS POTENTIAL ROLE IN MATURATION OF OLIGO/NEURO PROGENITORS

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Multiple sclerosis (MS) is an inflammatory, autoimmune disease characterized by massive demyelination associated with axonal damage and neuronal loss, typically accompanied with episodes of relapses/remissions. Both in MS and its murine model, experimental autoimmune encephalomyelitis (EAE), the development of central nervous system (CNS) inflammation was shown to be associated with robust upregulation of the CXCL12 expressed in the CNS. However, the dynamic of CXCL12 at the different stages of EAE and in particular at recovery and indeed, its expression by cells of neural lineages under such circumstances remains unclear. We noted that while CXCL12 expression in the CNS is increased in correlation with the development of severe pathological and clinical EAE, interestingly, the level of CXCL12 remains high during spontaneous clinical recovery associated with reduction of CNS inflammation. We show that not only astrocytes, but also newly generated neuronal and oligodendrocyte progenitors express CXCL12 in the CNS of mice at the peak of EAE and during recovery. CXCL12 immunoreactivity in neuronal and oligodendrocyte progenitor cells was further verified on in vitro differentiated adult neural stem/progenitor cells (aNPCs). In vitro studies further show that addition of CXCL12 enhances the differentiation of aNPCs towards neuronal and oligodendrocyte progenitors. Moreover, the addition of AMD3100, a CXCR4 antagonist, markedly inhibited the effect of both the exogenous and endogenous CXCL12 in promoting the differentiation of neuronal and oligodendrocyte progenitors, and their mature counterparts. Our data highlight the postinflammatory steady expression of CXCL12 by neuronal and oligodendrocyte progenitors and demonstrate its involvement in differentiation/maturation of aNPCs, implicating a role for CXCL12 in neural repair.
Disclosure of interest: None declared.

OC-029
ROLE OF TRANS-SIGNALING IN MEDIATING INTERLEUKIN-6 (IL-6) ACTIONS IN THE CENTRAL NERVOUS SYSTEM (CNS)

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IL-6 is implicated in the pathogenesis of various immunoinflammatory disorders of the CNS. IL-6 signals via binding to either the membrane bound IL-6Ra (classical signaling) or soluble (s)IL-6Ra (trans-signaling) that then form a complex with gp130 to activate the JAK/STAT and SHP2/MAPK pathways. Here we determined the relative importance of classical- versus trans-signaling in mediating IL-6 actions in the CNS. Biogenic mice were generated with CNS-restricted, astrocyte-targeted production of IL-6 and co-production of the specific inhibitor of IL-6 trans-signaling, sgp130-Fc (termed GFAP-IL6/sgp130 mice). Transgene-encoded IL-6 mRNA levels were similar in the brain of GFAP-IL6 and GFAP-IL6/sgp130 mice. However, GFAP-IL6/sgp130 mice had decreased pY-STAT3 in the brain due to a marked reduction in the total number of pY-STAT3-positive cells and the absence of detectable pY-STAT3 in specific cell types. These changes in pY-STAT3 were associated with a disproportionate reduction in the expression of various inflammatory markers as well as reduced vascular pathology and blood–brain barrier leakage. Degenerative changes in the cerebellum characteristic of GFAP-IL6 mice were almost absent in GFAP-IL6/sgp130 mice. The findings suggest that in the CNS: (1) sgp130-Fc is able to block IL-6 trans-signaling, (2) trans-signaling is important for IL-6 cellular communication with selective cellular targets and, (3) the blocking of trans-signaling may prove to be an effective means for alleviating the detrimental effects of IL-6. Support: NH&MRC grant 632754.
Disclosure of interest: None declared.

OC-030
BASOLATERAL MGLUR1 AMYGDALA-DRIVEN PREFRONTAL CORTICAL DEACTIVATION IN INFLAMMATORY PAIN

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The medial prefrontal cortex (mPFC) and the basolateral nucleus of the amygdala (BLA) are anatomically and functionally interconnected cooperating for the integration of emotionally salient sensory input. Specifically, the unpleasantness and affective component of chronic pain are driven by prelimbic and infralimbic (PL/IL) divisions of the mPFC and amygdala reciprocal connections. These regions can be profoundly affected by persistent pain generated by peripheral nerve injury or chronic inflammation in rodents. We show in this study that nociceptive responding BLA → mPFC pyramidal neurons undergo deactivation following carrageenan-induced inflammation together with downregulation of the endovanilloid/glutamate transmission (while GABAergic tone was enhanced) that can participate for impairing pain behaviour

mediated by BLA–mPFC circuits. In particular, it has been found here that pyramidal cells are inhibited by intra-plantar injection of carrageenan. We show unprecedented evidence of a neural mechanism by which selective metabotropic glutamate receptor subtype 1 (mGluR1) up-regulation in the BLA may impair mPFC function in inflammatory pain conditions. These results appear opposite to those ones found in neuropathic pain conditions (excitatory effect on pyramidal neurons). Thus amygdala can lead to differential changes in functionally distinct subsets of mPFC neurons in inflammatory versus neuropathic pain. In conclusion, we believe that the present data will help the understanding of the cross-talk between amygdala and mPFC, and the role of mGluR1 in the BLA for the functional changes of amygdalo-cortical interactions within the framework of the pain-related neuronal affective mechanisms.
Disclosure of interest: None declared.

OC-031
NECDIN MODULATES RESIDENT MICROGLIAL CELLS IN EXPERIMENTAL STROKE

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Necdin has been shown to promote neuronal survival, differentiation and restricts proliferation in hematopoietic stem cells. We tested the hypotheses that necdin is expressed in inflammatory cells and modulates inflammation in stroke. Necdin was specifically upregulated in cells derived from the ischemic hemispheres which was $CD11b^+$ sorted (60 min of middle cerebral artery occlusion). To corroborate, $necdin^+$/$Iba1^+$ double positive cells were observed and assumed the activated 'ameboid' morphology in brain sections. To test the role of necdin in growth arrest of inflammatory cells, we generated lentiviral particles (LVPs) to downregulate necdin at protein levels via RNA interference (RNAi). In concurrent with MCAO, LVPs were intra-arterially injected at the ipsilateral side. Cell counts show significantly higher amounts of $BrdU^+$, $Iba1^+$, $BrdU^+$/$Iba1^+$ double positive and $BrdU^+$/$Iba1^+$/LVP-$EGFP^+$ triple positive cells in the ischemic cortex in comparison to the control. Analysis of EGFP expressing bone marrow chimeric mice showed that $necdin^+$ cells were not derived from the peripheral macrophages invading the brain after MCAO. In summary, necdin is expressed in the hyperactivated subpopulation of microglia and restricts their proliferation.
Disclosure of interest: None declared.

OC-032
NUCLEAR RECEPTORS AS POTENTIAL TARGET FOR THE TREATMENT OF INFLAMMATION AND TISSUE INJURY ASSOCIATED WITH SPINAL CORD TRAUMA MICE

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The aim of the present study was to evaluate the contribution of Peroxisome Proliferator-Activated receptors (PPARs) and Liver X receptor (LXR) in an experimental model of spinal cord injury (SCI) in mice. To this purpose we used: (1) a high affinity ligand for PPAR-γ (15d-PGJ2); (2) a high affinity PPAR-β/δ agonist (GW0742); and (3) a potent LXR receptor ligand (T0901317). Moreover, to evaluate the contribution of PPAR-α, we used mice with a targeted disruption of the PPAR-α gene (PPAR-αKO). Spinal cord trauma was induced by the application of vascular clips (force of 24 g) to the dura via a four-level T5-T8 laminectomy. SCI in mice resulted in severe trauma characterized by oedema, neutrophil infiltration, and production of inflammatory mediators, tissue damage, and apoptosis. 15d-PGJ2, GW0742, and T0901317 treatments, 1 and 6 h after the SCI, significantly reduced: (1) the degree of spinal cord inflammation and tissue injury (histological score), (2) neutrophil infiltration (myeloperoxidase activity), (3) nitrotyrosine formation, (4) pro-inflammmaory cytokines expression, (5) NF-κB activation, (6) iNOS expression and (6) apoptosis (TUNEL staining, FasL, Bax and Bcl-2 expression). Moreover, 15d-PGJ2, GW0742, and T0901317 significantly ameliorated the recovery of locomotor function. In order to elucidate whether the protective effects of 15d-PGJ2 and GW0742 are related to activation of the PPAR-γ and PPAR-β/δ receptor respectively, we also investigated the effect of PPAR-γ and PPAR-β/δ antagonists on protective effects. Moreover, the absence of a functional PPAR-α gene mice resulted in a significant augmentation of inflammation and tissue injury events associated with spinal cord trauma. Our results clearly demonstrate that PPAR and LXR ligand treatments reduce the development of inflammation and tissue injury associated with spinal cord trauma.
Disclosure of interest: None declared.

OC-033
NEUROVEGETATIVE CONSEQUENCES AND INTESTINAL INFLAMMATION IN A LIPSKA-WEINBERGER NEONATAL MODEL OF SCHIZOPHRENIA

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The neonatal ventral hippocampus lesion (NVHL) model has been proposed for understanding the neurodevelopmental aspects of schizophrenia. With the conviction that a visceral control mismatch leaves functional traces, we propose observing, in vitro, some properties of gut of lesioned rats and sham operated rats. Ibotenic acid was delivered bilaterally in the ventral hippocampus of 7-day-old pups under anaesthesia. Lesion size was assessed by image analysis software. Adult animals were euthanized for in vitro experiments. Techniques of histology, MPO assay and QPCR were used. Abdominal organs and adipose tissue were weighed. Lesioned rats showed a decrease in weight. The mass of adipose tissue and liver were reduced in lesioned rats whereas stomach, adrenal glands and testicles weights were increased. MPO activity and the number of mast cells were increased in intestine of lesioned rats. The thickness of intestinal layers was decreased, whereas they were increased in the colon of lesioned rats. Finally, changes in GABA transporters (GAD67, GAT2, GAT3) were demonstrated. The present data show

that hippocampal lesions induce metabolic and neuronal alterations associated with intestinal inflammation.
Disclosure of interest: None declared.

OC-034
INHIBITION OF MESENTERIC LYMPHATIC VESSEL PUMPING BY THE NEURO-IMMUNO-MODULATORS VIP AND PACAP

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Efficient lymph flow is critically important during the inflammatory process to limit edema formation and to allow antigen-presenting cells and lymphocytes to migrate from the inflamed tissue to the draining lymph nodes and mount an adequate immune response. Lymph flow is an active process enabled by rhythmical, phasic constrictions of the collecting lymphatic vessels. This function, or lymphatic pumping, is intrinsic to the muscles embedded in the vessel wall and is modulated by many mediators present in the vicinity of the vessels during inflammation. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) are neuro-immuno-modulators with anti-inflammatory properties released by peptidergic nerves, and by inflammatory cells patrolling the interstitium and lymph. The aim of this study was to investigate the effects of VIP and PACAP on lymphatic pumping and the cellular mechanisms they activate. Pharmacological assessment of vessel diameter change and intracellular microelectrode electrophysiology demonstrated concentration-dependent inhibitions of lymphatic contraction frequency and smooth muscle hyperpolarization in response to VIP, PACAP27 and PACAP38. Quantitative real-time PCR and immunohistochemistry revealed mRNA and protein expression of PAC1, VPAC1 and VPAC2 receptors in lymphatics. Abolition of the inhibitory responses in the presence of the VPAC2/PAC1 antagonist PACAP6-38 and the VPAC1/VPAC2 antagonist VIP6-28 suggested a predominant involvement of VPAC2 in the peptide actions. Moreover, abolition of the VIP-induced responses with glibenclamide suggests K_{ATP} channel involvement. During inflammation, VIP and PACAP are likely to be released by nerve terminal and inflammatory cells in the vicinity of lymphatics. In this context, the potent lymphatic pumping inhibition these peptides cause may influence the inflammatory process by compromising lymph drainage, edema resolution and immune cell trafficking.
Disclosure of interest: None declared.

Cancer, genetics and inflammation (OC04)

OC-035
TUMOR PYRUVATE KINASE M2 AS BIOMARKER OF ESOPHAGEAL CANCER AND GERD

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Tumor M2-PK is known to be present in high concentrations in tumor tissue, plasma and other body fluids. Plasma determination of Tumor M2-PK has been shown to discriminate between benign/premalignant and malignant conditions. Tumor M2-PK has also been reported to be increased in some inflammatory conditions. The present study explores the ability of Tumor M2-PK expression to differentiate malignancy from chronic inflammatory condition (GERD) and assess it as a noninvasive biomarker in early diagnosis of esophageal cancer. The Tumor M2-PK concentration was quantitatively measured in EDTA-plasma of 218 individuals by a sandwich ELISA method (Schebo®Tech, Germany). Among them were 64 patients of histological confirmed esophageal cancer, 58 patients of GERD, 34 patients of breast cancer, and 62 healthy individuals as controls. The cut-off value to discriminate patients from controls was taken as 15 U/ml for Tumor M2-PK. In patients with esophageal cancer Tumor M2-PK mean values was 60.72 U/ml, in GERD was 32.28 U/ml, in breast cancer was 25.97 U/ml and in controls was 2.61 U/ml. Comparison of means showed a significant difference of esophageal cancer from GERD ($t = 6.544$, $P < 0.001$) and controls ($t = 16.405$, $P < 0.001$). GERD patients had non-significantly higher values than breast cancer patients ($t = 1.037$, $P > 0.05$). Patients at stage I of esophageal cancer and breast cancer had higher values of Tumor M2-PK than GERD patients. Sensitivity and specificity of Tumor M2-PK in differentiating malignancy from healthy controls was 94 and 95% in esophageal cancer and 67 and 90% in breast cancer. In differentiating GERD from esophageal cancer it was 94 and 64% respectively. Tumor M2-PK is a biomarker for malignancy and is a better marker for esophageal cancer than Breast cancer. It can differentiate early stages of esophageal malignancy from GERD but its ability to be a noninvasive preclinical biomarker for esophageal cancer needs further elucidation.
Disclosure of interest: None declared.

OC-036
THE MACROPHAGE MIGRATION ACTIVITY IS REQUIRED FOR TUMOUR CELL INVASION

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The presence of inflammatory cells like macrophages in tumours is often associated to enhanced invasiveness of tumour cells and formation of metastases and therefore, it is considered as a factor of bad prognosis. Thus we investigated the role of human macrophages in tumor cell invasiveness. First, we examined the migration modes used by human macrophages and breast carcinoma SUM159 cells when they infiltrate a thick layer of Matrigel® matrix. SUM159 cells used the mesenchymal migration mode in a matrix metalloproteinase (MMP)-dependent manner. In the presence of macrophages, SUM159 cells were no longer sensitive to MMP inhibitors: they followed macrophages which formed tunnels in the matrix in a collective and amoeboid migration mode. Next, we used tumour cell spheroids (TS) of SUM159 cells embedded into Matrigel, as a more complex set-up mimicking the tumour environment. Under these conditions, tumour cells were unable to invade the surrounding 3D

matrix unless macrophages infiltrated the TS. To determine if macrophage migration ability triggers invasion of tumor cells we used bone-marrow derived macrophages from wild-type or *hck−/−* mice. Hck is a phagocyte specific tyrosine kinase involved in mesenchymal 3D migration of macrophages. When TS were infiltrated by *hck−/−* macrophages deficient for Matrigel migration, tumour cells had a reduced invasive capacity indicating that the intrinsic migration ability of macrophages is critical for tumour cell invasiveness. Here, we developed a new in vitro model allowing the study of macrophage-tumour cell relationship in the invasion process. These results (1) demonstrate a MMP bypass of tumor cell migration in the presence macrophage, (2) support the pivotal role of the macrophage migration process in tumour cells invasion (3) highlight that inhibition of macrophage migration is a new pharmacological strategy.
Disclosure of interest: None declared.

OC-037
TARGETING MYELOID DERIVED SUPPRESSOR CELLS IN VIVO WITH 5-FLUOROURACIL REDUCES CANCER INCIDENCE IN THE INTERLEUKIN-10 MODEL OF COLITIS-ASSOCIATED CANCER

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The myeloid derived suppressor cell ($Gr1^+CD11b^+$, MDSC) is a recently described protumorigenic cell type with various immunosuppressive and proangiogenic affects. 5-Fluorouracil (5-FU), a pyrimidine analog chemotherapeutic drug has been shown to reduce MDSC recruitment in vivo in a xenograft model of lymphoma and to selectively kill MDSC in vitro. We have previously shown that MDSC are upregulated and correlate with neoplastic changes in the colon in the interleukin-10 deficient mouse (IL-$10^{-/-}$). The aim of this study was to test whether targeting MDSC in vivo with 5-FU affects cancer development in vivo in the IL-$10^{-/-}$ model of colitis associated cancer. 6-week-old IL-$10^{-/-}$ mice were given 6 weekly intraperitoneal injections of 50 mg/Kg 5-FU or saline control ($n = 5$). Animals were sacrificed at 12 weeks and colons were harvested. Inflammatory changes, polyps and dysplasia scores were evaluated macroscopically and histologically using previously published scoring systems. $Gr1^+CD11b^+$ cells per unit area of mucosa (cells/10^6 mm^2) were determined via immunohistochemical analysis of colon sections with anti-mouse antibodies. Student's T test was used to statistically compare groups. Both macroscopic (3.20 ± 0.83 vs. 2.67 ± 0.81) and histological (4.17 ± 0.69 vs. 3.84 ± 0.58) scores of inflammation were comparable between control and 5-FU treated mice. Polyp scores were reduced following 5-FU (0.33 ± 0.52) treatment as compared to controls (3.00 ± 0.00). 5-FU treatment reduced MDSC levels in the colon down to near wild type levels at 9.45 ± 1.59 cells/10^6 mm^2. 5-FU (0.17 ± 0.40) treatment reduced dysplasia scores from control levels (4.40 ± 1.82). Saline treated IL-$10^{-/-}$ mice had MDSC counts of 30.61 ± 4.23 cells/10^6 mm^2. Our data show that treatment with 5-FU does not affect inflammatory markers, but decreases neoplastic changes and MDSC recruitment in the IL-$10^{-/-}$ mouse. This is the first evidence that targeting MDSC in vivo in a model of colitis-associated cancer can reduce cancer incidence.
Disclosure of interest: None declared.

OC-038
POSITIVE CORRELATION BETWEEN ACUTE RESPONSE INFLAMMATORY AND HIGHER EXPRESSION OF HYPOXIC GENES AFTER ISCHEMIA AND REPERFUSION IN MICE

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Ischemia induce a hypoxia situation starting a sequence of events that are amplified after reperfusion leading to severe organ injury and dysfunction by production of reactive oxygen species, leukocyte infiltrate and protein extravasation. Inflammation has been considered the most important cause of injury in organs subjected to ischemia–reperfusion (I/R). The aim of this study was evaluate the endogenous mechanisms responsible for regulation of local and systemic inflammation after intestinal I/R in mice genetically selected for maximal (AIR_{max}) or minimal (AIR_{min}) local Acute Inflammatory Response. After 45 min of intestinal ischemia, mice were submitted to reperfusion (0, 1, 4 and 24 h). The extent of lung injury was evaluated by myeloperoxidase (MPO) activity and cellular infiltration in the lung parenchyma. Hypoxic genes and inflammatory cytokine expressions were evaluated in the lung by Real Time PCR. It was observed that AIR_{max} I/R mice had a progressive neutrophilia starting after ischemia and peaking at 4 h of reperfusion ($2.7 \pm 0.3 \times 10^6$ cell/ml). On the other hand, AIR_{min} I/R mice had a low infiltrate with equivalent levels compared to the control groups ($1.2 \pm 0.2 \times 10^6$ cell/ml). Corroborating these results the MPO activity was higher in the lungs of AIRmax I/R than AIRmin I/R mice ($p < 0.01$). A high expression of *Hif 1-α, Vhl, Il-1b* and *Il-6* was observed in AIRmax I/R mice. Conversely, AIR_{min} I/R mice presented low expression of these genes. The positive correlation found in AIR mice between the inflammatory reactivity and hypoxic genes expression suggest that the genes responsible for regulating the acute inflammatory response, fixed by selective breeding, can modulate the expression of genes involved in situations of hypoxia, and consequently modify the lung injury intensity hypoxia-dependent.
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OC-039
COMPLEMENT COMPONENT C5A RAPIDLY INCREASES AVAILABILITY OF TLR2 ON THE SURFACE OF GR1 + CD11B + MYELOID-DERIVED SUPPRESSOR CELLS INDUCED BY A TUMOR

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Tumor microenvironment components contribute to metastases of different carcinomas through TLR2-mediated stimulation of production of pro-tumorigenic cytokines by inflammatory myeloid cells. One of subsets within the inflammatory myeloid cells compartment is immature and includes myeloid-derived suppressor cells (MDSC), accumulating in tumor-bearing hosts in response to various inflammatory mediators. We supposed that TLR2 might become more readily available on the surface of MDSC in response to component of complement C5a, one of the most active inflammatory mediators, concentration of which is increased in a tumor microenvironment. In our experiments, C57BL/6.hMUC1 transgenic mice with growing subcutaneous pancreatic tumors PancO2.hMUC1 exhibited a significant accumulation of myeloid-derived suppressor cells (MDSC) with a phenotype $CD11b^{+}Gr1^{+}$ versus non-tumor-bearing control mice. The majority of MDSC (>70%) expressed inflammatory markers S100A9 and CD88 (C5a receptor). At a maximum dose of 100 ng/ml recombinant C5a increased the proportion of monocytic $S100A9^{high}$, $TLR2^{high}$ cells within the $CD11b^{+}$, $Gr1^{+}$ MDSC more than twofold during 6-h incubation ($P < 0.05$, three repeats with cells harvested from separate animals). Increased expression of TLR2 was associated with decreasing the expression of CD88 (presumably due to ligand-receptor internalization). We suggest that rapid C5a-induced increase in availability of TLR2 on the MDSC surface may promote metastazing through activation of MDSC known to tolerize anti-tumor T cells. Hypothetically, MDSC activation may be supported by TLR2 ligands hyaluronan and versican commonly overaccumulated within the tumor microenvironment. We also hypothesize that co-accumulation of $TLR2^{high}$ MDSC and versican/hyaluronan ligands within the same compartments may serve as candidate biomarker pattern for propensity of a tumor to metastasize. Authors appreciate the help of Dr. A. Hollingsworth, Mss. J. Anderson and Mr. T. Caffrey.
Disclosure of interest: None declared.

OC-040
MYELOTOXIC EFFECTS OF 7,12-DIMETHYLBENZ[A]ANTHRACENE ON BONE MARROW CELLS FROM MICE GENETICALLY SELECTED FOR INFLAMMATORY REACTIVITY

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Polycyclic Aromatic Hydrocarbons (PAHs), such as DMBA, induce a decrease in bone marrow cells (BMC) numbers and hematological alterations resulting in an immunosuppressive state. DMBA metabolism depends on the activation of the aryl hydrocarbon receptor (AhR). Mice genetically selected for high (AIR_{max}) or low (AIR_{min}) acute inflammatory response to s.c. injection of Biogel P100 presented a complete segregation of Ahr alleles endowed with low (Ahr^{d}) or high (Ahr^{b1}) affinity to PAHs, respectively. Accordingly, AIR_{max} are more resistant than AIR_{min} to DMBA induced skin and lung carcinogenesis. We investigated the effect of DMBA treatment on BMC of AIR selected mice and its possible impact on acute inflammatory response. AIR_{max} and AIR_{min} mice were treated with a single i.p. dose of 50 mg/kg DMBA in olive oil. Flow cytometric analysis was used to determine hematopoietic stem cells (HSC) ($Lin^{-}/Sca\text{-}1^{+}/c\text{-}Kit^{+}$) and neutrophils ($Gr\text{-}1^{hi}/CD11b^{hi}$). Proliferation index of BMC was determined in response to GM-CSF stimulus. Acute inflammation response was also evaluated after 24 h of Biogel P100 subcutaneous (s.c.) injection. DMBA treatment resulted in a significant ($p < 0.01$) decrease in neutrophil population and increase of HSC on bone marrow in AIRmin mice only. Blast cells from DMBA-treated AIRmin presented displastic nucleus, which is one of the distinguishing features of preleukemia, and myeloid cells showed low proliferation capacity after in vitro GM-CSF stimulation. These effects on myeloid BMC reflect on an impaired cellular migration to the inflammatory site 24 h after Biogel injection. This investigation demonstrates that AIR_{max} mice are protected and AIRmin are prone to acute bone marrow cytotoxic and presumable preleukemic effects of DMBA. The complete segregation of alleles at the *Ahr* locus found in AIR mice might contribute to their differential inflammatory responses and to the phenotypes of susceptibility and resistance of BMC to DMBA-induced effects.
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OC-041
EFFECTS OF IRON ON T-BET/GATA3 EXPRESSION AND EPIGENETIC REGULATIONS IN TH1 AND TH2 INFLAMMATORY MODELS

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At birth, immune system switches from a predominant Th2 profile to a balanced Th1/Th2 profile. This switch may be modulated by food factors which may participate in epigenetic regulation of immune orientation key genes expression. This study aims at identifying the incidence of daily ingestion of iron by juvenile mice on the orientation of immune response following the induction of an experimental colitis or food allergy. Six groups of male juvenile Balb/c mice were treated during 6 weeks with vehicle or iron solution (150 mg/kg/day–100 µl/day po). In parallel, half of the animals were submitted to sham or ovalbumin sensitization (1 mg/ml–100 µl/day po) and challenge (100 mg/ml–100 µl po). In the other half, we induced a sham or experimental TNBS colitis (40 mg/kg in 50% Ethanol 1 week follow-up) after the 6 weeks of iron treatment. Spleen expression of T-bet and GATA3 were evaluated by RT PCR. Their epigenetic regulations were assayed by the analysis of cytosine methylation levels of their respective promoter sequence. In Th1 model, T-bet/GATA3 ratio increase is mainly linked to T-bet promoter gene hypomethylation and thus gene overexpression. The Th2 model revealed upregulation of GATA3/T-bet ratio consecutive to GATA3 expression increase and T-bet expression inhibition. However, no modification of promoter gene methylation profile was detected. Iron prevented T-bet/GATA3 ratio upregulation and modulation of T-bet promoter gene methylation in the Th1 model. On the other hand, it prevented GATA3/T-bet ratio increase in the Th2 model but could not modulate methylation

profile. Chronic daily intake of iron in juvenile individuals interfere with T-bet and GATA3 gene expression reestablishing in both models the initial Th1/Th2 balance. In Th1 mediated immune response, it is linked to an epigenetic regulation of the promoter region methylation profile while the Th2 model revealed to be more complex and possibly, the resulting orientation will be associated to other epigenetic phenomenon.
Disclosure of interest: None declared.

OC-042
AGE-SPECIFIC FAMILIAL RISKS OF RHEUMATOID ARTHRITIS

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Objective: Familial risks of rheumatoid arthritis (RA) have been assessed in small case–control studies, usually based on reported, but not medically verified, RA in family members; thus the degree of familial clustering of these diseases remains to be established.
Methods: The Multigeneration Register, in which all men and women born in Sweden from 1932 onward are registered together with their parents, was linked to hospital admission data. Standardized incidence ratios (SIRs) were calculated as the ratio of the observed to the expected number of cases of men and women with mothers and/or fathers affected by RA, compared with men and women whose mothers and/or fathers were not affected by RA.
Results: A total of respectively 18,496 and 51,921 cases of RA were recorded in offspring and parents. The overall significant SIRs among men and women with a mother, father or both parents hospitalized for RA varied between 2.64 and 11.01. Marginally higher familial risk was found before age 40. The risk was not dependent on gender. The parental transmission of RA was similar for both men and women. The population attributable fraction of familial RA was 4.29%.
Conclusions: This study has provided the first data on age-specific familial clustering of RA, based on medically confirmed records. The risks were so high that hereditary factors were considered to be likely to contribute, possibly modified by environmental factors. Age-specific risk tables would be helpful for clinical counseling.
Disclosure of interest: None declared.

Inflammatory cells: neutrophils and macrophages (SY05)

OC-043
A NOVEL CROSS-TALK IN RESOLUTION: H2S ACTIVATES THE ANNEXIN A1 PATHWAY

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Hydrogen sulphide (H_2S), a gaseous mediator synthesized in several mammalian tissues by two main enzymes CBS and CSE, increases under inflammatory conditions or sepsis. Since H_2S and H_2S-releasing molecules afford potent inhibitory properties on the process of leukocyte trafficking, we tested whether endogenous Annexin A1 (AnxA1) could display intermediary functions. Treatment of human PMNs the H_2S donor NaHS (10–100 μM) provoked prompted and intense mobilization (>50%) of AnxA1 from the cytosolic pool to the cell surface, supporting the inhibitory effects of the gas in the flow chamber assay. Such in vitro actions could be translated in analyses of the inflamed microcirculation, where NaHS (100 μmol/kg s.c., −1 h) afforded marked inhibition of IL-1-induced cell adhesion and emigration in the mesenteric vessels of wild type, but not $AnxA1^{-/-}$, mice. Next, we investigated whether endogenous AnxA1 could modulate H_2S synthesis, indicating existence of a positive circuit. To this end, a marked increase in CBS and/or CSE expression in a variety of tissues (aorta, kidney, spleen) tested from $AnxA1^{-/-}$ mice, as compared to wild type animals, was quantified by qPCR. Moreover, NaHS was able to counteract the increase in expression in iNOS and COX2 (fourfold and sevenfold reduction, respectively) upon LPS-stimulation of bone marrow derived macrophages (BMDM), though it was totally inactive in cells prepared from $AnxA1^{-/-}$ mice. Taken together, these data strongly suggest—for the first time—the existence of a positive interlink between AnxA1 and the H_2S pathway, providing a novel mechanistic explanation for the exquisite properties of H_2S in the control of experimental inflammation. These finding may be relevant to innovative discovery programmes aiming at harnessing the biological properties of H_2S.

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Disclosure of interest: None declared.

Young Investigator Award Winner.
For mini paper see page 289

OC-044
CELLULAR MECHANISMS AND MOLECULAR DETERMINANTS OF HUMAN MACROPHAGE 3D MIGRATION

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Tissue infiltration of macrophages (Mph), critical for innate immunity, is also involved in pathologies, such as chronic inflammation and cancer. In vivo, Mphs migrate mostly in a constrained three-dimensional (3D) environment. We have recently shown that, depending on the extracellular matrix (ECM) architecture, human Mphs are able to migrate in 3D using two distinct migration modes: they use the protease-independent amoeboid migration mode (like other leukocytes) in porous ECM and the mesenchymal migration mode in non-porous ECM. In addition, we have identified the phagocyte specific tyrosine kinase Hck as an effector of Mph mesenchymal migration. Now we show that the mesenchymal migration of macrophages in ECM is matrix metalloproteinase (MMP)-independent, whereas it is MMP-dependent inside tumour cell spheroids. ECM degradation is the result of several cellular processes: (1) formation of 3D podosomes with proteolytic activity, (2) matrix compaction, and (3) matrix internalization. By using composite matrices, we show that a single Mph is able to switch from the amoeboid to the mesenchymal migration mode and

vice versa. To identify molecular determinants of MDM 3D migration, we have undertaken a transcriptomic analysis to compare gene expression profiles between 2D- and 3D-migrations and between 3D amoeboid versus 3D-mesenchymal migration. Analysis of transcriptomic signatures are currently under analysis. Characterization of Mph migration effectors will provide pharmacological targets to control deleterious Mph tissue infiltration.
Disclosure of interest: None declared.

OC-045
M-CSF IS NOT ESSENTIAL FOR B-1 CELL DERIVED PHAGOCYTE DIFFERENTIATION IN VIVO

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Mice with a naturally occurring Csfm(op)/Csfm(op) (op/op) gene mutation lack functional macrophage-colony stimulating factor (M-CSF) and are deficient of M-CSF-derived macrophages. They are severely monocytopenic and their remaining M-CSF-independent macrophages were shown to differ in differentiation and distinct functions when compared with phenotypically normal mice of the same background. Macrophages from op/op mice are able to sufficiently perform effector functions in vitro, such as phagocytosis and nitric oxide (NO) production. There are quantitative differences when peritoneal elicited macrophages were tested. Those from op/op mice showed a stronger response than those from control mice with regard to NO or IL-12 release. Interestingly, these same characteristics have been described to B-1 cell derived phagocytes. In this study, the peritoneal cell subpopulations of op/op mice and their littermates were analyzed. The aim was to investigate whether op/op peritoneal "macrophages" were B-1 cell derived phagocytes. The deficiency of cfms gene in op/op mice did not affect peritoneal B-1 cell population. Further, these cells are able to differentiate into phagocytes under LPS stimulus. The origin of these phagocytes was related to B-1 cells considering the VH11 Ig gene rearrangement detected in these cells. It also could be postulate that B-1 cell differentiation into phagocyte is independent of M-CSF. We also speculate that the MCSF-independent macrophage described in literature could be derived from B-1 cells. Investigations are being performed to provide more definitive evidence for the existence of B-1 cell derived phagocytes in vivo.
Financial support: FAPESP
Disclosure of interest: None declared.

Neuronal control of inflammation and pain (SY06)

OC-046
PROTEINASE ACTIVATED RECEPTOR-2 CAUSES A TRPV1-DEPENDENT SENSITISATION OF KNEE JOINT AFFERENTS IN NAIVE RATS

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Proteinase activated receptor-2 (PAR_2) is a G protein-coupled receptor that is activated by proteolytic cleavage. Previously, PAR_2 activation has been shown to cause pain in the joint (Helyes et al. 2010. Eur J Pain); however, the mechanisms responsible for this are unclear. To elucidate neuronal PAR_2 expression in joints, 10 μl of a 2% Fluoro-gold (FG) solution was injected into the knee joints of 4 naïve male Wistar rats to retrogradely label knee joint afferent neurones. Four days later, dorsal root ganglia (DRGs) from L3, L4 and L5 were removed, sectioned and stained with an anti-PAR_2 antibody. Cells positive for FG and for PAR_2 were counted and neuronal diameters measured. To measure joint nociception objectively, electrophysiological recordings were made from single unit afferent nerve fibres in the knee joints of male Wistar rats during both normal and noxious rotation of the joint. Afferent fibre firing rate was recorded during 10 s rotations made before and over a period of 15 min after close intra-arterial injection of the PAR_2 activating peptide, 2-furoyl-LIGRL-NH_2 (100 nmol/100 μl). Rats were either naïve ($n = 20$) or pretreated with the TRPV1 antagonist, SB366791 (500 μg/kg, i.p. 30 min prior to nerve recordings; $n = 6$) It was observed that $59 \pm 5\%$ of the FG positive cells in the DRGs were also positive for PAR_2, and PAR_2 was broadly expressed across all sizes of DRG neurones innervating the knee joint. 2-furoyl-LIGRL-NH_2 caused a significant increase in firing rate in joint afferent neurones during both normal and noxious rotation over a time period of 15 min. This effect could be blocked by pre-treatment with SB366791 ($p < 0.05$ compared to 2-furoyl-LIGRL-NH_2 alone, using two way ANOVA). In conclusion, PAR_2 is expressed in joint sensory neurones and activation of PAR_2 causes sensitization of these afferents via a TRPV1-dependent mechanism.
Disclosure of interest: None declared.

Young Investigator Award Winner.
For mini paper see page 289

OC-047
METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 7 AND 8 IN THE CENTRAL NUCLEUS OF THE AMYGDALA PLAY OPPOSITE ROLE IN INFLAMMATORY PAIN CONDITIONS

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Central nucleus of amygdala (CeA), "nociceptive amygdala", is a crucial area controlling the reciprocal interactions between chronic pain and its affective consequences. It is under glutamate- induced plasticity occurring after prolonged pain stimuli which leads to neuron hypersensitivity. CeA pain control pass through rostral ventromedial medulla (RVM) modulation. Metabotropic glutamate receptor subtype 7 and 8 (mGluR7 and mGluR8), which can be now selectively stimulated/inhibited with highly specific ligands, may "gently" affect glutamate transmission. AMN082 and (S)-3,4-DCPG, selective agonists for mGluR7 and mGluR8 receptors respectively, have been locally administered within CeA and behavioural and electrophysiological changes have been investigated in models of inflammatory chronic pain. mGluR7 and mGluR8 receptors play different, even opposite, actions on pain-control and induced changes in RVM. Moreover, mGluR7 shows to function only under normal conditions whereas mGluR8 requires a chronic pain-induced plasticity to play as analgesic. Having mGluR7 facilitated pain transmission, a selective mGluR7 antagonist, MPPIP has been tested.

It played an antinociceptive action, as expected. The activity of RVM putative "pronociceptive" ON and "antinociceptive" OFF cells has been also evaluated. Consistently with pain behaviour responses, (S)-3,4-DCPG inhibited ON and increased OFF cell activities whereas AMN082 increased the ON and reduced the OFF cell activity. MPIPP also changed the ON and OFF cell activity, consistently with behavioural analgesia. These results show that stimulation of mGluR8 and blockade of mGluR7 could be exploited for chronic pain control.
Disclosure of interest: None declared.

OC-048
ANTIGEN-DRIVEN T CELL RESPONSE INDUCES PERIPHERAL OPIOID-MEDIATED ANALGESIA IN MICE

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Painful sensation is a hallmark of the inflammatory response induced by pathogens or tissue damage. A large spectrum of molecules released within the inflamed tissue such as neuropeptides, prostaglandins or proteases induces pain by stimulating primary afferent neurons in situ. Activity of primary sensitive fibers can be counteracted by peripheral endogenous regulatory mechanisms involving local opioid release by leukocytes infiltrating the inflammatory site. Since pain is an inherent component of inflammation often accompanying immune response, we investigated the endogenous regulation of CFA-induced inflammatory pain in the context of adaptive T cell immune response. The nociceptive response to mechanical stimuli was studied using von Frey filaments in mice immunized with OVA in CFA. The nociceptive response of nude *versus* wild type mice was dramatically increased, demonstrating T cell deficiency associated with increased pain sensitivity. Based on adoptive transfer experiments of OVA-specific $CD4^+$ T lymphocytes into nude mice, we show that antigen-specific activated but not resting T lymphocytes are responsible for the spontaneous relief of inflammation-induced pain following antigen challenge. The analgesia was dependent on opioid release by antigen-primed $CD4^+$ T lymphocytes at the inflammatory site. Indeed, T cell-mediated analgesia was inhibited by local injection of an opioid receptor antagonist, unable to cross the blood–brain barrier. Notably, we found opioid precursor mRNA to be more than sevenfold increased in antigen-specific activated $CD4^+$ T lymphocytes, as compared to resting T lymphocytes in vivo. Taken together, our results show that $CD4^+$ T lymphocytes acquire anti-nociceptive effector properties when specifically primed by antigen and point out analgesia as a property linked to the effector phase of adaptive T cell response.
Disclosure of interest: None declared.

Rheumatoid arthritis: from pathophysiology to new targets (SY07)

OC-049
SYSTEMS APPROACH TO RHEUMATOID ARTHRITIS

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Objective: Recent findings based on basic and clinical research implicate important molecules and pathogenesis in rheumatoid arthritis (RA). However, there are still many unknown facts, due to the complexity of the disease and the numerous molecular players involved, and the integrated systems approach is needed to understand this disease.
Methods: A systems approach was performed to characterize the RA-associated lists of pathways, diagnostic markers, and therapeutic targets. Twelve global gene expression datasets obtained from the joint tissues or cell lines of patients with RA and osteoarthritis were analyzed through data integration with orthogonal non-negative matrix factorization, functional enrichment, and network modeling process.
Results: By the integrative analysis, we identified core RA-associated genes (RAGs). By mapping RAGs into functional pathways and diseases, we categorized key RA cellular processes, common pathological phenotypes, and their related diseases. Then, we reconstructed networks describing key RA processes and their inter-relationships based on their protein–protein and protein–DNA interaction. From these network models, we established cell type dominant signature, anti-TNF-alpha therapy perturbed domain, and putative regulatory mechanisms. Finally, we predicted essential molecules that can control or indicate such key processes. The list of potential markers and targets included 20 genes, which were selected based on the networks from RAGs identified from the integration of secretion data and cell/tissue specificity data.
Conclusion: We introduce lists of diagnostic markers and therapeutic targets identified using a systems approach. Also, suggested network models and panel of essential molecules can shed novel insights into RA pathogenesis and also serve as an important resource for discovery of therapeutic targets and diagnostic markers.
Disclosure of interest: None declared.

OC-050
SPECIFIC TARGETING OF IL-23 BY ACTIVE IMMUNIZATION IMPROVES INFLAMMATION AND DESTRUCTION IN A MURINE MODEL OF RHEUMATOID ARTHRITIS

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Background: IL-23 is a pro-inflammatory cytokine known to be essential for the differentiation of the Th17 lymphocytes, a subtype of T lymphocyte implied in auto-immunity. Its subunit, IL-23 p19, is specific of this cytokine. We had previously demonstrated for IL-1beta and TNF-alpha, that active immunization against these cytokines could be protective in animal models of arthritis.
Objectives: The aim of this study was to evaluate the effect of two vaccines targeting the IL-23 p19 subunit, IL23-K1 and IL23-K2, on collagen-induced-arthritis (CIA), a model of rheumatoid arthritis.
Methods: Using bioinformatics, we defined two peptides in IL-23 p19. Each peptide was coupled with keyhole limpet hemocyanin (KLH). Anti-murine IL-23 immunization was performed by injecting intramuscularly IL23-K1 or IL23-K2 formulated in incomplete Freund adjuvant (IFA), four times (D0, 7, 28, 49) in DBA/1 mice. Control groups received KLH or PBS at the same dates. CIA was induced by two subcutaneous injections of bovine type II collagen, the first at day 40 in Complete Freund Adjuvant, the second at day 61 in IFA. Anti-IL-23 and

anti-KLH antibody levels were assessed by ELISA. Pro and anti-inflammatory cytokines were quantified by qRT-PCR on the spleen and the synovium.
Results: The clinical scores show that mice treated with IL23-K1 develop less arthritis than the negative controls ($p < 0.05$). Mice vaccinated by IL23-K1 produced more anti-IL23 antibodies than the one vaccinated by KLH ($p < 0.001$). mRNA quantification showed that the IL23-K1 vaccination led to an increase of IL-10 in the spleen ($p < 0.05$ vs. KLH), without any effect on IL-17 level. Histology examination showed that IL23-K1 permitted a strong decrease of the joint destruction and inflammation ($p < 0.01$ vs. KLH and $p < 0.001$ vs. PBS).
Conclusion: These data show that targeting IL-23 p19 using a vaccination strategy may be efficient in CIA.
Disclosure of interest: None declared.

OC-051
GLPG0259, A SMALL MOLECULE INHIBITOR OF MAPKAPK5, DOSE-DEPENDENTLY REDUCES PRO-INFLAMMATORY CYTOKINES AND MMPS AND BLOCKS DISEASE PROGRESSION IN A MOUSE MODEL OF RHEUMATOID ARTHRITIS

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We identified the novel target kinase MAPKAPK5 as a mediator of matrix metalloprotease 1 (MMP1) production in synovial fibroblasts from rheumatoid arthritis (RA) patients (RASFs) [1]. GLPG0259 was selected as a clinical candidate out of a series of potent, small molecule MAPKAPK5 inhibitors.

Here we characterize the effect of GLPG0259 on pro-inflammatory cytokine and MMP production in vitro and ex vivo and evaluate GLPG0259's effect on disease progression in a collagen-induced arthritis (CIA) mouse model of RA. GLPG0259 and reference kinase inhibitors were assessed in human RASF cultures and in an ex vivo assay by measuring IL-6 and MMPs in synovial biopsies from CIA rats. GLPG0259 was thoroughly tested in the mouse CIA model (clinical score, histology, immunohistochemistry, X-ray radiography and μCT and in vivo imaging of protease activity). GLPG0259 concentration-dependently inhibited the release of IL-6 and MMPs in RASFs and synovial tissue, to levels better than or comparable to inhibitors of p38, JAK and SYK kinases. In contrast to p38 inhibitors, GLPG0259 inhibited MMP3 expression in synovial tissue. GLPG0259 did not block phosphorylation of JNK, ERK and p38 MAP kinases, indicating cytokine/MMP release is mediated by a downstream kinase. In the mouse CIA model, GLPG0259 reduced paw inflammation and bone destruction at doses of 1 mg/kg and higher. At 30 mg/kg, a well tolerated dose, GLPG0259 completely blocked disease progression, similar to the TNFα blocker Enbrel®. Immunohistochemistry and in vivo imaging showed that GLPG0259 reduced the expression and activity of MMPs within affected joints. GLPG0259, an orally bioavailable inhibitor of MAPKAPK5, has anti-inflammatory and bone protecting activity similar to or better than other therapeutically relevant inhibitors, thereby showing potential as a first-in-class candidate drug for the treatment of RA. GLPG0259 is currently being evaluated in a Phase II trial in RA patients.
[1] EULAR 2009: 2084.
Disclosure of interest: None declared.

Cell signaling and communication (SY08)

OC-052
ARACHIDONIC 5-LIPOXYGENASE ACTIVITY IS SUBJECT TO EPIGENETIC/ENVIRONMENTAL CONTROL IN INLFAMMATORY CELL POPULATIONS

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Objective: Our objective was to investigate putative epigenetic mechanisms regulating 5-LO expression in human mast cells (HMC-1), macrophages, neutrophils, and
RA-affected synovial fibroblasts (RASF).
Methods: 5-LO gene expression/activity in cultured cells were assessed by Western blotting, quantitative RT-PCR, and by lipid metabolite assays (ELISA). DNA/promoter methylation was analyzed by nested PCR/genomic DNA bisulfite sequencing. Histone (e.g., H3) methylation and acetylation patterns at promoter, exonic, and intronic sequences of the 5-LO gene were quantitated by chromatin immunoprecipitation (ChIP) assays. RT2 miRNA PCR Arrays were used to screen for 5-LO mRNA 3′UTR specific miRNA expression under study conditions.
Results: In untreated mast, macrophage and neutrophil-like cells, the degree of DNA CpG island methylation in the 5-LO gene proximal promoter correlated inversely with 5-LO expression. In contrast, RASF were 5-LO negative but both distal and proximal promoter regions were hypomethylated; ChIP analysis resolved this inconsistency when it was revealed that histone H3 is hypermethylated/hypoacetylated at Lys-9 and -27 residues in all promoter, exonic, and intronic sequences tested, favouring promoter silencing. Dexamethasone (DEX) treatment of HMC-1 cells increased basal expression levels of 5-LO, a process associated with reduced methylation but increased acetylation of histone H3 on lysines 9 and 27. These results were confirmed with cell-permeable histone deacetylase and methyltransferase inhibitors. RT2 miRNA PCR arrays revealed little correlation between the levels of 5-LO mRNA and protein expression and 32 miRNAs in RASF and HMC-1 mast cells.
Conclusion: DNA/histone modifications represent mechanisms governing both basal and dysregulated 5-LO gene expression. However, epigenetic mechanisms function in a cell specific manner making disease control targeting DNA/histone modification enzymes with specific pharmacotherapy challenging.
Disclosure of interest: None declared.

OC-053
INTERLEUKIN (IL)-31 ACTIVATES SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION FACTOR (STAT)-1/5, AND EXTRACELLULAR SIGNAL REGULATED KINASE (ERK) 1/2 AND MODULATES IL-12 PRODUCTION IN HUMAN MACROPHAGES FOLLOWING STIMULATION WITH STAPHYLOCOCCAL EXOTOXINS

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Background: IL-31 is a cytokine expressed by T cells following activation with cytokines or staphylococcal exotoxins. A major function of IL-31 in atopic dermatitis (AD) is the induction of pruritus in the skin. We recently showed that IL-31 induces pro-inflammatory cytokines following staphylococcal exotoxins stimulation in human macrophages. However, signalling pathways of IL-31 in activated human macrophages still remain unclear. The aim of the study was to investigate the signalling pathways of IL-31 receptor in activated macrophages as well as role of IL-31 in modulation of other cytokines.
Methods: Human macrophages were prestimulated with staphylococcal exotoxins (SEB, a-toxin) in order to up-regulate the IL-31 receptor with and without IL-31. Phospho-STAT (pSTAT) 1/3/5, phospho extracellular signal regulated kinase (ERK 1/2) as well as β-actin levels were determined by means of Western blot analysis. IL-12 secretion was assessed by using an ELISA.
Results: IL-31 activated strongly STAT-1 and 5 but not STAT-3 in human macrophages after up-regulation of IL-31 receptor with staphylococcal exotoxins. Furthermore, IL-31 down-regulated IL-12 secretion via ERK 1/2 phosphorylation in human macrophages following up-regulation of IL-31 receptor with staphylococcal exotoxins.
Conclusion: Th2 cytokine IL-31 induces pro-inflammatory effects in activated human macrophages via STAT-1 and 5 phosphorylation. IL-31 modulates Th1 cytokine IL-12 production via ERK1/2 phosphorylation. IL-31 induced ERK 1/2 activation contributes to the underlying mechanism of IL-12 suppression in macrophages. This mechanism may be relevant in Th2 inflammatory responses and may help to develop therapeutic strategies in IL-31 associated diseases such as AD.
Disclosure of interest: None declared.

OC-054
A TRIF-DEPENDENT NEGATIVE REGULATORY PATHWAY ORCHESTRATES SEPSIS ASSOCIATED ENDOTOXIN TOLERANCE IN MICE AND HUMANS

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Endotoxin tolerance is defined as the failure to respond to endotoxins as a result of prior exposure to low doses of endotoxin. Clinically, this is observed in monocytes/macrophages from sepsis patients and has been linked to sepsis-associated mortality. Gram-negative endotoxins (e.g. Lipid A, LPA) are recognized by Toll-like receptor 4 (TLR4) which signals through two adaptors, MyD88 and TRIF. The molecular mechanisms explaining endotoxin tolerance is poorly understood. Using systems biology approach, we identify a novel molecular mechanism for LPA/TLR4-induced endotoxin tolerance in macrophages. LPA-tolerized murine macrophages showed inhibited expression of MyD88-dependent inflammatory cytokine genes like TNFA, CCL3 and IL6, together with attenuated NF-κB activation. Computational modeling predicted a 'putative' TRIF-induced negative regulator(s) to mediate this event. Biochemical studies with TRIF-mutant macrophages identified that a functional TRIF pathway triggers the expression of negative regulators of TLR4 signalling which induce endotoxin tolerance. In vivo studies confirmed TRIF-silencing to inhibit endotoxin tolerance as well as block mortality in a mouse sepsis model. Our findings were also demonstrated in human monocytes, human natural mutants for TLR components (e.g. STAT1, UNC93B, MYD88) and in sepsis patient monocytes. These data demonstrate for the first time a negative regulatory role of TRIF-dependant pathway in orchestrating sepsis-associated endotoxin tolerance in mice and humans. Future targeting of TRIF-pathway may be helpful to modulate sepsis.
Disclosure of interest: None declared.

Sepsis and inflammation (OC05)

OC-055
ASSOCIATION OF PTEN TO TLR4 MEDIATES LPS-INDUCED INFLAMMATORY RESPONSE IN RAW264.7 MACROPHAGES

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LPS (lipopolysaccharide) can trigger inflammatory response by binding to toll like receptor 4 (TLR4) on innate immune cell through MyD88 dependent or independent pathway. Recent study suggests that the phosphatase PTEN (phosphatase and tensin homolog deleted

on chromosome ten) may enhance LPS/TLR4-dependent signaling and thus regulate LPS-induced inflammatory response. However, how PTEN plays its role in the TLR4-dependent signaling transduction pathway remains unclear till now. By performing immunoprecipitation assay we showed that PTEN was physically associated with TLR4 RAW264.7 cells after LPS stimulation. Interestingly, LPS stimulation also drove PTEN migrating to the cell membrane. This phenomenon suggested that interaction of PTEN with TLR4 may be involved in regulating TLR4-dependent signaling transduction pathway. In addition, LPS decreased the phosphorylation levels of PTEN, dephosphorylation of PTEN promotes its transfer to the cell membrane and induced its phosphatase activity to suppress Akt signaling, enhanced NF-kappa B activity and increased TNF-alpha production. This regulation was mediated by inhibiting Akt activity through lipid phosphatase of PTEN
Conclusion: This study reveals that PTEN in plasma translocate to the cell membrane and binds with TLR4 is a critical step to initiate LPS/TLR4 dependent inflammatory response.
Disclosure of interest: L. Li Grant/Research Support from: 973 project of China 2005CD522602 and Grant of The State Key Laboratory of Trauma Burns and Combined Injury SKLZZ200801.

OC-056
GALECTIN-3 INTERACTION WITH BACTERIAL LIPOSACCHARIDES (LPS) PROMOTES NEUTROPHIL ACTIVATION BY SUBOPTIMAL LPS CONCENTRATIONS

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Galectin-3 (Gal 3) is a glycan-binding protein that is secreted by tissue macrophages and mast cells at inflammation sites. In vivo experiments using Gal 3-deficient mice emphasized the critical role of this protein in regulating inflammatory responses in bacterial inflammatory diseases, mostly mediated by bacterial lipopolysaccharides (LPS). Although it is well established that Gal 3 can interact with LPS, the pathophysiological importance of LPS/Gal 3 interactions is not fully understood. We here demonstrate for the first time that the interaction of Gal 3, either via its carbohydrate binding C-terminal domain or via its N-terminal domain, with LPS from different bacterial strains, enhances the ability of LPS to activate neutrophils both in vitro and in vivo. Gal 3 allowed low LPS concentrations (1 μg/ml without serum, 1 ng/ml with serum) to upregulate CD11b expression on human neutrophils in vitro and drastically enhanced the binding efficiency of LPS to the neutrophil surface. These effects required LPS preincubation with Gal 3, before neutrophil stimulation and involved specific Gal 3/LPS interaction. A C-terminal Gal-3 fragment, which retained the lectin domain but lacked the N-terminal domain, was still able to bind both to E coli LPS and to neutrophils, but had lost the ability to enhance neutrophil response to LPS. This result emphasizes the importance of an N-terminus-mediated Gal 3 oligomerization induced by its interaction with LPS. Finally we demonstrated that Balb/c mice were more susceptible to LPS-mediated shock when LPS was pretreated with Gal 3. Altogether, these results suggest that multimeric interactions between Gal-3 oligomers with LPS potentiates its pro-inflammatory effects on neutrophils.
Disclosure of interest: None declared.

OC-057
INCREASED SOCS-1 INHIBITION OF MYD88 EXPRESSION CORRELATES WITH MILDER SEPSIS-INDUCED ALI IN DIABETIC RATS

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Introduction: The systemic inflammatory response induced by sepsis is the major cause of acute lung injury (ALI). Our previous work showed that in ALI induced by instillation of LPS, the NFkB nuclear translocation and cytokines production was reduced in diabetics. In the present study we investigated if the same occurs in ALI secondary to sepsis.
Methods: Diabetes was induced in male Wistar rats by alloxan and sepsis by cecal ligation and puncture surgery (CLP). After 6 h, bronchoalveolar lavage (BAL) was performed to measure cytokines by ELISA. Lung tissue was prepared for evaluation of edema, cell infiltration (HE staining), Transforming growth factor (TGF) β, α-smooth muscle actin (SMA) (immunohistochemistry), COX-2 (western blot) and for MyD88 and SOCS1 mRNA (RT-PCR).
Results: Morphological analysis of the lung after 6 h of CLP showed edema and mononuclear cell infiltrate in the BAL and parenchyma. Remodeling was evaluated by the increased number of cells expressing TGF-β and α-SMA. These parameters were lower in diabetics. Comparing the CLP with the sham operated group we found that non-diabetics exhibited an anti inflammatory profile (high IL10, TGFb and COX-2 and low IL-1b and TNFa). In diabetics this polarization was not observed and the concentration of mediators was much lower than in non-diabetics. Alveolar macrophages from diabetics with sepsis compared to non diabetics show lower expression of MyD88 mRNA and overexpression of SOCs1 mRNA.
Conclusion: The initial phase of ALI induced by CLP is characterized by edema, mononuclear cell infiltrate and signs of early remodeling. Concomitantly, the lung exhibited an anti inflammatory profile. These characteristics were less intense in diabetics. The impaired expression of MyD88 in macrophages from diabetics with sepsis can explain the milder lung inflammation observed in diabetics.
Financial support: FAPESP and CNPq
Disclosure of interest: None declared.

OC-058
FACTOR-VII ACTIVATING PROTEASE (FSAP) ACTIVATION IN HUMAN INFLAMMATION: A SENSOR FOR CELL DEATH

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Cell death is a central event in the pathogenesis of sepsis and is reflected by circulating nucleosomes. Circulating nucleosomes were suggested to play an important role in inflammation and were demonstrated to correlate with severity and outcome in sepsis patients. We recently showed that plasma can release nucleosomes from late apoptotic cells. Factor VII-activating protease (FSAP) was identified to be the plasma serine protease responsible for nucleosome release. The aim of this study is to investigate FSAP activation in patients suffering from various inflammatory diseases of increasing severity. We demonstrate FSAP to bind to apoptotic and necrotic cells, whereas it does not bind living cells. Furthermore plasma FSAP is activated upon contact with apoptotic and necrotic cells by an assay detecting complexes between FSAP and its target serpins α_2-antiplasmin, C1-inhibitor, and plasminogen activator inhibitor-1, respectively. By means of that assay we demonstrate FSAP activation in post-surgery patients, patients suffering from severe sepsis, septic shock and meningococcal sepsis. Levels of FSAP-inhibitor complexes correlate with nucleosome levels and correlate with severity and mortality in these patients. These results suggest FSAP activation to be a sensor for cell death in circulation and that FSAP activation in sepsis might be involved in nucleosome release thereby contributing to lethality.
Disclosure of interest: None declared.

OC-059
CONTRIBUTION OF INOS, COX-2 AND CYP4A1 TO THE PROTECTIVE EFFECT OF A SYNTHETIC ANALOG OF 20-HETE, 5,14-HEDGE, AGAINST ENDOTOXIN-INDUCED HYPOTENSION AND MORTALITY IN EXPERIMENTAL MODEL OF SEPTIC SHOCK IN RATS AND MICE

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Increased production of NO and prostaglandins associated with a decrease in the formation of a vasoconstrictor arachidonic acid product, 20-HETE, contributes to endotoxin (ET)-induced hypotension, vascular hyporeactivity, multiple organ failure, and high mortality rate. Therefore, we hypothesized that use of stable analogues of 20-HETE may provide an alternative strategy for the treatment of septic shock. To test this hypothesis, we investigated the effects of a synthetic analog of 20-HETE, N-[20-hydroxyeicosa-5(Z),14(Z)-dienoyl]glycine (5,14-HEDGE), on the ET-induced changes in iNOS-hsp90 complex formation, PKG and NADPH oxidase activity, peroxynitrite production, and COX-1/COX-2 and CYP4A1 protein expression in rats and mortality in mice. Blood pressure fell by 28 mmHg and heart rate rose by 47 beats/min in male Wistar rats treated with sublethal dose of ET (10 mg/kg, i.p.). ET-induced fall in blood pressure was associated with an increase in iNOS-hsp90 association, phosphorylated VASP, $gp91^{phox}$, $p47^{phox}$, nitrotyrosine and COX-2 protein expression, a decrease in COX-1 and CYP4A1 protein expression in heart, thoracic aorta, kidney and superior mesenteric artery, and elevated serum nitrotyrosine levels. All of the ET-induced changes, except for COX-1 protein expression, were prevented by 5,14-HEDGE given 1 h after ET injection. Treatment of male Balb/c mice with 5,14-HEDGE 1 h after injection of ET raised the survival rate from 84 to 98%. A competitive antagonist of vasoconstrictor effects of 20-HETE, 20-hydroxyeicosa-6(Z),15(Z)-dienoic acid, prevented the effects of 5,14-HEDGE on the ET-induced changes, except for iNOS-hsp90 association. These data suggest that 5,14-HEDGE reverses hypotension and improves survival during endotoxemia, and may be regarded as a potential therapeutic agent in the treatment of septic shock. [This study was supported by TUBITAK (SBAG- 109S121), USPHS NIH Grant HLBI-19134-34A1, NIH Grant GM31278, and the Robert A. Welch Foundation.]
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OC-060
TREATMENT OF ENDOTOXIN-MEDIATED SHOCK AND PSEUDOMONAS AERUGINOSA SEPSIS WITH THROMBIN-DERIVED HOST DEFENCE PEPTIDES

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Systemic infections leading to sepsis and septic shock are a major cause of mortality in intensive health care units. Gram-negative sepsis is accompanied by an overactivated innate immune response as well as excessive coagulation mainly induced by endoxins released from the bacteria. Due to rising antibiotic resistance and current lack of effective treatments there is an urgent need for new therapies. The aim of the present study was to explore the immunomodulatory effects and therapeutic potential of the thrombin-derived C-terminal peptides GKY25 (GKYGFYTHVFRLKKWIQKVIDQFGE) and the related HVF18 (HVFRLKKWIQKVIDQFGE) in regard to endotoxin-mediated shock and *Pseudomonas aeruginosa* sepsis. Both peptides abrogate endotoxin effects in vitro and in vivo. In human blood they interfere with coagulation by modulating tissue factor-mediated clotting. Furthermore, in a mouse model of *Pseudomonas aeruginosa* sepsis, treatment with GKY25 leads to reduction of the inflammatory

response, as well as decreased organ damage and reduced mortality. Taken together, the ability of these peptides to modulate both inflammation as well as coagulation, makes them attractive therapeutic candidates in the development of novel treatments for sepsis.
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OC-061
EFFECT OF NUTRITIONAL INTERVENTION ON SEPSIS INDUCED AUTOPHAGY IN MICE SEPTIC MODEL

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Background: Autophagy is the process cells recycle their own non-essential, redundant, or damaged organelles and macromolecular components. It is an adaptive response to sublethal stress, such as nutrient deprivation, that supplies the cell with metabolites it can use for fuel. The purpose of this study was to determine whether unbridled autophagy is related to sepsis induced organ dysfunction, and autophagy induced organ dysfunction can be compensated by nutritional intervention in mice cecal ligation and puncture model.
Methods: All experiments were conducted in accordance with the Institutional Care and Use Committee. Male C57BL/6 mice (20–23 g) were randomly allocated into four groups: Group C (sham operated), and Group S (food restriction for 12 h), Group CLP (cecal ligation and puncuture model), and Group CLP + 5%G (cecal ligation and puncture model treated with 5% glucose subdermal injection). Electron microscopic examination of liver were done for autophagosome and autolysosome detection. Immunoblotting of liver samples were done for detection of LC3. Blood samples were also examined such as ALT, AST, blood glucose, NEFA, and HMGB1. Statistical differences were determined by ANOVA and repeated measured ANOVA, followed by Dennett's test.
Result: AST, ALT and HMGB1 levels were highest in the Group CLP among the other groups; however, the Group CLP + 5%G was significantly lower than the Group CLP. On the other hand, autophagic levels detected by microscope and Immunoblotting was significantly increased in both Group S and Group CLP than other groups.
Conclusion: The results of our study show that autophagy was induced with cecal ligation and puncture model like as food restriction, and excessive autophagy may cause sepsis induced liver damage. 5% glucose administration can restore excess autophagy and liver damage. Nutritional intervention may improve autophagy associated organ dysfunction in several critical states.
Disclosure of interest: None declared.

OC-062
EVALUATION OF THE MINIMAL STRUCTURAL REQUIREMENTS IN THE GALACTOFURANOSE MOLECULE CAPABLE TO STIMULATE THE PHAGOCYTIC ACTIVITY AND EXACERBATES LETHALITY IN SEPTIC MICE

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Carbohydrate-containing molecules are distributed on external layers of all living organisms, being involved in many functions related to induction of the immune system. This work was focused on the effect of galactofuranosyl epitopes (carbohydrates usually observed in pathogenic microorganisms) on the phagocytic activity of peritoneal macrophages and also on the survival rate of mice with polymicrobial sepsis induced by cecal ligation and puncture (CLP). To synthesize galactofuranoside derivatives, galactose was converted to methyl and n-Octyl β- and α-galactofuranosides (GFB-Me, GFA-Me, GFB-O and GFA-O). The methyl galactofuranosides were converted to 5,6-O-isopropylidene derivatives (GFB-5,6-IPP and GFA-5,6-IPP). NMR and ESI–MS analysis confirmed the authenticity of all derivatives. GFB-Me and GFB-O had an immune stimulant effect at 5 μg/mL on macrophage phagocytic activity, with an enhancement of 35.12 and 11.90% respectively, but GFA-Me and GFA-O gave a low response. GFA-5,6-IPP and GFB-5,6-IPP had negative values relative to the control group −4.17 and −34.22%, respectively. These results showed that the free HO-5 and HO-6 and β-configuration are essential for the galactofuranosyl units to have phagocytic activity, which also stimulated peroxide, superoxide anion and nitric oxide production. GFB-Me and GFB-Me-5,6-IPP demonstrated the most significant effects on isolated macrophages and they have been selected to in vivo tests on septic mice. GFB-Me and GFB-Me-5,6-IPP increased significantly the mortality rate of septic mice. However, GFB-Me started killing earlier than GFB-Me-5,6-IPP. The treatment with GFB-Me elevated significantly the levels of all cytokines evaluated (TNF-α, IL-1β, IL-6 and IL-10). Both molecules were able to exacerbate mortality by sepsis in mice by a mechanism that depends on an increased levels of cytokines. These results reinforce the relevance of galactofuranosyl epitopes as components of pathogens and its virulence.
Disclosure of interest: None declared.

Autoimmunity, connective tissue diseases (OC06)

OC-063
ANTIBODIES TO VASCULAR SMOOTH MUSCLE CELLS IN PATIENTS WITH IDIOPATHIC AND SYSTEMIC SCLEROSIS-ASSOCIATED PULMONARY ARTERIAL HYPERTENSION

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Objectives: To investigate for antibodies directed at human VSMC in patients with systemic sclerosis (SSc) with or without PAH and idiopathic PAH (iPAH), and characterize their target antigens and their function**.**

Background: Pulmonary arterial hypertension (PAH) is characterized by a remodelling of pulmonary arteries with vascular smooth muscle cell (VSMC) proliferation.

Methods: Indirect immunofluorescence and 1-D immunoblotting were used to detect anti-VSMC antibodies in sera from healthy controls (HC) and patients with SSc without PAH, SSc-associated PAH (SSc-PAH) and iPAH. Target antigens were then identified by 2-D immunoblotting, then mass spectrometry. Finally, we analysed the contraction of a collagen matrix containing VSMC and sera from patients or HC or the corresponding purified IgG fractions.

Results: Anti-VSMC antibodies were detected in patients' sera using indirect immunofluorescence and 1-D immunoblotting. Then, 2-D immunoblots revealed a number of protein spots of interest and target antigens were identified by mass spectrometry, including stress-induced phosphoprotein 1 (STIP1), vimentin, α-enolase and proteins involved in smooth muscle contraction. Anti-STIP1 antibodies were detected by ELISA in sera from 84, 76 and 24% of patients with SSc without PAH, SSc-PAH and iPAH, respectively, versus from 3% of HC. Finally, IgG from SSc-PAH and iPAH patients were able to increase the contraction of VSMC in a collagen matrix as compared to IgG from HC, particularly when VSMC were pre-activated in the presence of TNF-a.

Conclusion: We have identified anti-VSMC antibodies in the serum of patients with SSc-PAH and iPAH. These antibodies might be pathogenic by modulating vascular contraction.

Disclosure of interest: None declared.

OC-064
INCREASED TH17 RESPONSE RELATED TO FUNCTIONAL DIFFERENCES IN CD4 + CD161 + CELLS IN GIANT CELL ARTERITIS AND POLYMYALGIA RHEUMATICA

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Th17 lymphocytes have recently been shown to be involved in the pathogenesis of giant cell arteritis (GCA). In this study, we investigated Th1, Th17 and Treg immune responses in 21 patients suffering from GCA (n = 14) or polymyalgia rheumatica (PR) (n = 7), at diagnosis and after 3 months of steroid treatment and in comparison with 24 age matched healthy volunteers. As human Th17 cells exclusively derive from $CD4^+$ $CD161^+$ cells, we also assessed the quantitative and qualitative characteristics of these cells. In comparison with control group, patients revealed decrease in Treg (3.23 vs. 4.71%; $p = 0.0003$) and Th1 cells (10.93 vs. 14.49%; $p = 0.0406$) and increase in Th17 cells (0.95 vs. 0.36%; $p < 0.0001$). Patients had also an elevated serum level of IL-6 (70.69 vs. 12.37 pg/mL; $p < 0.0001$). No difference was detected between GCA and PR patients. A dramatic infiltration by Th17 and Th1 cells with no presence of Treg was detected in positive temporal artery biopsies (TAB). No sign of immune imbalance was observed in negative TAB. After treatment, there was a significant decrease in the percentages of Th1 ($p = 0.0034$) and Th17 cells ($p = 0.0007$) whereas the percentage of Treg did not change. The functional analysis showed that $CD4^+$ $CD161^+$ cells from patients were able to produce significantly more IL-17 than those from controls irrespective of culture conditions (anti-CD3/CD28 + IL-2 with or without IL-1β, IL-23, TGF-β). The level of STAT1 phosphorylation was lower in patients than controls ($p = 0.0009$) with no difference in STAT3. After treatment, there was a dramatic decrease in IL-17 production in all the conditions, a downregulation of phosphorylated STAT3 ($p = 0.0145$) and an upregulation of phosphorylated STAT1 ($p = 0.0193$). This study demonstrates for the first time a decrease in Treg and confirms the increase in Th17 cells in GCA and PR patients, which is related to functional modifications of $CD4^+$ $CD161^+$ cells due to different phosphorylation profiles of STAT1 and STAT3.

Disclosure of interest: None declared.

OC-065
LACK OF IL-17RA SIGNALING PREVENTS AUTOIMMUNE INFLAMMATION OF THE JOINT AND GIVE RISE TO A TH2-LIKE PHENOTYPE IN COLLAGEN-INDUCED ARTHRITIS

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This study was performed to examine the role of the IL-17RA signaling in the development of CIA. Here we show that CII-immunized control mice developed CIA from day 24 onwards with an incidence between 40 and 60%. As expected, the IL-23p19−/− mice did not develop CIA. Interestingly, the IL-17RA−/− mice were completely protected and did not develop CIA even after a third CII/CFA injection. In contrast to the low percentage of IL-17^+ $CD4^+$ T cells in the IL-23p19−/− mice, there was a significant increase in the percentage of these cells in the IL-17RA−/− group compared to the control group at day 69. No significant difference was found in the percentage of IFN-gamma$^+$ $CD4^+$ T cells between all three groups.

Interestingly and in contrast to the IL-23p19 knockout mice, the IL-17RA deficient showed a Th2-like phenotype in splenic $CD4^+$ T cells at day 69. No difference was noted for FoxP3 expression in the splenic $CD4^+$ T cells between the three different mouse groups. Moreover, the CII-specific IgG2a levels in the sera of IL-17RA−/− was significantly lower compared to the control group at day 20 and lower although not statistically significant at day 69. At this latter time point, CII-specific IgG1 levels in the sera of IL-17RA−/− was increased although not statistically significant compared to the control. In conclusion, these data revealed a critical role for the IL-17RA signaling in the development of autoimmune inflammation of the joint. Moreover, these data show a Th2-like phenotype in IL-17RA−/− mice immunized with CII, suggesting that IL-17 receptor signaling is involved in the suppression of Th2 cytokines in autoimmune collagen arthritis.
Disclosure of interest: None declared.

OC-066
ISOLATION AND CHARACTERIZATION OF A NEW HUMAN T REGULATORY CELL SUBSET FROM PERIPHERAL BLOOD CD4 + T LYMPHOCYTE

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T regulatory (Treg) cells are specialized T lymphocyte subsets playing a role in sustaining peripheral tolerance. Treg cells produce a number of cytokines, such as IL-10 and TGF-β, and can be characterized by various markers including FOXP3, CD25 and cytotoxic T lymphocyte antigen-4 (CTLA-4). Moreover, glucocorticoid-induced TNF receptor family-related (GITR) has been indicated as a molecule involved in Treg cell activity regulation in mice (Nocentini et al. 2007). Aim of our study was to investigate GITR expression in human $CD4^+$ T lymphocytes and its possible role in Treg cell function. Results indicate that a subset of $CD4^+$ T cells, characterized by GITR expression and low levels of CD25 expression ($CD4^+CD25^{low}GITR^+$), is present in peripheral blood representing a low percentage of the entire $CD4^+$ T cell population (0.32–1.43%). These cells show Treg cell features since they express FOXP3 at intermediate level, IL-10 and TGF-β. Moreover, they are anergic and anergy is reversed by addition of IL-2. Most of $CD4^+CD25^{low}GITR^+$ cells shows a memory phenotype. Functional experiments, performed by removal of this cell population, demonstrated that $CD4^+CD25^{low}GITR^+$ cells have regulatory activity comparable with that of $CD4^+CD25^{high}$ cells. Moreover, similarly to $CD4^+CD25^+$ murine Treg cells, anti-GITR Ab inhibited their activity. Taken together, these data indicate that human $CD4^+CD25^{low}GITR^+$ cells represent a new Treg cell population.

Reference
Nocentini G, Ronchetti S, Cuzzocrea S, Riccardi C. GITR/GITRL: More than an effector T cell co-stimulatory system. Eur J Immunol. 2007; 37:1165–1169.
Disclosure of interest: None declared.

OC-067
AUTOIMMUNE INNER EAR DISEASE SUPPRESSES ION HOMEOSTASIS MECHANISMS

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It is generally assumed that autoimmune inner ear disease (AIED) is caused by cochlear inflammation and glucocorticoids restore hearing by immunosuppression. However, little attention has been given to the susceptibility of cochlear ion and water transport pathways in AIED and steroid control over their recovery. Therefore, MRL/lpr autoimmune disease mice were evaluated to determine how systemic disease and glucocorticoid treatment affect the ion homeostasis mechanisms in the ear. Mice were tested for hearing at either 2 months of age (prior to disease, N = 8) or 6 months of age (after hearing loss onset, N = 7). Additional 6 month old mice (N = 8) were given oral prednisolone for 2 weeks. Inner ears from all were collected for qRT-PCR of 24 genes responsible for endolymph homeostasis, including channels for transport of sodium, potassium, chloride, and water, as well as tight junctions and gap junctions. Mice developed hearing loss at 6 months and 22 of the 24 cochlear ion and water transport genes were significantly downregulated. Inflammatory cytokine genes were actually suppressed in the ear, not elevated. Steroid treatment significantly increased expression of several homeostasis genes, including the potassium channel Kcne1, the epithelial sodium channel, several gap junction proteins (Gja1, Gjb2, Gjb6), and aquaporin 3, all of which are involved in K^+ transport and endolymph production. Steroid treatment had no significant effect on inflammatory gene expression in the cochlea. It was concluded that circulating autoantibodies and immune complexes may cause hearing loss by disrupting the blood labyrinth barrier and genes required for endolymph homeostasis. Steroid treatment appeared to improve hearing by restoring these homeostatic genes without impacting inflammation. This provides new insight into one potential mechanism of AIED and how glucocorticoid therapies may help to restore hearing. [Supported by NIH-NIDCD R01 DC05593].
Disclosure of interest: None declared.

OC-068
LYMPHOCYTE IL-13 PRODUCTION IS UP-REGULATED BY TGF-B IN SYSTEMIC SCLEROSIS AND IS DOWN-REGULATED IN HEALTHY CONTROLS VIA GATA-3

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Systemic sclerosis (SSc) is an auto-immune disease characterized by skin fibrosis and internal organ dysfunction. Transforming growth factor-β (TGF-β) and Interleukin-13 (IL-13) two cytokines from Thelper 2 (Th2) lymphocytes, are closely involved in inflammation and fibrosis associated with SSc pathogenesis. TGF-β activates

collagen synthesis whereas IL-13 promotes fibrosis by stimulating TGF-β production. The aim of the present study was to determine the effect of TGF-β on IL-13 expression by peripheral blood lymphocytes (PBLs) from SSc patients (n = 8) compared to healthy donors (n = 10). Jurkat cell line was used as a Th2 model. Cells were treated or not with TGF-β 5 ng/ml for 2–48 h in the presence or not of inhibitors for Smad and MAPkinase pathways. Quantification of IL-13 mRNA levels and mRNA stability were studied by real-time PCR. Jurkat cells were also transiently transfected with IL-13 reporter gene. Smad and MAPkinase pathways as well as GATA-3 expression were assessed by immunoblot. GATA-3 nuclear transactivation was analyzed by electrophoretic mobility shift assay and ChIP assays. Our results showed that TGF-β inhibited IL-13 mRNA steady state levels by 20–40% without affecting mRNA stability in Jurkat cells and PBLs form healthy donors. Inhibition of IL-13 production involved both Smad- and p38-dependent pathways and repression of GATA-3 transcription factor binding capacity to IL-13 promoter. In contrast to the inhibition detected in Jurkat cells and healthy PBLs, TGF-β increased IL-13 mRNA and protein expression by 35–43% in PBLs from SSc patients as compared to PBLs from healthy donors. This study demonstrates that TGF-β downregulates IL-13 production via GATA-3 as a consequence of signaling through both Smad and p38 pathways in physiological situation whereas it becomes a potent activator in SSc pathological situation. Inhibition of GATA-3 expression or its IL-13 promoter-DNA binding ability might provide a new approach for SSc treatment.
Disclosure of interest: None declared.

OC-069
GRANULOCYTE–MACROPHAGE COLONY-STIMULATING FACTOR REGULATES SYSTEMIC AND LOCAL MYELOID CELL SUBPOPULATIONS IN INFLAMMATORY ARTHRITIS

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Even though there are clinical trials assessing granulocyte-macrophage colony-stimulating factor (GM-CSF) blockade in rheumatoid arthritis (RA), questions remain as to how GM-CSF acts as a proinflammatory cytokine. For the regulation of arthritis progression by GM-CSF the aims of this study were to determine (1) whether there are systemic effects, (2) the changes in synovial tissue leukocyte populations and (3) the arthritis model dependence on GM-CSF. The collagen-induced arthritis (CIA) and K/BxN serum transfer arthritis models were tested in GM-CSF−/− mice and/or wild-type mice using an anti-GM-CSF mAb. Cell populations were assessed by differential staining and flow cytometry. GM-CSF blockade, while ameliorating CIA development, was found to have systemic effects, limiting the increase in circulating $Ly6C^{hi}$ monocytes and neutrophils. GM-CSF blockade also led to fewer synovial macrophages (both $Ly6C^{hi}$ and $Ly6C^{lo}$), neutrophils and lymphocytes. In the absence of GM-CSF, K/BxN serum transfer arthritis initially developed normally; however, the numbers of $Ly6C^{hi}$ monocytes and synovial macrophages (both $Ly6C^{hi}$ and $Ly6C^{lo}$) were again reduced, along with the peak disease severity and maintenance. Thus GM-CSF is a key player in two arthritis models, participating in interactions between hemopoietic cells, locally and even systemically, to control myeloid cell number as well as presumably to "activate" them. These results could be useful for the analysis of the current clinical trials targeting GM-CSF in RA.
Disclosure of interest: None declared.

OC-070
INFLAMMATORY CYTOKINES IL-21 AND IL-15 ENABLE THE TRIGGERING NAÏVE AUTOREACTIVE CD8 T LYMPHOCYTES BY LOW AFFINITY TCR LIGANDS

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The cellular and molecular events preceding the initial activation of autoreactive CD8 T cells in several autoimmune diseases remain unclear. Recent reports have implicated IL-6, IL-21 and IL-7 in the triggering of autoreactive CD8 T cells in mouse models of autoimmune diseases. We have shown that IL-21 and IL-6 synergize with IL-7 or IL-15 to induce antigen-independent proliferation of naive CD8 T cells, with a concomitant increase in antigen responsiveness. We postulated that the cytokine-induced augmentation of TCR sensitivity (referred herein as 'cytokine priming') might enable stimulation of potentially autoreactive CD8 T cells by low affinity self antigens. Here, we show that cytokine priming enables naive TCR transgenic CD8 T cells to respond to low affinity TCR ligands, resulting in proliferation and display of effector functions. We have used a transgenic mouse model of autoimmune type 1 diabetes to show that cytokine-primed autoreactive CD8 T cells acquire the capacity to cause disease following stimulation with weak TCR agonists, and that the diabetogenic potential of these cells is dependent on the continuous availability of IL-15. These findings demonstrate a novel mechanism by which inflammatory cytokines could contribute to the triggering of autoreactive CD8 T cells and thus have important implications for autoimmune diseases associated with microbial infections and chronic inflammation.
Disclosure of interest: None declared.

Inflammatory cells recruitment and activation (OC07)

OC-071
DIFFERENTIAL ACTIVATION OF COCULTURED ENDOTHELIAL CELLS BY CD14 + CD16- AND CD14 + CD16 + MONOCYTES REVEALS A ROLE FOR IL-6 IN CROSSTALK BETWEEN SUBSETS

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Monocytes can be subdivided into discrete populations using surface markers, and in humans these fall into two subsets, the $CD14^{+}CD16^{-}$ ($\sim$90%) and $CD14^{+}CD16^{+}$ ($\sim$10%) cells. We have previously demonstrated that in co-culture a mixed population of monocytes can activate endothelial cells, leading to the recruitment of flowing neutrophils. Here we extend this work to study the inflammatory activity of purified subsets. $CD14^{+}CD16^{-}$ and $CD14^{+}CD16^{+}$ monocytes were cultured on 0.4 μM pore inserts with endothelial cells for 24 h. The ability of the endothelial cells to recruit neutrophils was then tested in

a flow based adhesion assay. In some experiments, monocyte conditioned medium, anti-IL-6 antibody or human recombinant IL-6 were added to cocultures. Cytokine secretion in cocultures was measured by luminex (Millipore). Mixed monocytes and the $CD14^+CD16^-$ population both induced endothelial cell activation resulting in moderate levels of neutrophil recruitment from flow, while $CD14^+CD16^+$ monocytes induced significantly higher levels of neutrophil recruitment. Mixed monocytes and $CD14^+CD16^-$ both generated high levels of IL-6 in co-culture, whereas $CD14^+CD16^+$ generated significantly less IL-6. Addition of IL-6 rich supernatant from $CD14^+CD16^-$ coculture to $CD14^+CD16^+$ cocultures significantly reduced the neutrophil recruitment driven by $CD14^+CD16^+$ cells. Addition of recombinant IL-6 to CD14+ CD16+ cocultures also reduced the level of neutrophil recruitment. These observations suggest that IL-6 plays a role in crosstalk between monocyte subsets and is capable of modulating the inflammatory response of endothelial cells.
Disclosure of interest: None declared.

OC-072
INTERLEUKIN-18 FACILITATES NEUTROPHIL MIGRATION ACROSS HUMAN COLONIC EPITHELIA VIA MLCK-DEPENDENT DISRUPTION OF TIGHT JUNCTIONAL OCCLUDIN

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Compromised epithelial barrier function and tight junction alterations are hallmarks of Inflammatory Bowel Disease (IBD). Several studies have identified a role for proinflammatory cytokines such as TNFα and INFγ in epithelial barrier defects observed in the context of IBD. Interleukin (IL)-18 is the latest member of the IL-1 family of cytokines to be identified. Elevated levels of IL-18 have been reported in mucosal tissue from IBD patients. Furthermore, neutralization of IL-18 was shown to reduce disease severity in several murine models of colitis, suggesting a role for this cytokine in the pathogenesis of IBD. Nevertheless, the role of IL-18 in intestinal epithelial barrier function remains unclear. The major aims of our study were to determine the effect of IL-18 on epithelial tight junction structure and function, and to investigate the mechanism involved in this process. Colonic epithelial Caco-2 monolayers were challenged with IL-18 (50 ng/mL) for 24 h and samples were processed for immunocytochemistry, immunoblotting, and permeability measurements. Our findings demonstrated that treatment with IL-18 selectively disrupts tight junctional occludin, without affecting the distribution of claudin-4 or -5. This effect is accompanied by a significant increase in the expression and activity level of myosin light chain kinase (MLCK). Further experiments revealed that inhibition of MLCK by ML9 prevents IL-18-induced loss of occludin protein levels. Structural alterations induced by IL-18 coincided with a significant increase in neutrophil migration across Caco-2 monolayers, without affecting epithelial permeability to ions and macromolecules. Finally, inhibition of MLCK abrogated IL-18-induced neutrophil transmigration. In conclusion, IL-18 may potentiate inflammation associated with IBD by facilitating neutrophil transepithelial migration via MLCK-dependent disruption of occludin.
Disclosure of interest: None declared.

OC-073
RESOLVIN D1 LIMITS PMN RECRUITMENT TO INFLAMMATORY LOCI: RECEPTOR DEPENDENT BIOACTIONS

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Many endogenous mediators act in concert to actively resolve inflammation, a concept that stems from the identification of novel anti-inflammatory and pro-resolving lipid mediators biosynthesized from omega-3 fatty acids (DHA and EPA) termed resolvins and protectins. The biological properties of resolvins are mediated via specific G-protein coupled receptors (GPCRs). Recent studies identified two separate GPCRs that RvD1 specifically binds on human leukocytes, the lipoxin A4/Annexin A1 receptor (FPR2/ALX) and the orphan receptor GPR32 (which has no known murine orthologue). Using zymosan stimulated peritonitis—an acute model of inflammation—RvD1 reduced 4 h peritoneal PMN infiltration, by 27 and 44% at doses as low as 1 and 10 ng/mouse, respectively. Importantly, this action of RvD1 was no longer observed in ALX/FPR2 null mice, indicating the crucial role of this GPCR in RvD1 mediated PMN responses in the mouse. RvD1 (1 and 10 nM) also displayed potent actions on human PMN recruitment to endothelial cells under shear conditions, RvD1 significantly reduced PMN capture, rolling and adhesion quantified using an in vitro flow chamber system. Interestingly, receptor specific antibodies blocked these anti-inflammatory actions of RvD1, with low concentrations being sensitive to GPR32 blockade, whilst the high concentration appeared to be ALX/FPR2-specific. Together these results indicate that RvD1 acts via both GPCRs to regulate and prevent overzealous PMN recruitment to sites of inflammation. Defective mechanisms and pathways in resolution may underlie our current understanding of the inflammatory phenotype(s) that characterize some prevalent human diseases. Mapping these resolution circuits can provide innovative drug discovery opportunities for inflammatory pathologies. Supported by the Arthritis Research UK (Foundation Fellowship 18445 to LVN); this work was supported in part by the National Institutes of Health USA GM38765 and DE019938 to CNS.
Disclosure of interest: L. Norling: None Declared, J. Dalli: None Declared, C. Serhan Other: CNS is inventor on patents assigned to BWH and Partners HealthCare on the composition of matter, uses, and clinical development of anti-inflammatory and pro-resolving lipid mediators and related compounds. These resolvins are licensed for clinical development to Resolvyx Pharmaceutical. C.N.S. retains founder stock in the company, M. Perretti: None Declared.

OC-074
THE ADAPTOR LNK (SH2B3) IS A KEY REGULATOR OF INFLAMMATORY- AND INTEGRIN-SIGNALING CONTROLLING ENDOTHELIAL CELL DYSFUNCTION

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Lnk (SH2B3) belongs to a family of SH2-containing proteins and is a signaling adaptor molecule that plays regulatory functions in the homeostasis and proliferation of immune cells. We previously showed that Lnk is expressed in endothelial cells (EC) and phosphorylated in response to TNF (1, 2). We further demonstrated that, in EC, Lnk is a negative regulator of TNF signaling that inhibits E-selectin and VCAM-1 expression in response to TNF (3). Mechanistically, Lnk activates PI3-kinase through Akt phosphorylation. Sustained Lnk-mediated activation of PI3-kinase in TNFα-activated ECs correlated with the inhibition of ERK1/2 phosphorylation, while phosphorylation of p38 and JNK MAPKs was unchanged. ERK1/2 inhibition decreases VCAM-1 expression in TNFα-treated ECs. In the present study we show that integrin ligation, using anti-CD29 Abs, also promotes Lnk activation and regulation in EC whereas integrin-mediated signaling (Akt and GSK3β phosphorylation) consecutive to CD29 ligation is abrogated in EC when Lnk is not expressed. We found that Lnk binds to ILK and activates FAK and paxillin. Overall, Lnk causes F-actin stress fibers, cytoskeleton reorganization and increases both the number and density of focal adhesions. Functionally, Lnk expression dramatically increases EC adhesion, slows down EC migration and inhibits apoptosis induced by anoïkis. Transfection of mutants and SiRNA identified alpha-parvin as a molecular partner of Lnk mediating the negative regulation of EC migration. Thus, the adaptor Lnk is an effective key regulator of inflammatory- and integrin-signaling controlling EC activation, migration and death, all critical in vascular remodeling and regeneration. Our current work in animal models also proposes to modulate Lnk in graft EC to provide a cytoprotective signal in organ transplantation. (1) Boulday G. et al. Circ. Res. (2001) 2;88:430; (2) Boulday et al. Transplantation (2002);4(9):1352; (3) Fitau J. et al. J. Biol. Chem.(2006); 281:20148.
Disclosure of interest: None declared.

OC-075
A NOVEL PATHOGENIC ROLE OF THE ER CHAPERONE BIP/GRP78 IN INFLAMMATORY ARTHRITIS

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The accumulation of misfolded proteins can trigger a cellular survival response in the endoplasmic reticulum (ER). In this study, we found that BiP, an ER chaperone, was highly expressed in RA synovium and synoviocytes. The proinflammatory cytokines, such as TNF-a and IL-1b, but not IL-10, increased the BiP expression in synoviocytes. Thapsigargin and tunicamycin, ER stressors, increased the BiP and Bcl-2 expression in synoviocytes, which was greater in RA synoviocytes than in OA synoviocytes. Downregulation of BiP transcripts using siRNA increased apoptosis of RA synoviocytes, while abolishing the TNF-a- and TGF-b-induced synoviocyte proliferation and cyclin D1 up-regulation. Moreover, BiP siRNA inhibited $VEGF_{165}$-stimulated proliferation, capillary tube formation, wounding migration, chemotaxis of endothelial cells in vitro. Downregulation of BiP expression in RA synoviocytes that engrafted into immunodeficient mice also significantly reduced synoviocyte proliferation and angiogenesis. BiP siRNA also significantly impeded synoviocyte proliferation and angiogenesis in Matrigel implants that engrafted into immunodeficient mice. Conversely, a selective BiP-inducer, BIX, prevented synoviocyte apoptosis induced by thapsigargin, and increased synoviocyte proliferation in mice with experimental osteoarthritis when injected intra-articularly. In summary, BiP is crucial to the synoviocyte proliferation and angiogenesis, suggesting that ER chaperone BiP response contributes to RA pathogenesis.
Disclosure of interest: None declared.

OC-076
OVEREXPRESSION OF PHOSPHOLIPASE C EPSILON IN KERATINOCYTES RESULTS IN IL-23 PRODUCTION LEADING TO SKIN INFLAMMATION ACCOMPANIED BY IL-22-PRODUCING T CELL INFILTRATION

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Phospholipase Cε (PLCε) is an effector of Ras and Rap small GTPases. We have for the first time shown by using PLCε-deficient mice that PLCε plays a critical role in the induction of cytokine production in non-immune cells in a variety of inflammatory reactions, such as those involved in tumor promotion. The role of PLCε in tumorigenesis is suggested also in humans by recent genome-wide studies that have identified SNPs in the PLCε gene (PLCE1) as a risk factor for gastric adenocarcinoma and esophageal squamous cell carcinoma. Here, to further investigate its role in inflammation, we create transgenic mice overexpressing PLCε in epidermal keratinocytes. The transgenic mice spontaneously develop chronic skin inflammation sharing the features of human psoriasis, such as adherent silvery scale formation and aberrant infiltration of IL-22-producing $CD4^+$ T cells. The keratinocytes in the PLCε transgenic mice highly produce IL-23, which the skin symptom development depends on as demonstrated by the reversion of the skin phenotype upon its neutralization. Importantly, overproduction of IL-23 is induced even in wild-type keratinocytes in vitro by stimulating with the conditioned medium from those overexpressing PLCε. These results suggest that increased IL-23 expression in the PLCε transgenic mouse skin is mediated by certain autocrine/paracrine factors secreted by PLCε-overexpressing keratinocytes.
Disclosure of interest: None declared.

OC-077
TLR5 ACTIVATION/IL-1B SIGNALING: A NEW PATHWAY FOR ALVEOLAR MACROPHAGE PHAGOCYTOSIS AND BACTERIAL KILLING OF PSEUDOMONAS AERUGINOSA

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A deficit in early lung clearance of bacteria, such as Pseudomonas aeruginosa (P.a), is suspected to be key in nosocomial pneumonia or in

chronic lung infections. Although Toll-like receptor (TLR) 4 and TLR5 are respectively implicated in the recognition of LPS and flagellin of P.a and in the control of lung infections, very little is known about their roles in the early activation of alveolar macrophages (AMs). Here, we show that flagellin/flagellum-TLR5 interaction is essential to induce P.a clearance by AMs. In contrast to WT PAK (a wild-type P.a strain), unflagelled PAK strain or a strain expressing a flagellin mutated into TLR5-recognition site were not killed by either MH-S cells or primary AMs. We showed reduced uptake of unflagelled or flagellin-mutated PAK in MH-S cells and primary AMs, when compared to WT PAK. Furthermore, primary AMs from TLR5−/− mice were unable to kill PAK, when compared to primary AMs from WT mice. A deficit in PAK phagocytosis by primary TLR5−/− AMs was also observed. The importance of recognition by TLR5 for AM-mediated clearance of PAK is recapitulated in vivo in mice. We showed that TLR5-mediated PAK recognition and uptake by AMs were associated with high IL-1β synthesis, which was reduced with PAK mutants or with primary TLR5−/− AMs. We demonstrated that IL-1β release was associated with the ability to kill PAK since adding recombinant IL-1RA inhibited significantly the early clearance of PAK by AMs. In conclusion, we demonstrated for the first time that TLR5 plays a major role in the early clearance of P.a by AMs and that the flagellum-TLR5 interaction is required for effective phagocytosis of P.a and for the induction of IL-1β synthesis. Our data described a role of IL-1β in the control of P.a killing by AMs, a mechanism that we are currently further dissecting.
Disclosure of interest: None declared.

Lipid mediators and metabolic diseases (OC08)

OC-079
ARACHIDONIC ACID AND 2-ARACHIDONOYL-GLYCEROL PROMOTE HOST DEFENSE BY ENHANCING THE FUNCTIONS OF HUMAN NEUTROPHILS

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Background: We recently reported that endocannabinoid 2-arachidonoyl-glycerol (2-AG) activates human neutrophils independently of CB receptor activation. We found the effects of 2-AG were mediated by an autocrine activation loop involving arachidonic acid (AA) and its subsequent metabolism to leukotriene (LT) B_4. In the present study, we tested whether 2-AG and AA could also stimulate neutrophil functions linked to host defense, more specifically the release of microbicidal peptides as well as their bacterial and virucidal activities.
Results: We initially tested if AA and 2-AG were triggering the release of the microbicidal peptides α-defensins and LL-37. We found that both 2-AG and AA stimulated the release of a large amount (μg) of α-defensins as well as a limited amount (ng) of LL-37. This release occurred rapidly and paralleled the biosynthesis of LTB_4. Importantly, the supernatants of neutrophils activated with nanomolar concentration of AA, 2-AG or LTB_4 almost completely inhibited the infectivity of herpes simplex virus-1 (HSV-1). Additionally, the supernatants of 2-AG- and AA-stimulated neutrophils strongly attenuated the growth *Escherichia coli and Staphylococcus aureus*. Importantly, all the effects of AA and 2-AG mentioned above were prevented by inhibiting the biosynthesis of LTB_4 with the 5-lipoxygenase-activating protein antagonist MK-0591 or by blocking LTB_4 receptor 1 with CP 105,696.
Conclusion: The rapid conversion of 2-AG to AA and their subsequent metabolism into LTB_4 promote 2-AG and AA as multifunctional activators of neutrophils, mainly exerting their effects by activating the LTB_4 receptor 1. The low concentration of AA or 2-AG needed to impair HSV-1 infectiveness likely reflects the physiological importance 2-AG and AA might have in promoting host defense in vivo (*supported by grants to NF from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada*).
Disclosure of interest: None declared.

OC-080
MODULATION OF FCR-MEDIATED PHAGOCYTOSIS IN MACROPHAGES BY TLRS AGONISTS: INVOLVEMENT OF 5-LO PRODUCTS

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Introduction: Toll-like receptors (TLRs) are pattern recognition receptors which recognize molecules in microorganisms. Leukotrienes (LTs), lipid mediators derived from 5-lipoxygenase (5-LO) pathway, has been recognized as modulators of FcgR-dependent phagocytosis. Taking this information into account, our aim was to evaluate the participation of TLRs ligands on FcgR-mediated phagocytosis.
Methods: Rat alveolar macrophages (AMs) or mouse (SV129 or 5-$LO^{-/-}$) peritoneal macrophages (PMs) were treated with TLR ligands and challenged with IgG-sRBCs. After 90 min, phagocytosis was evaluated by a colorimetric assay. Lipid mediators (LTB_4 and cys LTs) were measured by EIA. Western blotting was also used.
Results: Except for CpG, all TLR agonists tested (TLR2, TLR3, and TLR4) were able to amplify phagocytosis of IgG-sRBC by AMs in a concentration-dependent manner. Phagocytosis amplification induced by TLR3 ligand was a time-dependent, fact not observed for TLR2 and TLR4 ligands. AMs treatment with zileuton also impaired TLR4 ligand-induced phagocytosis amplification, suggesting the participation of 5-LO products in this phenomenon. In order to confirm LTs involvement in TLRs-induced FcgR-mediated phagocytosis amplification, we observed that PMs obtained from 5-$LO^{-/-}$ mice did not presented any difference on phagocytosis when stimulated with TLR2, 3, and 4 ligands. LTB_4 production induced by IgG engagement was amplified by TLR2 and TLR4 ligands, but not by TLR3. Similar results were obtained when cys-LTs were quantified, whose production was increased by TLR2, TLR3, and TLR4. ERK1/2 and p38, mitogen-activated proteins kinases (MAKs), phosphorylation induced by IgG engagement with FcgR was enhanced by TLRs agonists.
Discussion: Our data suggest that TLR ligands amplify phagocytosis in a time- and concentration-dependent manner, via 5-LO products. ERK1/2 and p38 presented a greater activation when cells were incubated with TLRs agonists and challenged with IgG-sRBC
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OC-081
HUMAN GROUP X SECRETED PHOSPHOLIPASE A2 INDUCES DENDRITIC CELL MATURATION THROUGH THE RELEASE OF LDL-DEPENDENT AND INDEPENDENT LIPID MEDIATORS

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Secreted phospholipases A2 (sPLA2) are a family of enzymes which catalyze the hydrolysis of glycerophospholipids liberating lysophospholipids and fatty acids. Among sPLA2 s, the human group X (hGX)-sPLA2 has the highest catalytic activity towards phosphatidylcholine (PC) the major phospholipid of cell membranes and blood lipoproteins. Increased sPLA2 activity has been documented in several inflammatory disorders and the expression of the sPLA2 groups IIA, III, V and X has been detected in human atherosclerotic lesions, indicating that sPLA2s are an important link between lipids and inflammation, both involved in atherosclerosis. This pathology linked to chronic inflammation of the arterial wall is characterized by the accumulation of Low-Density Lipoproteins (LDL) and foamy cells within the subendothelial space. Recently the presence of dentritic cells (DC), the most potent antigen presenting cells in atherosclerotic lesions raised the question about their role in disease progression. We have previously shown that modified lipoproteins can modulate DC function, interfering with their ability to stimulate T cells. In this study, we show that hGX-sPLA2-treated LDL induced DC maturation resulting in a characteristic mature DC phenotype and in enhanced DC ability to activate IFNγ secretion from T cells. hGX-sPLA2 phospholipolysis of LDL produced high levels of lipid mediators, such as lysophosphatidylcholine and free fatty acids, which also modulated DC maturation. Our results indicate that hGX-sPLA2 can induce DC maturation, both directly by hydrolyzing PC from cell membranes and indirectly by LDL phospholipolysis, though resulting in different cytokine secretion profiles, pointing to differential modulation of DC function. In conclusion, the secretion of hGX-sPLA2 in inflamed tissues may contribute to the local activation of DC through the production of lipid mediators from LDL and/or cell plasma membranes.

Disclosure of interest: None declared.

OC-082
MONOCYTE CHEMOATTRACTANT PROTEIN-1 SECRETION IS INHIBITED BY A SYNTHETIC ANALOG OF 15-EPI-LIPOXIN A4 IN HUMAN STIMULATED MONOCYTES: INVOLVEMENT OF P38 MAP KINASE

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Introduction: Lipoxins (LX) and 15-epi-lipoxins (ATL) are arachidonic acid metabolites with well recognized anti-inflammatory and pro-resolution activities, although little is known about the mechanisms that trigger these effects. We investigated the role of ATL-1, a synthetic analog of 15-epi-lipoxin A_4, on the modulation of monocyte chemoattractant protein-1(MCP-1) secretion by stimulated monocytes.

Methods: U937 cells were incubated with ATL-1 (1–100 nM) for 15 min to investigate ERK-2 and p38 MAPK phosphorylation, or previously treated with SB203580, a p38 MAPK inhibitor, followed by treatment with ATL-1 for 12 h to investigate heme oxygenase (HO)-1 induction using a western blot assay. Lipoxin A_4 receptor (ALX) expression was evaluated using RT-PCR analysis and western blot assay. MCP-1 levels were determined by ELISA in cells previously treated with SB203580 or SnPPIX, a HO-1 inhibitor, followed by treatment with ATL-1 and LPS stimulation for 24 h.

Results: We characterized ALX expression in U937 cells, via the assessment of gene expression and protein. The receptor functionality was confirmed through the analysis of ERK-2 activation, which makes this cell line a suitable tool to study the mechanism of action of LX on monocytes. Furthermore, we demonstrated that ATL-1 increases the expression of HO-1 in U937 cells via activation of p38 MAPK and decreases the secretion of MCP-1, a chemokine involved in the recruitment of monocytes to the inflammatory focus, in LPS-stimulated U937 cells. MCP-1 inhibition by ATL-1 was reverted by SB203580 indicating that this effect is dependent on the activation of the p38 MAPK pathway.

Conclusion: ATL-1 reduces MCP-1 secretion via p38 MAPK activation. This study clarifies some of the mechanisms involved in the activation of monocytes by LX and may lead to new approaches for the control of different pathologies where the inflammatory component is relevant.

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Disclosure of interest: None declared.

OC-083
URIC ACID SIGNALLING VIA ADAPTOR MOLECULE MYD88 AND M1/M2 MACROPHAGE BALANCE IN THE DEVELOPMENT OF RENAL FIBROSIS

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The chronic renal failure is an immune mediated disease characterized by renal fibrosis. The injured tissue releases molecules, such as uric acid, resulting from extracellular matrix degradation or dying cells, which can activate Toll-like receptors (TLRs), and leads to translocation MyD88 in many cell types. This immune system modulation interferes in the macrophage and TCD4 + cell activity, with the Th1/Th2 paradigm considered a possible effector mechanism of fibrosis.

Objective: We aimed to investigate the role of uric acid signaling via TLR 2, TLR 4 and MyD88 and the function of M1/M2 macrophage in the development of renal fibrosis.

Methods: We used the Unilateral Ureter Obstruction (UUO), where the animals were sacrificed at 7 days after the surgery. Some animals were treated with allopurinol, a xanthine oxidase inhibitor. Proteinuria and uric acid levels were measured in wild-type (C57Bl/6) and IL-12, IL-4, TLR2, TLR4 and MyD88 knockout (KO) mice. TGF-β

Elisa assay and hydroxyproline quantification of kidneys tissues were done. Macrophage culture was supplemented with uric acid and Th1/Th2 cytokines was quantified by qPCR and Elisa assay.
Results: UUO increases macrophage entrance in obstructed kidneys, as seen by flow cytometry. IL-12 KO mice presented higher levels of TGF-β compared to WT mice. Besides, TGF-β and type 1 collagen mRNA was decreased in TLRs KO mice, compared to WT mice. Allopurinol treated animals showed preserved renal function and decreased fibrosis formation. MyD88 KO mice showed a renal protection. Uric acid stimulated pro-fibrotic cytokines production by macrophage in vitro. These data were corroborated by Sirius red staining and hydroxyproline quantification
Conclusion: Uric acid crystals are responsible to stimulate Th2 immune response, which leads to fibrosis. This suggests future therapeutic strategies against renal fibrosis should be based on uric acid formation blockage and finally, in the Th1/Th2 balance. Support CNPq and Fapesp
Disclosure of interest: None declared.

OC-084
CONTRIBUTION OF ARACHIDONIC ACID METABOLITES IN HYPERSENSITIVITY ASSOCIATED WITH IRRITABLE BOWEL SYNDROME (IBS)

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Transient Potential Receptor Vanilloid (TRPV4), a calcium channel can be activated in vitro by arachidonic acid metabolites. We have previously reported that TRPV4 activation induces visceral hypersensitivity in response to colorectal distension (CRD) in mice. However, the potential endogenous activators of TRPV4 present in the digestive tract as well as the potential role of this receptor channel in the context of IBS still have to be addressed. As we have previously reported, we have used supernatants of incubated biopsies from IBS patients to induce hypersensitivity symptoms in mice. 4 Biopsies from control and IBS patients were immersed in Hank's solution. Groups of mice received intracolonically (IC) biopsy supernatant. Abdominal muscle contractions in response to CRD were recorded before and 3 h after biopsy supernatant administration. The same approach was used in mice that had received intrathecal injections of TRPV4 SiRNA. Lipids from mouse colon treated with DSS to induce colitis were extracted and EET, HETE and diHETE quantified by mass spectrometry after HPLC. In a last set of experiments, the extracted metabolites were tested on calcium mobilization response in HEK-TRPV4 transfected cells and on sensory neurons harvested from mouse dorsal root ganglia. We detected an increase of EET and HETE in DSS treated mice. Only the 5,6-EET provoked an increase in calcium flux in HEK-TRPV4 cells. Metabolite extracts from mouse colon and 5,6-EET also induced calcium signal in sensory neurons. Intracolonic administration of supernatants from IBS induced visceral hypersensitivity in response to CRD in mice. Three days after SiRNA treatment, the hypersensitivity to CRD distention induced by IBS patient biopsy supernatants was inhibited. Our study shows that TRPV4 agonists are present in the mouse colon and increased under colitis. TRPV4 activation plays a central role in IBS patient biopsy supernatants-induced visceral hypersensitivity.
Disclosure of interest: None declared.

OC-085
INFLAMMATORY CROSS-TALK BETWEEN ADIPOCYTES AND ENDOTHELIAL CELLS IN A 3D CULTURE MODEL

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Introduction: Obesity, a chronic inflammatory status, is associated with a cellular remodeling of white adipose tissue (WAT) with the presence of inflammatory cells [1]. Visceral WAT which hypertrophy is associated with metabolic complications, is more vascularized and inflammatory than subcutaneous WAT [2]. We hypothesized that endothelial cells from the visceral WAT through their inflammatory secretions could alter adipocyte biological functions.
Methods: To reproduce tissue architecture, we developed a new experimental 3D model where human mature adipocytes are included in a peptidic hydrogel. In this 3D gel, the adipocytes maintained their metabolic functions including adipokine secretion (leptin and adiponectin) for 1 week. We performed co-culture of these cells both isolated from visceral WAT of obese subjects in our 3D experimental design.
Results: Endothelial cells provoke a decrease of adipokine secretions (-50% for leptin and -20% for adiponectin, $p < 0.01$). An increased secretion of inflammatory cytokines including IL-6, CXCL-8, CCL-5 and G-CSF was observed in co-culture adipocytes/endothelial cells. By studying the secretory profile of angiogenesis-related molecules, we also highlighted the increased secretion of IGFBP-3 in these experimental conditions.
Conclusion: Increased secretion of cytokines and chemokines as IL-6 and CCL-5, with conversely decreased secretion of the anti-inflammatory adiponectin could perpetuate a vicious cycle in hypertrophied and inflammatory WAT. Inflammation and angiogenesis being interrelated processes, G-CSF, CXCL-8 and IGFBP-3, all involved in endothelial precursor recruitment, could promote neovascularization contributing to tissue repair.

References

1. Cancello R, Taleb S, Poitou C, Tordjman J, Lacasa D, et al. J Annu Diabetol Hotel Dieu. 2006:115–128.
2. Villaret A, Galitzky J, Decaunes P, Esteve D, Marques MA, et al. Diabetes. 2010;59:2755–2763.

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OC-086
EVIDENCE BIOCHIP ARRAYS FOR THE MULTIPLEX ANALYSIS OF BIOMARKERS RELATED TO METABOLIC SYNDROME AND INFLAMMATION

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Inflammation is frequently associated with metabolic syndrome (MetS). Analytes such as adiponectin, C-peptide, CRP, cystatin C, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNFα, take part in physiological processes such as insulin secretion, glucose regulation, fatty acid metabolism, inflammation, iron homeostasis, energy homeostasis/endocrine regulation, fibrin regulation. Other molecules involved in the inflammation response are the adhesion molecules. Biochip array technology enables the determination of multiple analytes from a single sample and this approach offers more information than single analyte determination as provides a patient's profile at a single point in time. This is illustrated here with examples of analytical parameters of biochip arrays for the determination of analytes related to the MetS and inflammation. Simultaneous chemiluminescent immunoassays are employed for biomarker detection and the microarrays of capture ligands define the discrete test areas for each of the analytes in each biochip (9 × 9 mm). The immunoassays were applied to the Evidence Investigator™ analyser. The MetS Array I enables the multiplex determination of C-peptide, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNFα and the MetS Array II measures simultaneously adiponectin, CRP and cystatin C with sensitivity values ranging from <3.5 pg/ml (IL-1α) to <1 mg/l (CRP). For all the assays the intra-assay and inter-assay precision, expressed as %CV, were typically <15%. The adhesion molecules array allows simultaneous measurement of the soluble forms of L-, P-, E-selectins, VCAM-1 and ICAM-1 with analytical sensitivity values ranging from 0.1 ng/ml (E-Sel) to 4.1 ng/ml (VCAM-1) for neat sample. The data indicate applicability of biochip array technology to the multiplex determination of a broad range of biomarkers with the generation of quantitative patient profiles. It represents a useful tool for application in clinical research settings.
Disclosure of interest: None declared.

Resolution and inflammatory concert (SY09)

OC-087
ANTI-ALLERGIC CROMONES INHIBIT HISTAMINE AND EICOSANOID RELEASE FROM HUMAN MAST CELLS THROUGH AN ANNEXIN 1 DEPENDENT MECHANISM

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Mast cells, basophils and eosinophils represent the first line of host defence in allergic inflammation reaction. We investigated the mechanism by which the anti-allergic drugs di-sodium cromoglycate and sodium nedocromil inhibit histamine and eicosanoid release from human mast cells (HMCs) as well as peripheral blood basophils (PBBs) using biochemical techniques. Cord derived HMCs were cultured in vitro and PBBs obtained from human donors or cardiac puncture in mice. Subsequent antigen challenged led to a prompt release of histamine and prostaglandin D_2 (PGD_2), which was inhibited by cromoglycate, nedocromil, dexamethasone and human recombinant Annexin-A1 (Anx-A1). Treatment of HMCs with cromoglycate or nedocromil induced a rapid phosphorylation on the Ser^{27} residue of Anx-A1, hence its secretion, secondary to inhibition of phosphatase activity and activation of PKC. Treatment of the HMCs or PBBs with a specific neutralising anti-Anx-A1 (but not irrelevant) monoclonal antibodies reversed the inhibitory effect of these drugs on histamine and PGD_2 –released. To highlight the crucial importance of Anx-A1 in this rapid effect of these drugs we tested the effect of nedocromil in Anx-A1 null mice. Cromones did not inhibit the release of histamine nor prostaglandin D2 from basophils from Anx-A1 null mice.To translate these new findings clinically, we have analyzed Anx-A1 pattern of expression in tears from patients with Vernal Kerato Conjuntivitis (VKC). While this seasonal disease is associated with an increased amount of the clipped- or inactive- form of Anx-A1, VKC patients treated with the cromoglycate-like Alomide (or Lodoxamide) show an augmentation of the total-or active- form of the protein. Altogether, the use of in vitro, in vivo investigations and the translational outcome of VKC allergic disease, suggest strongly the anti-inflammatory Anx-A1 protein to be a component of rapid cromone actions on mast cells, basophils and eosinophils.
Disclosure of interest: None declared.
Young Investigator Award Winner. For mini paper see page 289

OC-088
SATIATED-EFFEROCYTOSIS: A NOVEL FUNCTIONAL PROPERTY FOR RESOLUTION-PHASE MACROPHAGES REGULATED BY RESOLVINS, GLUCOCORTICOIDS, AND THE CHEMOKINE-SCAVENGING RECEPTOR D6

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Background: The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for homeostasis and results in macrophage reprogramming/immune-silencing.
Materials and methods: To characterize pro-resolving properties of $CD11b^{low}$ macrophages and their modulation by pro-resolving agents, zymosan A-initiated peritonitis was used, followed by immunostaining and FACS sorting to distinct macrophage subpopulations. Sorted $CD11b^{high}$ and $CD11b^{low}$ macrophages were analyzed for protein markers by Western blot, efferocytic scores using fluorescent microscopy, and secretion of cytokines ex vivo using ELISA.
Results: Here, we show $CD11b^{high}$ macrophages convert to $CD11b^{low}$ ones and stop efferocytosing apoptotic PMN after reaching an engulfment threshold in vivo. In addition, $CD11b^{low}$ macrophages are distinct from either M1 or M2 in their protein expression signature and express pro-resolving properties, such as diminished responses to different TLR ligands ex vivo and propensity to emigrate from resolving inflammation sites to lymphoid organs. Of interest, we found the pro-resolving lipid mediators resolvin (Rv) E1 and RvD1, as well as the glucocorticoid dexamethasone (Dex), to enhance satiated-efferocytosis and consequently $CD11b^{low}$ macrophage conversion from their $CD11b^{high}$ counterparts, whereas genetic deficiency in the chemokine scavenging receptor D6 resulted in delayed satiation and reduced immune-silencing of macrophages, and inhibits their departure from resolving inflammation sites.
Conclusions: We suggest satiated-efferocytosis is a novel phagocyte property displayed by $CD11b^{low}$ macrophages and regulated by pro-resolving mediators. Moreover, satiated-efferocytosis is required for $CD11b^{low}$ macrophage emigration from resolving inflammation sites and the return of tissue homeostasis. Thus, satiated-efferocytosis is essential for the completion of timely- and spatially-coordinated resolution of acute inflammation.
Disclosure of interest: None declared.

OC-089
EOSINOPHILS CONTRIBUTE TO THE RESOLUTION OF ACUTE PERITONITIS THROUGH THE 12/15-LIPOXYGENASE PATHWAY

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Acute inflammation in healthy individuals is self-limiting and has an active termination program. The mechanisms by which acute inflammation is resolved are still of interest. In murine zymosan-induced peritonitis, we found that eosinophils are recruited to the inflamed loci during the resolution phase of acute inflammation. In vivo depletion of eosinophils caused a resolution deficit, namely impaired lymphatic drainage with reduced appearance of phagocytes carrying engulfed zymosan in the draining lymph node, and sustained numbers of polymorphonuclear leukocytes in inflamed tissues. Liquid chromatography-tandem mass spectrometry (LC–MS/MS)-based lipidomics of the resolving exudates revealed that locally activated eosinophils in the resolution phase produced 12/15-lipoxygenase-derived lipid mediators including protectin D1 (PD1) from docosahexaenoic acid. The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the administration of PD1. Eosinophils deficient in 12/15-lipoxygenase were unable to rescue the resolution phenotype. The present results indicate that eosinophils and eosinophil-derived lipid mediators including PD1 have a role in promoting the resolution of acute inflammation, expanding the roles of eosinophils in host defense and resolution.
Disclosure of interest: None declared.

Damps and pamps; critical endogenous and exogenous danger signals (SY10)

OC-090
ENTEROHEMORRHAGIC ESCHERICHIA COLI, A NON-CYTOSOLIC PATHOGEN ACTIVATING CYTOSOLIC NLRP3 AND AIM2 INFLAMMASOMES THROUGH RELEASE OF BACTERIAL PRODUCTS INTO THE CYTOSOL

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Enterohemorrhagic *Escherichia coli* (EHEC) induces elevated levels of IL-1beta in infected patients. However, the molecular mechanisms involved in the activation of IL-1beta by EHEC are unknown. Inflammasomes are multiprotein complexes in the cytosol that process proIL-1beta to bioactive IL-1beta. To identify the inflammasomes involved in IL-1beta induction by EHEC, we infected macrophages derived from wild type and mice deficient in inflammasome components with EHEC and analyzed the processing and release of mature IL-1beta. EHEC-induced IL-1beta production was dependent on caspase-1 and the adaptor ASC. Notably, both NLRP3, a cytosolic sensor for microbial and endogenous products, and AIM2, a cytosolic sensor for DNA, were absolutely essential for EHEC-induced IL-1beta production. Though EHEC is a non-cytosolic pathogen, bacterial products such as DNA were observed in the cytosol of infected cells, providing a potential means by which cytosolic inflammasomes might be activated. This also indicated that phagosomal degradation of bacteria is a prerequisite for inflammasome activation by EHEC. Supporting this hypothesis, blocking lysosomal acidification and type I interferon signaling that are essential for bacterial lysis in phagosome, led to a marked decrease in EHEC-induced IL-1beta secretion. Thus, our results demonstrate that EHEC potently activates multiple inflammasomes as a result of release of bacterial products from phagosomes into the cytosol.
Disclosure of interest: None declared.

Young Investigator Award Winner. For mini paper see page 289

OC-091
REACTIVE OXYGEN SPECIES-MEDIATED INFLAMMASOME ACTIVATION AND IL-1B ARE ESSENTIAL FOR HOST RESISTANCE TO DENGUE VIRUS PRIMARY INFECTION

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Dengue virus (DENV) infection is the most important mosquito-borne disease on Earth. In the past few years, several studies have shown the importance of inflammasome complex activation during infectious processes. Then, we evaluated the role played by inflammasome activation during host response to primary DENV infection. To this end a mouse experimental model, utilizing a DENV-2 strain that causes a severe disease in mice was used. Wild-type (WT) infected mice showed increased levels of cleaved Caspase-1 in spleen. However, Casp-1 cleavage in spleen of DENV-2-infected $ASC^{-/-}$ mice was reduced. These animals, as well as Casp-$1^{-/-}$ mice, presented a more severe disease manifestation after DENV infection. In addition, viral loads in spleen of $ASC^{-/-}$ and Casp-$1^{-/-}$-infected mice were markedly elevated and lethality rates after infection were strikingly higher. Of note, both groups showed reduced IL-1βproduction after DENV infection, when compared to WT-infected mice. Hence, IL-1ra-treated mice showed marked susceptibility to DENV-2 infection when compared with infected-WT mice, showing similar alterations to that seen in $ASC^{-/-}$ and Casp-$1^{-/-}$-infected mice. Of note, there was marked increase in reactive oxygen species (ROS) after DENV infection in WT mice, and inhibition of NADPH oxidase by Apocynin (APO) treatment or infection of gp91phox$^{-/-}$ mice resulted in reduced ROS production and in inhibition of IL-1βproduction after DENV infection. In a similar way to the response seen in mice deficient for inflammasome components and in IL-1ra treated mice, APO-treated and gp91phox−/− mice showed more marked susceptibility to DENV infection. These data suggest that ROS-mediated inflammasome activation is essential for host ability to deal with DENV infection, in part through promotion of IL-1β production and control of viral replication.
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Disclosure of interest: None declared.

OC-092
MSU CRYSTAL-RECRUITED NON-INFLAMMATORY MONOCYTES DIFFERENTIATE INTO M1-LIKE MACROPHAGES CAPABLE OF DRIVING INFLAMMATION IN A MODEL OF GOUT

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Acute gout is an intensely painful form of inflammatory arthritis caused by the formation of monosodium urate (MSU) crystals in the joint and characterised by the infiltration of leukocytes including monocytes. Currently, the role of MSU-crystal recruited monocytes in the progression of the inflammatory response is unclear with monocyte differentiation into macrophages being linked with either anti-inflammatory or pro-inflammatory responses to MSU crystals in vitro. Using a murine model of acute MSU crystal-induced inflammation we profiled the differentiation pathway of MSU crystal-recruited monocytes in vivo. Our findings showed that newly recruited monocytes exhibited poor phagocytic capacity, expressed low levels of pro-IL-1β and failed to produce pro-inflammatory cytokines alone, or in response to MSU crystal restimulation ex vivo. However, over time MSU crystal-recruited monocytes exhibited increased phagocytic capacity and expression of CD14, and the accumulation of intracellular pro-IL-1β, active caspase-1 and intracellular IL-1β, indicative of differentiation into an M1-like macrophage. Consistent with this, MSU crystal restimulation triggered the production of pro-inflammatory cytokines by these cells ex vivo, and initiated IL-1β production and leukocyte recruitment in vivo. MSU crystal-recruited monocytes differentiate into pro-inflammatory M1-like macrophages in vivo. These data show for the first time that differentiation of MSU crystal-recruited monocytes into a pro-inflammatory M1-like macrophage in vivo, combined with ongoing MSU crystal deposition, may play a significant role in abrogating resolution and perpetuating inflammation in gout.
Disclosure of interest: None declared.

Vasculitis-autoimmunity (SY11)

OC-093
THE LEADING ROLE OF THE TARGET: ENDOTHELIAL CELLS FROM DIFFERENT TISSUES DIFFERENTIALLY INFLUENCE IMMUNE ACTIVATION

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Wegener's granulomatosis (WG) is an autoimmune disease characterized by granulomatous inflammation of the respiratory tract and systemic vasculitis. Vasculitis involves small- to medium- size vessels and mostly affects lungs and kidneys. Given the current lack of a mouse model for this human disease, it is important to conceive alternatives to investigate the pathogenesis of this syndrome. We have previously described [1] a peculiar NK-like CD4 T cell subset probably involved in WG vascular damage. We here investigated the role of the endothelium, target of the vascular injury. We analyzed the phenotype and functional features of human microvascular endothelial cells (MECs) derived from renal glomerula, lung and skin. We observed consistent differences between the three tissues in the basal expression of activation and adhesion markers (ICAM-1; CX3CL1; MHC-I) along with NK cell receptor (NCR) ligands (MICA/B-; ULBPs; PVR). Moreover the MECs stimulation with different factors induced distinct changes in phenotype and cytokine production (IL-8; IL-6; IL-15; CX3CL1). Finally, we showed that MECs derived from the three tissues have different ability to stimulate NK cells. Of note, renal- and lung-derived MECs elicited NK cell response more efficiently than the skin-derived. Our findings demonstrate that endothelial cells depending on their tissue origin can differently stimulate cells of the immune system. These data could contribute to explain why vasculitis may selectively affect specific organs, as for Wegener's disease, in which lung and kidney vessels are particularly concerned.
1. de Menthon et al. Excessive IL-15 transpresentation endows NKG2D + CD4 T cells with innate-like lytic capacity to vascular endothelium in Wegener's vasculitis. Arthritis & Rheumatism (in press).
Disclosure of interest: None declared.

OC-094
A LOSS-OF-FUNCTION VARIANT OF THE ANTIVIRAL MOLECULE MAVS IS ASSOCIATED WITH A SUBSET OF SYSTEMIC LUPUS PATIENTS

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Dysregulation of the antiviral immune response may contribute to autoimmune diseases. Here, we hypothesized that altered expression or function of MAVS, a key molecule downstream of the viral sensors RIG-I and MDA-5, may impair antiviral cell signalling and thereby influence the risk for systemic lupus erythematosus (SLE), the prototype autoimmune disease. We used molecular techniques to screen non-synonymous single nucleotide polymorphisms (SNPs) in the MAVS gene for functional significance in human cell lines and identified one critical loss-of-function variant (C79F, rs11905552). This SNP substantially reduced expression of type I interferon (IFN) and other proinflammatory mediators and was found almost exclusively in the African-American population. Importantly, in African-American SLE patients, the C79F allele was associated with low type I IFN production and absence of anti-RNA-binding protein autoantibodies. These serologic associations were not related to a distinct, functionally neutral, MAVS SNP Q198K. Hence, this is the first demonstration that an uncommon genetic variant in the MAVS gene has a functional impact upon the anti-viral IFN pathway in vivo in humans and is associated with a novel sub-phenotype in SLE.
Disclosure of interest: None declared.

OC-095
IFN ALPHA CAUSES SLE AND ITS IMPLICATION TO SELF-ORGANIZED CRITICALITY THEORY OF AUTOIMMUNITY

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Interferonα (IFNα) has been hypothesized to cause SLE, however, direct proof for this is lacking. We previously found that overstimulation of CD4 T cells with antigen led to the development of autoantibody-inducing CD4 T (*ai*CD4 T) cell capable of inducing autoantibodies including anti-dsDNA antibody and lupus tissue disease including IC-mediated glomerulonephritis. Notably, *ai*CD4 T cell is induced via de novo TCR revision, but not by cross-reaction to antigen. We test here the role of IFNα in relation to generation of *ai*CD4 T cell by making IFNα transgenic (Tg) mice.
Methods: Mouse IFNa (mIFNa) gene controlled under TetOp promoter (TetOp-mIFNa) was microinjected into fertilized eggs of C57BL/6 to generate TetOp-mIFNaTg mice. We mated this mice with EμSR-tTA Tg mice, expressing tetracycline-transactivator (tTA) gene under the control of Ig heavy chain enhancer and SRα promoter, to obtain double Tg (TetOp-mIFNa/EμSR-tTA; +/+ (IFNaTg) mice. The littermate (TetOp-mIFNa/EμSR-tTA; ∓) was used as a control.
Results: Solely by increasing IFNα in doxycycline (Dox)-inducible IFNα Tg mice, anti-dsDNA antibody, immune complex (IC) and tissue injuries identical to human SLE were induced. Serum anti-dsDNA raised 12 weeks (wks) after cessation of Dox, and anti-ds DNA antibody reached maximum at 20 wks. IFNα was increased in 7/14 (50%) mice at 28 wks. Serum IC was raised at 28 wks, and present in 7/11 (63.6%) mice at 40 wks. Lesions consisted of IC-deposited glomerulonephritis, interstitial lung disease, alopecia, epidermal liquefaction, positive skin - lupus band test, splenic onion skin lesion, and inflammatory infiltrates to salivary gland and bile duct. Effector $IFN\gamma^{+}CD8^{+}$ T cell (CTL) and activated $CD3^{+}CD4^{-}CD8^{-}$ double negative (DN) T cell were increased, and DN T cell not only infiltrated to glomeruli of Tg mice, but also induced de novo glomerulonephritis and alopecia when transferred into naïve recipients.
Conclusions: IFNα causes SLE, likely mediating *ai*CD4 T cell function.
Disclosure of interest: None declared.

Osteoarthritis (SY12)

OC-096
CARTILAGE-SPECIFIC PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARΓ) DEFICIENT MICE EXHIBIT ACCELERATED CARTILAGE DESTRUCTION IN A MICE MODEL OF OSTEOARTHRITIS (OA)

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Purpose: OA is characterized by cartilage destruction, subchondral bone remodelling and synovial membrane inflammation. Studies suggest that the PPARγ activation is a therapeutic target for OA. In vitro activation of PPARγ can reduce the synthesis of various catabolic and inflammatory factors implicated in OA pathophysiology. However, the specific in vivo role PPARγ in OA pathophysiology is still unknown. Here, for the first time, my study examines the specific in vivo role of PPARγ in OA using cartilage-specific PPARγ knockout (KO) mice.
Methods: Cartilage-specific PPARγ KO mice were generated using LoxP/Cre system. 10 weeks old WT and PPARγ KO mice were subjected to model of OA. 10 weeks post-surgery, mice were sacrificed, hind limbs were dissected and embedded in paraffin. Sections were stained with Safranin-O and blindly graded by OARSI scoring method. Cartilage explants studies were conducted using hip cartilage from 3 to 4 weeks old control and PPARγ KO mice. Cartilage was extracted, cultured and stimulated with IL-1ß, RNA was isolated and RT-PCR was conducted.
Results: Cartilage-specific PPARγ KO mice exhibited accelerated OA compared to WT mice. OARSI scoring of joint sections showed that PPARγ KO mice (10 weeks post-surgery) exhibit greater matrix depletion, hypocellularity, fibrillation and structural damage compared to WT mice. So, loss of PPARγ in the cartilage results in greater cartilage destruction associated with OA. RT-PCR analysis further indicated that PPARγ KO mice exhibit significantly reduced expression of aggrecan and collagen II and increased expression of MMP-13 compared to control mice. In addition, IL-1β-stimulated PPARγ KO cartilage exhibited significantly increased expression of inflammatory enzymes (iNOS-2 and COX-2) compared to WT mice.
Conclusion: These results, for the first time, demonstrate that loss of PPARγ in the cartilage leads to accelerated OA in mice, suggesting a key role of PPARγ in the pathophysiology of OA.
Disclosure of interest: None declared.

OC-097
IMPACT OF MECHANICAL LOADING OF OSTEOBLASTS ON THE CHONDROCYTES PHENOTYPE: A NOVEL MURINE MODEL FOR ASSESSING BONE/CARTILAGE COMMUNICATION

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Osteoarthritis (OA) is the most common form of arthritic disease and a major cause of disability in elderly. This disease, which is characterized by joint degeneration, leads to a progressive loss of articular cartilage in response to mechanical and biochemical factors. However, OA is a complex disease not limited to cartilage degeneration and recently, experimental animal models suggest that subchondral bone remodeling could initiate and/or contribute to cartilage loss in OA through bone/cartilage interplay. The aim of this study was to demonstrate the ability of soluble mediators released by loaded osteoblasts to shift the articular chondrocyte phenotype from resting to pro-degradative through a novel and unique bone/cartilage communication model. Thanks to a three dimensional (3D) culture model, murine calvaria osteoblasts were submitted to compression in Biopress Flexercell plates (1.7 MPa, 1 Htz during 24 h). Then media from loaded or unloaded osteoblasts was used to treat primary mouse articular chondrocytes for 24 h. Chondrocyte

expression and secretion of matrix metalloproteinases (MMP-3 and -13), aggrecanases (ADAMTS-4 and -5) and theirs inhibitors (TIMP-1, -2 and -3) were analyzed by RT-PCR, ELISA and Immunoblotting. Media from compressed osteoblasts highly induced MMP-3 (50-fold) and MMP-13 (18-fold) chondrocyte expression. It also significantly induced MMP-3 and MMP-13 chondrocyte release. Media from compressed osteoblasts also increased chondrocyte ADAMTS-4 and -5 expression (respectively 8.3-fold and 1.7-fold). Moreover, compressed osteoblast media regulated TIMPs chondrocyte expression. It enhanced TIMP-1 expression (5.2-fold) whereas it strongly inhibited TIMP-2 and -3 expression (respectively by 55 and 89%). These results indicate that soluble mediators released by compressed osteoblasts promote a pro-degradative phenotype in chondrocytes. Identification of these soluble mediators should lead to new therapeutical targets and/or new biomarkers in OA.
Disclosure of interest: None declared.

OC-098
ANTIINFLAMMATORY EFFECTS OF CO-RELEASING MOLECULE-2 IN OSTEOARTHRITIC SYNOVIOCYTES

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CO-releasing molecules (CORM) can reproduce the effects of CO derived from heme oxygenase-1 (HO-1) activity and show anti-inflammatory properties in a range of diseases with an inflammatory component. We have demonstrated previously the protective actions of CORM-2 in osteoarthritic (OA) cartilage. However, little is known about the effects of these molecules in OA synovitis. In this study we explored the effects of CORM-2 in OA synoviocytes stimulated with interleukin (IL)-1β. Synovial tissue samples were obtained from 15 OA patients undergoing total knee joint replacement. Synoviocytes were obtained by digestion with collagenase and cultured until third passage. Cells were stimulated with IL-1β (10 ng/ml) in the presence or absence of CORM-2 at various concentrations (50–200 μM) or $RuCl_3$ (200 μM) as negative control. CORM-2 markedly decreased cell proliferation induced by IL-1β and it did not modify HO-1 expression, thus discarding the participation of this protein in the effects of CORM-2. This agent was able to reduce both protein and mRNA levels of IL-8, CCL2 and CCL20, effects that were accompanied by a marked reduction in the migration promoted by IL-1β. At the highest concentration employed, this treatment also showed beneficial effects by decreasing reactive oxygen species generation. Interestingly, CORM-2 also reduced, in a concentration-dependent manner, the activity and expression of degenerative mediators such as MMP-1 and MMP-3. Further analyses revealed that CORM-2 significantly counteracted the activation of mitogen-activated protein kinases and nuclear factor κB signalling pathways, which could be responsible, at least in part, for the protective effects elicited by CORM-2 in this cellular system.

In conclusion, CORM-2 exerts anti-inflammatory and anti-degenerative effects on OA synoviocytes. CO-releasing molecules represent new and interesting strategies to counteract synovial inflammation during osteoarthritis.
Disclosure of interest: None declared.

Cytokines, cell signaling (OC09)

OC-099
SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)3, AND AT A LOWER EXTENT SOCS1, CONTRIBUTE TO THE ANTI-APOPTOTIC PROGRAMS OCCURRING IN IFN-GAMMA/TNF-ALPHA-ACTIVATED PSORIATIC KERATINOCYTES BY SUSTAINING AKT PATHWAY

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Due to their genetically-determined capacity to respond to pro-inflammatory stimuli, keratinocytes have a role in the pathogenesis of psoriasis. Upon IFN-γ and TNF-α exposure, psoriatic keratinocytes express exaggerated levels of inflammatory mediators, and show aberrant hyperproliferation and terminal differentiation. However, the thickening of psoriasic skin also results from a resistance of keratinocytes to cytokine-induced apoptosis. Recently, we found that SOCS1 and SOCS3 counteract IFN-γ-induced effects in keratinocytes and are very highly expressed by psoriatic cell strains. Due to the well-known anti-apoptotic effects of SOCS1 and SOCS3, we investigated on their possible involvement in protecting psoriatic cells from IFN-γ/TNF-α-induced apoptosis through the PI3 K/AKT signaling, which has definite anti-apoptotic functions in keratinocytes. Other than expressing increased SOCS1 and SOCS3, psoriatic keratinocytes showed high levels of phosphorylated AKT in response to IFN-γ and TNF-α. Moreover, the expression of the anti-apoptotic Bcl-X_L and Bcl-2 genes could not be down-regulated by IFN-γ/TNF-α in psoriatic keratinocytes, differently to what observed in healthy cells. Keratinocytes stably transfected with SOCS3 were more resistant to IFN-γ/TNF-α-induced apoptosis compared to control clones, and showed high levels of Bcl-X_L, Bcl-2 and phosphorylated AKT. Vice versa, SOCS3 silencing resulted in enhanced IFN-γ/TNF-α-induced apoptosis, correlating with impaired AKT phosphorylation, and reduced Bcl-X_L and Bcl-2 levels. SOCS1 manipulation in keratinocytes had effects similar to that of SOCS3 even though less significant. Our data indicate that SOCS3 and SOCS1 can contribute to the anti-apoptotic programs occurring in IFN-γ/TNF-α-activated psoriatic keratinocytes by sustaining AKT activation and Bcl-X_L and Bcl-2 expression. We are currently investigating whether SOCS3 and AKT inhibition in psoriatic keratinocytes renders these cells sensitive to apoptosis.
Disclosure of interest: None declared.

OC-100
INTERPLAY BETWEEN TNF AND REGULATORY T CELLS IN A TNF-DRIVEN MODEL OF ARTHRITIS, AND THE IMPACT OF PASSIVE OR ACTIVE ANTI-TNF THERAPIES

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TNF plays a major inflammatory role in rheumatoid arthritis (RA), and anti-TNF targeted treatments are highly effective in this disease. In the present study, we aimed to elucidate the interplay between TNF and $CD4^+$ $CD25^+$ $FoxP3^+$ regulatory T cells (Treg), and the impact of anti-TNF therapies on those cells. We used a TNF-driven disease, specifically human TNF transgenic (TTg) mouse model of arthritis. In the first part of our study, we observed that, in 7 weeks old-TTg mice, hTNF overexpression induced lower Treg proportions in secondary lymphoid organs, as compared to age-matched wild type (wt) mice. However, the frequency of Treg cells increased with time in TTg mice, reaching the same proportion than wt mice at 24 weeks. TNFR2 expression by Treg, also increased with time in TTg mice. In the second part of our study, TTg mice were treated with either passive (infliximab) or active (TNF-K: vaccination against TNF) anti-TNF. Compared to untreated mice, hTNF blockade with either infliximab or TNF-K resulted in an increased Treg frequency. In lymph nodes (LN), our study showed that hTNF blockade induced an expansion of induced Treg cells defined by the $CD4^+$ $CD25^+$ $FoxP3^+$ $CD62L^-$ phenotype, an up-regulation of CTLA-4 expression by Treg cells, accompanied by an increased Treg suppressive activity on effector T cell proliferation. In conclusion, in a strictly hTNF dependent inflammation model, we show for the first time that TNF can have different effects on Treg, depending on the duration of exposure and on disease phase. Our work also shows that TNF blockade either by TNF-K or infliximab could depend not only on TNF neutralization but also on Treg upregulation. Disclosure of interest: J. Biton: None Declared, L. Semerano: None Declared, L. Delavallée: None Declared, M. Laborie: None Declared, G. Grouard-Vogel: None Declared, D. Lemeiter: None Declared, M.-C. Boissier Grant/Research Support from: UCB, N. Bessis: None Declared

OC-101
IL-1B-DEPENDENT DOWN REGULATION OF MIR-24 PARTICIPATES TO PREMATURE AGING OF CHONDROCYTES IN OA

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Osteoarthritis (OA) is characterized by chondrocyte premature aging involving altogether an hypertrophic phenotype, a DNA damage accumulation and presence of senescence-mediated secretory factors such as Il-1b, Il-8 and MMPs. Molecular mechanisms involved in OA are still under investigation. Here we identified by genome wide miR-array analysis that p16INK4A-regulatory microRNA, hsa-miR-24 is one target of three ex vivo osteoarthritic mimicking models namely Il-1b, IL-8 and oxidative stress-treated chondrocytes. hsa-miR-24 expression is repressed upon IL-1 treatment while p16INK4A accumulates. Furthermore, we show by RT-qPCR that hsa-miR-24 expression increases during early steps of ex vivo chondrogenic differentiation of mesenchymal stem cells and decreases while p16INK4A increases correlating with an hypertrophic stage. Finally, preliminary results based on specific antagomir approach suggest that hsa-miR-24 down regulation is sufficient to trigger p16INK4A-dependent premature aging on chondrocyte and expression of hypertrophic markers such as MMP13. Altogether, we proposed that hsa-miR-24 is one of the first microRNA in human having an central anti-aging role by preventing the onset of one age-related disease.
Disclosure of interest: None declared.

OC-102
REGULATION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 4 ACTIVITY BY PROTEASE-ACTIVATED RECEPTOR AGONISTS AND HYDROGEN SULFIDE

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We have previously demonstrated that Transient Receptor Potential Vanilloid 4 (TRPV4) activation in intestinal epithelial cells caused intracellular calcium mobilization and chemokine release. Further, TRPV4 activation in mouse intestine induced colitis, suggesting that this channel could participate to intestinal inflammation. Many mediators are released at inflammatory sites, including serine proteases known to activate Protease-Activated Receptors (PAR), but also endogenous gas, such as hydrogen sulfide (H_2S). We hypothesized that such mediators are able to regulate TRPV4 functions in intestinal epithelial cells
Methods: TRPV4 response was evaluated by measuring calcium flux with a Fluo-8 probe in Normal derived Colon Mucosa (NCM460) cells. Cells were exposed to PAR_1- (TFLLRN-NH_2), PAR_2- (SLIGRL-NH_2), or PAR_4-activating peptide (AYPGFK-NH_2), Sodium Hydrogen Sulfide (NaHS) a H_2S donor, or their vehicle, 3 min before a second stimulation with the specific agonist of TRPV4, 4αPDD (4α-phorbol-12,13-didenoate) or its vehicle.
Results: TRPV4 agonist induced a dose-dependent calcium flux in NCM460 cells. The response to 10 μM of 4αPDD was significantly increased when cells were pre-incubated with PAR_1-(100 μM) PAR_2-(10 μM), but not PAR_4-activating peptide (200 μM). Intestinal epithelial cells did not respond to low concentration (1 μM) of 4αPDD. However, if cells were pre-treated with the PAR_1- (100 μM) or the PAR_2- (10 μM), but not the PAR_4 agonist, they became responsive to 1 μM of the TRPV4 agonist. Incubation of NCM460 cells with NaHS (200–1,000 μM) dose-dependently decreased the calcium response to pro-inflammatory dose of TRPV4 agonist (50 μM).
Conclusions: Activation of PAR_1, PAR_2 but not PAR_4 potentiated the cellular response of TRPV4 but also sensitized the receptor, potentially enhancing its pro-inflammatory effects. However, NaHS exposure decreased cellular response to TRPV4 agonist, thereby potentially exerting anti-inflammatory effects in the gut.
Disclosure of interest: None declared.

OC-103
LIPOTOXIC STRESS AND MITOCHONDRIAL DYSFUNCTION IN ENDOTHELIAL CELLS ACTIVATED WITH PHOSPHOLIPOLYZED LDL

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Secreted phospholipases A2 (sPLA2), are now considered attractive therapeutic targets and potential biomarkers, as they contribute in inflammation through lipoprotein-dependent and independent mechanisms. We have previously shown that hGX-sPLA2 is expressed in human atherosclerotic lesions and that the phospholipolysis of LDL by hGX sPLA2 results in a proinflammatory LDL particle (LDL-X), which perturbs endothelial function through the induction of endoplasmic reticulum (ER) stress. Alteration in ER homeostasis triggers the unfolded protein response (UPR) which regulates the balance between survival and apoptosis. In the present study we aimed to identify the molecular pathways directing LDL-X-stress responses to survival or apoptosis in endothelial cells. Endothelial cells (HUVEC), activated with LDL-X induced all three branches of the UPR response with concomitant activation of apoptotic and survival pathways (BCL2 family of proteins). WB and RT-QPCR analysis showed a significant decrease in cyclinD1 expression, suggesting that the regulation of cyclinD1 in response to LDL-X induced-ER stress is a checkpoint control, which prevents cell-cycle progression until homeostasis is restored. Furthermore, immunofluorescence analysis of the mitochondria in the LDL-X treated cells showed altered morphology and shrinkage indicating mitochondrial dysfunction. In parallel, we observed upregulation of antioxidant/detoxifying enzymes, with concomitant activation of NRF2, whose main role is to protect cells from oxidative stress. As LDL-X is not oxidized, we postulated that in addition to the modification of LDL, the lipid mediators produced from LDL hydrolysis, arachidonic acid and Lyso-PC, activate the antioxidant response and this was further confirmed by WB analysis, RT-QPCR and immunofluorescence experiments of HUVEC cells stimulated with the lipid mediators.
Conclusion: LDL-X induces inflammation in endothelial cells through a lipotoxic stress which implicates mitochondria.
Disclosure of interest: None declared.

OC-104
NLRP3 INFLAMMASOME-MEDIATED NEUTROPHIL RECRUITMENT AND HYPERNOCICEPTION DEPENDS ON LEUKOTRIENE B4 IN A MURINE MODEL OF GOUT

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Introduction: Deposition of monosodium urate (MSU) crystals in the joint promotes an intense inflammatory response and joint dysfunction. We have evaluated the role of the inflammasome and 5-lipoxygenase (5-LO)-derived leukotriene B_4 (LTB_4) in driving tissue inflammation and hypernociception in a murine model of gout
Methods: Gout was induced by injecting MSU crystals in the joint of the mice. Wild type (WT) mice and mice deficient in NLRP3, ASC, Caspase-1, IL-1β, IL-1R1 IL-18R, MyD88 and 5-LO were used. Neutrophil influx, LTB_4, cytokine production (IL-1β, CXCL1) (ELISA), intravital microscopy and hypernociception were evaluated. Expression of caspase-1, cleaved IL-1β (Western Blot) and production of reactive oxygen species (ROS) (fluorimetric assay) were analyzed in macrophages.
Results: Injection of MSU in the knee joints of mice induced neutrophil influx and neutrophil-dependent hypernociception. MSU-induced neutrophil influx was CXCR2-dependent and relied on the induction of CXCR2 acting chemokines in a NLRP3/ASC/Caspase-1/IL-1β/MyD88-dependent manner. LTB_4 was produced rapidly after injection of MSU, and was necessary for Caspase-1-dependent IL-1β production and consequent release of CXCR2-acting chemokines in vivo. In vitro, macrophages produced LTB_4 after MSU crystals and LTB_4 was relevant for MSU-induced IL-1β maturation. Mechanistically, LTB_4 drove MSU-induced production of ROS and ROS-dependent activation of the inflammasome.
Conclusion: We show the role of the inflammasome in mediating MSU-induced joint inflammation and dysfunction, and highlight a previously unrecognized role of LTB_4 in driving inflammasome activation in response to MSU crystals both in vitro and in vivo.
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Disclosure of interest: None declared.

OC-105
PURINERGIC SIGNALING: A COMMON PATHWAY FOR CRYSTALS-MEDIATED INFLAMMASOME ACTIVATION

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Deposition of uric acid crystals in joints causes gout disease. Prolonged airway exposure to silica crystals leads to silicosis development. Aluminum hydroxide (alum) salts are frequently used as vaccine adjuvant. These micrometer size particles activate innate immunity through the Nlrp3 inflammasome resulting in IL-1β production. The inflammasome is activated not only by particles derived from endogenous aggregates or environmental pollutants but also by a broad spectrum of situations, from metabolic stress to pathogen-related molecules. Particles-mediated inflammasome activation has been linked to reactive oxygen species involvement and lysosome destabilization. In an other hand, ATP is present in all living cells and extracellular ATP is a well known soluble danger signal able to activate the Nlrp3 inflammasome through P2X7 receptor and pannexin-1 pore formation. Here we establish a link between ATP and particles signaling. First, we show that uric acid, silica and alum particles trigger endogenous ATP release depending on functional P2X7 receptor and pannexin-1 hemichannel proteins. Then, we observe that maturation and secretion of IL-1β were dramatically impaired in presence of purinergic receptors or pannexin-1 pore antagonists. This demonstrates that crystals are competent to induce

maturation and secretion of IL-1β through a process that involves as a first event the extracellular release of endogenous ATP. Moreover, we show that not only ATP but also ADP and UTP are able to stimulate IL-1β production through multiple purinergic receptors signaling, rendering even more interesting to better decipher purinergic-mediated inflammatory processes. These data demonstrate that nucleotides are endogenous danger signal generated in response to particle exposures and provide a new molecular mechanism to explain how chemically and structurally diverse stimuli can activate the Nlrp3 inflammasome and trigger IL-1β maturation and secretion.
Disclosure of interest: None declared.

OC-106
PLATELET-DERIVED IL-27 AND SOX40L ASSOCIATED WITH ACUTE TRANSFUSION REACTIONS

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IL-27 and sOx40L are produced mainly by antigen presenting cells and are powerful immunomodulatory molecules. For instance, IL-27 enhances B cell proliferation and OX40L reduces Th2 response. To date platelets were not reported to be able to release neither IL-27 nor sOX40L. We sought to examine the levels of IL-27 and sOx40L in supernatants of apheresis platelet concentrate preparations (PCs) during storage and in left over products involved in transfusions that induced acute transfusion reactions (ATR). Samples from PCs were taken for analysis each day during their 7-day storage. We recorded 4 ATR episodes for which PC left over products could be analyzed. Two fractions (supernatant and lysates of platelets) from each ATR-associated PC (and 10 controls) were prepared. *A proteomic* approach was used to determine inflammatory factors involved in ATR and ELISA was used for quantify these factors. After a 1-day storage of PCs we found elevated levels of IL-27 and sOx40L (respectively at 18,020 $\pm$ 5,844 and 374 $\pm$ 50 pg/mL/10^9 unit) that increased respectively by 2.2 and 2.25-fold at storage day 7 versus day 0. Moreover, supernatants of ATR-associated PCs contained higher and corresponding platelet lysates contained lower, IL-27 and sOx40L levels, compared to control PCs ($P < 0.05$). We exposed B and T cells to supernatants and cell lysates of ATR-associated PCs and observed an increase of CD86 and CD69 expression only for PCs supernatants. This over-expression of activation markers was abolished when B and T cells were preincubated with IL-27 and sOx40L blocking antibodies. We demonstrated that platelets contain and release IL-27 and sOx40L that are implicated in ATR. These data emphasize the importance of investigating, in a multi-center collaborative study, larger series of ATR-associated PCs to determine specific reagents or processes to reduce IL-27 and sOx40L production during collection and storage of platelet components.
Disclosure of interest: None declared.

Inflammatory processes in cardiovascular and renal diseases (OC10)

OC-107
ABERRANT PRESENTATION OF MPO INDUCES TOLERANCE AND PROTECTS FROM EXPERIMENTAL ANTI-MPO GLOMERULONEPHRITIS

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Glomerulonephritis (GN) is a major cause of renal failure. Crescentic GN is the most destructive form of this disease. Most cases result from autoimmunity to myeloperoxidase (MPO) causing severe glomerular inflammation. We have defined an immunodominant and nephritogenic T cell epitope from MPO (20 amino acids; known as peptide 52 [pep52]). This work examines whether aberrant presentation of MPO could attenuate autoimmunity and anti-MPO glomerulonephritis. Autoimmunity was induced by immunizing mice (with MPO or pep52) then disease was triggered with low dose anti-GBM globulin which induced glomerular neutrophil influx and MPO deposition. Aberrant presentation of MPO was performed by intranasal insufflation of pep52 or immunization with MPO-pulsed bone marrow-derived CD40−/− dendritic cells (DCs). Nasal insufflation of pep52 over 3 consecutive days protected mice from the development of autoimmunity when subsequently immunized with 200 μg pep52, compared with control mice given nasal insufflation with an irrelevant peptide. The frequency of CD4+ cells responding to MPO by producing IFNγ was 457 $\pm$ 107 (per 10^6, control mice) versus 289 $\pm$ 90 (tolerized mice) and IL-17A-producing cells was 231 $\pm$ 154 versus 49 $\pm$ 32; $p < 0.001$. Anti-MPO IgG titres were also reduced. When pep52 immunized mice were challenged with anti-GBM globulin, GN was reduced in tolerized mice with reduced glomerular segmental necrosis, glomerular neutrophils, macrophages, CD4+ cells and proteinuria. Immunization of C57BL/6 mice with MPO-pulsed CD40+/+ DCs induced strong anti-MPO autoimmunity which induced GN when triggered with anti-GBM globulin. However, immunization with CD40−/− DCs protected from the development of autoimmunity and GN. Draining lymph node cells isolated from CD40−/− DC-immunized mice showed higher levels of IL-10 suggesting the induction of Tr1 regulatory T cells. Aberrant presentation of immunodominant peptide or MPO autoantigen tolerizes and protects from autoimmune anti-MPO GN.
Disclosure of interest: None declared.

OC-108
INFLAMMATORY RESPONSE IN IGA NEPHROPATHY IS ASSOCIATED WITH PI3 K/AKT/M-TOR PATHWAY ACTIVATION

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In IgA nephropathy (IgAN), the commonest primary glomerulonephritis worldwide, mesangial proliferation and renal inflammation are key pathological features for disease progression. Our group has identified the transferrin receptor 1 (TfR1 or CD71) as a mesangial IgA1 receptor and we had shown that in cultured mesangial cells TfR1 activation by polymeric IgA1 (pIgA1) induces IL-6 production and cell proliferation. However the mechanism underlying this cytokine production and cellular proliferation is still unclear. Herein, we developed a new inducible mouse model of IgAN, in which patients' IgA induced mesangial cell proliferation, IL-6 and TNF-α production, monocytes/macrophage renal infiltration and renal dysfunction. These responses were associated with PI3K/Akt/m-TOR pathway activation, once sustained PI3K/Akt pathway activation in PTEN± mice amplified patients' IgA1 effects, whereas blocking of this pathway by rapamycin was inhibitory. Concomitant with PI3K/Akt/m-TOR pathway activation we observed an increased TfR1 expression and platelet-derived growth factor receptor-beta (PDGFRß) expression and phosphorylation. Interestingly, PDGFR inhibition abrogated mesangial cell proliferation but not the inflammatory cytokine production. In vitro overexpression of TfR1 increased cell sensitivity to PDGF-BB suggesting that mesangial cell proliferation is due to a convergence of TfR1 and PDGFR signaling. We conclude that PI3 K/Akt/m-TOR pathway is pivotal to IgAN inflammation by controlling renal inflammatory cytokines production and is also important to mesangial cell proliferation via a modulation of PDGFR signaling threshold.
Disclosure of interest: None declared.

OC-109
CD44-SPLICE VARIANT 3 ATTENUATES TUBULAR INJURY IN THE EARLY STAGE OF CHRONIC OBSTRUCTIVE NEPHROPATHY AND REDUCES THE PRO-FIBROTIC EFFECTS OF TGF- B1

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Renal tubulointerstitial fibrosis is the end result of chronic inflammation and it is regulated by a complex network of cytokines, including the pro-fibrotic factor TGF-β1 and the two anti-fibrotic cytokines BMP-7 and HGF. One of the molecules that might orchestrate the balance between TGF-β1 and HGF/BMP-7 is CD44, which is a surface glycoprotein involved in inflammation and whose expression is up-regulated on tubular epithelial cells upon kidney injury. All CD44 isoforms differ in the extracellular domain and are therefore interacting with different molecules. The CD44 standard (CD44s) isoform can facilitate TGF-β1 signaling, while the CD44-variant 3 (CD44v3) is capable of binding HGF and BMP-7 due to its attached heparan sulfate moieties. Using transgenic mice over-expressing either CD44s or CD44v3, specifically on proximal tubular epithelial cells (TECs), we studied the progression of obstructive nephropathy (UUO), as a model of chronic kidney diseases. Wt and transgenic mice (n = 8/group) were subjected to UUO, and sacrificed at 1, 3, 7, and 14 days. Primary TECs were isolated from Wt and transgenic mice (n = 3/group), and stimulated with r-TGF-β1. Statistics were done with the Mann–Whitney test. At day 1 upon UUO the CD44 s transgenic mice showed significantly more tubular damage compared to the Wt and the CD44v3 mice, while no differences were found at later time-point. At day 1, the CD44v3 UUO kidneys revealed significantly less myofibroblasts accumulation together with less TGF-β1 signaling and increased BMP-7 signaling compared to the Wt and CD44 s kidneys. In vitro stimulations of primary TECs with TGF-β1 showed that, in response to TGF-β1, CD44v3-TECs synthesize less
α-SMA, CTGF, TGF-β1 and collagen type1 mRNAs then the Wt-, and CD44 s-TECs. In conclusion, these results suggest that CD44v3 decreases tubular injury and myofibroblast accumulation in early stage of chronic obstructive nephropathy through downregulation of TGF-β1 pro-fibrotic signaling.
Disclosure of interest: None declared.

OC-110
MONOCYTE/MACROPHAGE CHEMOKINE RECEPTOR CCR2 MEDIATES DIABETIC RENAL INJURY

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Introduction: Monocyte/macrophage recruitment correlates strongly with the progression of renal impairment in diabetic nephropathy (DN). CC chemokine receptor (CCR)2 regulates monocyte/macrophage migration into injured tissues. However, the direct role of CCR2 in the progression of DN remains unknown. We hypothesize that deficiency or blockade of CCR2 confers kidney protection in DN.
Methods: Experiments were conducted in $Ins2^{Akita}$ mice with or without the selective CCR2 antagonist, RS504393 (2 mg/kg/day via osmotic minipump) for 12 weeks or in CCR2 deficient mice ($CCR2^{-/-}$) and their wild type littermate ($CCR2^{+/+}$) following STZ induced diabetes for 6 weeks.
Results: Blocking CCR2 using RS504393 in $Ins2^{Akita}$ mice significantly attenuated albuminuria ($p < 0.005$), histological changes and kidney macrophage recruitment ($p < 0.005$) compared to $Ins2^{Akita}$ + vehicle. Furthermore, mice lacking CCR2 ($CCR2^{-/-}$) mimicked CCR2 blockade by reducing albuminuria to normal range, diabetes-mediated histological changes, kidney fibronectin mRNA expression, and inflammatory cytokine production (TNF-α and IFN-g) compared to $CCR2^{+/+}$ mice. Bone-marrow derived monocytes from $CCR2^{+/+}$ mice adoptively transferred into $CCR2^{-/-}$ mice reversed the renal tissue protective effect in diabetic $CCR2^{-/-}$ mice. In vitro, podocytes grown on high glucose (HG) media are associated with significant increase in macrophage migration from those grown in normal glucose (NG) media using transwell migration assay and this effect was blocked with the addition of anti-MCP-1 antibody. In addition, M1 macrophages induced podocyte permeability; but not M2 macrophages.
Conclusion: (1) pharmacological blockade or genetic deficiency of CCR2 reduced kidney macrophage recruitment, cytokines and the

extent of fibrosis in DN; (2) podocytes directly mediate macrophage migration; (3) macrophage-dependent soluble factors directly induce podocyte injury through the pro-inflammatory M1 but not the anti-inflammatory M2 subsets.
Disclosure of interest: None declared.

OC-111
ANTI-INFLAMMATORY AND ANTI-ATHEROGENIC FUNCTION OF THE COP9 SIGNALOSOME

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Inflammatory processes play a crucial role in all stages of atherogenesis: from early endothelial activation to the eventual rupture of the atherosclerotic plaque. Activation of the canonical NF-kB signal trans-duction pathway, mediated by phosphorylation and degradation of IkBa by the IkB-kinase (IKK) complex, is a crucial step in pro-inflammatory target gene expression such as that of chemokines or adhesion molecules, which have been implicated in atherosclerosis. The role of the COP9 signalosome (CSN), a multi-functional protein complex with functions in NF-kB and b2-integrin signaling, in atherosclerosis has been unknown. Here, super-complex formation between IKK and the CSN was indicated for by IKK2/CSN5, IKK2/CSN3 and NEMO/CSN2 coimmunoprecipitation in human umbilical vein endothelial cells (HUVECs). The CSN interacts with IKKs and attenuates IKK phosphorylation as observed by siRNA knockdown (KD) of *CSN5* or *CSN2* under pro-atherogenic conditions. Next, a TNFa-dependent degradation of IkBa was markedly enhanced (52%) by *CSN5*- or *CSN2*-siRNA treatment (KD efficiency: $\sim$40%) and a concomitant increase of NF-kB activity between 60 and 70%, as readout by reporter gene and binding activity assay, respectively in HUVECs. Inversely, transient overexpression of CSN5 using CTAP-CSN5 fusion construct led to an increase in IkBa levels and a reduction in NF-kB activity. Furthermore, CSN5-depleted cells stimulated with TNFα showed an increase in MCP1, VCAM1, E-selectin and also ICAM1 expression when compared to control cells. This was translated into increase monocytes arrest on the surface of *CSN5* siRNA-treated HUVEC monolayers. Finally, double-immunostaining for CSN5 and CD31 in human atherosclerotic plaque showed the presence of CSN5 in endothelial cells. Taken together, the CSN controls the activity of NF-kB in atherogenically stimulated endothelial cells and might have anti-inflammatory and anti-atherogenic functions.
Disclosure of interest: None declared.

OC-112
HYDROGEN SULFIDE INHIBITS CARDIOMYOCYTIC AUTOPHAGY INDUCED BY ISCHEMIA/REPERFUSION INJURY

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Hydrogen sulfide (H_2S) has been implicated in cardioprotection during ischemia/reperfusion (I/R) injury. Autophagy is the primary pathway for degrading and recycling long-lived proteins, and its activation has recently been shown to mediate myocardial injury in response to I/R injury. In this study we investigated the ability of H_2S preventing autophagy as a potential mechanism underlying its cardioprotective effects against I/R injury. Male rats were subjected to myocardial ischemia for 45 min followed by 2 h reperfusion. NaHS, a H_2S donor, at doses of 10, 30, 100 μmol/kg was injected i.p. at 30 min before ischemia. The autophage related genes beclin 1 and ATG5 mRNA were elevated in the area at risk (AAR) of hearts subjected to I/R. NaHS reduced myocardial infarct size and attenuated the increase of beclin 1 and ATG5 expressions. In vitro, cultured neonatal rat cardiomyocytes were subjected to 24 h hypoxia followed by 2 h reoxygenation, and NaHS at doses of 10, 30, 50, 100 μmol/L were added 30 min prior to hypoxia. Detection of microtubule-associated protein 1 light chain 3 (LC3) conversion, i.e., LC3-I to LC3-II, and monodansylcadaverine (MDC) staining were also used for the determination of autophagy activity in cardiomyocytes. Autophagy was induced in cardiomyocytes subjected to hypoxia/reoxygenation (H/R), as indicated by the increases of beclin 1 and ATG5 mRNA expression, LC3 conversion as well as MDC staining. NaHS pretreatment reduced cardiomyocyte death induced by H/R. At meantime, NaHS dose-dependently attenuated the H/R-induced autophagy activity. Our results indicate that inhibition of autophagy might be one of the mechanisms underlying H_2S cardioprotective effects against ischemia reperfusion injury.
Keywords: Hydrogen sulfide, cardiomyocyte, autophagy, ischemia reperfusion.

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Disclosure of interest: None declared.

OC-113
ABSENCE OF MICRORNA-155 PROTECTS AGAINST ADVERSE CARDIAC INFLAMMATION AND HYPERTROPHY

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Cardiac hypertrophy, accompanied by progressive inflammation, and consequent heart failure continue to burden Western society. In the current study, we show that miR-155, an inflammatory cell-expressed microRNA, stimulates the secretion of pro-hypertrophic and -inflammatory factors by macrophages and thereby causes adverse inflammation and heart failure. We subjected miR-155 KO and WT mice to 1 and 4 weeks of angiotensin II (AngII) to induce cardiac hypertrophy, inflammation and failure. Whereas AngII significantly increased both cardiac mass and hypertrophic signalling in WT mice, the absence of miR-155 inhibited this hypertrophic response. Moreover, absence of miR-155 prevented inflammatory cell influx in the heart following AngII, more specifically the influx of macrophages. These data were independently confirmed using antagomiRs against miR-155 in AngII-treated mice. In situ hybridization predominantly identified cardiac miR-155 in macrophages and very little was detected in cardiac myocytes. Therefore, we hypothesized that macrophage miR-155 mediates pro-hypertrophic signalling towards cardiac myocytes. Indeed, both bone marrow transplantation with miR-155 KO and WT mice and in vitro experiments independently confirmed that miR-155 in the macrophage mediates cardiac hypertrophy. We found that miR-155 represses its direct target Suppressor of Cytokine Signalling-1 in the macrophage, leading to increased IL-6 secretion and consequent STAT3 activity, implicated in cardiac inflammation and hypertrophy. These data reveal miR-155 expression in the macrophage to be mandatory to allow cardiac hypertrophy, inflammation and failure, by permitting cytokine signalling, identifying miR-155 as a crucial inflammatory regulator of cardiac hypertrophy.
Disclosure of interest: None declared.

Young Investigator Award Winner.
For mini paper see page 289

OC-114
ADIPOSE TISSUE-DERIVED STEM CELL TREATMENT PREVENTS RENAL DISEASE PROGRESSION

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Adipose tissue-derived stem cells (ASCs) are an attractive source of stem cells with regenerative properties that are similar to those of bone marrow stem cells. Here, we analyze the role of ASCs in reducing the progression of kidney fibrosis. Progressive renal fibrosis was achieved by unilateral clamping of the renal pedicle for 1 h, after that, the kidney was reperfused immediately. Four hours after the surgery, 2×10^5 ASCs were intraperitoneally administered, and animals were followed for 24 h post-treatment and then at some other time interval for the next 6 weeks. Also, animals were treated with 2×10^5 ASC at 6 weeks after reperfusion and sacrificed 4 weeks later to study their effect when interstitial fibrosis already is present. At 24 h after reperfusion, ASC-treated animals showed reduced renal dysfunction and enhanced regenerative tubular processes. Renal mRNA expression of IL-6 and TNF was decreased in ASC-treated animals, whereas IL-4, IL-10 and HO-1 expression increased despite a lack of ASC in the kidneys as determined by SRY analysis. As expected, untreated kidneys shrank at 6 weeks, whereas the kidneys of ASC-treated animals remained normal in size, showed less collagen deposition and decreased staining for FSP-1, type I collagen and hypoxyprobe. The renal protection seen in ASC-treated animals was followed by reduced serum levels of TNF-α, KC, RANTES and IL-1α. Surprisingly, treatment with ASC at 6 weeks, when animals already showed installed fibrosis, demonstrated amelioration of functional parameters, with less tissue fibrosis observed and reduced mRNA expression of type I collagen and vimentin. ASC therapy can improve functional parameters and reduce progression of renal fibrosis at early and later times after injury, mostly due to early modulation of the inflammatory response and to less hypoxia, thereby reducing the epithelial-mesenchymal transition.
Disclosure of interest: None declared.

Respiratory diseases (OC11)

OC-115
REPRESSION OF THE NUCLEOSIDE TRANSPORTERS 1 AND -2 REDUCES INFLAMMATORY ACUTE LUNG INJURY

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Acute lung injury (ALI), a devastating disorder characterized by hypoxemia and overwhelming pulmonary inflammation, remains a primary factor of morbidity and mortality in critically ill patients. Extracellular adenosine has been implicated as central signalling molecule during conditions of limited oxygen availability (hypoxia), important in attenuating tissue damage, acute inflammation and the preservation of pulmonary barrier function. During periods of acute inflammation extracellular levels of adenosine are increased in the affected tissues, yet the molecular mechanisms are presently unknown. The Equilibrative Nucleoside Transporters (ENTs) have significant impact on regulation of the levels of extracellular adenosine. During periods of hypoxia, the expression levels of ENT1 and -2 are significantly reduced, leading to the attenuation of adenosine uptake and resulting in an increased extracellular adenosine concentration. Cellular hypoxia is part of the pathophysiological correlate of an acute inflammatory process, yet in addition a variety of cytokines are released upon the stimulation of the immune system. Given this, we investigated consequences of pulmonary acute inflammation on the expression of ENTs. Our initial studies with endothelia and pulmonary epithelia demonstrate attenuation of adenosine uptake as result of diminished expression of ENTs during acute inflammation in vitro. Studies with siRNA confirmed the major contribution of ENT2 as main adenosine transporter in lung. Furthermore, examination of the ENT2 promoter suggests nuclear factor-kappa B in ENT2 repression. Additional in vivo studies using a

murine model for ALI showed that pharmacological inhibition of ENTs results in improved pulmonary barrier function, attenuated neutrophil accumulation and reduced release of pro-inflammatory cytokines during ALI. Taken together, these findings reveal transcriptional repression of ENTs as an innate protective response during acute pulmonary inflammation.
Disclosure of interest: None declared.

OC-116
IN VIVO IMAGING OF NF-KB PATHWAY IN ACUTE LUNG INFLAMMATION MOUSE MODEL CAN PREDICT A PHARMACOLOGICAL RESPONSE

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NF-kB plays a central role in immunity, inflammation, development, cell survival and has been indicated under a number of pathological conditions of lung disease, including asthma, chronic bronchitis, and chronic obstructive pulmonary disease (COPD). In this study, we assessed the in vivo activation of NF-kB signaling in lung tissue using a bioluminescence imaging system (IVIS) to monitor activation of an NF-kB promoter in response to lipopolysaccharide (LPS) stimulation. A plasmid contained responsive elements (RE) of NF-kB and luciferase as a reporter gene has been delivered intravenously in nude mice at the concentration of 40 ug per mouse using in vivo-jetPEI^{TM} from Polyplus as a transfectant agent. One week after DNA delivery the transient transgenic mice had been imaged in order to check the baseline activation of the NF-kB pathway. The day after, the mice have been treated with LPS 15 ug per mouse intratracheally and the lungs imaged using bioluminescence (BLI) at 2, 4, 7 and 24 h. The ability of the IKK2 inhibitor MLN120B orally administered at the dose of 300 mg/kg to counteract NF-kB activation has been evaluated. The maximum peak of NF-kB activation was reached at 4 h with seven-tenfolds of induction in comparison to the saline group and at 24 h the signal dropped down at basal level. In the group treated with MLN120B was observed a 50% inhibition of LPS-induced NF-kB stimulation, an effect that was in good agreement with the inhibition of p65 nuclear translocation evaluated ex vivo in lung homogenates. In this experiment we showed that is feasible to monitor NF-kB activation in vivo in lung tissue in a non-invasive way by BLI and create a new in vivo tool for drug discovery process.
Disclosure of interest: None declared

OC-117
RESOLVING INFLAMMATION DURING INFECTION: ANTIBIOTIC-INDUCED NEUTROPHIL APOPTOSIS AND INHIBITION OF PROINFLAMMATORY SIGNALING IN A MODEL OF RESPIRATORY DISEASE

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The accumulation of neutrophils (PMN) and potent chemoattractants, such as interleukin-8 (CXCL8) and leukotriene B_4 (LTB_4), are characteristic markers of inflammatory disease. Clearance of apoptotic PMN and inhibition of pro-inflammatory signaling are important mechanisms in resolving inflammation. Immunomodulation by antibiotics can have direct anti-inflammatory benefits. Tulathromycin (TUL), a new antibacterial agent for treating Bovine Respiratory Disease (BRD), offers superior clinical efficacy for reasons not fully understood. The aim of this study was to use TUL as a model to characterize anti-inflammatory and pro-resolution properties of antibiotics.
Methods: Bronchoalveolar lavages were collected from calves challenged intra-tracheally with *Mannheimia haemolytica*, a causative agent of BRD, or pro-inflammatory zymosan A, and treated with vehicle or TUL. Whole blood PMN and monocyte-derived macrophages of healthy steers were used in vitro.
Results: TUNEL staining and ELISA revealed that TUL-treatment increased PMN apoptosis and reduced LTB_4 in *M. haemolytica*- and zymosan-challenged calves. In vitro, TUL induced PMN apoptosis in a capase-3-dependent manner. In calcium (ionophore A23187)-activated PMN, TUL inhibited secretion of LTB_4 and release of free fatty acids by phospholipase A_2. Immunoblotting and real-time RT-PCR showed that TUL reduced levels of phospho-IκB and mRNA levels of CXCL8 in lipopolysaccharide (LPS)-stimulated PMN. TUL also inhibited secretion of CXCL8 in LPS-stimulated macrophages. Lastly, the induction of PMN apoptosis by TUL was associated with increased macrophage efferocytosis.
Conclusions: The findings describe novel mechanisms through which TUL confers both anti-inflammatory and pro-resolution benefits, independent of its antibacterial properties (e.g. in zymosan-inflamed lungs). The data suggest that drugs with both antimicrobial and pro-resolution properties would be most beneficial for treating infectious inflammatory diseases.
Disclosure of interest: None declared.

OC-118
NEW INSIGHTS IN CYPRESS POLLEN ALLERGEN REPERTOIRE: WHAT FOR? HOW FAR?

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Introduction: Allergen repertoire analysis allows the distinction between co sensitization and cross reactivity and is at the basis of the improvement of allergy diagnosis. About 10% of children are affected by allergy to cypress pollen in Mediterranean area and the prevalence is growing. The symptoms are sometimes more severe than for a pollinosis to grass. Objective: (1) To decipher the repertoire of pollen allergens from Italian cypress (*Cupressus sempervirens*), (2) To correlate clinical characteristics to circulating specific IgE profiles.
Methods: A proteomic approach was used. Allergens were located by serum IgE immunoblotting after 2D separation of water or detergent pollen soluble extracts. Allergens were then identified by mass spectrometry.

Results: Two different IgE reactivity profiles were observed by immunoblotting in 80 cypress pollen allergic patients. A first group recognized allergens with high relative molecular masses (HMW > 30 kDa) and a second group recognized peptidic epitopes on a basic 14 kDa protein. The major allergen Cup s 1 (43 kDa) was found among the HMW allergens as well as Cup s 2, a polygalacturonase (43 kDa, basic), newly described, with a 70% prevalence. Among three candidates for the 14 kDa protein (a Lipid Transfer Protein, cytochrome c or histones), histone is the most probable one on the basis of preliminary data. Because of its monomorphic nature, such reactivity would put the patient at the frontier between allergy and autoimmunity. The data obtained by immunoblot were compared with IgE profile obtained using ImmunoCAP ISAC (Phadia), a miniaturized multiarray system.
Conclusion: The investigation of this model of allergy leads to both analytical (description of new allergens) and mechanistic (inflammation and autoimmunity) results and hypothesis. The definite identification of the 14 kDa protein, as well as its clinical relevance in inflammation, autoimmunity and IgE-mediated basophil mediator release remain to be unraveled.
Disclosure of interest: None declared.

OC-119
DUAL ROLE OF IL-22 IN ALLERGIC AIRWAY INFLAMMATION AND ITS CROSS-TALK WITH IL-17A

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Rationale: Interleukin-22 (IL-22) has both pro- and anti-inflammatory properties and its role in allergic lung inflammation has not been explored.
Objectives: To investigate the expression and roles of IL-22 in the onset and resolution of experimental allergic asthma and its cross-talk with IL-17A.
Methods: IL-22 expression was assessed in patient samples and in the lung of ovalbumin-immunized and challenged mice. IL-22 functions in allergic airway inflammation were evaluated using IL-22-deficient mice or anti-IL-22 neutralizing antibodies. Moreover, the effects of recombinant IL-22 and IL-17A neutralizing antibodies were investigated. Measurements and main results: Increased pulmonary IL-22 expression is found in the serum of asthmatic patients and in wild-type mice immunized and challenged with ovalbumin. Allergic lung inflammation is IL-22 dependent since eosinophil recruitment, Th2 cytokine including IL-13 and IL-33, chemokine production, airway hyperreactivity and mucus production are drastically reduced in IL-22-deficient mice or by IL-22 antibody neutralization during immunization of wild-type mice. By contrast, IL-22 neutralization during antigen challenge enhanced allergic lung inflammation with increased Th2 cytokines. Consistent with this, recombinant IL-22 given with allergen challenge protects mice from lung inflammation. Finally, we show that IL-22 may regulate the expression and proinflammatory properties of IL-17A in allergic lung inflammation.
Conclusions: IL-22 is required for the onset of allergic asthma, but functions as a negative regulator of established allergic inflammation. Our study revealed that IL-22 contribute to the proinflammatory properties of IL-17A in experimental allergic asthma.
Disclosure of interest: None declared.

OC-120
ANTI-INFLAMMATORY EFFECTS OF INOSINE IN A MURINE MODEL OF OVALBUMIN-INDUCED ASTHMA: EVIDENCE FOR THE INVOLVEMENT OF A2 ADENOSINE RECEPTORS

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Introduction: Inosine has been suggested to exert anti-inflammatory effects in a wide range of inflammatory conditions, including acute lung inflammation.
Objective: This study intended to investigate the possible involvement of adenosine receptors in inosine anti-inflammatory effects in a murine model of ovalbumin-induced asthma.
Methods: Female balb/c mice, sensitized (day 0) and boosted (day 7) subcutaneously with OVA (10 μg) were challenged at day 14 and 15 with aerosolized OVA (1%) and treated intraperitoneally 30 min prior with inosine (0.001–10 mg/kg) or with the selective adenosine receptors antagonists A_1 (DPCPX), A_{2A} (ZM241385), A_{2B} (alloxazine) or non-selective (caffeine), 30 min before inosine injection (10 mg/kg). After 24 h, the bronchoalveolar lavage (BAL) was obtained to perform the cell counting. Lungs fragments were obtained and cultured-24 h (explant) for determination of cytokines
Results: Inosine treatment reduced the number of total leukocytes ($85 \pm 2\%$) with ID_{50} of 0.094 (0.023–0.37) mg/kg, macrophages ($89 \pm 11\%$), lymphocytes ($92 \pm 3\%$) and eosinophils ($97 \pm 2\%$) recovered in the BAL at 10 mg/kg, when compared with the allergic control group. Pre-treatment with ZM241385 and caffeine, reverted 70 ± 5 and $97.6 \pm 8.1\%$ the inosine effect on the total cell count, and $86.5 \pm 7.9\%$ and $96.2 \pm 4.1\%$ the macrophage count, respectively. Also, caffeine treatment reverted the lymphocyte cell count in $68.0 \pm 14.5\%$. DPCPX or alloxazine treatment did not cause any effect. Inosine also reduced the levels of IL-4 ($67 \pm 8\%$) and IL-5 ($55 \pm 8\%$) in explants, an effect reverted in 96.5 ± 3.4 and $78.0 \pm 8.4\%$ by pre-treatment with caffeine
Conclusions: Inosine causes a dose-dependent inhibition of cell migration in the BAL, an effect that might involve A_{2A} receptor, as demonstered by pre-treatment with ZM241385 and caffeine. Moreover, inosine effects can involve an inhibition on cytokine release that might be related to A_2 and/or A_3 adenosine receptors.
Disclosure of interest: None declared

OC-121
OPPOSITE ROLES OF BRADYKININ RECEPTORS IN BLEOMYCIN-INDUCED PULMONARY INFLAMMATION AND FIBROSIS IN MICE

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Bradykinin (BK) acts through B_1 and B_2 receptors. B_2R is constitutively expressed in healthy tissues and B_1R is induced upon inflammation. Bleomycin (BLEO) is an anticancer drug, which its main side effect is lung toxicity correlated with fibrosis. In mice, BLEO is used to study Pulmonary Fibrosis. Here we evaluated the role of BK receptors in lung inflammation and fibrosis. B_1R, B_2R and B_1B_2R deficient mice or C57Bl6/j (WT) were intra-tracheally instilled with (BLEO 3.75U/Kg). WT mice showed 30% of survival (d = 21) and 20% of weight loss. B_1RKO succumbed with 100% of mortality (d = 14), however B_2RKO mice were protected form lethality and weight loss. The B_1B_2RKO showed the same phenotype as WT mice. BLEO induced marked PMN influx into lung and airways with protein leakage (d = 7) in WT, B_1RKO and B_1B_2RKO, but not in B_2RKO mice. B_2RKO displayed reduced blood hematocrit than WT, B_1RKO and B_1B_2RKO mice. Lung levels of IL-1b, IL-6 and CXCL1 were increased in WT, B_1RKO and B_1RB_2RKO, but not in B_2RKO mice. However, TGF-b_1 was elevated only in WT and B_1B_2RKO, and B_2RKO mice showed increased IL-10 lung levels (d = 7). By real-time PCR no differences were found in the kinetic of B_1R expression between WT and B_2RKO mice. However, B_1RKO mice had increased B_2R expression than WT (d = 4.7). iNOS mRNA expression was progressively increased in B_1RKO compared to WT (d = 4.10), but it was blunted in B_2RKO mice. B_2RKO showed a reduced expression of eNOS, von Willebrand Factor, a-SMA and Col1a1 (d = 10) compared to WT or B_1RKO mice. WT and B_1B_2RKO presented increased lung hydoxyproline content, but not B_2RKO mice (d = 21). Histopathology confirmed the lung integrity of B_2RKO when compared to WT or B_1B_2RKO mice (d = 21). We showed that different BK receptors could establish the susceptibility to lethality induced by BLEO. We conclude that B_2R is more important for lung inflammation and fibrosis than B_1R, and B_2R inhibitors may be beneficial for Pulmonary Fibrosis treatment.

Support: CNPq and FAPEMIG.

Disclosure of interest: None Declared.

OC-122
METABOLIC CONDITIONING AND REPROGRAMMING ARE HALLMARKS OF NEUTROPHILIC INFLAMMATION IN CYSTIC FIBROSIS

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Background: Massive recruitment of neutrophils and extracellular release of their azurophil granules (AG) leads to chronic proteolytic and oxidative stresses in cystic fibrosis (CF) airways. Previously, we demonstrated that neutrophils are reprogrammed upon entry into CF airway (via cAMP and mTOR pathways), and then go to release AG actively. Better understanding of these reprogrammed neutrophils is essential to develop efficient treatments for fatal CF airway disease.
Methods: We used novel retroviral envelope probes for FACS analysis of surface metabolite transporters on CF airway neutrophils (glucose transporter Glut1, inorganic phosphate transporters Pit1 and Pit2, and neutral amino acid -AA- transporter ASCT2), as well as mass spectrometry to characterize AA metabolites (assumingly, derived from proteolysis) in CF airway fluid.
Results: We found increased expression of Glut1 (+81%) and PiT1 (+77%) on CF airway neutrophils, compared to blood neutrophils. Moreover, CF airway neutrophils with high AG release compared to those with low AG release (as identified in our previous studies), displayed major increases in Glut1 (+29%), PiT1 (+315%), ASCT2 (+145%) and PiT2 (+259%), consistent with the idea that AG release and metabolic reprogramming occur concomitantly. Next, we found that CF airway fluid is highly enriched in AA metabolites, starting in newborns and infants. CF airway fluid contained 300 + AA metabolites, with >95% of them increased tenfold or more compared to airway fluid from healthy controls. AA metabolites correlated highly with neutrophil count and anabolic AA, some of which are transported by ASCT2, were very high in CF airway fluid.
Conclusions: Our results suggest that neutrophils not only proteolyze the airway tissue and thereby condition the extracellular fluid towards high AA content, but that they also use these AA, and other nutrients, to drive their reprogramming. This new pathway may be amenable to therapeutic modulation.

Disclosure of interest: None declared.

Reactive oxygen species and proteases (OC12)

OC-123
NF-KB ACTIVITY IS REDUCED BY INOS-DERIVED NO IN HUMAN CHONDROCYTES: CONTRIBUTION TO SUSTAINED LOW GRADE INFLAMMATION IN OSTEOARTHRITIS

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NF-kB mediates important chondrocyte inflammatory and catabolic responses that ultimately lead to cartilage destruction, the hallmark of osteoarthritis (OA). This work aimed at identifying the role of the neuronal and inducible Nitric Oxide Synthases (nNOS and iNOS, respectively) as sources of NO in normal and OA human chondrocytes and at elucidating the role of endogenous iNOS-derived NO on the regulation of NF-kB activity. For this, we compared nNOS and iNOS protein levels by western blot and NF-kB activity by ELISA in normal and OA human chondrocytes and measured spontaneous NO production. Then, we evaluated the effect of inhibiting iNOS on the ability of interleukin-1b (IL-1) to induce NF-kB. Chondrocytes were isolated from human knee cartilage obtained from normal multi-organ donors or OA patients undergoing total knee replacement surgery. The results showed that nNOS was expressed in 83% of the normal and 65% the OA chondrocytes, whereas the opposite was found for iNOS (33 vs. 65%). The average nitrite concentration in the supernatants from normal or OA chondrocyte cultures that did not express iNOS was 3.6 ± 1.0 mM, whereas in cells expressing iNOS it was 9.4 ± 2.9 mM ($P < 0.01$). Although IkB-a was present in all the normal samples and in the majority (88.5%) of the OA samples, NF-kB activity in OA chondrocytes (1.82 ± 0.23) was approximately twofold higher than in normal ones (0.91 ± 0.05). Treatment with a general NOS inhibitor, after induction of iNOS expression, was sufficient to induce IkB-a

degradation and NF-kB-DNA binding and also potenciated IL-1-induced NF-kB activation. These findings favor an inhibitory role of iNOS-derived NO on the regulation of NF-kB activity in human chondrocytes. This seems to contribute to sustain a moderate level of NF-kB activity and NF-kB-dependent gene expression over time, thus perpetuating inflammation and cartilage destruction. Grants: PTDC/SAU-OSM/67936/2006 and PTDC/EME-PME/103578/2008
Disclosure of interest: None declared.

OC-124
MITOCHONDRIAL REACTIVE OXYGEN SPECIES PROMOTE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN THE TNFR1-ASSOCIATED PERIODIC FEVER SYNDROME

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Reactive oxygen species (ROS) have an established role in inflammation and host defense. However, how they contribute to the pathogenesis of autoinflammatory diseases is not clear. Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is a familial autosomal dominant periodic fever syndrome characterized by prolonged febrile episodes, serositis, dermal inflammation and an elevated risk of amyloidosis. Mutations in the extracellular domain of TNFR1 associated with TRAPS cause misfolding and accumulation of mutant TNFR1 protein in the endoplasmic reticulum. PBMC from TRAPS patients and myeloid cells from mice heterozygous for TRAPS-associated TNFR1 mutations exhibit enhanced basal MAPK activity and enhanced production of TNF, IL-1 and IL-6 in response to LPS that is dependent on p38 and JNK activity. Production of TNF and IL-6 in response to LPS is independent of the NALP3 inflammasome, IL-1 or Caspase-1. TNFR1 mutant heterozygous mice do not develop spontaneous fevers but are hypersensitive to LPS-induced endotoxic shock. We hypothesized that ROS might sustain MAPK activation and consequent inflammatory responses in cells from TRAPS patients. Indeed, we found that a variety of antioxidants could significantly reduce inflammatory cytokine production by cells from TRAPS patients and knock-in mice. Rather than NADPH oxidases, we find that mitochondrial respiration provides the ROS necessary for enhanced cytokine production in TRAPS. TNFR1 mutant cells exhibit enhanced mitochondrial oxidative capacity and ROS generation. Pharmacological agents reduce inflammatory cytokine production after LPS stimulation in both cells from TRAPS patients and healthy controls in proportion to their ability to block mitochondrial ROS production but not simply respiration. These findings suggest that mitochondrial ROS are required for optimal inflammatory responses and may be a novel therapeutic target in TRAPS and other autoinflammatory diseases.
Disclosure of interest: None declared.

OC-125
REACTIVE OXYGEN AND NITROGEN SPECIES ARE ESSENTIAL FOR HOST RESISTANCE TO DENGUE VIRUS PRIMARY INFECTION BY PEROXYNITRITE GENERATION

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Dengue is one of the most important mosquito-borne viral diseases. There are no treatments or vaccines available and mechanisms involved in host response to infection are poorly understood. However, there are several reports documenting the generation of free radicals after viral infections. Then, we evaluated the role played by reactive oxygen species (ROS) and nitric oxide (NO) generation during host response to primary DENV infection. To this end a mouse experimental model, utilizing a DENV-3 strain that causes a severe disease in mice was used. Wild-type (WT) infected mice showed increased levels of ROS, NO and peroxynitrite ($ONOO^-$) in spleen. Inhibition of NADPH oxidase by Apocynin (APO) treatment or infection of gp91phox$^{-/-}$ mice and inhibition of iNOS by infection of iNOS$^{-/-}$ mice resulted in reduced ROS and NO production followed by $ONOO^-$ decreased generation. These animals presented a more severe disease manifestation after DENV infection, showing increased thrombocytopenia, marked hemoconcentration, elevated plasmatic transaminases activity, enhanced neutrophil arrest and tissue injury in liver and elevated systemic production of TNF-a and IL-6. In addition, viral loads in spleen of all knockout and treated mice were markedly elevated and lethality rates after infection were strikingly higher. Of note, APO treated, gp91phox$^{-/-}$ and iNOS$^{-/-}$ mice showed reduced $ONOO^-$ production after DENV infection, when compared to WT-infected mice, suggesting this specie is of great importance during host response to DENV infection. Hence, FeTPPs (a Peroxynitrite scavenger) treated mice showed marked susceptibility to DENV-3 infection when compared with infected-WT mice. These data suggest that ROS and NO generation are essential for host ability to deal with DENV infection, in part through promotion $ONOO^-$ production and control of viral replication. Financial Support: INCT em Dengue, CAPES, CNPq and FAPEMIG
Disclosure of interest: None declared.

OC-126
SERINE PROTEASE INHIBITION REDUCES POST ISCHEMIC NEUTROPHILS RECRUITMENT IN A MODEL OF INTESTINAL ISCHEMIA/REPERFUSION INJURY IN MICE

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Increasing evidence showed that proteases and proteinase-activated receptor (PARs) activation are involved in several intestinal inflammatory conditions. We hypothesized that serine proteases could modulate the injury induced by acute mesenteric ischemia/reperfusion.
Methods: C57/Bl6 mice were subjected to 90 min of intestinal ischemia followed by 0 or 2 h of reperfusion (I/R). Mice were treated with FUT-175, a potent serine-protease inhibitor or with the PAR_1 antagonist SCH-79797. $PAR_2^{-/-}$ mice were subjected to I/R and compared to $PAR_2^{+/+}$. Serine-protease activity was determined in plasma and intestinal tissues. Myeloperoxidase (MPO) activity (an index of granulocyte recruitment), chemokine expression and western blot were performed on intestinal tissues.
Results: After the ischemic period, plasma and tissue serine protease activity levels were increased compared to the activity measures in tissues from sham, and this increase was maintained for 2 h after reperfusion. Trypsin 25 kDa band was detected in I/R tissue. Mouse treatment with FUT-175 increased survival after I/R, inhibited tissue protease activity and also significantly decreased MPO activity, KC and MCP-1 intestinal expression. We investigated whether serine proteases modulate granulocyte recruitment by

a mechanism dependent on PARs activation. MPO activity levels were significantly reduced in I/R group treated with the PAR1 antagonist SCH-79797 and in I/R $PAR_2^{-/-}$ compared respectively to vehicle group and $PAR_2^{+/+}$ mice.
Conclusion: This study showed that increased proteolytic activity and PAR activation play an important role in intestinal I/R injury. Inhibition of PAR-activating serine proteases could be beneficial to reduce post-ischemic tissue inflammation.
Disclosure of interest: None declared.

Young Investigator Award Winner.
For mini paper see page 289

OC-127
PROTEASE-ACTIVATED RECEPTOR 2 PROMOTES COLONIC HEALING AFTER DSS-INDUCED COLITIS BY ENHANCING EPITHELIAL CELL SURVIVAL

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Aims: Activation of protease-activated receptor 2 (PAR_2) has been shown to increase proliferation of colonic epithelial cells and inhibit apoptosis in airway cells, actions that are consistent with the healing of damaged tissue. We hypothesized that PAR_2 was important in resolving colitis by modulating the survival of colonic epithelial cells.
Methods: (1) Colitis was induced in wild-type (WT) and $PAR_2^{-/-}$ C57Bl/6 mice by the oral administration of 2.5% dextran sodium sulfate (DSS) for 1 wk. Mice were either killed at the end of DSS treatment or maintained on regular drinking water for 4 more weeks. Colonic MPO activity and levels of PGE_2 and PGD_2 were determined for mice killed at the end of DSS treatment. (2) HT-29 colonic epithelial cells were treated with PAR_2 activating peptide 2-furoyl-LIGRLO (2fLI; 5 μM) followed by IFNγ and TNFα to induce apoptosis. Western blotting for cleaved caspase-3 and PARP, as well as Annexin-V/PI flow cytometry, were used to quantify apoptosis.
Results: (1) $PAR_2^{-/-}$ mice lost weight earlier than WT after DSS treatment compared to controls and had significantly higher MPO levels than WT at the earlier time point. Colonic PGE_2 was increased in both strains after DSS treatment, while PGD_2 was only increased in WT. $PAR_2^{-/-}$ mice showed increased levels of cellular infiltration and loss of architecture at the later time point compared to WT. (2) Pretreatment with 2fLI, but not the reverse peptide, was able to significantly reduce levels of IFN-γ + TNF-α-induced apoptosis by all methods of detection. 2fLI decreased levels of cleaved caspase-8 and but did not change levels of Bax, Bcl-2 or Bcl-xL as determined by Western blot.
Conclusions: PAR_2 promotes the resolution of inflammation by stimulating the production of the pro-resolution eicosanoid PGD_2 and by reducing cytokine-induced epithelial apoptosis. Our findings may represent important physiological mechanisms whereby proteases facilitate colonic healing after inflammation.
Disclosure of interest: None declared.

OC-128
ORAL ADMINISTRATION OF RECOMBINANT LACTIC ACID BACTERIA DELIVERING ANTIPROTEASES AT THE MUCOSAL LEVEL PROTECTS MICE FROM COLITIS

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Imbalance between proteases and their endogenous inhibitors such as Elafin or Secretory Leukocyte Protease Inhibitor (SLPI) has been identified in colonic tissues from inflammatory bowel disease (IBD) patients. We tested here whether the administration of recombinant food-grade lactic acid bacteria (LAB) expressing these protease inhibitors could restore the proteolytic balance and thus decrease inflammation in a mouse model of IBD.
Methods: Colitis was induced in mice with DSS (5% in drinking water) for 7 days. During DSS exposure, mice were orally treated daily with either recombinant LAB (either *Lactococcus lactis* or *Lactobacillus casei*) expressing Elafin, SLPI or IL-10, or with wild-type (wt) LAB as a control. A *L. lactis* strain inactivated for the extracellular housekeeping protease HtrA was also used to express Elafin and tested in the same model, to potentially increase the levels and quality of secreted proteins and thus activity of recombinant Elafin. At day 7, colonic tissues were analyzed for macroscopic damage scores, cytokines secretion and myeloperoxydase activity. Proteolytic activity (trypsin-like and elastase) was also assessed in colon washes.
Results: Mice were significantly protected against colitis by recombinant LAB strains producing protease inhibitors, while wt LAB failed to modify any parameters of inflammation. All LAB strains producing Elafin and SLPI were more efficient at decreasing intestinal inflammation induced by DSS treatment than the ones producing IL-10. The protective effects of *L. lactis* expressing Elafin were enhanced when *htrA* gene was inactivated.

Conclusion: Oral treatments with recombinant LAB producing Elafin or SLPI restored the proteolytic balance and protected mice from colitis. Food-grade LAB could be used as carriers of beneficial proteins such as protease inhibitors (Elafin and SLPI) for their delivery in the gut. Such recombinant bacteria could be considered as effective treatments to reduce inflammation in IBD.
Disclosure of interest: None declared.

OC-129
APOPTOSIS-INDUCED PROTEINASE 3 MEMBRANE EXPRESSION IMPAIRED APOPTOTIC NEUTROPHIL CLEARANCE BY INTERFERING WITH THE CALRETICULIN "EAT-ME SIGNAL" AND TRIGGER PRO-INFLAMMATORY CYTOKINES RELEASE

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Background: Proteinase 3 (PR3) is a serine-proteinase contained in azurophilic granules. PR3 is the specific target of anti-neutrophil cytoplasm antibodies (ANCA) in Wegener's granulomatosis. When PR3 is expressed at neutrophil plasma membrane, ANCA can bind to their antigen and activate neutrophils thus amplifying inflammation. PR3 can be externalized during neutrophil apoptosis. Apoptosis-induced PR3 membrane expression interferes with macrophage phagocytosis.
Objectives: To investigate how PR3 could impair apoptotic neutrophils clearance.
Methods and results: Confocal microscopy analysis of neutrophils under basal or apoptotic conditions showed that PR3 colocalized with calreticulin (CRT) a protein involved in apoptotic cell recognition and macrophage phagocytosis. Co-immunoprecipitation demonstrated that PR3 was associated with CRT in neutrophils. Direct interaction was observed between PR3 and CRT globular domain by Surface Plasmon Resonance spectrometry. In order to understand the molecular mechanisms, we have used a model of stably PR3 transfected rat basophilic cell lines (RBL). Flow cytometry analysis showed that (1) PR3 and CRT were co-expressed at the plasma membrane during apoptosis but not after RBL-PR3 degranulation; (2) PR3 and CRT membrane expression were closely related with phosphatidylserine externalization. Phagocytosis assay and PR3 binding on macrophages allow us to determine that PR3 impaired apoptotic RBL phagocytosis by interacting with CD91 (CRT receptor on macrophages). Pro-inflammatory cytokines secretion by macrophages increased after apoptotic RBL expressing PR3 phagocytosis. In addition, PR3 impaired apoptotic RBL uptake by intra-peritoneal macrophages in vivo. Moreover, neutrophils from Wegener patients who expressed a high level of membrane PR3 are less eliminated by macrophages.
Conclusion: PR3 might interfere with the safe disposal of apoptotic neutrophils. We demonstrated that PR3 amplified inflammation through a novel mechanism.
Disclosure of interest: None declared.

OC-130
A NOVEL MUTATION OF PROTEASOME SUBUNIT CAUSES DECREASE OF PROTEASOME ACTIVITY IN NAKAJO-NISHIMURA SYNDROME (FAMILIAL JAPANESE FEVER)

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Nakajo-Nishimura syndrome (NNS) (MIM 256040) is a disorder that segregates as an autosomal recessive fashion and shows autoinflammatory symptoms including periodic fever, partial lipodystrophy, contracture of joints, skin rash and calcification of basal ganglia. We detected the gene locus using the SNP microarray-based homozygosity mapping following to determine the affected gene by direct sequencing. A mutation was found in a gene encoding the proteasome subunit in NNS patients. As predicted by structural modeling, fractioned analysis revealed assembly defect of proteasome and decrease of all 3 different peptidase activities (chymotrypsin-like, trypsin-like, and caspase-like activities). Accumulation of ubiquitinated proteins were detected in the cells and skin biopsy sections from patients with NNS by western blotting and immunohistochemistry. The productions of inflammatory cytokines were increased in cultured NNS cells compared with controls, as increased concentrations in sera from patients with NNS. Our findings reveal that decrease of proteasome activity is associated with a novel mutation of the proteasome subunit in NNS. The ubiquitin–proteasome pathway might play an important role for inflammation.
Disclosure of interest: None declared.

Yes, we have new opportunities for anti-inflammatory drug discovery (SY13)

OC-131
S100A9, A NEW POTENTIAL THERAPEUTIC TARGET IN CHRONIC INFLAMMATORY DISEASES

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The Ca^{2+}-binding proteins S100A9 and S100A8 are predominantly expressed by neutrophils and monocytes/macrophages. Once secreted, these proteins act as damage-associated molecular patterns (DAMPs) by triggering inflammatory responses. Indeed, previous studies showed that S100A9 induces leukocyte activation and promotes their migration toward inflammatory sites. Interestingly, high levels of S100A8 and S100A9 are found in the serums and at inflammatory sites of patients with chronic inflammatory diseases like rheumatoid arthritis, and these levels correlate with disease activity. In this study, neutralizing antibodies were used in the murine collagen-induced arthritis model to investigate the importance of S100A8 and S100A9 in rheumatoid arthritis. Anti-S100A8 or anti-S100A9 mAbs were injected twice a week starting 1 day before arthritis induction. Anti-S100A9 injections led to a 50% reduction of disease intensity while anti-S100A8 injection caused a worsening of the disease. The anti-S100A8-associated results are consistent with the

anti-inflammatory properties of S100A8 protein after its oxidation. Lower bone destruction was observed in anti-S100A9-treated animals compared to the isotype control group. Cytokine production was also decreased as exemplified by a marked reduction of IL-6 in serum of anti-S100A9-treated animals. Considering that human S100A9 induces the production and secretion of various cytokines like IL-6, MCP-1, MIP-1α, IL-1β, and TNF-α by human peripheral blood mononuclear cells, we suggest that S100A9 amplify the immune response through the production of pro-inflammatory soluble factors. Treatment with monoclonal anti-S100A9 may breach the amplification of the immune response by reducing pro-inflammatory cytokine secretion and leukocyte migration, leading to less tissue destruction. Our results show that S100A9 is a potential therapeutic target in inflammatory diseases unlike S100A8 which seems to be relevant in the resolution of inflammation.
Disclosure of interest: None declared.

OC-132
THE FIRST DEMONSTRATION OF CLINICAL ACTIVITY BY A SMALL MOLECULE SIRT1 ACTIVATOR: SRT2104 REDUCES CYTOKINE RELEASE AND COAGULATION ACTIVATION IN A HUMAN ENDOTOXEMIA MODEL

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Background and Aim: SRT2104 is a highly selective small molecule activator of the silent information regulator transcript (SIRT1) that has broad anti-inflammatory effects in cell cultures and rodents. The aim of the current study was to determine the effect of SRT2104 on the inflammatory and coagulation responses in normal healthy male subjects after exposure to LPS.
Methods: This double-blind, placebo-controlled study consisted of three treatment arms (N = 8 per arm): (1) Oral SRT2104 (2 g/day) for seven consecutive days; (2) placebo on days 1–6 and SRT2104 (2 g) on day 7; (3) placebo for seven consecutive days; on day 7, all subjects received intravenous LPS 3 h after dosing with SRT2104/placebo.
Results: SRT2104 significantly attenuated LPS-induced release of IL-6 ($p = 0.0777$ and 0.0318 for groups 1 and 2 vs. group 3, respectively) and IL-8 ($p = 0.0470$ and 0.0249, respectively). There was a trend toward lower TNFα and IL-10 levels. SRT2104 attenuated coagulation activation reflected by significantly lowered plasma levels of the prothrombin fragment F1 + 2 concentration ($p = 0.0384$ and 0.0368, respectively). SRT2104 did not impact LPS-induced changes in gene expression profiles of blood leukocytes as determined by whole genome arrays performed before and 4 h after LPS. CRP levels measured 24 h post-LPS were significantly lower in the SRT2104 group 2 versus placebo ($p = 0.0179$). Clinical signs associated with LPS tended to be reduced after SRT2104 administration. AEs were primarily those expected following LPS.
Conclusion: This is the first clinical study to demonstrate biological responses consistent with the activation of SIRT1 by a small molecule. SRT2104 was shown to significantly attenuate LPS-induced IL-6 and IL-8 release and activation of coagulation. The effects on coagulation are differentiated from those previously observed for steroid agents in a similar setting and suggest a unique potential as a therapeutic strategy for inflammatory disease.
Disclosure of interest: None declared.

OC-133
NEXT GENERATION THERAPEUTICS FOR THE TREATMENT OF RESPIRATORY DISEASES—DISCOVERY AND CHARACTERIZATION OF AN INHALABLE HIGHLY POTENT AND SPECIFIC ANTI-IL-4RA SMALL PROTEIN ANTAGONIST

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Anticalins are a new class of therapeutic proteins based on human lipocalins. We reasoned that given the diversity of compounds that these molecules can naturally bind, it should be possible to engineer and identify Anticalins with specificity for IL-4Ra, a receptor known to play a key role in allergy and asthma pathogenesis.
Methods and results: Phage display technology was used to isolate a human tear lipocalin-derived Anticalin to specifically recognize human IL-4Ra with low picomolar potency. The protein can be expressed at high yield using a scalable E.coli process and exhibits drug-like properties (Tm of 64°C + refolding after thermal denaturation) and resistance to multiple stress conditions. A dissociation constant of Kd = 14 pM was measured for the interaction with human IL-4Ra by surface plasmon resonance (SPR). The cross-reactivity profile, competitive antagonistic properties and potency towards both the IL-4Ra/gamma and the IL4Ra/IL13Ra receptor complexes were confirmed in FACS-based binding studies using transfected cells and multiple disease relevant cell-based assays. Furthermore, Anticalins can also be effectively delivered by inhalation to the lung, due in part to their relative small size of 17 kDa and robust biophysical properties, and exhibit an extended pulmonary half-life in rodents.
Conclusions: Our data describe the discovery and characterization of an inhalable IL-4Ra Anticalin (PRS-060), a potential therapeutic for the treatment of allergic diseases, including asthma. PRS-060 displays numerous differentiating features posited to be relevant for the proposed therapeutic concept, including, but not limited to inhibition of IL-4 and IL-13-dependent pathways with high potency, local administration and action in the lung via the inhaled route, favourable safety and tolerability (human scaffold, lack of immune effector cell interactions due to the absence of an Fc domain), robustness (dry powder formulation) and low production costs.
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Anticalin patents, M. Huelsmeyer Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, Patent licensing of: Inventor on several Anticalin patents, K. Kirchfeld Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, M. Kolodziejczyk Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, G. Matchiner Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, Patent licensing of: Inventor on several Anticalin patents, B. Rattenstetter Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, S. Trentmann Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG, Patent licensing of: Inventor on several Anticalin patents, L. Audoly Grant/Research Support from: BMBF, BiochancePlus, Employee of: Pieris AG.

POSTERS

Inflammatory cells: adhesion, migration, differentiation

P-001
UP-REGULATION OF IL-1R1 AND CHEMOKINE EXPRESSION IN HUMAN NEUTROPHILS FOLLOWING IN VIVO EXTRAVASATION AND IL-1 STIMULATION

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The aim was to scrutinize gene activation following neutrophil in vivo extravasation and IL-1 in vitro stimulation in healthy study subjects and to address the question if neutrophils render a potential to tune the composition of inflammatory mediators. In vivo extravasated neutrophils were collected by the skin chamber method and compared to circulating neutrophils following a density separation. Gene expression was assessed by gene array and confirmed by quantitative PCR. Protein expression of selected markers was assessed by flow cytometry, immunoelectron microscopy and ELISA. Expression of the IL-1 receptor type 1 (IL-1R1) increased following extravasation at both gene and protein level. Flow cytometry indicated an increased surface expression that was further induced following stimulation. Immunoelectron microscopy confirmed an increased pool of IL-1R1 both at the cell surface as well as intracellular, following extravasation. Furthermore, NFkB1 and several chemokines regulated by NFkB such as CCL3, CCL4, CCL20 and CXCL2 were up-regulated following extravasation, and the corresponding chemokines were locally produced at high concentrations at the inflammatory site. In addition, IL-1 in vitro activation of purified granulocytes induced an increased gene expression of NFkB, CCL3, CCL4, CCL20 and CXCL2, similar to gene activation following extravasation. Furthermore, a release of CCL3 and CCL4 was detected following 5 h of in vitro IL-1 stimulation. The present results indicate that neutrophils could tune the pro-inflammatory milieu following extravasation and IL-1 stimulation by the production of chemokines. This renders the neutrophil a potential to bridge the consecutive accumulation of mononuclear leukocytes.
Disclosure of interest: None declared.

P-002
ACTION ON P-SELECTIN AND ICAM-1 EXPRESSION CONTRIBUTE TO POTENTIAL ANTI-INFLAMMATORY PROPERTIES OF CROTOXIN, A TOXIN FROM RATTLESNAKE VENOM

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Crotoxin, the main toxin of *Crotalus durissus terrificus* rattlesnake venom presents long-lasting anti-inflammatory properties. A single dose of crotoxin inhibits paw edema, leukocyte–endothelium interaction and consequently cell migration induced by carrageenan. Leukocyte–endothelium interaction induced by carrageenan involves adhesion molecules expression, such as P-Selectin and ICAM-1. The aim of this study was to demonstrate histologically the inhibitory effect of crotoxin on cell migration to the subcutaneous tissue of footpads injected with carrageenan, and investigate the effect of this toxin on ICAM-1 and P-Selectin expression at this tissue. A single dose of crotoxin (0.89 μg/50 μL s.c.) or saline (control) was administered 1 h before carrageenan (300 μg/50 μL) or saline intraplantar injection in mice. For histological analysis, the footpad was removed 6 h after carrageenan. The tissue was processed and sections were stained with H/E. To evaluate P-Selectin and ICAM-1 expression, the footpad of the treated animals with crotoxin was removed 30 min or 1 h after carrageenan and analyzed by immunohistochemical. Tissues were incubated with antibody against these adhesion molecules and incubated with secondary antibody peroxidase conjugate. Histological analyses showed that the treatment with crotoxin (1 h) reduced edema and cellular infiltration when compared to control. The same treatment decreased the expression of P-Selectin and ICAM-1 when compared to control. These results suggest that the inhibitory effect of crotoxin on cell migration involves the action of this toxin on the expression of these adhesion molecules plays a key role in inflammation. These results contribute to characterization of anti-inflammatory properties of the crotoxin and reinforce that this toxin is a potential natural product in controlling inflammatory diseases.
Supported by: CAPES, FAPESP and INCCTOX.
Disclosure of interest: None declared.

P-003
AMBLYOMIN-X, A KUNITZ-TYPE SERINE PROTEASE INHIBITOR, IMPAIRS IN VIVO VEGF-INDUCED ANGIOGENESIS

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Introduction: The fundamental role of serine proteases and their inhibitors on pathophysiological processes has been evidenced. The mechanisms of action of Amblyomin-X, a Kunitz-type serine protease inhibitor designed from the cDNA library of the *Amblyomma cajennense* tick, were here characterized in the vascular endothelial growth factor (VEGF)-induced angiogenesis.
Methods: In vivo intravital microscopy assay was carried out in the dorsal skin chamber in male Swiss mice and optical microscopy evaluation was performed in the chick embryo chorioallantoic membrane (CAM). t-End cell lineage was simultaneously treated with amblyomin-X and VEGF and employed to quantify cell migration and tube formation by optical microscopy; cell adherence to matrigel by colorimetric assay; apoptosis, necrosis, proliferation, cell cycle and adhesion molecules expression by flow cytometry.
Results: Topical application of Amblyomin-X, simultaneously to VEGF, inhibited the VEGF-induced angiogenesis in the mice dorsal subcutaneous microvasculature and in the CAM. Treatment of t-End with Amblyomin-X delayed the cell cycle by maintaining them in G0/G1 phase; inhibited cell migration, adherence, proliferation, ICAM-1, VCAM-1 and PECAM-1 protein expressions.
Conclusion: Amblyomin-X blocks the in vivo VEGF-induced angiogenesis by affecting endothelial cells functions involved in different steps of the process. Besides contributing to the comprehension of pathophysiological mechanisms of Kunitz type-serine protease inhibitors, data herein have shown support further studies focusing on Amblyomin-X as a scientific tool to the design of therapeutic molecules.
Financial support:FAPESP (08-57850-8; 08/56072-1).
Disclosure of interest: None declared.

P-004
IN VITRO BLOOD IMMUNE CELL ADHERENCE TEST TO WOUND TISSUE REVEALS SUBSETS PARTICIPATING IN HEALING

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Circulating polymorphonuclear leucocytes are recruited in the wound by soluble mediators, causing cell adherence, transcapillary migration and chemotaxis. In a later stage T cells play a modulatory role in wound healing, although the full range of their effects is incompletely understood. There is no evidence that B lymphocytes play a significant role in wound healing. Aim. The question arises which and when immune cells and their specific subsets accumulate in the fast and delayed healing wounds. This applies particularly to lower limb non-healing ulcers. Methods. In order to define which blood immune cells reveal predilection for wound cells or matrix, an in vitro adherence test was worked out. Briefly, cryopreserved tissue sections were covered with blood leukocyte suspension at 4°C, incubated for 30 min, the non-adherent cells were washed out. This test allowed to show (a) adhesion to fibroblasts, keratinocytes, endothelial cells, matrix, (b) phenotypes of adhering cells stained with monoclonal antibodies. Venous ulcer edge biopsy specimens obtained from 15 patients and skin fragments of five healthy undergoing various vein surgery were studied. Results: Ulcers. Neutrophils adhered to granulation tissue around capillaries and to matrix, forming clusters. Few adhered to fibroblasts. They also stuck to epidermis. CD68+ve and elastase+ve monocytes were evenly distributed close to capillary lumen. Few scattered irregularly distributed CD3 T cells were seen on granulation tissue but none on epidermis. On normal skin section adherence of neutrophils, monocytes and lymphocytes was almost nil. Interestingly, all cell types strongly adhered to glass. Conclusions. Preponderance of neutrophils and monocytes over lymphocytes on granulation tissue sections suggests presence of a signal for their accumulation as in the scavenging phase of wound healing. The signal might be microbes and autoimmune tissue-antigen-specific cohorts of granulocytes and lymphocytes.
Disclosure of interest: None declared.

P-005
MICRORNA MIR-150 REGULATES NKT CELL DEVELOPMENT AND FUNCTION

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CD1d-restricted invariant natural killer T (*i*NKT) cells play an important role in the regulation of diverse immune responses, including allergy, autoimmunity, and cancer. MicroRNAs (miRNAs), a class of 21–25 nt single-stranded non-coding small RNAs, are increasingly being recognized as important regulators of gene expression through the inhibition of effective mRNA translation. We recently reported that miRNAs expressed in *i*NKT cell lineage are potent regulators of *i*NKT cell development, maturation, and function. However, the role of specific miRNAs in the *i*NKT cells has not been addressed yet. Using miRNA arrays, we found that the expression of miR-150 was significantly upregulated in mature *i*NKT cells compared to immature *i*NKT cells in the thymus. Interestingly, mice with miR-150 deficiency have a defect in *i*NKT cell development in the thymus and *i*NKT cell maturation was blocked at developmental stage 1 and 2, while the peripheral *i*NKT cells maturated normally. Furthermore, miR-150-deficient peripheral *i*NKT cells produce more cytokine interferon-gamma compared to that of wild type after PMA/ionomycin stimulation. Thus, our results suggest that miR-150 controls *i*NKT cell thymic maturation and regulates *i*NKT cell function.
Disclosure of interest: None declared.

P-006
EFFECTS OF METFORMIN ON ANGIOGENESIS ASSESSED AS PROLIFERATION AND MIGRATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Introduction: Most pharmacological effects of Metformin, the antidiabetic drug is mediated through activation of Adenosine Monophosphate-activated Protein Kinase (AMPK). AMPK is a key sensor of cellular AMP/ATP ratio. AMPK activation by Metformin has a number of other biological effects including inhibiting of mammalian Target of Rapamycin (mTOR) as a key signaling process in cells that regulates cell growth, cell proliferation, cell motility, protein

synthesis, and transcription. Recent epidemiological studies demonstrate that Metformin lowers the risk for several types of cancer in diabetic patients. Concerning the critical role of angiogenesis in the incidence and progression of tumors, we investigated the effect of Metformin on Human Umbilical Vein Endothelial Cells (HUVECs) proliferation and migration and its possible mechanism by inhibiting AMPK signaling using Compound C.
Method: We studied the effect of Metformin on morphology and viability of HUVECs using MTT and LDH assays, proliferation by MTT and cell numbering, and migration by wound repair method. Moreover, the effect of Metformin on VEGFR2 expression was evaluated by flow cytometry. Metformin at concentrations of 0.5–3 mM effectively reduced the number of endothelial cells by 5.5–55%, without being cytotoxic to these cells. Similarly, the cell proliferation and migration were markedly ($P < 0.001$) inhibited by Metformin. The inhibitory effects of Metformin on the endothelial cells number and migration were reversed partially by Compound C ($P < 0.01$). Interestingly, compound C (10 mM) alone showed a slight but significant inhibitory effect on the endothelial cells number (16%) and migration (16.5%). Metformin at the studied concentrations had no significant effect on VEGFR2 expression. This study indicates that Metformin exerts potent anti-angiogenic effects on HUVECs and AMPK activity, at least in part, is required for this effect.
Keywords: Metformin, Angiogenesis, Migration, Proliferation, Endothelial cells
Disclosure of interest: None declared.

P-007
HUMAN M-FICOLIN (FICOLIN-1) BINDS TO LEUKOSIALIN CD43 ON THE NEUTROPHIL SURFACE AND INDUCES NEUTROPHIL ACTIVATION.

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Ficolins are a family of pattern-recognition molecules, which interact with microorganisms surface and trigger complement activation. M-ficolin has been identified in lung epithelial cells, in monocytes and in easily mobilizable granules of neutrophils. A peculiarity of M-ficolin is its ability to bind sialylated ligands on autologous neutrophils or monocytes, but the physiological function of this binding is unknown. Our aim was to analyze the responses induced by M-ficolin interaction with neutrophils and to evaluate whether leukosialin (CD43), a membrane sialoglycoprotein related to cell adhesion, aggregation and migration, is a potential target receptor for the M-ficolin. We confirmed that biotinylated rM-ficolin binds to neutrophils via a calcium-dependent interaction inhibited by *N*-acetylglucosamine (GlcNAc), thus involving its capacity to bind acetylated ligands including sialic acid. Interestingly, binding of rM-ficolin inhibited neutrophils labeling with anti-CD43 mAb, but not with antibodies to another membrane glycoprotein, CD66. The effect of rM-ficolin on CD43 immunolabeling was abolished by GlcNAc but not by lactose, thus confirming the steric hindrance of CD43 by exogenous M-ficolin. Fluorescence microscopy showed an even distribution of biotinylated-rM-ficolin on resting neutrophil surface at 4°C but a polar redistribution at 37°C, similar to the antibody-induced capping of CD43. Exposure to recombinant M-ficolin triggered neutrophil functions, such as aggregation, adhesion and respiratory burst in a dose-dependent manner, effects already described for CD43 antibody cross-linking. The Y271F mutant of M-ficolin, unable to bind sialic acid, was unable to enhance neutrophil activation and did not prevent access of anti-CD43 mAb to its antigen. These results strongly suggest that CD43 is a receptor for M-ficolin and show that M-ficolin interaction with the cell surface, possibly via the cross-linking of CD43, induces potent neutrophil activation.
Disclosure of interest: None declared.

P-008
NEUTROPHILS AND COMPLEMENT ACTIVATE EACH OTHER'S. AN INFLAMMATION AMPLIFICATION LOOP FURTHER ENHANCED BY ANCA

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Knock-out mice from experimental inflammatory diseases shed new light on an important participation of the complement alternative pathway in neutrophil-mediated diseases such as rheumatoid arthritis, membranoproliferative glomerulonephritis or vasculitis with anti-neutrophil cytoplasmic autoantibodies (ANCA). We have recently described an activation of the complement alternative pathway by TNF-stimulated neutrophils (PMN). We now analyzed the consequences of this activation. PMN, stimulated or not with TNF, were incubated with normal human serum or sera immunodepleted in C5, C2 or factor B, to distinguish classical and alternative pathways involvement. PMN activation was assessed by flow cytometry measurement of CD11b expression or oxidative burst. Complement activation on the surface of PMN, stimulated by coagulation-derived products or by TNF, further activated PMN, enhancing the degranulation and oxidative burst. Complement-induced PMN activation was inhibited by a C5aR-antagonist and in C5-depleted serum, emphasizing the role of C5a and possibly C5b-9 complexes, detected on TNF and serum-activated PMN. Anti-MPO or –PR3 ANCA amplified complement activation exclusively on TNF-stimulated adherent PMN a condition known to allow the access of ANCAs to their antigens. This activation involved the classical pathway but amplification by the alternative pathway was required. Microparticles, released by PMN in vitro and highly present in plasma of acute vasculitis patients, efficiently activated the complement alternative pathway. In conclusion, PMN activate the complement alternative pathway, which, in turn, amplifies PMN pro-inflammatory responses, thus representing a new amplifying loop in the inflammation process. This mechanism probably explains the complement involvement in PMN-mediated diseases such as ANCA-associated vasculitis.
Disclosure of interest: None declared.

P-009
EXPRESSION OF ADHESION MOLECULES BY DIFFERENT METALLOPROTEASES ISOLATED BY BOTHROPS: ROLE OF DIFFERENT DOMAINS

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Snake venom metalloproteinases (SVMP) are classified as P1–P3, according to their domains composition and are involved in local inflammatory reactions observed in envenomed victims. Using three SVMPs, we evaluated the participation of different domains in alterations of the leukocyte–endothelial interactions induced by *Bothrops* venoms in the microcirculation of the cremaster of mice. We also evaluated, by immunofluorescence and ELISA, the expression of ICAM-1 (CD54) and PECAM-1 (CD31), molecules responsible by leukocytes adhesion and migration, respectively. The toxins used were: Jararhagin (JAR), a P3 SVMP with a strong hemorrhagic activity, which has catalytic, disintegrin-like, and cysteine-rich domains; JAR-C, a degraded form of JAR devoid only of the catalytic domain, with no hemorrhagic activity; and BnP1, a weakly hemorrhagic P1 SVMP, which has only the catalytic domain. Toxins (0.5 μg) or PBS (100 μL) were injected into the scrotal bag of mice. Adhered and emigrated leukocytes were counted in a post-capillary venule by intravital microscopy 2, 4 or 24 h after the injections. The number of adhered and emigrated cells increased in all times studied. Adhered cells diminished after 24 h of the toxins injection when compared to 2 h, but emigrated cells significantly increased in this period. There was a significant increase in the expression of CD54 after 2 and 4 h, followed by a decrease 24 h after the injection. We observed a time-related increase in expression of CD31 in endothelial cells during the period studied. The number of adhered and migrated cells was consistent with the expression of CD54 and CD31, respectively. Despite the differences in hemorrhagic activities and in domain compositions of the toxins used, all of them induced similar alterations in leukocyte–endothelial interactions. The results suggest that the alterations induced by these SVMPs in the microcirculation are associated to the expression of CD54 and CD31.

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P-010
TSPO LIGAND AGONIST ALTERS NEUTROPHIL ADHESION MOLECULE EXPRESSION DEPENDENT ON CALCIUM CHANNEL

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Introduction: Translocator protein (TSPO) is found in peripheral cells, as immune and endothelial cells, and may be related to the benzodiazepine actions on immune system. This worked investigated TSPO-binding drugs effects on leukocyte–endothelial interactions and on adhesion molecules expressions.

Methods: Intravital microscopy assays were performed in the mesentery microcirculation of male Wistar rats after topical application of TSPO ligands (PK 11195 or Ro 5-4864; 100 nM) and fMLP (10^{-8} M; topical application). In other set of assays, circulating leukocytes were in vitro incubated with TSPO ligands (100 nM) and 1 h after incubated with fMLP (10^{-8} M) to quantify adhesion molecules expressions by flow cytometry. Cells were also pre-treated with nifedipine (100 nM) to investigate the role of intracellular calcium on TSPO ligands effects on L-selectin expression. All procedures were performed according to protocols approved by the Brazilian Society of Science of Laboratory Animals for proper care and use of experimental animals.

Results: fMLP stimulation decreased rolling cells and increased adherent cells, and Ro5-4864 pre-treatment abolished these effects. fMLP in vitro stimulation reduced and enhanced L-selectin and β2-integrin expression on neutrophils, respectively, and Ro5-4864 treatment inhibited the reduction on L-selectin expression caused by fMLP. Pre-treatment with nifedipine reversed Ro5-4864 effects on L-selectin expression after fMLP stimulation.

Conclusions: Results herein presented show that TSPO ligand alters in vivo leukocyte–endothelial interactions evoked by fMLP. And more, Ro5-4864, but not PK11195, blocks the fMLP effect on neutrophil L-selectin expression depending on intracellular calcium levels.

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P-011
INVOLVEMENT OF TSPO AND CYTOSOLIC GLUCOCORTICOID RECEPTORS ON ADHESION MOLECULES EXPRESSION BY LEUKOCYTES

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Introduction: Translocator protein (TSPO) involvement on benzodiazepine's actions on immune system seems to be dependent on endogenous glucocorticoids actions. This work evaluated the effects of TSPO-binding agents and the participation of the cytosolic glucocorticoid receptor (CGR) on leukocyte–endothelial interactions.

Methods: RU 38486 (10 mg/kg; i.p.; 18 and 1 h before experiments; RU) or vehicle-treated (VT) adult male Wistar rats were anaesthetized and intravital microscopy assays were performed in the mesentery microcirculatory network after topical application of RPMI or TSPO ligands PK 11195 (100 nM) or Ro 5-4864 (100 nM). In other set of assays, circulating leukocytes were obtained from VT or RU-treated animals, and in vitro incubated with TSPO ligands (100 nM) to quantify adhesion molecules expressions by flow cytometry. All procedures were performed according to protocols approved by the Brazilian Society of Science of Laboratory Animals for proper care and use of experimental animals.

Results: RU treatment enhanced the number of rolling and adhered cells, and Ro5-4864 treatment reduced only the number of rolling cells. RU treatment also enhanced L-selectin and decreased β2-integrin expressions in circulating neutrophils, and only L-selectin enhancement was reversed by both TSPO ligands. RU treatment did not affect adhesion molecules expression on circulating lymphocytes, but additional incubation with in vitro Ro5-4864 decreased L-selectin and b2-integrin expressions.

Conclusions: TSPO ligands interfere with in vivo leukocyte–endothelium interactions evoked by RU treatment, which may be dependent on alterations on adhesion molecules expression. Therefore, an interaction of CGR and TSPO may be proposed on leukocyte–endothelial interactions.

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Disclosure of interest: None declared.

P-012
DNA METHYLOME AND TRANSCRIPTOME IN ANTIGEN-SPECIFIC MEMORY CD4 T CELLS

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The development of $CD4^+$ memory T lymphocytes during immune responses is not completely understood. In this study, to define the molecular signature of $CD4^+$ memory T cells, we investigated the gene expression profile and methylome of antigen specific memory $CD4^+$ T cells generated using T cells from DO11.10 OVA specific TCR transgenic mice. More than 12 million 5′-end tags were sequenced from naïve effector and memory T cell libraries with a next generation sequencer, corresponding to approximately 12,000–14,000 different protein-coding genes in this single cell type. Several novel up- and down-regulated genes were found in memory $CD4^+$ T cells, which are possible candidate genes related to development and maintenance of the memory phase. In addition, when the genome-wide DNA methylation profile was investigated in memory CD4 T cells, 466 DMRs (differentially methylated regions) in 438 genes were identified. Interestingly, most DMRs in naïve and memory T cells were demethylated during T cell differentiation. Moreover, the majority of the DMRs were localized to intronic sites and not to CpG islands. Among these DMRs, only 5% were correlated with expressed genes that related to "signal transduction", "cell communication" and "immune response". Moreover, these DMR were different between various T cell subsets, indicating that the methylation status of T cell subsets reflects T cell conditions. Our findings indicate that the genes associated with DMR are key genes related to memory phenotype that undergo variable changes in DNA methylation during $CD4^+$ T cell differentiation.
Disclosure of interest: None declared.

P-014
CONCANAVALIN A-INDUCED PAW EDEMA: THE CONTRIBUTION OF C-SENSORY NEURONS AND MAST CELLS

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The present study examined the diverse role of mediators from C sensory neurons and mast cells (MC) in paw inflammation induced by intraplantar (i.pl.) injection of Concanavalin A (Con A) in Dark Agouti (DA) male rats. Depletion of C sensory neurons was performed by neonatal administration of capsaicin, while systemic depletion of MC was performed by successive injections of increasing doses of compound 48/80 in adult rats. The result showed that neonatal depletion of C sensory neurons diminished Con A-induced increase in paw diameter and plasma extravasation, while MC depletion increased both parameters. Although opposing effects on paw diameter and plasma extravasation were recorded, both treatments similarly reduced the influx of immune cells to inflamed paws. However, both treatments led to the decrease in proportion of T cells and increase in proportion of resident macrophages in draining lymph nodes (DLN). According to results, neuropeptides secreted from activated C sensory neurons have pivotal role in acquiring local inflammatory response, influencing both vascular and cellular components. On the other hand, mediators released from MC are not indispensable for inflammatory edema formation, but are critical for cell recruitment to the inflammatory focus. Mediators secreted either from C sensory neurons or MC also affect microenvironment in DLN during Con A-induced inflammation.
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Disclosure of interest: None declared.

P-015
HEME INDUCES INFLAMMATORY RESPONSES IN ALVEOLAR MACROPHAGE: IMPLICATIONS FOR LUNG HEMORRHAGIC EPISODES

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Diverse evidence supports the hypothesis that in hemorrhagic and hemolytic episodes free heme might be responsible for the inflammatory response in the lung. Starting to the premise that the alveolar macrophages (AM) is the main defender of lung environment, we investigated the effects of free heme (ferriprotoporphyrin IX) in alveolar macrophage activation. Heme stimulation is able to increases AM responsiveness, enhances IL-1β, IL-6 and IL-10 release, induces HO-1 expression and stimulates ROS production by rat AM. In addition, heme stimulation induces $p47^{phox}$ phosphorylation, 5-LO and FLAP expression, as well as NF-kB nuclear translocation, suggesting that these proteins can be related to pro-inflammatory effects by heme in AM. All these events are directly involved in microbicidal functions of macrophages and prompted us to investigate the role of heme in these functions. In this study, we observed that heme induces phagocytosis and bacterial killing, key events responsible by pathogens removal. Taken together, these results suggest a role for heme as a proinflammatory agent able to induce antimicrobicidal activity in alveolar macrophage. The understanding of the effect of heme in pulmonary inflammatory processes can lead to the establishment of new strategies to ameliorate tissue damage associated with hemorrhagic episodes and severe hemolysis.
Disclosure of interest: None declared.

P-016
GALECTIN-9: SWEETENING LEUKOCYTE RECRUITMENT

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Galectins are structurally related proteins characterised by their carbohydrate recognition domains and an affinity for β-galactosides. Galectin-9 (Gal-9) was initially characterized as a potent eosinophil chemoattractant but has subsequently been found to have profound immunosuppressive effects in models of collagen-induced arthritis, acute graft versus host disease and carrageenan paw oedema. Its effects on neutrophil recruitment and behaviour during inflammation have not been thoroughly investigated. The objectives of the present study were therefore to investigate whether Gal-9 modulates neutrophil recruitment during the acute inflammatory response. To determine the effect of Gal-9 on leukocyte trafficking in vivo a model of zymosan peritonitis was performed. Mice were pre-injected i.p. with Gal-9 (1–30 μg) followed by 0.2 mg zymosan. Peritoneal lavages were collected and leukocyte influx assessed. To pinpoint whether Gal-9 affects a specific step of the leukocyte recruitment cascade flow chamber assays were performed. Freshly isolated neutrophils were incubated with Gal-9 (3-30 nM) and perfused over endothelial monolayers. The number of interacting neutrophils were quantified in six random fields. The effect of Gal-9 on neutrophil annexin V expression, ERK phosphorylation and adhesion molecule expression was also assessed. Pre-treatment with Gal-9 resulted in a significant increase in the numbers of neutrophils and monocyte/macrophages recruited into the peritoneal cavity. The flow chamber experiments identified a specific effect of Gal-9 on neutrophil transmigration with a concomitant reduction in neutrophil rolling, significant at 10 nM. In contrast to other galectins, Gal-9 was found to induce Annexin V exposure in the absence of other activating stimuli and also induced ERK phosphorylation. These data indicate a role for Gal-9 in neutrophil recruitment during acute inflammation.
Disclosure of interest: None declared.

P-018
MICROARRAY PROFILING OF MOUSE AND HUMAN MACROPHAGES

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Macrophage-CSF (M-CSF or CSF-1) and granulocyte macrophage-CSF (GM-CSF) can induce the survival, proliferation, activation and differentiation of monocyte/macrophage lineage cells. Murine bone marrow-derived macrophage populations are often used as models of human monocyte-derived populations; it is unknown how similar the various cell populations are when differentiated in the presence of M-CSF or GM-CSF. We have used microarray to determine how similar are the respective gene expression profiles of murine M-CSF-induced bone marrow-derived macrophages (BMM) and GM-CSF-induced BMM (GM.BMM), on the one hand, to the respective profiles of human M-CSF-induced monocyte-derived macrophages (MDM) and GM-CSF-induced MDM (GM.MDM), on the other. We found that there were 4,206 genes (20%) different between BMM and GM-BMM, but only 17% of these differentially expressed genes were identical across the species. By gene ontology (GO) classification, 34 and 41% of differentially expressed GO categories in the mouse and human gene lists, respectively, were common across the species; however, 84% of the most significant 50 differentially expressed GO categories were common. By molecular pathway analysis using the Pathway Interaction Database (http://pid.nci.nih.gov/), 57 (77%) NCI-Nature and 37 (82%) BioCarta differentially expressed pathways were common across the species. RT-PCR was used to confirm these patterns. In conclusion, BMM and GM-BMM would appear to be reasonable models for human monocyte-derived macrophage populations when relative gene expression in response to M-CSF and GM-CSF is analyzed at the "pathway" level rather than at the level of individual genes.
Disclosure of interest: None declared.

P-019
CHARACTERIZATION OF VISFATIN AS A NOVEL ATHEROGENIC TARGET: VISFATIN PROMOTES THE DEVELOPMENT OF ATHEROSCLEROSIS BY INDUCING SCAVENGER RECEPTOR EXPRESSION AND MACROPHAGE TRAPPING

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Objective: Visfatin is a novel proinflammatory adipokine secreted from adipocytes, which has been linked to atherosclerotic plaque formation, recently. However, controversial results exist about the role of visfatin in the pathogenesis of atherosclerosis. Therefore, we investigated whether visfatin could regulate monocyte/macrophage differentiation and expression of scavenger receptors (SRs), containing CD36, SR-A, and lectin-like oxidized LDL (oxLDL) receptor-1 (LOX-1), responsible for foam cell formation in macrophages, a critical step in atherogenesis.
Methods and Results: The THP-1 monocytic cells were differentiated into a macrophage-like phenotype by incubation with phorbol-12-myristate-13-acetate (PMA) in the presence or absence of visfatin. In this study, we show that visfatin enhanced the oxLDL uptake by inducing CD36 and LOX-1 expression, but not SR-A, and led to the production of inflammatory cytokines such as IL-1b, IL-6, and TNF-a. We also found that PMA-induced THP-1 cells became more adherent with a concomitant increase of ICAM-1 protein in the presence of visfatin. Moreover, immunofluorescence microscopy revealed that visfatin induces peripheral membrane ruffling, spreading and actin polymerization, suggesting that visfatin alters morphology and cytoskeletal organization. Visfatin-induced effects were involved with JNK pathway as determined by inhibition with JNK inhibitor, SP600125 and we also observed activation of JNK by visfatin.
Conclusions: Taken together, these data suggest that visfatin promote PMA-induced THP-1 cell differentiation into trapping macrophage phenotype and contribute to macrophage foam cell formation by increasing CD36 and LOX-1 expression and inflammatory cytokine production, consequently leading to the progression of atherosclerosis.
Disclosure of interest: None declared.

P-020
NICOTINAMIDE TREATMENT INHIBITS NEUTROPHIL ADHESION, ROLLING AND THE NEUTROPHIL MIGRATION IN THE PLEURAL CAVITY OF MICE

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There are evidences supporting anti-inflammatory properties of nicotinamide, a component of vitamin B_3. However, its role on the

regulation of the leukocyte migration in response to inflammatory stimuli has not been fully elucidated. We assessed the effects of nicotinamide on neutrophil recruitment in the pleural cavity of mice, and the microcirculation of the murine cremaster muscle using intravital microscopy. BALB/c mice were treated with nicotinamide per os (Nic, 1,000 mg kg^{-1}) 30 min before and 1 h after challenge with an intrapleural injection of carrageenan (Cg, 200 μg/cavity), zymosan (Zy, 200 μg/cavity), or leukotriene B_4 (LTB_4, 500 ng/cavity). Neutrophil migration was assessed 4 h after the challenge through pleural wash. To perform intravital microscopy studies, BALB/c mice were pretreated with Nic (1,000 mg kg^{-1}) 30 min before and 1 h after challenge with Cg (100 μg directly added to cremaster muscle). Rodamine 6G (0.1 mg/ml; i.v.) was injected prior to light fluorescence microscopy. The number of rolling and adherent leukocytes was determined during video analysis. Nic (1,000 mg kg^{-1}) inhibited the neutrophil recruitment induced by Cg (PBS, 1.9 ± 0.6; Cg, 27.1 ± 5.6; **Cg + Nic, 5.4 ± 0.6), Zy (PBS, 0.04 ± 0.01; Zy, 113.7 ± 22.9; ***Zy + Nic, 0.18 ± 2.7), or LTB_4 (PBS, 0.08 ± 0.02; LTB_4, 6.4 ± 1.5; ***Nic + LTB_4, 0.8 ± 0.2). Moreover, Nic also inhibited the rolling of neutrophils (PBS, 69.0 ± 17.1; Cg, 137.5 ± 6.1; *Cg + Nic, 36.2 ± 7.3 cells/min) and neutrophil adhesion (PBS, 5.6 ± 0.6; Cg, 29.5 ± 4.4; *Cg + Nic, 2.7 ± 0.2 cells/100 μm). Values are expressed as mean ± SEM $\times 10^3$ neutrophils/cavity. ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ANOVA followed by Bonferroni's post test). Nic was able to inhibit the neutrophil migration in response to different inflammatory stimuli, in addition to its ability to reduce neutrophil rolling and adhesion. Thus, Nic could be useful since its parameters of biosecurity and pharmacokinetics have already been defined.
Disclosure of interest: None declared.

P-021
EGFR MEDIATED INFLAMMATION AND PROLIFERATION IN LUNG CELL (A549)

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Exposure to cadmium is associated with the development of pulmonary damage such as emphysema and lung cancer. This metal is also a powerful inducer of different proinflammatory and cell cycle regulatory proteins in many biologic models. Previously we showed that prolonged exposure of low concentration of cadmium resulted in up regulation of proinflammatory cytokines and cell cycle regulatory molecules in mice lung cell. The present study was undertaken to determine molecular mechanism of inflammation and its relation to cell proliferation in a transformed human lung adenocarcinoma epithelial cell line (A549) in response to cadmium chloride. In comparative studies, we examine that short-duration exposure to lower doses of cadmium significantly increase the growth of A549 cells, whereas, higher doses are toxic and cause cell death. We also observed that cadmium induced elevated expression of epidermal growth factor receptor (EGFR) along with different proinflammatory cytokines like IL-1β, TNF-α, IL-6. The possible occurrence of cell proliferation events was evaluated via analysis of the physical state of the DNA and the expression of Ki67 and PCNA. We also checked the pattern of expression of different cell cycle regulatory molecules involved in the onset of cell proliferation. Our results indicate that cadmium treatment appears to induce inflammatory and growth responses in transformed A549 cell line by activating epidermal growth factor receptor and its downstream modulators. These results may contribute to better understand the toxic mechanism of cadmium moreover; the expression profile of cadmium induced regulatory molecules could provide potential biomarkers for cadmium exposure.
Disclosure of interest: None declared.

P-022
INDUCTION OF TRANSDIFFERENTIATION OF HUMAN KIDNEY EPITHELIAL CELLS WITH REACTIVE OXYGEN SPECIES

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Chronic allograft nephropathy (CAN) is a major obstacle to the establishment of survival of the transplanted kidney. The trait most associated with the decline of renal function in this pathology is the interstitial tubule fibrosis (FTI) and tubular atrophy (TA). Recent studies suggest that certain interstitial myofibroblasts derive from renal tubular cells that have undergone the process of epithelial-mesenchymal transdifferentiation (EMT). In this study, the objective was to induce the transdifferentiation of epithelial cells derived from human proximal tubular epithelial (HK-2), using as inducer hydrogen peroxide. Methods: To this end, epithelial cells were incubated with Keratinocyte-SFM medium (GIBCO) with 5% fetal calf serum (GIBCO), deprived of serum during the experiment. Cells were treated with concentrations of H_2O_2 (0.10, 0.25, 0.50, 1.0 and 2.0 mM/mL) and evaluated in the time of incubation for 24, 48 and 72 h for induction of EMT by oxidative stress. TGF-b was used in concentration (10 ng/mL) as positive control. Cells without treatment were used with negative control. Evidence was given by EMT microscopy, cell viability, cell motility and immunocytochemistry of molecules related to the process, such as cytokeratin, E-cadherin, vimentin and α-SMA. Results: We observed the cell viability assay that the better dose to sublethal condition was 0.25 mM/mL at 24, 48 and 72 h in relationship to control, but when tested with cell motility, the doses of 0.25 and 0.50 mM H_2O_2, when done for 48 and 72 h, provided the best results of migration, supported by immunocytochemistry analysis, in which cells are subjected to oxidative stress by altering the expression of surface proteins of epithelial cells begin to express proteins mesenchymal cells.
Conclusion: This study provides that H_2O_2 can be used to induce transdifferentiation of epithelial cell in the dose of 0.50 mM in the time of 72 h.
Disclosure of interest: None declared.

P-023
STRUCTURAL REQUIREMENTS FOR REGULATION OF MACROPHAGE AND OSTEOCLAST DEVELOPMENT AND ACTIVATION BY PSTPIP2

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Studies from our laboratory have identified the *m*acrophage F-*a*ctin-associated and tyrosine phosphorylated *p*rotein (MAYP), also known

as *p*roline, *s*erine, *t*hreonine, *p*hosphatase-*i*nteracting *p*rotein *2* (PSTPIP2), as a regulator of actin bundling, filopodia formation and directed motility in macrophages (Chitu et al. 2005). Two missense mutations, I282N(*Lupo*) and L98P(*cmo*), in the MAYP/PSTPIP2 gene lead to autoinflammatory disease in mice characterized by skin inflammation, cartilage and bone destruction (Grosse et al. 2006; Chitu et al. 2009). This phenotype closely resembles human chronic recurrent multifocal osteomyelitis (Ferguson et al. 2006). PSTPIP2 is expressed predominantly in macrophages and osteoclasts (Chitu and Stanley 2007) and regulates their differentiation and activation in vitro and in vivo. Absence of PSTPIP2 in *cmo* mice leads to the expansion of myeloid progenitors thus priming macrophage and osteoclast differentiation. In addition, compared to wt macrophages, *cmo* macrophages produce more osteoclastogenic cytokines in response to LPS challenge. Structure–function studies reveal that interactions of PSTPIP2 with PTP-PEST and membrane phospholipids are critical for its ability to control the development and activation of macrophages and osteoclasts.
Disclosure of interest: None declared.

P-024
THE CRITICAL REQUIREMENT OF TLR/MYD88 SIGNALING IN THE ANTI-INFLAMMATORY ACTIVITY OF NATTERINS

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Introduction: TLRs expressed in endothelial cells and leukocytes are pattern recognition receptors that detect invading microorganisms and sterile molecules to trigger immune and inflammatory responses. TLR activity is closely linked to the risk of many inflammatory diseases and immune disorders. Combined proteomic and transcriptomic approaches applied to analyze *Thalassophryne nattereri* venom complexity revealed the identity of the major toxins as a family of new proteins displaying kininogenase activity, the Natterins.
Objective: In this study, we investigated the modulation of TLR activity by Natterins and the underlying mechanism.
Methods and results: Interaction of leukocytes with cremaster microvessels was monitored by using an intravital microscope in Swiss, C57BL/6 (WT) and TLR2 *KO* and MyD88 *KO* and C3H/HePas (WT) and C3H/HeJ (*TLR4* mutant) mice. LPS (0.02 μg/mL), KC (0.01 μg/mL) or PAR4 agonist (0.02 μg/mL) in 20 μL were administered topically in cremaster muscle after 6 h of treatment with Natterins (0.02 μg/mL) injected by intraescrotal route. When rolling behavior was determined after topical application of LPS or KC in mice pre-treated with Natterins, the proportions of rolling leukocytes in venules were almost the same in control-mice. In contrast, no inhibitory effect was observed in Natterins-treated mice challenged with topical application of PAR4 agonist. The anti-inflammatory effect of Natterins was not modified by metyrapone or ZnPP IX. Finally, the rolling of leukocytes induced by LPS or KC was not impaired in Natterins-treated TLR2 *KO*, MyD88 *KO* or TLR4 mutant mice, indicating that TLR2- or TLR4-MyD88-mediated signals are required for the anti-inflammatory effect induced by Natterins.
Conclusion: These data provide a molecular level explanation for the anti-inflammatory function of Natterins in LPS- or KC-induced inflammation that is dependent on TLR2- and TLR4-MyD88 signaling.
Support: FAPESP and CNPq.
Disclosure of interest: None declared.

P-025
ANGIOMOTIN EXPRESSION INCREASES DURING ALKALI INDUCED INFLAMMATORY CORNEAL NEOVASCULARIZATION IN MICE

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Corneal neovascularization is a side effect of numerous inflammatory and non-inflammatory ocular surface disorders. During corneal injury, angiogenic factors are released from both corneal cells and infiltrating inflammatory immune cells. The angiogenic factors induce endothelial cells proliferation, migration, and tubulogenesis. Angiomotin, an angiostatin binding protein, is involved in endothelial migration during angiogenesis. Angiomotin protein is a key factor for endothelial migration. Therefore, blockade of Angiomotin function will leads to inhibition of angiogenesis. In this study we evaluated the expression of Angiomotin in alkali induced inflammatory corneal neovascularization in mice. A total of 20 BALB/c mice divided into two groups for this study. First, animals were anesthetized by administration of ketamine and xylazine combination intraperitoneally. Further topical anesthesia induced by 0.5% proparacaine hydrochloride on animals right cornea. Alkali burn corneal neovascularization induced by a silver nitrate cauterization technique. Mice were sacrificed 3 and 6 days post injury and eyes were enucleated for analysis. Corneas were removed and RNA was extracted using TRIzol reagent. The first-strand cDNA was synthesized from 1 μg of total RNA. Real-time PCR was performed using TaqMan gene expression master mix and specific primers and probes of Beta-actin and Angiomotin genes. The results showed that Angiomotin expression increases during corneal neovascularization. Angiomotin expression increased in both groups of animals and its expression was higher in day 6 in comparison to day 3. The results of this study confirm the over-expression of Angiomotin during inflammatory induced corneal neovascularization. Based on these results, we here propose this idea that Angiomotin can be a new target for designing new therapeutic option for corneal neovascularization.
Disclosure of interest: None declared.

P-026
REGULATION OF NEUTROPHIL FUNCTION BY NAMPT DURING INFLAMMATION

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Neutrophil apoptosis is required for the effective resolution of inflammation, and defects in the regulation of this process are implicated in inflammatory disease. Nampt [nicotinamide phosphoribosyltransferase, pre-B-cell colony-enhancing factor (PBEF) or visfatin] is reported to regulate neutrophil apoptosis during inflammatory diseases such as rheumatoid arthritis. However, Nampt also functions in the regulation of NAD metabolism. In view of the importance of NAD in the control of neutrophil activities, such as the action of the NADPH oxidase, we investigated the role of Nampt in neutrophil apoptosis and the control of functions such as the respiratory burst.

Exogenously added Nampt (100 ng/mL) delayed both constitutive neutrophil apoptosis, and the turnover of the anti-apoptotic protein Mcl-1. However, it did not have any significant effect on functions such as chemotaxis and the respiratory burst. Inhibition of the enzymatic activity of Nampt (using the inhibitor FK866/APO866 at concentrations from 1 to 100 nM) inhibited the activity of the respiratory burst in a time and dose-dependent manner. Inhibition of Nampt also significantly inhibited transcription of inflammatory cytokines such as TNFα. These data confirm the importance of Nampt in regulating neutrophil apoptosis, but reveal a new function for Nampt in controlling functions that would contribute to the inflammatory process. Therapeutic inhibition of Nampt in inflammatory diseases would be predicted to decrease the release of tissue-damaging reactive oxygen species and the release of pro-inflammatory cytokines from activated neutrophils.
Disclosure of interest: None declared.

P-027
THE PEPTIDE FRACTION ISOLATED FROM BOTHROPS JARARACA SNAKE VENOM INDUCES LUNG LEUKOCYTE INFLUX

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Bothrops jararaca snake venom (BjV) is a complex mixture of biologically active proteins, such as proteinases, phospholipase A2, cysteine-rich secretory protein, lectin-like protein, C-type lectin and L-amino acid oxidase. Besides them, it have been identified a variety of pharmacologically active peptides in the low molecular weight fraction (Bj-LMWF) from BjV, such as Bradykinin-potentiating peptides, natriuretics peptides and disintegrins. It has been shown that BjV induces a significant leukocytes accumulation, manly neutrophils, at the local tissue damage. In the present study we investigate the contribution of Bj-LMWF to induce lung leukocyte influx after gastrocnemius intramuscular injection. The Bj-LMWF was obtained through a Millipore centrifuge filter device with a molecular weight cut-off 10KDa. To confirm the absence of proteolytic enzymes or others proteins (>10 kDa) of the venom, the filtrate contained the LMWF was analysed by SDS-PAGE silver stained, gelatinolytic activity and MALDI-TOF mass spectrometry. The lung leukocyte influx was induced in male Swiss mice by injection of BjV (1.2 mg/kg) or Bj-LMWF (0.24 mg/kg), in gastrocnemius muscle. Cell influx was evaluated by bronchoalveolar lavage at 15 min, 1, 6 and 24 h after venom or Bj-LMWF injection. Results showed that BjV and Bj-LMWF evoked a significant cell accumulation in the lung from 6 up to 24 h following the injection. Differential cell counts from BjV mice demonstrated that neutrophils were the predominant cells accumulating at 6 h and mononuclear cells at 24 h. Bj-LMWF significantly increased the number of neutrophils at 6 h as well as mononuclear cells at 6 and 24 h. In conclusion, both BjV and Bj-LMWF have a similar activity as pro-inflammatory response to lung leukocyte migration. The fact that Bj-LMWF induces inflammation suggests that non-enzymatic peptides elicit inflammatory events perhaps by interacting with specific cell membrane acceptors.
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Disclosure of interest: None declared.

P-028
BOTHROPS JARARACA HIGH MOLECULAR MASS KININOGEN (BJHK) INHIBITS ALTERATIONS OF LEUKOCYTE–ENDOTHELIAL INTERACTIONS INDUCED BY B. JARARACA SNAKE VENOM METALLOPROTEINASES

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Severe local inflammatory symptoms are common in envenoming induced by *Bothrops jararaca* (Bj) snakebites. It is known that metalloproteases is the main class of toxins involved in the inflammatory activity of this venom. A protein related to mammals high molecular mass kininogen (HK), purified from the plasma of Bj snakes was tested on inflammatory activity of Bj venom in vivo, using intravital microscopy. Bj venom (1 μg) or Jararhagin (JAR) (0.5 μg), a hemorrhagic metalloproteinase isolated from Bj venom, incubated or not with BjHK (2 μg) were injected (100 μL) into the scrotal bag of mice. The microcirculation of cremaster muscle was analyzed 2 or 24 h after the injections. We analyzed a segment of a post-capillary venule during 5 min and counted adhered and emigrated leukocytes, comparing with the observed in control group injected with sterile buffered saline. Results showed that, in groups injected with Bj venom or JAR, adhered and emigrated leukocytes were significantly increased in all times studied. Adhered cells were diminished after 24 h of the toxins injection, when compared to 2 h, but emigrated cells were significantly increased. When incubated with BjHK, the Bj venom or JAR did not induce alterations of leukocyte–endothelial interactions in the microcirculation. This inhibition was similar to the observed when Bj venom and JAR were treated with *o*-phenanthroline, despite of the mechanism of action of BjHK on the catalytic activity of JAR not be like a chelating agent. We can conclude that BjHK inhibits the inflammatory activity of the Bj venom by inhibiting the activity of their metalloproteinases.
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Disclosure of interest: None declared.

P-029
INHIBITORY EFFECT OF CROTALUS DURISSUS TERRIFICUS (RATTLESNAKE) VENOM ON THE MULTINUCLEATE GIANT CELL FORMATION BY IMPAIRMENT IN ACTIN DYNAMICS

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In human envenoming by South American rattlesnakes there are no inflammatory reactions at the site of the bite. Experimentally, the venom of the *Crotalus durissus terrificus* (CdtV) shows a significant anti-inflammatory action, inhibiting the acute inflammatory reaction induced by thioglycolate or carrageenan. Still, the *Cdt*V alters some functions of macrophages, cells that in chronic inflammatory processes are fused to form multinucleated giant cells (MGC). This process depends on the participation of actin filaments (F-actin). Our objectives were to evaluate the effect of the *Cdt*V on the dynamic of

F-actin rearrangement on the formation of MGC in mice subjected to a chronic inflammatory stimulus and to assess the fraction of the venom responsible for the inhibitory effect on the formation of MGC. After an ion exchange chromatography of *Cdt*V, three different fractions are obtained: PI, PII (corresponding to the pure crotoxin-CTX, the main toxin of this venom) and PIII. We implanted round glass cover slips on the s.c of mice pretreated with a non-toxic doses *Cdt*V or fractions. After 4, 7, 14 and 21 days of the implantation, cover slips were removed, stained with hematoxylin/eosin in order to count the formed MGC or processed for immunohistochemical identification of F-actin. The formation of MGC was significantly inhibited in cover slips removed after 4, 7 and 14 days of the group treated with the crude venom as well as in the group treated with crotoxin, when compared to saline treated groups. The immunostainings for F-actin were also significantly inhibited in all times studied when compared to control groups. The inhibition of F-actin expression was positively correlated with the inhibition of the giant cells formation observed previously in cover slips implanted in the same period and stained with h/e. The compiled data shows a significant inhibitory action of the *Cdt*V on the progression of the chronic inflammatory response.
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Disclosure of interest: None declared.

P-030
MODULATION OF NEUTROPHIL FUNCTIONS BY EXTRACELLULAR GALECTIN-3

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Galectin-3, an endogenous beta-galactoside-binding lectin with both intracellular and extracellular localizations, is expressed and secreted by various inflammatory cells. In vivo experiments with galectin-$3^{-/-}$ mice emphasized the critical role of this lectin in inflammatory responses but did not allow distinguishing extracellular and intracellular effects. Modulations of polymorphonuclear neutrophils (PMN) responses by galectin-3 have been described, but with contradictory results regarding the need of neutrophil priming.
Objective: The aim of this study was to analyze galectin-3 extracellular effects on human PMN functions.
Results: Although galectin- 3 is highly expressed intracellularly in neutrophils, as shown by western blot analysis of cell lysates, we could not detect galectin-3 on the surface of resting or activated neutrophils. Exogenous recombinant galectin-3 was able to bind to PMN via its lectin site (inhibited by lactose) and this binding was significantly increased (60%) when PMN were activated with fMLP. A homogeneous binding of biotinylated galectin 3 was observed by immunofluorescence on PMN at 4°C. Transfer to 37°C resulted in a capping of cell-bound galectin 3, which was co-localized with a known galectin 3 ligand, CD66 and with another major membrane glycoprotein, leukosialin CD43, but not with the CD11b/CD18 integrin. Galectin-3 (0.8 mM) triggered PMN degranulation, as shown by CD11b upregulation. Moreover, it was able to further enhance the CD11b up-regulation triggered by suboptimal TNF-α concentrations (1 ng/ml). Finally, galectin-3 induced the adhesion of PMN to gelatin-coated plates in a dose-dependent manner. Gal 3 preincubation with polymyxin B did not modify its ability to activate neutrophils, excluding an effect of contaminating LPS.
Conclusion: These results show that exogenous galectin-3 activates both naive and primed PMN, possibly via an interaction with CD66 and CD43, thus confirming its pro-inflammatory function.
Disclosure of interest: None declared.

P-031
ANTI-INFLAMMATORY ACTION OF ETHANOL EXTRACT AND ITS SUBSTANCES OBTAINED OF LEAVES FROM AN ANACARDIACEAE FAMILY SPECIE

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Plants from Anacardiaceae family occur from Brazil. Its leaves and stem bark are used in folk medicine against general inflammations. The aim of this study was to evaluate the anti-inflammatory action of the ethanol extract and substances obtained from Anacardiaceae leaves (EFl). Pleurisy was induced by an intra-thoracic injection of zymosan (Zym 100 μg/cav.) or carrageen (Car 300 μg/cav.) in mice oral pre-treated with EFl (12.5–400 mg/kg), and purified substances: gallate acid (GA), methyl gallate (Mg) and penta-galloylglucose (Pg). Pleural wash was used to evaluate leukocyte migration, protein extravasation, cytokines KC and IL-6 production and LTB4. Mice received an intra-articular (i.a.) injection of Zym. (500 mg/cav.) and the knee-joint swelling was evaluated by measurement of left knee joints using a digital caliper. Knee synovial cavities were washed to analyze leukocyte migration. The experiments were realized under approval of Committee on Ethical Use of Laboratory Animals of Fundação Oswaldo Cruz (licence n. L0052/08. The statistically analyzed was realized by one-way ANOVA and Student–Newman–Keuls post-test ($p < 0.05$). The pre-treatment with EFl reduced in dose–response way the total leukocyte influx (100–400 mg/kg) in pleurisy induced by Zym. The pre-treatment with EFl was also able to inhibit the neutrophil influx and protein extravasation in the pleurisy induced by Car. The EFl, GA, Mg and Pg showed an anti-inflammatory effect on pleurisy induced by Zym reducing the LTB4, KC and IL-6 production. EFl also inhibited neutrophil influx and the oedema in Zym. induced arthritis. The results describe the anti-inflammatory activity to EFl suggesting a putative new phytomedicine.
Disclosure of interest: None declared.

P-032
PROTEINASE-ACTIVATED RECEPTOR (PAR)-4 PLAYS AN IMPORTANT ROLE ON THE CARRAGEENAN-INDUCED NEUTROPHIL RECRUITMENT INTO THE PLEURAL CAVITY OF MICE

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Although proteinase-activated receptor (PAR)-4 has been implicated in inflammation, it's role in regulating the neutrophil recruitment in response to chemoattractants has not been demonstrated. To investigate the contribution of serine-proteinases and PAR-4 activation to the neutrophil migration in response to carrageenan or trypsin, we examined the effects of aprotinin or PAR-4 antagonist tcY-NH_2 on the neutrophil migration induced by these chemoattractants. BALB/c mice were treated with intrapleural (i.pl.) injections of trypsin (Tryp, 0.1–10 μg), and the number of infiltrating neutrophils evaluated 4 h after trypsin injection through pleural wash. Mice were also treated with aprotinin i.pl. (Aprot, 0.01–0.1 μg) or tcY-NH_2 (0.1–10 ng) 1 h before the i.pl. injection of carrageenan (Cg, 30 μg) or trypsin (10 μg). Neutrophil migration was assessed 4 h after the challenge through pleural wash. Trypsin induced a dose-dependent neutrophil recruitment (PBS, 0.02 ± 0.01; Tryp 0.1 μg, 0.06 ± 0.01; Tryp 1.0 μg, 0.3 ± 0.05; **Tryp 10 μg, $1.2 \pm 0.4 \times 10^4$ neutrophils/cavity). This recruitment was inhibited by tcY-NH_2 pretreatment (PBS, 3.0 ± 0.2; Tryp, 11.2 ± 0.3; ***tcY-NH_2 + Tryp, $4.6 \pm 0.7 \times 10^4$ neutrophils/cavity). tcY-NH_2 or Aprotinin treatment inhibited the neutrophil recruitment induced by Cg in a dose-dependent manner (PBS, 1.6 ± 0.2; Cg, 7.9 ± 0.4; ***tcY-NH2 0,1 μg + Cg, 2.6 ± 0.4, ***tcY-NH_2 0.3 μg + Cg, 4.2 ± 0.3, ***tcY-NH_2 1 μg/cavity + Cg. $1.9 \pm 0.3.10^4$ neutrophils/cavity), (PBS + PBS, 1.9 ± 0.3; PBS + Cg, 11.2 ± 1.1; *Aprot 0.01 μg/cavity + Cg, 7.6 ± 1.0, *Aprot 0.03 μg/cavity + Cg, 8.3 ± 0.9, ***Aprot 0.1 μg/cavity + Cg, $5.2 \pm 0.8 \times 10^4$ neutrophils/cavity). ANOVA followed by Tukey-test, ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). These data demonstrate that trypsin is an important component to mediating the neutrophil migration in carrageenan-induced pleurisy, and that the activation of PAR-4 plays an important role in regulating this migration.

Disclosure of interest: None declared.

P-033
FUNCTIONAL IMPLICATION OF THE LNK ADAPTOR PROTEIN IN STEM CELL FACTOR-DEPENDENT MAST CELLS MIGRATION

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The Lnk adaptor protein is mainly expressed in the haematopoietic system. It possesses a potential dimerization (DD), a PH and SH2 functional domains and a conserved C-terminal tyrosine phosphorylation site that allow its interaction with different signalling effectors. Mice deficient for this protein have demonstrated its role as a negative regulator of signalling pathways controlling the proliferation of haematopoietic stem cells (HSC), myeloid and B-lymphoid progenitors. This phenotype is partly due to hypersensibility to several cytokines and growth factors, notably Stem Cell Factor (SCF). Our previous studies have implicated for the first time Lnk as an important inhibitor of SCF-dependent migration of primary mast cells. However, the molecular mechanism underlying this regulation has not been identified. To analyze the functional contribution of the different Lnk domains to Kit–dependent cell migration, we used *Lnk*-deficient bone marrow-derived mast cells (BMMCs) expressing wild-type or Lnk mutated forms as our cellular system. First, we examined the effect of Lnk on the actin reorganization and cell spreading by immunofluorescence. Our results showed that the Lnk SH2 domain is important for inhibiting SCF-mediated actin polymerization and cell spreading of BMMC. In order to dissect at the molecular level the mechanism by which Lnk negatively regulates Kit–dependent cytoskeleton reorganization and cell migration, we examined the activation of two signalling effectors involved in these processes, the SHP-2 phosphatase and the Vav1 protein. Our results showed that Lnk down-regulates SCF-mediated activation of both molecules. Interestingly, our preliminary data suggest that Vav1 and Lnk interact in a SCF-independent fashion; we are currently confirming this association. Altogether, our findings suggest that Lnk down-regulates specific SCF-dependent signalling pathways, implicated in cytoskeleton rearrangement and migration of primary mast cells.

Disclosure of interest: None declared.

P-034
CHARACTERIZATION OF CELL ADHESION MOLECULES AND CYTOKINES PROFILE IN BLOOD DONORS INFECTED WITH HEPATITIS C VIRUS

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HCV is a highly immunogenic infectious agent leading to the development of an intense humoral and cellular response. The HCV infection of cells rapidly triggers intracellular signaling events leading to cytokine production and changes in the pattern of expression of cell adhesion molecules, providing a barrier to replication and viral spread. The aim of this study was to characterize the expression of adhesion molecules and cytokine profile in peripheral blood from blood donors of the Foundation HEMOAM HCV positive. We analyzed blood samples from 50 individuals that were reactive in serological screening test for HCV, and 53 samples negative of healthy blood donors. The expression of cell adhesion molecules in leukocytes was analyzed by flow cytometry and serum cytokine was performed by ELISA. The prevalence of HCV infection in the population of blood donors of the Foundation HEMOAM living in Manaus, from August/2009 to July/2010, was 0.1% and was greater prevalent in the group of first-time blood donors, compared to repeat blood donors. It was observed that the expression of cell adhesion molecules on leukocytes in peripheral blood is influenced by HCV infection, resulting in changes in the pattern of leukocyte recruitment to the liver and thus alters the course of the disease. There is evidence that the immune response against HCV in the population of blood donors, provide a toward Th1 profile, characterized by a high concentration of IL-12 and IL-10 in peripheral blood. It was found that the concentration of cytokines in peripheral blood from donors infected with HCV correlated with the expression pattern of cell adhesion molecules of leukocytes. However, our results reinforce the importance of the relationship between cell adhesion molecules, cytokine production and HCV infection.

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Disclosure of interest: None declared.

P-035
DIFFERENT STIMULI REMOTE FROM THE PERITONEAL CAVITY ALTERS THE PATTERN OF PERITONEAL CELLS IN MICE

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It is well documented that irritant inoculation into either the peritoneal cavity (PC) or other sites of mice generate a factor/s that stimulate bone marrow production of monocytes. We hypothesized that cells from the peritoneal cavity might also respond to remote inflammatory stimuli. Searching for data to support this hypothesis, LPS, BCG or Ehrlich ascitic tumor were inoculated in the footpad of Balb/c mice and B-1a and B-1b cells, macrophages and B-2 cells were analyzed by flow cytometry after different time intervals of irritant inoculation. Results show that the peritoneal macrophage population increased in the PC with a simple needle puncture in the foot pad after 24 and 48 h. When tumor was inoculated, macrophage population also increased persisting for 72 h. The number of B-1 cells increased after 48 h of tumor inoculation and B-2 cell population declined in the three periods studied. Therefore, we may suggest that alteration in PC cells is remote-stimulus dependent since these modifications were not observed after BCG and LPS inoculation. Only B-1 cell population increased 24 h after BCG stimulus. Also, animals were inoculated with Ehrlich tumor in the paw and 24 h after, CFSE solution was inoculated in the foot pad or in PC. 16 h after, the presence of $CFSE^+$ cells in spleen, bone marrow, popliteal lymph node and PC was evaluated. Results show that cells in the paw migrate to the popliteal lymph node and PC. Yet, cells in the CP migrate to the same lymph node. $CFSE^+$ cells were not detected in bone marrow or spleen. Data obtained so far show that the peritoneal cavity is not physiologically limited to itself but interacts with pathological manifestations that occur remote to its limits by mechanisms under investigation in our laboratory.

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Disclosure of interest: None declared.

Chemokines and cytokines

P-036
INTERLEUKIN-6 IMPAIRS CHRONOTROPIC RESPONSIVENESS TO CHOLINERGIC STIMULATION AND DECREASES HEART RATE VARIABILITY IN MICE

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Heart rate variability (HRV) is reduced in several clinical settings associated with systemic inflammation. The underlying mechanism of decreased HRV during systemic inflammation is unknown. It appears that the inflammatory cytokines might play a role, since epidemiologic studies has shown that circulating levels of interleukin-6 (IL-6) correlate significantly with indices of depressed HRV in various clinical conditions. Since there is no report to show a cause-effect relationship between IL-6 and decreased HRV, the present investigation was carried out to study the peripheral and central effects of IL-6 on heart rate dynamic in mice. Adult male BALB/c mice were used in the study. RT-PCR was performed to study the expression of IL-6 receptor in mouse atrial and the results showed that gp130 mRNA was detectable in the atrium. The effect of IL-6 was also studies on chronotropic responsiveness of isolated atria to adrenergic and cholinergic stimulations. Incubation of isolated atria with 10 ng/ml of IL-6 was associated with a significant hypo-responsiveness to cholinergic stimulation (log IC_{50} of carbacholine changed from -6.26 ± 0.1 in controls to -5.59 ± 0.19 following incubation with IL-6, $P < 0.05$). The chronotropic responsiveness to adrenergic stimulation was identical with or without incubation with IL-6. To examine the systemic effects of IL-6 on HRV, studies were performed in two separate experiments. Intraperitoneal injection of IL-6 (200 ng/mouse) was associated with a significant decrease in HRV parameters (SDNN, SD1, and SD2). While intracerebroventricular injection of IL-6 (50 ng/mouse) had no significant effect on HRV parameters. These data are in line with a peripheral role for IL-6 in the genesis of decreased HRV during systemic inflammation. Understanding the interaction between cytokines and cardiovascular regulatory system could potentially lead to development of strategies for treatment and management of patients with inflammatory diseases.

Disclosure of interest: None declared.

P-037
ELEVATED SERUM LEVELS OF CX3CL1 IN ADULT-ONSET STILL'S DISEASE; POTENTIAL INVOLVEMENT IN HEMOPHAGOCYTIC SYNDROME

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Objective: To investigate the prevalence and characteristics of patients with adult-onset Still's disease (AOSD), serum levels of cytokines/chemokines were measured and examined the association of systemic manifestations, especially hemophagocytic syndrome (HPS) in complicated with AOSD,

Methods: Seventeen patients diagnosed as having AOSD and 20 healthy controls, with informed consents, were enrolled. We analyzed clinical and laboratory findings retrospectively. Serial serum samples were obtained from patients with active and inactive AOSD and controls. Cytokines/chemokines, including IL-18, CXCL8, CXCL10, CCL2 and CX3CL1 were determined by ELISA. Multivariate analysis was used to evaluate the correlation between serum chemokine levels and disease activity and clinical features of AOSD.

Results: Significantly higher serum levels of all cytokines/chemokines (IL-18, CXCL8, CXCL10, CCL2 and CX3CL1) were observed in patients with active AOSD compared with healthy controls. The elevated CX3CL1 levels seen in AOSD patients correlated positively with clinical activity, as well as with CRP and ferritin levels, although there were no significant correlation between serum levels of other chemokines and either CRP or ferritin levels. In 17 patients with AOSD, 4 patients were complicated with HPS. Levels of serum CX3CL1 and ferritin were all significantly higher in AOSD patients with HPS than in those without HPS. Notably, serum CX3CL1 levels were significantly diminished following successful treatment and clinical improvement.

Conclusion: The serum CX3CL1 level may be used as a clinical marker to assess disease activity of AOSD. We suggest that high

levels of serum CX3CL1 and ferritin encountered in AOSD reflect the presence of HPS. The association between levels of chemokine profiles and distinct clinical manifestations and various patterns of disease course suggest the heterogeneity of pathogenesis in AOSD.
Disclosure of interest: None declared.

P-038
FUNCTIONAL IMPAIRMENT OF FOXP3+ CELLS IN ACTIVE RHEUMATOID ARTHRITIS PATIENTS

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In a limited number of patients with active RA, we undertook a pilot study to characterize the phenotype and function of effector and Treg cells obtained from the synovial fluid of affected joints and compared them with that of the autologous peripheral blood. Multi-color flow cytometry is employed for this purpose. Our preliminary data reveals significant enrichment of both Th17 (IL-17A+) and Treg cells (FoxP3+) in the local disease site of RA patients (Synovium) over their peripheral blood. The most interesting observation we made was the significantly reduced IL-10 production by CD4+FoxP3+ Treg cells derived from the local disease site(affected articular joints).However, under same in vitro condition higher percentage of Treg cells(CD4+CD25hi, FoxP3+) from the peripheral blood of the same patients could produce IL-10. Our results suggest (1) intact homing of Treg cells (in parallel with Th17 cell enrichment) to the diseased synovial fluid and (2) there is definitive functional impairment of the locally recruited Treg cells, at least in terms of IL-10 production. We also studied the expression of chemokine receptors such as CCR4, CCR6, etc. on the Treg cells which may be involved in their selective recruitment to the pathologic site of RA. We feel that these preliminary findings are quite interesting and require further studies with more elaborate experiments to understand the possible mechanism(s) contributing to the languidness of locally recruited Treg cells in terms of suppressive cytokine production (IL-10). Identifying the complex immune interplay between T effector and T reg cells might help in restoration of locally recruited Treg cell's function for clinical application in rheumatoid arthritis.
Disclosure of interest: None declared.

P-039
TWEAK MODULATES TNFALPHA INDUCED CYTOKINE PRODUCTION IN HUMAN ADIPOCYTES

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Context: Tweak and TNFα cytokines are involved in the ongoing pro-inflammatory process found in obesity. Their respective receptor activation (Fn14 for Tweak and TNFR1 and 2 for TNFα) results in the recruitment of TRAF adaptor proteins and activate NF-κB and MAPKinases pathways. Recently, it has been found that Tweak pre-treatment can attenuate the production of TNFα induced pro-inflammatory cytokines in human cancer cell lines.
Objectives: To determine if TWEAK priming could affect the production of TNFα induced pro-inflammatory cytokines in human adipocytes.
Design: In Tweak primed TNFα treated SGBS and human primary adipocytes, we measured IL-6, IL-8 and AdipoQ cytokine production by ELISA; analyze by western blot NF-κB and MAPK signalling pathways and cellular levels of TRAF2, TNFR1 and TNFR2. In addition, serum levels of TWEAK were measured in a human cohort covering a wide range of obesity.
Results: We found that Tweak priming modulates the production elicited by TNFα of IL-6, IL-8 and AdipoQ cytokines; modulates TNFα signalling mainly through the NF-κB and p38 MAPK pathways in part by reducing TRAF2 levels. Serum levels of Tweak were significantly reduced in severely obese subjects when compared to a matched control group ($p < 0.003$).
Conclusion: Tweak modulates TNFα signalling in adipocytes.
This study was supported by the following grants: PI 08/0733; PN: BFU2008-04043 and German Research Foundation (WA 1096/3-3 to MW). Dr Matilde R. Chacón is supported by a fellowship from the Fondo de Investigación Sanitaria (FIS) CP 06/00119. Both CP06/00119 and PI08/0733 are co-finance by the European Regional Development Fund (ERDF).
Disclosure of interest: None declared.

P-040
CIGARETTE SMOKE UPREGULATES CXCL8 EXPRESSION BY ENHANCING MRNA STABILIZATION VIA P38 MAPK SIGNALING IN AIRWAY SMOOTH MUSCLE CELLS

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CXCL8/Interleukin-8 is an important neutrophil chemoattractant known to be elevated in the airways of cigarette smokers and in patients with chronic obstructive pulmonary disease (COPD). We examined here the acute effect of aqueous cigarette smoke extract (CSE) on CXCL8 expression in primary cultures of normal human airway smooth muscle cells (ASMC). In parallel, CXCL8 released in the culture medium was measured by ELISA. We found that CXCL8 mRNA levels markedly increased (up to 100-fold) upon CSE exposure in a concentration and time-dependent manner and such effect was accompanied by the accumulation of the CXCL8 protein in the culture medium. CSE-evoked elevation of CXCL8 mRNA was mimicked by the smoke component acrolein and was fully prevented by the α,β-unsaturated aldehydes scavenger *N*-acetylcysteine (NAC). Inhibition of protein synthesis with cycloheximide (CHX) *per se* caused a super-induction of CXCL8 mRNA levels, indicating that gene products maintaining basal CXCL8 mRNA at low levels are constitutively expressed in ASMC. Upregulation of CXCL8 mRNA levels evoked by CSE and acrolein, but not by TNF-alpha, was negatively modulated by CHX indicating the involvement of secondary response genes. By western blot analysis we observed that CSE-evoked upregulation of CXCL8

mRNA was preceded and accompanied by phosphorylation of p38 MAPK. In cells simulated with CSE, and subsequently treated with actinomycin D to block mRNA neosynthesis, pharmacological inhibition of p38 MAPK strongly accelerated the decay of CXCL8 mRNA levels, indicating that p38 MAPK signalling enhances the stability of CXCL8 mRNA. In sum, p38 MAPK-mediated stabilization of mRNA underlies upregulation of CXCL8 elicited by CSE. Given the pivotal role of CXCL8 in neutrophil chemotaxis and activation, this study sheds light on the mechanisms through which cigarette smoke can initiate inflammatory responses in the lung.
Disclosure of interest: None declared.

P-041
EFFECT OF RESISTIN AND TNF-A ON INSULIN RESISTANCE IN NORMAL PREGNANCY

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Background and objective: Pregnancy is related to glucose metabolism disorders and insulin resistance Insulin Resistance May Facilitate Supply of Appropriate nutrients particularly of glucose to fetus for fetal growth and metabolism. The mechanism responsible for insulin resistance has not been clearly stated. The purpose of this study was to evaluate the role of resistin and in insulin resistance during pregnancy.
Approach: Serum resistin and TNF-α concentrations were measured by ELISA in 86 healthy pregnant women (26, 23 and 37 of them in the 1st, 2nd and 3rd trimesters, respectively) and in 21 healthy non pregnant women in a cross sectional study.
Results: Resistin concentration was significantly higher in the third trimester (9.5 ± 3.3 ng mL^{-1}) as compared with non pregnant women (7 ± 3.3 ng mL^{-1}). Serum TNF-α level were also significantly increase in pregnant women (2.6 ± 1.9 pg mL^{-1}) as compared with maternal healthy controls (0.8 ± 0.7 pg mL^{-1}). There were significant correlation between gestational age and BMI ($r = 0.28$, $p = 0.01$), resistin ($r = 0.36$, $p = 0.002$) and TNF-α ($r = -0.44$, $p < 0.0001$). There was not significant correlation between gestational age and insulin resistance (IR). We also did not found correlation between IR and resistin as well as between IR and TNF-α in pregnant women.
Conclusion: TNF-α and resistin do not appear to contribute greatly to pregnancy induced insulin resistance in healthy pregnancy.
Disclosure of interest: None declared.

P-042
HUMAN SKIN TISSUE FLUID/LYMPH CYTOKINES AND GROWTH FACTORS: THEIR ROLE IN SKIN WOUND HEALING AND INFECTIONS

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Objective: Tissue fluid/lymph (TF) contains cytokines, chemokines, growth factors originating from blood, parenchymatous and infiltrating cells. These proteins regulate immune processes but also influence cellular events in lymph nodes draining inflammatory tissues.
Aim: To measure concentration of pro- and anti-inflammatory cytokines and chemokines in human lower limb skin tissue fluid/lymph in normal subjects, patients with rheumatoid arthritis, obstructive lymphedema without and with bacterial dermatitis.
Methods: TF was collected from lymphatics in lower leg and cytokines measured with ELISA.
Results: (1) pro- and anti-inflammatory (IL1β, TNFα, IL1Rα, MIP1α, MCP1, IL6, IL12, TGF β), (2) regulating epidermal and dermal cellular (KGF, MMP9, TIMP 1 and 2, PDGF BB) and (3) lymphatic structure (VEGF, VEGF C, CCL21 and 27) were measured in patients: (A) without any dermal conditions (N), (B) with rheumatoid arthritis (RA), (C) lymphedema without dermatitis (LD), (D) lymphedema complicated by dermatitis (L). (1) Level of proinflammatory cytokines was highest in RA. In all patients groups it was higher than in N. IL10 and 12 levels were low. (2) KGF, MMP9 and TIMPS concentration was significantly higher than in N in all groups, (3) VEGFs and CCL21 and 27 were much elevated in lymphedema but not so much in RA.
Conclusions: Concentration of cytokines in tissue fluid/lymph varies depending on the type of processes in skin. Most of cyto- and chemokines are produced locally and their level exceeds that of serum. Measuring humoral factors in TF gives insight into tissue events that is not possible with measuring serum concentrations.
Disclosure of interest: None declared.

P-043
VITAMIN D SUPPRESSES TH PATHOGENIC BEHAVIOR OF PRIMARY TH17 CELLS FROM PATIENTS WITH EARLY RA IN SYNOVIAL FIBROBLAST ACTIVATION

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Objective: This study was performed to identify the effect of vitamin D on the pathogenic behavior of primary Th17 cells on synovial fibroblast (RASF) activation both from patients with early RA. Here, we show in Th17-RASF co-culture experiments an increase of IL-6, IL-8, and MMP-1 and MMP-3. Vitamin D significantly suppressed the production of IL-6 and MMP-3 in these co cultures. Interestingly, the specific enhanced autocrine production of IL-17 due to this Th17-RASF interaction was significantly inhibited by vitamin D. In addition, markedly suppressed expression of RORgammat and significantly enhanced expression of GATA3 was noted in the presence of vitamin D. No effect of vitamin D was observed on T-bet and FoxP3 expression. In addition, lower IL-22 and enhanced IL-10 production was found in the presence of vitamin D. The regulatory effects of vitamin D on IL-17 production in the Th17-RASF co culture was comparable to neutralizing IL-17 and on IL-6 production comparable to neutralizing TNF or IL-17. In conclusion, these data show that vitamin D modulates the pathogenic behavior of primary Th17 cells in their activation of synovial fibroblasts. In addition, vitamin D suppresses the proinflammatory IL-17 loop between Th17 and RASF cells. These data suggest that the activation of the vitamin D pathway may have therapeutic potential for the treatment or even prevention of persistent arthritis.
Disclosure of interest: None declared.

P-044
IL-8/RANKL INCREASED BY EXCESSIVE ORTHODONTIC FORCE IN PERIODONTAL LIGAMENT INDUCES ORTHODONTICALLY-INDUCED INFLAMMATORY ROOT RESORPTION

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Objectives: Orthodontically-induced inflammatory root resorption (OIIRR) is an unavoidable pathologic consequence of orthodontic tooth movement. OIIRR is a sterile inflammatory process that is extremely complex and involves various disparate components, including mechanical forces and certain known biologic messengers. Interleukin (IL)-8, a member of the CXC chemokine family of cytokines, is potent chemoattractants for neutrophils. Recent studies demonstrated that IL-8 and RANKL contributed to alveolar bone remodeling during orthodontic tooth movement. However, little is known about the relationship between OIIRR and IL-8/RANKL. The aim of this study was to investigate how IL-8 and RANKL contribute to root resorption during orthodontic tooth movement.
Materials and methods: Forty male 6-week-old Wistar rats were subjected to orthodontic force of 10 or 50 g to induce a mesially tipping movement of the upper first molars for 7 days. We determined the expressions of CINC-1/CXCR2 (cytokine-induced neutrophil chemoattractant; CINC-1/CINC-1 receptor) and RANKL proteins in root resorption area using immunohistochemistry. Furthermore, we investigated the effects of compression forces (CF) on IL-8, and IL-8 on RANKL production by human periodontal ligament (hPDL) cells for 48 h. We observed an effect of chemokine treatment on rat odonto/osteoclasts in dentin slices that recapitulated root resorption.
Result: The immunoreactivity for CINC-1/CXCR2 and RANKL was detected in odontoclasts and PDL fibroblasts by the orthodontic force of 50 g on day 7. CF increased the secretion and the expression of mRNA of IL-8 from hPDL cells in a magnitude-dependent manner. Addition of IL-8 increased the expression of mRNA of RANKL from hPDL cells. Moreover, CINC-1 stimulated osteoclastogenesis from rat osteoclast precursor cells.
Conclusion: IL-8/RANKL may facilitate the process of root resorption due to excessive orthodontic force.
Disclosure of interest: None declared.

P-045
HSP70 INHIBITS THE COMPRESSION FORCE-INDUCED TNF-A AND RANKL EXPRESSION

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Objective: The aims of this study were to investigate the expression of heat shock protein 70 (HSP70) in periodontal ligament (PDL) in vivo and in vitro, and to examine the effect of HSP70 on the mRNA expression of tumor necrosis factor-alpha (TNF-α) and receptor activator of nuclear factor-kappa B ligand (RANKL) induced by compression force (CF) in human PDL (hPDL) cells.
Materials and methods: In vivo study, 10 g of orthodontic force were applied to the molars to induce experimental tooth movement in rats. Immunohistochemical staining with HSP70 was performed. In vitro study, hPDL cells were subjected to 1.0, 2.0, or 4.0 g/cm^2 of CF for 24 h, and were treated with recombinant human HSP70 for 12 h. The mRNA expression of HSP70, TNF-α, and RANKL from hPDL cells subjected to CF were determined by real-time PCR. Effects of HSP70 on the CF-induced TNF-α and RANKL expression were determined.
Results: In vivo study, on 1 day after tooth movement, the immunoreactivity of HSP70 was weakly. On 2, 3 and 4 days, HSP70 positive reaction in fibroblasts was recognized more than on 1 day. Further, HSP70 positive reaction was increased on 7 and 14 days. In vitro study, the mRNA expression (CF 4.0 g/cm^2) of HSP70 markedly increased to 12 h. In particular, the increase from 9 to 12 h was significantly higher than other duration. In contrast, the mRNA expression (CF 4.0 g/cm^2) of TNF-α and RANKL significantly increased after the application and peaked at 9 h. However, the mRNA expression of TNF-α and RANKL gradually decreased with the increasing level of HSP70 at 12 h after the application. Furthermore, exogenous HSP70 partially inhibited the CF-induced TNF-α and RANKL expression in a dose-dependent manner at 6 and 12 h.
Conclusions: These results indicated that HSP70 inhibited the expression of TNF-α and RANKL in hPDL cells in response to CF. In conclusion, HSP70 may function as a homeostatic mechanism to compensate for PDL cell changes occurring during orthodontic tooth movement.
Disclosure of interest: None declared.

P-046
COMPARISON OF INFLAMMATORY CYTOKINE PROFILE IN PLASMA OF PATIENTS SUBMITTED TO SURGERY UNDER PROPOFOL OR ISOFLURANE ANAESTHESIA

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Background: Although is known that surgery and anaesthesia can influence the release of mediators in patients, the role of volatile and venous anaesthetics on the modulation of cytokines in patients submitted to minor elective surgeries is not well elucidated. We evaluated and compared pro- and anti-inflammatory cytokines in patients submitted to non-invasive elective surgeries under isoflurane anaesthesia or total intravenous anaesthesia (TIVA) with propofol.
Methods: ASA I adult patients submitted to otorhinolaryngology surgery were randomly allocated to receive anaesthesia with isoflurane 1 MAC (minimum alveolar concentration) (n = 16) or propofol 3–5 mg mL^{-1} (n = 18). Venous blood samples were drawn from each patient at baseline (T1), 120 min after anaesthesia induction (T2) and on the first postoperative day (T3). Plasma concentration of interleukins IL-1b, IL-6, IL-8, IL-10 and IL-12 and tumor necrosis factor (TNF-a) were measured by Cytometric Bead Array (CBA) using flow cytometry.
Results: The pro-inflammatory IL-6 concentration significantly increased in isoflurane group at T2 (4.55 pg mL^{-1}) and T3 (8.10 pg mL^{-1}) versus baseline (2.10 pg mL^{-1}) whereas in propofol group this increasing occurred only at T3 (12.80 pg mL^{-1}) versus baseline (2.10 pg mL^{-1}). Besides, in TIVA group IL-8 had also raised just on the first postoperative day T3 (8.20 pg mL^{-1}) versus

baseline (5.65 pg mL^{-1}). There were no significant differences of IL-1β, IL-10, IL-12 and TNF-α within or between groups.
Conclusions: ASA I patients submitted to non-invasive surgeries isoflurane anaesthesia promotes prompt and progressive IL-6 release while TIVA with propofol promotes late IL-6 and IL-8 release.
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Disclosure of interest: None declared.

P-047
INFLAMMATORY CYTOKINES PROFILE IN PATIENTS SUBMITTED TO NON-INVASIVE SURGERIES UNDER SEVOFLURANE ANESTHESIA

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Patients submitted to surgical procedure have inflammatory response because of the trauma of the surgery and possibly due to the anesthetics. The role of volatile anesthetics on the modulation of cytokines in patients submitted to non-invasive surgey is not well elucidated. Sevoflurane is widely used for inhalation anesthesia. This study aimed to evaluate the cytokines (pro-inflammatory: IL-1beta, IL-6, IL-8, IL-12 and TNF-alpha and anti-inflammatory IL-10) in plasma from ASA I patients scheduled for otorhinological surgery under inhalation anesthesia with sevoflurane at Botucatu Medical School Hospital, UNESP. Patients with a disease, smokers, alcoholics, obeses, those who were occupationally exposed, those who had any kind of infection or inflammation within the previous 30 days, those who were under medication or who had received radiation were excluded. After approval of The Ethical Committee for Human Research, the study was conducted in 15 patients of both genders, aged from 18 to 44, who were submitted to surgery with 1.0–1.5 minimum alveolar concentration of sevoflurane. Blood samples were drawn at four time points: before anesthesia induction (T1-baseline), before the beginning of the surgery (T2), 120 min after anesthesia induction (T3) and on the first post-operative day (T4). Cytokines were evaluated using the kit BD Human Inflammation Cytometric Bead Array by flow cytometry. Friedman test was used to compare cytokine profile among four sampling times. There were no significant differences in the concentrations of all cytokines among the four time points evaluated ($P > 0.05$), with exception of IL-6, that increased on the first post-operative day (T4) in relation to baseline (T1) and T2 ($P < 0.001$). In conclusion, sevoflurane anesthesia does not change concentrations of anti- or pro-inflammatory cytokines during surgical procedure, but promotes late IL-6 release after otorhinological surgery in adult ASA I patients.
Financial support:FAPESP (2009/51110-5).
Disclosure of interest: None declared.

P-048
THE PARTICIPATION OF INTERLEUKIN 17 AND RANKL IN ROOT RESORPTION OF THE ALLERGIC MOUSE

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Objective: Orthodontically induced inflammatory root resorption (OIIRR) is an unavoidable pathological consequence of orthodontic tooth movement. Cause of OIIRR has not yet been conducted. IL-17 from rheumatoid arthritis (RA) patient enhances local inflammation and increases the production of inflammatory cytokines, which further promote RANKL expression and activity. We reported that compressed periodontal ligament cells obtained from tissues with severe external apical root resorption produced a large amount of IL-17 and RANKL in vitro. Therefore, in the present study, the expressions of RANK, RANKL, IL-17, IL-17 receptor, IL-23 receptor and Cathepsin-K were investigated using immunohistochemical analysis, and presence of Th17 cells was confirmed using immunofluorescence confocal analysis in RA model mice in root resorption during experimental tooth movement.
Materials and methods: 8-week-old male BALB/cAJcL mice (control) and SKG/Jcl mice (RA) were subjected to an orthodontic force of 10 g with a closed coil spring ligated to the maxillary first molar cleat by a stainless steel ligature wire to induce a mesial tipping movement of the maxillary first molars for 7 days. Each sample was sliced into 4 μm continuous sections in a horizontal direction. The expression of RANK, RANKL, IL-17, IL-17 receptor, IL-23 receptor and Cathepsin-K evaluated by immunohistochemistry, and the presence of Th17 cells was confirmed by Immunofluorescence confocal microscopy for CD4 and IL-17 colocalization in root resorption area.
Results: The number of positive cells of RANK, RANKL, IL-17, IL-17 receptor, IL-23 receptor and Cathepsin- Kin root resorption area increased significantly in RA model mice compared with that in control mice on day 7 after application of the appliance. Moreover, presence of Th17 cells in root resorption area was confirmed.
Conclusions: These results indicate that Th17 cells may be involved in the process of root resorption during tooth movement by RA model mice.
Disclosure of interest: None declared.

P-049
IL-17 STIMULATES IL-6 PRODUCTION AND OSTEOCLASTOGENESIS IN HUMAN PERIODONTAL LIGAMENT CELLS

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Objective: Orthodontically induced root resorption (OIRR) is one of side-effects in orthodontic treatment, and this resorbed root cannot be recovered again. The occurrence of severe root resorption is higher in patients with allergies or asthma, therefore the immune factors may be involved with the occurrence of root resorption. Further, we also reported that orthodontic patients with severe root resorption have high level proinflammatory cytokines such as receptor activator of NF-κB ligand (RANKL) and interleukin (IL)-17 in gingival crevicular fluid (GCF).Recently, it has been revealed that IL-17 plays an important role in bone destruction of rheumatoid arthritis by inducing IL-6 production in fibroblast-like synoviocytes. However, the relationship among IL-17, IL-6 and OIRR is not clear. Therefore, we investigated the effects of IL-17 on IL-6 production from human periodontal ligament (hPDL) cells. Furthermore, the expression of IL-17 and IL-6 in root resorptive lacunaes were observed by immunohistochemical staining.

Materials and methods: To induce experimental tooth movement in rats, 50 g of orthodontic force was applied for 7 days. Immunohistochemical staining with TRAP, IL-17, IL-17R, and IL-6 was performed. In vitro study, hPDL cells were stimulated by IL-17 (0–100 ng/ml) for up to 72 h. Real time PCR and ELISA were estimated the expression of IL-6. In addition, the effect of IL-17 on osteoclastogenesis was assessed by TRAP staining and activity of actin ring.
Result: In immunohistochemical staining, TRAP, IL-17, IL-17R and IL-6 positive cells were significantly increased in the root resorption area at 7 days. In vitro study, IL-17 induced IL-6 production from hPDL cells in a dose-dependent manner. Furthermore, the number of TRAP positive cells and osteoclasts with actin ring significantly increased.
Conclusion: These results suggest that IL-17 may stimulate osteoclastogenesis (odontoclastogenesis) through the expression of IL-6 in occurrence of OIRR.
Disclosure of interest: None declared.

P-050
EFFECT OF IL-17 ON THE EXPRESSION OF RANKL FROM HUMAN DENTAL PULP CELLS

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Objective: Orthodontically induced inflammatory root resorption (OIIRR) is an unavoidable pathological consequence of orthodontic treatment. Orthodontic forces are known to produce mechanical damage and inflammatory reactions in the dental pulp and periodontal ligament. Interleukin (IL)-17 is an inflammatory cytokine which T helper (Th) 17 cell derived. However, the relationship between IL-17 and osteoclast development is not fully understood. Our previous study found that the level of IL-17 in gingival crevicular fluid (GCF) obtained from orthodontic patients with severe root resorption increased. We examined that the effects of IL-17 on the expression of receptor activator of nuclear factor-B ligand (RANKL) by human dental pulp (hDP) cells in vitro, and the expression of IL-17, IL-17 receptor, IL-6 and RANKL in root resorption area during experimental tooth movement in rats.
Materials and methods: hDP cells were stimulated by IL-17 (0–100 ng/ml) for up to 48 h. The expression of RANKL and OPG mRNA was determined by using real-time PCR. In animal study, forty 6-week-old male Wistar rats were subjected to an orthodontic force of 10 or 50 g with a closed coil spring (wire size: 0.005 in., diameter: 1/12 in.). Experimental tooth movement was undertaken for 7 days. Each sample was prepared for haematoxylin and eosin (H and E) and immunohistochemistry staining for tartrate-resistant acid phosphatase (TRAP), IL-17, IL-17R, IL-6 and RANKL in root resorption area.
Results: Stimulation with IL-17 increased the levels of RANKL mRNA expression from hDP cells in a dose-dependent manner. On days 7, immunoreactivity for IL-17, IL-17R, IL-6 and RANKL was detected in odontoclasts with an orthodontic force of 50 g, but not 10 g.
Conclusions: These results indicate that IL-17 may modulate the mRNA expression of RANKL in hDP cells.IL-17 might be involved in the progress of inflammation in pulp tissue and the incidence of severe root resorption during orthodontic treatment.
Disclosure of interest: None declared.

P-051
ASSOCIATION BETWEEN INTERLEUKIN-6 AND PSMA–PSA CO-EXPRESSION IN HUMAN HYPERPLASIA AND PROSTATE CANCER

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The interleukin-6 is released in response to inflammation and cancer can function to promote growth and facilitate invasion of several forms of tumours including that of the prostate. The PSMA and PSA are implicated in the development and progression of prostate cancer. The increased secretion of IL-6 and co-expression of PSMA–PSA in prostate tumorigenesis may be correlated. The aim of this study was to relate the expression of IL-6 and their receptors with PSMA–PSA profiles in pathologic prostate tissues to investigate their role in prostate tumorigenesis. The study was carried out in 9 benign prostatic hyperplastic (BPH) and 17 cancerous human prostates (PC). Immunohistochemical analysis was performed. Our results showed that in BPH, (PSMA+, PSA+) was positive in 4/9 (44.5%), (PSMA+, PSA−) was present in 3/9 (33.3%), (PSMA−, PSA+) was absent, and (PSMA−, PSA−) was present in 2/9 (22.2%) of patients. While, in PC, (PSMA+, PSA+) was present in 12/17 (70.58%), (PSMA+, PSA−) was present in 4/17 (23.52%), (PSMA−, PSA+) was present in 1/17 (5.88%) of patients and (PSMA−, PSA−) was absent. Concerning (PSMA+, PSA+) profile, IL-6 and it's receptor IL-6Rα was expressed in all the patients with BPH and in the most of PC patients (75 and 91.66%, respectively), whereas it's second receptor gp130 was present in 50% of BPH and in 83.3% of PC. Alternatively, IL-6 and it's receptors were expressed in the most of patients in the other profiles in BPH and also in PC. Among (PSMA+, PSA+, IL-6) profile, PSA is more expressed than PSMA (16.25 ± 2.49 and 31.28 ± 5.3, respectively) in BPH. Inversely, PSMA is more expressed than PSA in PC patients within the same profile (32.69 ± 7.63 and 20.45 ± 1.31, respectively).There was an association between the high expression of IL-6 and PSMA–PSA co-expression in BPH and PC. A better understanding of biologic mechanism of this association connecting with the role of several signaling pathways within PSMA–PSA profiles will be interesting.
Disclosure of interest: None declared.

P-052
DIPEPTIDYL PEPTIDASE IV ACTIVITY IN RAT PLASMA AT CYCLOPHOSPHAMIDE INDUCED CYSTITIS: ROLE OF PROLINE-RICH NEUROPEPTIDE

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Dipeptidyl peptidase IV (DPPIV) is a protease preferentially cleaving N-terminal dipeptides from peptides with Pro or Ala in the penultimate position. It regulates the activities of physiologically important

compounds: chemokines, neuropeptides, hormones and growth factors, and is expressed on the surface of various cell types and is found also in soluble form. To gain new insight into the physiological role of DPPIV in experimental model of inflammation, we explored blood plasma DPPIV activity during cyclophosphamide (CPA)-induced cystitis in rats. We compared the DPPIV activity in blood plasma of CPA injected rats with and without treatment by proline rich peptide (PRP), a cytokine from neurosecretory granules of neurohypophysis. PRP had been demonstrated as a unique regulator of activity of neurons, strong antibacterial agent, and mediator of the hypothalamus-neurohypophysis-bone marrow-thymus axis. In the present work, the Wistar line white rats (130–160 g) were divided on three groups. Two experimental groups were intraperitoneally administrated CPA (25 mg/animal), the control group received saline. On the next day, one of experimental groups was injected with PRP (20 μg/animal). At 3rd, 5th, 7th, 9th, 11th, 13th days after CPA administration, the animals were anaesthetized, per 1 ml of blood were taken by cardiopuncture, and plasma was separated from the erythrocytes by centrifugation during 5 min at 2,000 rpm. In CPA injected animals, along with the cystitis development, we observed the time dependent inhibition of DPPIV activity in blood plasma peaked at 60% drop on the 7th day and the recovery of this activity after 11th day. In the PRP treated animals the inhibition of DPPIV activity peaked at 40% drop, and the occurrence of cystitis was milder. In conclusion, it seams the using of PRP along with CPA as a chemotherapeutic agent diminished the side effects of CPA as cystitis and on the other hand, protected the activity of important protease, DPPIV.
Disclosure of interest: None declared.

P-053
FATTY ACID PROFILE ALTERATIONS IN PERINODAL ADIPOSE TISSUE CAN CONTRIBUTE TO LYMPH NODE CELL ACTIVATION DURING INTESTINAL INFLAMMATION

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Colitis induced by the hapten trinitrobenzene sulfonic acid (TNBS) with reactivation is a good experimental model for studying inflammatory bowel disease pathogenesis and appropriate therapeutics, mainly Crohn's disease (CD). TNBS administration repeatedly provides induction of colitis relapse and remission periods and the establishment of chronic disease features, such as the mesenteric adipose tissue (MAT) alterations observed in CD. Lymph node activation and the role of perinodal adipose tissue (PAT) have been poorly studied in this model. Thus, the aim of this work was study the interactions of lymph nodes and PAT during reactivated TNBS-colitis in Wistar rats. The alterations of PAT evaluated were fatty acid profile and adipokine production by gas chromatography and ELISA, respectively. Lymph node cells were cultured and cytokines production evaluated by ELISA. Fatty acid analysis of PAT reveals an increase of ω-6 polyunsaturated fatty acids during colitis, such as linoleic acid, gamma-linolenic acid and arachidonic acid, resulting in a ratio $\omega 6/\omega 3$ different from healthy controls. ω-6 arachidonic acid level and ratio $\omega 6/\omega 3$ were not modified in lymph node cells or serum. PAT also produces elevated levels of pro- and anti-inflammatory adipokines during colitis. Lymph node cells release high levels of IFN-γ and TNF-α but not IL-10, characterizing the predominant Th-1 response associated with this disease. Additionally, T cells collected from lymph nodes from animals with colitis demonstrated increased IFN-γ production via a COX-2-dependent pathway after supplementation with ω-6 arachidonic acid. Our results suggesting that PAT modification could contribute to the lymph node cell activation observed during colitis, increasing the Th-1 response.
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Disclosure of interest: None declared.

P-054
CXCL8 IS INVOLVED IN ZEBRAFISH NEUTROPHIL RECRUITMENT

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In mammals the chemokine system modulates leukocyte migration during inflammatory process. Neutrophil recruitment depends mainly of the CXC chemokine family, such as CXCL8 and its receptors CXCR1 and CXCR2. The zebrafish (zf) inflammatory process also involves leukocyte recruitment. Sequence analysis studies have identified several members of CC and CXC chemokine families. Additionally, recent studies have shown that teleost have two distinct lineages of CXCL8, with distinct expression profiles at early inflammatory stages: the CXCL8-Lineage 1 (CXCL8-L1) and the CXCL8-Lineage 2 (CXCL8-L2). Both chemokine lineages seem to have a powerful chemotactic activity in carp. Here we address the role of zfCXCL8-L1 and L2, in neutrophil recruitment, under inflammatory conditions. We have been employing splice morpholinos (MOs) designed to block zfCXCL8 expression in the Tg(mpx:GFP)i114 transgenic line. To monitor neutrophil recruitment two different stimuli were used: tail transection and injection of fluorescent bacteria. Briefly, one-cell stage Tg(mpx:GFP)i114 embryos were microinjected with the zfCXCL8 MOs or with MO standard control. Next, tg larvae with 3 days post-fertilization (dpf) were tail transected or injected with fluorescent bacteria. Larvae were mounted in 1% of agarose low-melting point and time-lapse images were taken at different time points to address the total neutrophil number recruited to the injuries. Furthermore, a gene expression study of cxcl8-l1, cxcl8-l2, cxcr1 and cxcr2 was made by qPCR using extracted RNA from 3dpf larvae in different times of inflammation. Our data show that cxcl8-l1 and l2 genes are over-expressed under inflammatory conditions. Additionally, in zfCXCL8 morphants the in vivo time-lapse images have shown a significant decrease in the neutrophil number recruited to the inflamed area. These results support the idea that also in zebrafish CXCL8 has an important role in modulation of neutrophil recruitment under inflammatory conditions.
Disclosure of interest: None declared.

P-055
INFLAMMATORY MEDIATORS PRODUCTION BY HUMAN MONOCYTES IS MODULATED BY BIPHASIC CALCIUM-PHOSPHATE COATING

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Introduction: Hydroxyapatite (HA) is widely used for prosthesis coating and is known for its generation of wear particles. This

material release mounts an inflammatory response which could result in implant loss. To improve the bioactivity of the implant, electrodeposited biphasic ceramic containing 50% HA and 50% tricalcium phosphate (TCP) was elaborated. In this work, we have studied the regulation of inflammatory mediators production induced by the biphasic biomaterial compared to standard HA coating or titanium alloys (Ti6Al4V).
Material and methods: Freshly elutriated human monocytes were exposed to the materials for 4 and 24 h. IL-1β, -6, -8, -10, TNF-α, TIMP-1 and -2 concentrations were measured with ELISA. Gelatinolytic activities of conditioned media were examined by gelatin zymography. Total mRNAs were extracted and variations of mRNA production were measured by RT-qPCR.
Results and discussion: Pro-inflammatory mediators production study had highlighted an up-regulation of IL-1β and TNF-α production, and an increased IL-6 mRNA level by HA-stimulated cells. IL-8 was more produced by HA/TCP-stimulated cells and HA coating induced an increased IL-10 concentration. Moreover we had stressed that HA-induced MMP-2 mRNA production and activity were decreased by comparison to HA/TCP condition whereas MMP-9 did not exhibit any variations at either mRNA or enzymatic activity levels. Tissular inhibitors of MMPs (TIMP)-1 and -2, did not exhibit any variations between the two studied coatings. Our data have highlighted that HA/TCP had a lesser inflammatory potential than HA coating leading to a decreased in inflammatory cells activation. Therefore, this regulation of inflammation process was counterbalanced with an increased in ECM turnover.
Conclusion: These results suggest that HA/TCP might be used as a novel biomaterial for prosthesis coatings to improve implant integration and lifespan.
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Disclosure of interest: None declared.

P-056
CXCL2: A NEW ADIPOKINE, CONTRIBUTES TO THE INFLAMMATION OF HUMAN VISCERAL ADIPOSE TISSUE

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Introduction: During obesity, a chronic inflammatory state, white adipose tissue (WAT) undergoes profound cellular remodeling with accumulation of immune cells like macrophages and lymphocytes. Visceral WAT which development is associated with obesity-related complications, is more inflammatory than subcutaneous WAT [1]. Using cDNA microarray analysis, we identified CXCL2 among the most over-expressed genes in human inflammatory preadipocytes. We aimed at precising the role of CXCL2 that we characterized for the first time in human WAT.
Results: The circulating levels of CXCL2 measured by Elisa assay were significantly higher in obese than in lean age-matched subjects ($p < 0.05$). CXCL2 mRNA levels determined by RT-PCR, were also increased in WAT of obese subjects ($p < 0.05$). CXCL2 secretion was significantly higher in visceral compared to subcutaneous WAT (156 vs. 24 ng/ml, $p < 0.001$). In visceral WAT, CXCL2 expression was positively correlated to M1 (TNF and IL-6) while not to M2 (CD206 and AMAC-1) macrophage markers. Adhesion/transmigration of human monocytes to/through endothelial cells from visceral WAT performed as described in [2] was enhanced by CXCL2. Immunohistological analysis showed a higher number of activated neutrophils adherent to the endothelium of visceral WAT from obese compared to lean subjects. In vitro adhesion of neutrophils to endothelial cells from visceral WAT was also increased by CXCL2. The same stimulatory effect was observed with conditioned media from visceral WAT whereas neutralizing antibody of CXCL2 abolished it.
Conclusion: CXCL2 could be considered a new adipokine upregulated in WAT during human obesity. CXCL2 could participate in the inflammation of visceral WAT by recruiting blood monocytes and neutrophils.
1. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, et al. Diabetes 2006;55:1554–61.
2. Keophiphath M, Rouault C, Divoux A, Clement K, Lacasa D.. Arterioscler Thromb Vasc Biol 2010;30:39–45.
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P-057
TS2 AND TS6 FRACTIONS OF TITYUS SERRULATUS VENOM INDUCED INFLAMMATORY MEDIATORS IN PERITONEAL CAVITY

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Introduction and objectives: Scorpion envenomation is a common medical problem in many countries, and it is an important cause of morbidity and mortality. Animal venom is able to induce systemic alterations similar to those observed in acute-phase inflammatory responses. There are many studies regarding the venom actions, however little is known about the interactions the fractions IX (Ts2) or X (Ts6) with the immune system. The aim of this study was to investigate in vivo, the ability of Ts2 and Ts6 induce cytokines and nitric oxide release, as well as lipid bodies (LB) formation in peritoneal cavity of mice.
Methodology and results: Ts2 or Ts6 (250 μg/kg) or PBS were intraperitoneally injected in the peritoneal cavity of 129sv mice and after 4, 24, 48 and 96 h the lavage was collected. The cytokines were measured by ELISA and nitric oxide (NO) by Greiss method. Ts2 or Ts6 toxins were not able to stimulate NO, however they stimulated the release of inflammatory cytokines, such as, IL-6, TNF-α, TGF-β, IL-1β and IFN-γ in different manner depending of the time analyzed. To evaluate LB, cells were stained by osmium tetroxide and counted in optical microscope. LB formation was increased after 24 h of the injection of toxins. Neutrophils were increased in peritoneal cavity between hours 4 and 96 after injection of toxins.
Conclusion: Our results show that Ts2 and Ts6 induced recruitment of inflammatory cells (neutrophils) and inflammatory mediators. Ts2 and Ts6 induced the formation of LB, which could be related with eicosanoids production.
Disclosure of interest: None declared.

P-058
DOUBLE-EDGED-SWORD-LIKE ROLE OF VITAMIN D IN COMBATING CORNEAL INFECTIONS: ANTI-INFLAMMATORY AND ANTI-MICROBIAL

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Purpose: Corneal infections are the main cause for worldwide preventable blindness. Corneal cells respond to infections by triggering an inflammatory response through toll-like receptors (TLRs). Vitamin D ($1,25(OH)_2D_3$) modulates immune responses in certain cells. We hypothesise $1,25(OH)_2D_3$ plays an immunomodulatory role and there exists a coordinated cross-talk between vitamin D and TLR signalling to reduce inflammation and remove infection.
Methods: Three different cell types- primary corneal epithelial cells (PHCEC), corneal fibroblasts (HKF) and scleral fibroblasts (HSF) were generated from each donor corneal-scleral button remnants from transplant surgery. The mRNA and protein expression of VDR, vitamin D metabolizing enzymes 1α-OHase and 24-OHase and their regulation with TLR activation was investigated by RT-PCR, immunohistochemistry, real-time PCR and superarray. The culture supernatants were analysed for the production of cytokines using ELISA and multiplex bead immunoassays.
Results: Immunolocalisation studies identified VDR, 1α-OHase and 24-OHase as cytoplasmic in the epithelial cells and keratocytes. Only the PHCEC converted the inactive ($25(OH)D_3$) to active $1,25(OH)_2D_3$. Following TLR activation (TLR3 and 4), the mRNA and protein expression of pro-inflammatory cytokines and chemokines and anti-inflammatory cytokine (IL-10) were up-regulated in corneal cells. Under these conditions, the mRNAs for 1α-OHase and 24-OHase were also up-regulated. $1,25(OH)_2D_3$ inhibited the cytokine production upon TLR activation. Only PHCEC showed stimulation in anti-microbial peptide-cathelicidin expression with TLR stimulation which was enhanced by vitamin D.
Conclusions: In the cornea vitamin D acts as a 'double-edged sword' where on one hand it offers immuno-suppression by up-regulating anti-inflammatory cytokines and down-regulating pro-inflammatory cytokines and on the other hand it up-regulates anti-microbial peptides in defence against microbial infections.
Disclosure of interest: None declared.

P-059
CYTOKINE CONCENTRATION MEASURED BY MULTIPLEX BEAD ARRAY IS UNAFFECTED BY SAMPLE TYPE (PLASMA OR SERUM) OR FREEZE–THAW CYCLE

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Background: Although measurements of inflammatory factor concentrations are thought to be affected by testing procedures, little is known about the effect of sample type. This study compared (a) the extraction of selected inflammatory factors from human plasma and serum samples, and (b) the effect of thaw-freeze cycles on the concentrations of these factors in plasma and serum.
Methods: Participants enrolled in a study examining the role of acute inflammation in the development of Complex Regional Pain Syndrome following a wrist fracture, consented to an assessment that included a blood test. Serum and plasma samples were collected from the uninjured arm within 7–14 days of fracture and stored at −80°C. Alternate samples were exposed to 0, 1 or 2 prolonged thaw–freeze cycles. A Bio-Rad multiplex-17 bead array cytokine assay and Luminex technology were used to test for the cytokines IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-10, IL-12p70 and the chemokines MCP-1 and MIP-1β. Out of range low values were replaced with the established minimal detectable value for the test.
Results: Samples were collected from 25 participants (11 female and 14 male, mean age 43.96 ± 17.26). Concentrations were below the detectable limit of the test for many of the inflammatory markers. Concentrations of IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-10, MCP-1 and MIP-1β were higher in serum than in plasma; IL-12p70 was measured as higher in plasma than in serum. However, paired sample *t* tests revealed no significant group differences between concentrations obtained from plasma and from serum ($p < 0.05$). Inflammatory factors were not affected by freeze–thaw cycles, except for IL-8 ($p < 0.05$).
Conclusions: Inflammatory factor concentrations measured by multiplex bead array are not affected by the sample medium (plasma or serum) or by one or two thaw-freeze cycles (except IL-8). Other factors, such as handling requirements, should be considered when selecting a sample medium for testing of inflammatory factors.
Disclosure of interest: None declared.

P-060
THE ROLE OF INFLAMMATORY CELLS, ADHESION MOLECULES, INTERMEDIATE FILAMENTS AND CHEMOKINE RECEPTORS IN THE PATHOGENESIS OF NASAL POLYPS

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The pathogenesis of development of nasal polyposis is incompletely understood. The aim of this study was the investigation of the role of inflammatory cells, adhesion molecules, intermediate filaments and chemokine receptors in the development of nasal polyposis. Totally, 35 patients were enrolled (Group 1, 10 patients with Samter syndrome; Group 2, 10 patients with diffuse polyposis without signs of Samter syndrome; Group 3, 5 patients with solitary nasal polyps; Group 4, 10 controls). Expression of CD105, CD106, CD62E, CD4, CD8, CXCR4, CD147, CD90, CD104, BF45, vimentin, pancytokeratin and muscle specific actin (MSA)from all patients' specimens were determined. Expression of CD4, CD8 and CD106 were similar between the groups. Ratio of patients expressing CD4 in Group 1, Group 2 and Group 3 were higher than the controls. Ratio of patients expressing CD8 antigen were significantly higher in all three groups than the control group. Expression of CD147 in Group 3 and Group 4 was significantly higher than in Group 1 and Group 2. CD98 expression was higher in Group 1, Group 2 and Group 3 than in Group 4. The ratio of patients expressing vimentin in Group 1, Group 2 and Group 3 were significantly higher than in Group 4. Immunostaining for pancytokeratin was positive in all

patients. In conclusion, inflammatory cell, adhesion molecule, intermediate filament and chemokine receptor profiles in nasals polyps differ among both different patient groups and control subjects. Our findings suggest that inflammatory cells, adhesion molecules, intermediate filaments and chemokine receptors may play a role in the pathogenesis of nasal polyps.
Disclosure of interest: None declared.

P-061
ACTIVITY OF A NEW ELECTROMAGNETIC DEVICES (TAMMEF SYSTEM) AND ELF ON LPS INDUCED TNF-A RELEASE IN HUMAN LYMPHOMONOCYTES CELL CULTURE

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Electro-magnetic fields (EMFs) interact with inflammatory mechanisms. To investigate the activity of EMFs on the release of TNF-α we did dose in a monocyte culture isolated from the blood of eight healthy donors. We studied "in vitro" the effects of TAMMEF System (Therapeutic Application of a Musically Modulated Electromagnetic Field), Electromagnetic Low Frequency ELF or untreated cells on LPS induced TNF-α release. ELF apparatus modulate the frequency of 100 Hz, sinusoidal waveform; TAMMEF System produces a field whose parameters are modified in time and varying within the respective range, so that in a single application all possible codes can occur. We used a pair of electromagnetic plates controlled by an audio tape player, recorded with a classical excerpt. The human lymphomonocytes culture were positioned between the polar expansions and exposed at ELF and TAMMEF electromagnetic field for an hour each 24 h 4 days before LPS stimulation. Sham group was positioned away from magnetic fields. TNF-α determination was made by Elisa test; the data were analyzed by Student's "*t*" test and ANOVA. The baseline release of TNF-α did not differ between the three study groups (intergroup difference, +34.1, +33.1, +38.3% for Sham, ELF and TAMMEF treated groups, respectively). The increase of TNF-α was greater after ELF $$treatment (+440, +387, +370, +407% at 24th 48th 72th and 96th hours) versus TAMMEF groups (+159, +278, +298, +256% in the same times) in comparison to the Control group (+247, +356, +216, +311%). There was a statistical significant difference ($p = 0.01$) between ELF and TAMMEF whereas the difference between control vs ELF and control vs TAMMEF were not significant ($F = 0.2$ and 0.1, respectively). LPS induces a stronger TNF-a release in culture undergone to ELF in comparison to TAMMEF, in each time of the experimental protocol, and this seems to indicate that TAMMEF is able to induce a complex modulation of LPS induced TNF-a release.
Disclosure of interest: None declared.

P-062
MODULATION OF STEROID RESPONSIVENESS BY COMBINATION OF IFN-GAMMA AND TNF-ALPHA IN HUMAN AIRWAY EPITHELIAL CELLS

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Airway epithelial cells are a major source of proinflammatory cytokines and chemokines and have a key role in the pathogenesis of respiratory diseases such asthma and COPD. The severity of these diseases is associated with increased levels of IFNγ and TNFα and with a reduced sensitivity to steroids. Recently, it has been demonstrated that combination of TNFα and IFNγ impairs the ability of steroids to inhibit the expression of pro-asthmatic genes, such as IL6 and IL8, in airway smooth muscle cells. It has not been studied whether a similar phenomenon occurs in the airway epithelial cells. The aim of this study was therefore to investigate whether IFNγ, in combination with TNFα, reduces steroid sensitivity in A549 human lung epithelial cells and human primary bronchial epithelial cells (NHBE cells). IFNγ synergistically enhanced the expression of TNFα-induced inflammatory mediators such as IL6 and Rantes, but did not affect TNFα-induced IL8 expression in A549 cells. The ability of the steroid fluticasone propionate (FP) to inhibit the secretion of these cytokines was similar in response to TNFα alone or in response to TNFα in combination with IFNγ, suggesting that IFNγ doesn't affect the steroid trans-repression activity. Moreover, in A549 cells stably transfected with a glucocorticoid response element (GRE)-driven reporter gene, increasing concentrations of IFNγ, up to 200 ng/ml, did not alter steroid trans-activation activity. However, combination of TNFα and IFNγ produced high expression of IP10 and ICAM1 that is completely steroid insensitive. Same trend of steroid sensitivity for TNFα- and IFNγ -induced inflammatory genes has been observed in primary NHBE cells from different donors. We conclude that the reduction of steroid responsiveness by combination of TNFα and IFNγ is cell type specific and that there is a set of IFNγ-induced genes intrinsically steroid insensitive.
Disclosure of interest: B. Maschera employee of: GSK and participate in share scheme, K. Simpson employee of: GSK and participate in share scheme.

P-063
EVALUATION OF CD MARKERS IN EXERCISERS COMPARED TO NON EXERCISERS

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Objectives: Exercise has been introduced as a model of the stress response hence having some impact on immune system reactions. To obtain information about lymphocyte turnover in cells recruited during exercise, we recently analyzed the interactions between exercise stress and the immune system to provide an opportunity to link basic and clinical physiology and to evaluate the role of stress like activities and immune related sub population of t-cells.
Methods: The study was performed using sera samples of 34 exercisers as the case group and sera samples of 15 non-exercisers as the control group. Two blood samples were taken before and after exercise of which serum was extracted. Cell blood count (CBC), CD3, CD4 and CD8 were measured for all participants. Data were analysed using ANOVA and Spearman analysis tests.
Results: The mean ± SD of CD3, CD4, CD8 and CD4/CD8 were $64.7 \pm 6.3, 41.25 \pm 5.07, 21.75 \pm 5.49$ and 2.08 ± 0.63 in case group while 64.33 ± 4.56, 42.86 ± 5.44, 20.33 ± 4.93 and 2.34 ± 0.76 in control group, respectively. There were a significant correlation

between CD3 (r = 0.424, p = 0.01) and CD8 (r = 0.661, p = 0.000) with WBC count in case group, while this correlation was not found for control group. The mean CD8 level was significantly higher in $$women (24.66) compared to men (19.50) (95% CI −8.82–1.50, P = 0.008). The CD4/CD8 ratio was increased significantly in control than case group but this relationship was not significant.
Conclusion: The mean OD value of CD3 and CD8 and the CD4/CD8 ratio were all significantly different among exercisers compared to non-exercisers indicating that immune system can be affected by exercise depending on the nature and quality of exercise. The results of the current study indicated that changes in the case of mean values for each immunological parameter such as CD3, CD4, CD8, CD19 and CD56 are explaining the impact of exercise on immune response.
Keywords: Inflammation, cytokine, exercisers, non-exercisers.
Disclosure of interest: None declared.

P-064
THE PROTEASOME INHIBITOR MG132 BLOCKS IL-1BETA-INDUCED CYCLOOXYGENASE 2 EXPRESSION AND IL-8 SECRETION IN HUMAN OSTEOARTHRITIC MENISCUS CHONDROCYTES

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Objective: The objective of this study was to examine the effects of proteasome inhibitor MG132 on cyclooxygenase 2 (COX-2) induction and IL-8 secretion induced by IL-1beta in human osteoarthritic meniscus.
Methods: Human meniscal chondrocytes were enzymatically isolated from osteoarthritic knee cartilage. The cultured meniscus cells were characterized by immunocytochemistry. Western blotting was used to assess the induction of COX-2 by IL-1beta stimulation (10 ng/ml), in the presence or absence of MG132 (35 μM) or NF-kB SN50 peptide (100 μg/ml). IL-8 production was assayed by ELISA.
Results: Primary cultures of human meniscal chondrocytes demonstrated the fibrochondrocytic nature with expression of collagen type I and II, but not CD34 by immunocytochemistry. COX-2 was significantly upregulated following IL-1beta stimulation from 6 to 24 h tested ($p < 0.05$). COX-2 protein induction was inhibited by MG-132, but not SN-50. IL-1beta-induced IL-8 secretion by meniscal chondrocytes was also inhibited by MG-132 (control meniscus cells 585 ± 179 pg/ml, IL-1beta stimulation group 7,721 ± 2568 pg/ml, MG132 incubation only 432 ± 164 pg/ml, and IL-1beta/MG132 group 491 ± 176 pg/ml).
Conclusion: The proteasome inhibitor MG132 may block IL-1beta-induced COX-2 expression and IL-8 secretion in human osteoarthritic meniscus chondrocytes. It may be of value in treatment of degenerative meniscus disease.
Disclosure of interest: None declared.

P-065
EFFECT OF PRE-EXERCISE CARBOHYDRATE MEALS WITH DIFFERENT GLYCEMIC INDEX ON CIRCULATING LEUKOCYTES, HORMONAL AND CYTOKINE RESPONSES DURING SUBSEQUENT ENDURANCE EXERCISE

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Purpose: Carbohydrate ingestion during prolonged strenuous exercise attenuates the leukocytosis and subsequent neutrophilia and lymphopenia by maintaining plasma glucose concentration and minimizing stress hormone and inflammatory cytokines responses. The purpose of this study was to determine the effect of pre-exercise high carbohydrate meals with high glycemic index (HGI) or low glycemic index (LGI) on blood leukocyte redistribution during subsequent endurance exercise.
Methods: 24 male subjects (age 24.8 ± 0.35 years, body mass 76.1 ± 3.5 kg, height 1.77 ± 0.02 m, body fat percentage 11.1 ± 3.23, VO2max 51.18 ± 0.65 mL kg^{-1} min^{-1}; mean ± SEM) performed two 90-min runs on a treadmill at 70% VO2max 2 h after ingesting a HGI or LGI meal. Each isocaloric test meal contained 1 g kg^{-1} body mass of carbohydrate and the glycemic index values were 94 and 40, respectively. Trials were separated by at least 7 days in counterbalanced order. Results were analyzed using a two-factor (trial × time) repeated measures ANOVA with post hoc (Bonferroni) comparison as appropriate.
Results: Plasma glucose concentrations were significantly higher in LGI than HGI immediately after exercise. However, there were no significant differences in circulating leukocyte and neutrophil counts, plasma cortisol, interleukin-6 concentrations between trials.
Conclusion: Ingestion of a LGI meal 2 h before sub maximal exercise maintains plasma glucose concentration during subsequent exercise when compared with ingestion of a HGI meal. However, it seems to have limited effects on circulating leukocyte and neutrophil counts, circulating stress hormone and cytokine responses to exercise.
Keywords: High Glycemic Index (HGI), Low Glycemic Index (LGI), leukocyte redistribution, cortisol, interleukin-6(IL-6).
Disclosure of interest: None declared.

P-066
THE CARDIAC FIBROBLAST AS AN INFLAMMATORY SUPPORTER CELL IN HEART FAILURE: INCREASED CHEMOTAXIS AND ACTIVATION OF INFLAMMATORY CELLS DUE TO PARACRINE ACTION OF PATHOLOGICAL CARDIAC MYOFIBROBLASTS IN PATIENTS WITH HEART FAILURE

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Background: Inflammation is one pathology in the progression of heart failure. Here, we investigate the chemotactic properties of myofibroblasts as a stimulator for cardiac inflammation.
Methods: Endomyocardial biopsies were obtained and analyzed in regard to inflammation and the number of myofibroblasts in patients with heart failure. Moreover, a human cardiac fibroblast cell culture system using cells from these heart failure patients was used to investigate the response of the fibroblasts to different stress stimuli in regard to the expression of chemokines, migratory activity and induction of inflammatory cell activation.
Results: We show here that heart failure patients have increased numbers of myofibroblasts in their biopsies and importantly,

increased numbers of inflammatory cells. Myofibroblasts did express chemokines (CCL2, CCL7 and CCL12) as well as proinflammatory cytokines (TNF-a und Il1b). Using supernatant from myofibroblasts in migration assays against inflammatory cells, we could document that the chemotactic properties of myofibroblasts were increased significantly by 300% compared to supernatant from unstimulated fibroblasts. Moreover, investigating the adhesion of inflammatory cells to myofibroblasts, we show an increased adhesion of inflammatory cells on myofibroblasts (+150%). These inflammatory cells were also activated by the supernatant of myofibroblasts and showed an increase in degradatory activity, allowing for easier transendothelial migration through the basal membrane.
Conclusions: Myofibroblasts, known as the main contributor for extracellular matrix proteins were also highly chemotactic active and increased the number of inflammatory cells in patients with heart failure. This makes the myofibroblasts an inflammatory supporter cell and may be one explanation for cardiac inflammation in heart failure in general.
Disclosure of interest: None declared.

P-067
CYTOKINE PROFILE IN SERUM AND CERVICAL SECRETION OF WOMEN WITH LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION, HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION AND INVASIVE SQUAMOUS CELL CARCINOMA

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Introduction: High-risk type HPV is a marker for tumour progression from low-grade squamous intraepithelial lesion (LSIL) to an invasive squamous cell carcinoma (ICC) via a high-grade squamous intraepithelial lesion (HSIL). The host immune response is involved in viral persistence and progression of lesions. Most HPV infections likely involve a balance of Th1 and Th2 type immune responses.
Objective: To measure serum and cervical levels of IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ and TNF-α from women with LSIL, HSIL and ICC.
Patients and methods: The study included 109 women with LSIL (n = 16), HSIL (n = 40), ICC (n = 13) and 40 women with suspected of HPV-induced disease, but no pathological changes on cervix biopsy (control group) attending in the Colposcopy Clinic of the Botucatu Medicine School (UNESP) and in the Preventive Gynecology, Hospital Amaral Carvalho, Jaú, SP. The cytokines concentrations were determined in cervical secretion and in serum by enzyme-linked immunosorbent assay. The search and genotyping for HPV in the fragments of the biopsies were performed by polymerase chain reaction using specific primers. The socio-demographic data was obtained by interview and study was approved by the local ethics committee.
Results: A total of 78.9% of the patients were of Caucasian origin, 23.8% completed the second degree, 37.6% were smokers, 57.8% had at least three sexual partners lifetime and 8.3% reported a history of previous Sexually Transmitted Diseases. HPV DNA was detected in 90.0% in control group, 93.7% in LSIL, 100.0% in HSIL and 84.6% in the ICC. The HPV type 16 was the prevalent in all groups. The levels of IL-4, IL-6 and IL-10 in cervical secretion were significantly increased in patients with ICC. In the serum, the level of cytokines was not significantly different among the studied groups.
Conclusion: These results suggest that the increased of cervical and serum Th2 cytokines of women with ICC is related to the progression of intraepithelial lesions.
Disclosure of interest: None declared.

P-068
DEVELOPMENT OF ANTI-INFLAMMATORY DRUG TARGETING CHEMOKINE RECEPTOR AND FROUNT INTERACTION WHICH REGULATES CHEMOTAXIS SIGNALING

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Chemotaxis, which is crucial for the trafficking of leukocytes during inflammatory and immune responses, is achieved by chemokine signaling through a chemokine receptor that belongs to the GPCR family. The precise mechanism underlying the connection between the chemokine-stimulated receptor and cytoskeletal reorganization is not fully understood. A cytoplasmic protein FROUNT binds to C-terminal region of chemokine receptor CCR2 and regulates monocyte and macrophage infiltration to sites of inflammation. Here we aim to reveal the molecular mechanism of FROUNT–chemokine receptor interaction in the regulation of chemotaxis signaling.

We identified the minimum sequence in the C-terminal region of CCR2 for binding with FROUNT. We exploited the sequence to develop a novel screening method to identify inhibitors of FROUNT-CCR2 interaction. We identified selective inhibitors by screening small-molecule libraries. In a cell-based assay, some of such inhibitors tuned out to block CCR2-mediated chemotaxis. Our results indicate the potent possibility of this interaction between chemokine receptor and FROUNT as a therapeutic target for controlling the chemotaxis signaling. One appeal of our strategy is that it can avoid the undesired side effects caused by conventional approaches targeting receptor itself at the cell surface.
Disclosure of interest: None declared.

P-069
ARE TNF-A AND IL-6 FROM MONOCYTES RELATED WITH FUNCTIONAL ACTIVITY IN HEALTHY ELDERLY?

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Introduction: It is known that TNF-α and IL-6 may influence the development of aging-related disorders and the mononuclear cells can modulate the immune activity. The aim of this study was to analyze the release of these cytokines from peripheral blood monocytes and its correlation with physical function and longevity in healthy elderly.

Methods: A study with 19 healthy elderly (12 women and 7 men) aged 84 ± 0.58 years and average co-morbidities of 5.31 ± 0.59 has been conducted at Federal University of São Paulo. It was evaluated the independence of instrumental activities of daily living (IAD), grip strength (GS), ability to sit and stand up in seconds (ASS) and IL-6 and TNF-α from monocytes stimulated with LPS. Data were expressed as mean ± SE and the Pearson' test between variables was used.
Results: 63% of healthy elderly were independent IAD and 37% partial dependent, good GS (21.42 ± 1.69) and normal ASS (17.0 ± 1.21). TNF-α and IL-6 levels in culture supernatants of stimulated monocytes were 1,152.0 ± 113.70 and 1,045.0 ± 12.33 pg/ml, respectively. There was no correlation between cytokines levels and physical function or co-morbidities.
Conclusion: Although low grade inflammation may be a marker of functional limitations in older people, we could not find any correlation between IL-6 and TNF from stimulated monocytes and physical function. It is possible that monocytes are not related to the functional activity of this elderly and that these healthy elders have a defense mechanism against the physical function disorders.
Disclosure of interest: None declared.

P-070
CAN FISH OR OLIVE OIL TO PROMOTE CHANGES IN MEDIATORS OF INFLAMMATION AND HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)?

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Introduction and objective: Studies have shown that supplementation with fish oil may modify cardiovascular risk and inflammatory markers. The studies with olive oil supplementation, however, are controversial yet. The aim of this study was to evaluate the effect of fish or olive oil supplementation on systolic blood pressure (SBP) and some mediators of hypertension and inflammation, using SHR.
Procedures: Male SHR (12 weeks) received olive oil (Aceites Borges.) (OO, 1.5 g/kg), fish oil (Sigma) (FO, 1.5 g/kg) or vehicle (C) by gavage for 8 weeks, once a day. SBP was measured once a week by tail cuff plethysmography. ACE activity was determined in serum fluorimetrically and in cardiac and renal tissue using Hipuril-His-Leu (HHL) and Z-Phe-His-Leu (ZPhe-HL) as substrates. Cholesterol HDL and glucose were measured in serum and serum cytokines (TNF-α, IL-1β and IL-6) were determined by ELISA.
Results: Both oils decreased SBP, ACE activity in serum and the ZPhe-HL/HHL ratio in the cardiac tissue. Only the FO decreased Cholesterol HDL but increased all studied cytokines. FO also increased Glucose levels in relation to C group. The Heart weight, however, was lower only in OO group.
Conclusion: These data shows that FO and OO supplementation decrease the SBP associated with a reduction on ACE activity, in SHR. The decreased ZPhe/HHL ratio by both oils, however, may indicate decrease of N-domain ACE, able to cleave angiotensin 1-7, which has antiproliferative and vasodilatadores effects. The decrease of HDL and the increased glucose and cytokines levels in FO group can suggest insulin resistance, which could promote cardiovascular hypertrophy and hyperplasia.
Disclosure of interest: None declared.

P-071
AMYLOID BETA ENHANCES COMPLEMENT ALTERNATIVE PATHWAY ACTIVATION IN AGE-RELATED MACULAR DEGENERATION

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We preciously reported the potent role of Amyloid β (Aβ) on complement activation in pathogenesis of age-related macular degeneration (AMD) in WCI 2009. Using in vitro and in vivo studies, we further investigated the effect of Aβ on complement alternative pathway (CAP) activation. Activities of CAP inhibitors; factor H and factor I were examined by cofactor assay. iC3b production was determined in Aβ stimulated retinal pigment epithelium (RPE) and NEP mice which over accumulate Aβ in subretinal space. Gene expression of CAP activator; factor B in RPE was analyzed by real-time PCR. Aβ bond to factor H, factor I and inhibited activity of factor I to cleave C3b into iC3b. Aβ stimulation decreased iC3b production in RPE. iC3b generation in NEP mice decreased compared to wild type mice. Aβ did not alter factor B expression in RPE. In co-cultured RPE and microphage/microglia, Aβ caused up-regulation of factor B in RPE, neutralizing antibodies against IL-1β and TNF-α abolished factor B up-regulation caused by Aβ. finally, factor B was identified present in subretinal deposit of NEP mice. Combined mechanism of Aβ-induced CAP activation: dysfunction of factor I and cytokine-induced up-regulation of factor B in subretinal space. Non-specifically activated CAP may result in the development of AMD.
Disclosure of interest: None declared.

P-072
ESSENTIAL ROLES OF CCL3–CCR1 AXIS IN THE PATHOGENESIS OF ANTIGEN-INDUCED ARTHRITIS

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We examined the pathophysiological roles of CC chemokines in antigen-induced arthritis (AIA) by using $CCL3^{-/-}$, $CCR1^{-/-}$ and $CCR5^{-/-}$ mice. Administration of methylated BSA (mBSA) into the intra-articular of the knee joint induced severe arthritis in wild-type (WT) mice, whereas the absence of CCL3 and CCR1, but not CCR5, attenuated the severity of AIA. Histopathologically, the infiltration of macrophages and T cells, in

the mBSA-treated joint of CCL3$^{-/-}$ and CCR1$^{-/-}$ mice significantly reduced compared with WT mice. Furthermore, severe bone destruction was markedly suppressed in CCL3$^{-/-}$ and CCR1$^{-/-}$ mice with attenuated osteoclast differentiation, compared with WT mice. In vitro study, RANKL-induced osteoclastogenesis was significantly enhanced by CCL3 addition in WT-derived bone marrow cells, and anti-CCL3 Abs significantly suppressed these CCL3 effects on osteoclastogenesis. Supportingly, the enhancement failed to be observed in CCR1$^{-/-}$-derived bone marrow cells. These observations indicated that CCL3–CCR1 axis would be essential for the osteoclastogenesis as a co-stimulator. Thus, the absence of CCL3–CCR1 interaction reduced macrophage recruitment from the bone marrow, and local osteoclastogenesis, and eventually ameliorated AIA. Collectively, these observations indicated that CCL3–CCR1 interaction might be a molecular target for the treatment of inflammatory arthritis.
Disclosure of interest: None declared.

P-073
ESSENTIAL ROLE OF CHEMOKINE RECEPTOR CX3CR1 IN BLEOMYCIN-INDUCED PULMONARY FIBROSIS THROUGH REGULATION OF BONE MARROW-DERIVED FIBROCYTE INFILTRATION

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Bone marrow (BM)-derived cells are known to play important roles in repair/regeneration of injured tissues, but their roles in pathological fibrosis are less clear. Here, we report a crucial role for CX3C chemokine receptor 1 (CX3CR1) in the recruitment of lung fibrocytes. An intratracheal injection of bleomycin (BLM) into wild-type (WT) mice caused a massive infiltration of leukocyte subsets, such as neutrophils, T lymphocytes, and macrophages, followed by the development of diffuse pulmonary fibrosis (PF) with accumulation of fibrocytes, characterized by CD45^{+}/collagen type I^{+} cells. Intrapulmonary CX3CR1 and its ligand CX3CL1 expression were enhanced significantly and remained at elevated levels until PF developed. Compared with WT mice, collagen deposition was attenuated in CX3CR1$^{-/-}$ mice with reduced number of lung fibrocytes although BLM increased leukocyte infiltration to a similar extent in WT and CX3CR1$^{-/-}$ mice, implying that less intrapulmonary recruitment of fibrocytes directly correlated with decreased collagen deposition in CX3CR1 deficiency. Furthermore, BM transplantation from CX3CR1$^{-/-}$ to WT mice, but not that from WT to CX3CR1$^{-/-}$ mice, recapitulated the phenotypes in CX3CR1$^{-/-}$ mice. These results demonstrated that CX3CL1–CX3CR1 interaction was involved in BLM-induced recruitment of BM-derived fibrocytes and subsequent development of PF.
Disclosure of interest: None declared.

P-074
IN VITRO FERTILIZATION: ANTIINFLAMMATORY FACTORS AND TOTAL ANTIOXIDATIVE CAPACITY

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Infertility is an important health problem throughout the world and repeated in vitro fertilization (IVF) failure is distressing for the couples seeking reproductive assistance. Since among about one-fourth of these couples no identifiable cause for infertility can be found, an immunologic basis has been attributed to unexplained losses for years. The aim of this study is to investigate the effects of antiinflammatory parameters (AIPs) as well as total antioxidative capacity (TAC) on the outcome of IVF therapy. Serum samples were obtained prior to and after IVF treatment from 26 women selected according to the appropriate criteria among 70 infertile women and 26 fertile women as the control group. Interleukin 10 (IL-10), IL-1 receptor antagonist, fetuin A, adiponectin as the AIPs were determined by ELISA. TAC was measured spectrophotometrically. SPSS was used for statistical analyses. IVF clinical pregnancy and live birth rates were determined as 30.8 and 23.1%, respectively. During the therapy, adiponectin and IL-10 levels decreased ($p \leq 0.001$); insulin and TAC levels increased ($p \leq 0.02$). Increases observed in fetuin A levels were not statistically significant. No correlation was detected in control group. However, significant correlations were observed between fetuin A and adiponectin as well as TAC in samples obtained prior to IVF therapy ($p \leq 0.05$). For the pregnant in this group, important correlations were detected between fetuin A and adiponectin as well as TAC, and between IL-10 and fetuin A ($p \leq 0.05$). There was also an association between fetuin A and TAC following IVF ($p \leq 0.01$). No correlation of fetuin A with these parameters was detected in nonpregnant group. It was concluded that the relationship between fetuin A and the other parameters and particularly TAC during IVF therapy may be important to improve IVF success.
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Disclosure of interest: None declared.

P-075
ASSOCIATION BETWEEN PRESENCE OF MYCOPLASMA HOMINIS AND UREAPLASMA UREALYTICUM AND PRO- AND ANTI-INFLAMMATORY CYTOKINE LEVELS IN AMNIOTIC FLUID OF TERM PREGNANCIES

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Microbial invasion of the amniotic cavity has been described in term deliveries, and its role in immune modulation is of interest to better understand the underlying labor processes
Objective: The aim of this study was to determine the prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in the amniotic fluid of term pregnancies and to evaluate its influence on cytokine production at the end of pregnancy.
Patients and methods: A cross sectional study was conducted with 55 pregnant women out of labor with intact membranes and gestational age between 37 and 41 weeks seen at the Bom Jesus Hospital in Ariquemes, Rondônia, between June 2009 and May 2010. Amniotic fluid samples and fragments of chorioamniotic membranes were collected at cesarean section. *M. hominis* and *U. urealyticum* detection was performed by PCR, and Interleukin (IL)-1β, IL-6, IL-8, IL-10 and Tumor Necrosis Factor (TNF)-α levels

were determined by ELISA. Chorioamniotic membranes were submitted to histopathological analyses.
Results: Presence of *M. hominis* was detected in 36.4% of amniotic fluid samples, and none of them was positive for *U. urealyticum*. Regarding cytokines levels, 63.6 and 90.9% of samples did not show detectable concentrations of TNF-α and IL-1β, respectively. The median concentration of IL-6 and IL-8 were 107.9 pg/mL (0–517.1) and 208.1 pg/mL (0–1897.4), respectively. Interleukin-1β, IL-6, IL-8 and TNF-α concentrations were not associated with the presence of *M. hominis* in amniotic fluid, regardless the gestational age. None of the samples had detectable IL-10 levels. The histopathological analyses did not show chorioamnionitis in any of the membranes, and only a discrete mononuclear infiltration in the decidua was observed in 40.4% of the samples.
Conclusion: The amniotic cavity can be colonized by *M. hominis,* and IL-6 and IL-8 production was not modulated by the presence of this microorganism in the amniotic cavity in term pregnancies, regardless the gestational age.
Disclosure of interest: None declared.

P-077
BACTERIAL VAGINOSIS: PROINFLAMMATORY CYTOKINES LEVELS AND SIALIDASE ACTIVITY

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Background: Bacterial vaginosis (BV) is the most common type of abnormal vaginal flora. Although BV stimulates local cytokine production, it is still not well established its cytokine profile. It is believed that sialidase activity, a microbial hydrolytic enzyme, may modify the local innate immunity in BV and interfere in cytokine production.
Objective: To determine whether differences in cytokine profile in BV cases are correlated with the sialidase activity.
Methods: A total of 120 women attending outpatient clinics were included and were sampled for vaginal and cervical swabs, and vaginal wash with sterile buffer. All women had confirmed BV by microscopic classification of GRAM stained vaginal smears by Nugent criteria. Additionally, they were tested for other infections to assure that only cases of solely BV were included. Absence of Candidiasis was confirmed by microscopy, possible presence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were excluded by culture and PCR, respectively. Sialidase activity was qualitatively determined by incubation of the samples of vaginal washes with the fluorogenic substrate 2′-(4-methylumbelliferyl)-aL-D-*N*-acetylneuraminic acid. The levels of IL-1β, IL-6, IL-8 and TNF-α were measured in vaginal washes by ELISA. All women provided written informed consent term and study was approved by the local ethics committee.
Results: Among the 120 women with exclusive BV, the median values (range) of IL-1β, IL-6, IL-8 and TNF-α levels were 52.1 (0.0–751.5) pg/mL, 0.0 (0.0–324.4) pg/mL, 242.8 (0.0–1985.0) pg/mL and 0.0 (0.0–18.6) pg/mL, respectively. Sialidase activity was detected in 42 (35.0%) cases. When comparing group of women with BV positive for sialidase with the ones without detection of this enzyme, no differences in IL-1β ($p = 0.38$), IL-6 ($p = 0.78$), IL-8($p = 0.89$) or TNF-α ($p = 0.99$) levels were found.
Conclusion: The enzymatic activity of sialidases do not correlate with different vaginal proinflammatory cytokine levels in women with BV.
Disclosure of interest: None declared.

P-078
IL-17+ GAMMA DELTA T LYMPHOCYTE MIGRATION DURING ALLERGY IS MEDIATED BY CCL25 AND ALPHA4BETA7 INTEGRIN

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CCL25 is a homeostatic chemokine expressed in lymphoid organs and small intestine. CCR9 (CCL25 receptor) is mainly expressed on alpha$_4$beta$_7$-integrin$^+$ T lymphocytes and dictates their homing to intestinal mucosa. Here we show that CCL25 and alpha$_4$beta$_7$-integrin mediates gamma delta T cell migration into inflamed tissue during allergy. The intra-pleural (i.pl.) injection of ovalbumin (OVA) induced the migration of gamma delta T cells expressing CCR9 and alpha$_4$beta$_7$-integrin into the pleural cavities of immunized C57BL/6 mice. CCL25 levels increased in the pleural washes recovered from OVA-challenged mice (OPW). The in vivo neutralization of CCL25 did not inhibit gamma delta T cell migration to pleural cavities after OVA challenge; however, it selectively inhibited alpha$_4$beta$_7$-integrin$^+$ and IL-17$^+$ gamma delta T lymphocyte migration into inflamed pleura. Accordingly, we observed that the majority of pleural alpha$_4$beta$_7^+$ gamma delta T cells from OVA-challenged mice express CCR9. Furthermore, CCL25 i.pl. injection induced the migration of alpha$_4$beta$_7^+$gamma delta T cells to mouse pleura and triggered IL-17 production by those cells. CCL25 induced IL-17$^+$ gamma delta T lymphocyte in vitro chemotaxis, even though it failed to alter IL-17 production by gamma delta T cells after in vitro stimulation. In addition, alpha$_4$beta$_7$-integrin blockade inhibited in vitro transendothelial migration of gamma delta T cells induced by CCL25 and OPW. Moreover, the in vivo blockade of alpha$_4$beta$_7$-integrin inhibited the migration of IL-17$^+$ gamma deltaT lymphocytes into OVA-challenged mouse pleura. In contrast, anti-CCL25 mAb and anti-alpha$_4$beta$_7$-integrin mAb failed to inhibit the migration of alpha beta T lymphocytes, suggesting that the CCL25/alpha$_4$beta$_7$-integrin pathway is selective for gamma delta T cells. Our results reveal that, during the allergic reaction, CCL25 drives IL-17$^+$ gamma deltaT cell mobilization to inflamed tissue via alpha$_4$beta$_7$-integrins and modulates IL-17 levels.
Disclosure of interest: None declared.

P-079
IN VIVO FUNCTIONAL ANALYSIS OF PRIMATE-SPECIFIC CASPASE-4 IN MICE

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Caspase-4 has been proposed to be a member of the inflammatory caspases. We have previously found that caspase-4 mRNA is significantly upregulated in the brain of Alzheimer disease subjects. Other studies also implicate its role in the neuropathologies such as Alzheimer's disease. Despite numerous studies implicating the role of the gene in apoptosis and inflammation, the fact that the gene is only represented in the primate genome makes it difficult to characterize its physiological and pathological function in vivo.

To study physiological function of caspase-4, we have generated transgenic mice expressing human caspase-4 under the control of its authentic promoter using bacterial artificial chromosome (BAC). Tissue expression analysis revealed the prominent level of caspase-4 expression in immune tissues such as spleen and thymus, while those in the brain and liver are relatively low, mirroring well the previous studies of mRNA distribution in human tissue. Unexpectedly we found significant portion of caspase-4 are in the processed form, although transgenic mice were viable and fertile. The role of caspase-4 in cytokine secretion becomes apparent upon lipopolysaccharide (LPS) challenge, as transgenic mice exhibited hyper-sensitivity to intraperitoneally injected LPS. At high dose, transgenic mice died within 24 h due to cytokine storm in the serum as well as in the brain. Quantitative gene expression analysis in the brain revealed the moderate upregulation of caspase-4 expression. At low dose, transgenic mice rapidly secreted higher levels of proinflammatory cytokines including interleukin-1β (IL-1β) and interleukin-18 (IL-18), both of which are known substrates of caspase-1. To investigate whether caspase-4 acts in a manner independent or dependent on caspase-1, caspase-4 transgenic mice are studied with caspase-1 null background. Our study indicates that caspase-4 acts in caspase-1 dependent manner for IL-1β and IL-18 secretion and endotoxemia.
Disclosure of interest: None declared.

P-080
SERUM AMYLOID A IN PLACENTAL VILLOUS EXPLANTS: EFFECT OF HYPOXIA

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Serum amyloid A (SAA) is a major acute-phase protein in mammals with proinflammatory and angiogenic activities. Although SAA is mainly synthesized by liver, extrahepatic synthesis occurs in several tissues including the placenta. In the early stages of pregnancy the hypoxia is essential to induce cell proliferation and angiogenesis in the placenta and organogenesis in the embryo. The hypoxia is also related to placental diseases. Interestingly, the serum levels of SAA are increased in preeclampsia. The possibility that hypoxia may control the expression of SAA was recently observed by our lab in adipose tissue cells. Herein we evaluated whether hypoxia affects the expression and intracellular concentration of SAA in placental villous explants. For this purpose, placental villous explants were cultured at <1% (hypoxia) and 21% (normoxia) of oxygen tension during 24 h. The placental villous explants from normal term placenta were lysate to measure SAA intracellular by commercial ELISA kit. The RNA total was extracted by Tryzol® and analyzed by RT-PCR using specific primers for SAA1 and SAA4. We also evaluated the mRNA and protein expression for hypoxia-inducible factor-1alpha (HIF-1α) by western blotting. In the experimental conditions used it was observed an increase mRNA and protein expression for HIF-1α. On the contrary, the hypoxia decreased the SAA1 and SAA4 mRNA levels. Also the intracellular concentrations of SAA in lysate of placental villous explants were diminished. According MTT test and consumption of glucose the viability of the cultured placental villous explants was maintained. Compared to adipocyte, in that it was observed that hypoxia increases SAA expression, the decrease of SAA induced by hypoxia in placental villous explants may contribute with a local physiological compensatory response in the first trimester of pregnancy or may be involved to abnormal remodeling of the placental vasculature found in preeclampsia.
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Disclosure of interest: None declared.

P-081
ACTIVATION OF A CD14/TLR4-INDEPENDENT CHEMOKINE PATHWAY ENHANCES BACTERIAL CLEARANCE IN ACUTE INFECTION

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Sepsis remains an important clinical problem with a high mortality rate. Our long-term goals are to define new targets for therapy. We have previously shown that CD14-deficient mice are resistant to infection with clinical isolates of *E. coli* and that this resistance is accompanied by efficient clearance of the bacteria; in contrast, wild-type (WT) mice die from such infection and are unable to clear the bacteria. To define the mechanisms that regulate resistance, we have extended our studies to TLR4-deficient mice, the signaling molecule for the CD14/TLR4 receptor complex, and have designed experiments to define these mechanisms. Mice [wild-type (WT), $CD14^{-/-}$ or $TLR4^{-/-}$] were injected with live *E. coli* and parameters of their response to acute infection were measured, including survival, bacterial clearance, chemokine production and cell recruitment. Similar to $CD14^{-/-}$ mice, $TLR4^{-/-}$ mice were resistant to infection and cleared the bacteria more efficiently than WT mice. Bacterial clearance was due to an early recruitment of neutrophils to the site of infection that occurred in response to activation of a unique chemokine pathway that is activated independently of both CD14 and TLR4. Our results show a pathogenic role for the CD14/TLR4 pathway in severe Gram-negative infection resulting in high levels of proinflammatory cytokines; however this response may be secondary to a more important insidious role resulting in high levels of chemokines preferentially in blood, preventing PMN transmigration to infected tissue and facilitating the dissemination of bacteria from a localized site of infection. In contrast, activation of a second chemokine pathway that is independent of CD14 and TLR4 results in a chemokine gradient that promotes PMN recruitment to the site of infection, resulting in enhanced bacterial clearance. Supported by NIH/NIAID R01 AI023859 to SMG and by a grant to the RCMI program to The City College of New York from NIH/NCRR, G12RR03060.
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P-082
CYTOKINE PRODUCTION BY HUMAN ODONTOBLASTS UPON TOLL-LIKE RECEPTOR 2 ACTIVATION

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Human odontoblasts are neural crest-derived, dentin-producing mesenchymal cells aligned at the periphery of the dental pulp. They become exposed to oral bacteria as these demineralise enamel then dentin to gain access to the pulp during the caries process. Given their situation at the dentin-pulp interface, odontoblasts are the first cells encountered by invading pathogens and/or their released components, and represent, in the tooth, the first line of defence for the host. We have focussed our study on the effects of Gram-positive bacteria as these largely dominate the carious microflora in initial and moderate dentin caries lesions. We have shown that odontoblast TLR2 engagement in vitro by lipoteichoic acid (LTA), a cell wall component of Gram-positive bacteria, triggers TLR2 up-regulation, NF-kB nuclear translocation, production of chemokines and cytokines including CCL2, CXCL1, CXCL2, CXCL8, CXCL10, IL-6 and IL-10, while promoting immature dendritic cell recruitment. Responses to LTA were modulated by lipopolysaccharide-binding protein and CD14. Together these data suggest that odontoblasts may elicit innate immunity and related inflammation. Studies are ongoing to hinder Gram-positive bacteria sensing by odontoblast TLR2 and downstream signal transduction in order to prevent excessive inflammatory and immune responses that lead to the necrosis of the human dental pulp challenged with cariogenic bacteria. This work was supported by University Lyon 1, CNRS, IFRO and Région Rhone-Alpes.
Disclosure of interest: None declared.

P-083
TREATMENT OF HUMAN MONOCYTES WITH INTERLEUKIN (IL)-18 INCREASES TLR4 AND MANNOSE RECEPTOR EXPRESSION AND MODULATES CYTOKINE PRODUCTION

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Introduction: Interleukin-18 is a proinflammatory, proapoptotic, and proatherogenic cytokine belonging to the interleukin-1 family of cytokines. The cytokine exerts many unique immunologic and biological effects. It is produced as a biologically inactive and leaderless precursor protein, which must be cleaved into its mature form by caspase-1. The caspase-1 also exists in an inactive precursor in the cytosol and needs proteolytic auto-cleavage, which is catalyzed by the assembly of a multi-protein complex called inflammasome. During inflammation, interleukin (IL)-18 is produced by macrophages and other cell types such as keratinocytes and damaged endothelial cells.

Objectives: To explore the role of IL-18 in inflammatory innate immune responses we investigated their impact on toll-like receptors (TLR2 and TLR4) and mannose receptor (MR) expression by human peripheral blood monocytes, and its effect on TNF-alpha, IL-12, IL-15 and IL-10 production.

Subjects and methods: Monocytes from healthy donors were stimulated or not with IL-18 during 18 h, and then TLR2, TLR4 and MR expression as well as intracellular TNF-alpha, IL-12 and IL-10 production were assessed by flow cytometry and the levels of TNF-alpha, IL-12, IL-15 and IL-10 on culture supernatants were measured by ELISA.

Results: IL-18 treatment was able to increase TLR4 and MR expression by monocytes. The production of TNF-alpha and IL-10 was also increased by the treatment with the cytokine. However, IL-18 was unable to induce neither IL-12 nor IL-15 production by these cells.

Conclusion: Taken together, these results show an important role of IL-18 on the initial of inflammatory response, promoting the expression of some pattern recognition receptors (PRRs) during microbes recognition phase. Financial support: FAPESP (2007/04042-9 and 2007/00755-0).
Disclosure of interest: None declared.

P-084
EXPRESSION OF CYTOKINE RECEPTORS IN CD11C+ AND CD11B+ CELLS OF MICE WITH DISTINCT ABILITY TO ELABORATE A PROTECTIVE IMMUNE RESPONSE AGAINST MYCOBACTERIUM TUBERCULOSIS INFECTION

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Introduction: Protection in tuberculosis has been associated with lymphocyte and macrophage activation, secretion of IL-12, IFN-gamma and TNF-alpha. On the contrary, IL-4 and IL-10 production, expansion and activation of regulatory T cells have been correlated with progression of infection. Our group showed that the frequency and activity of regulatory T cells during *M. tuberculosis* experimental infection are influenced by genetic background of infected host.
Objectives: The aim of our study was to evaluate whether the expression of IFN-gamma receptor as well as IL-12 and IL-10 receptors would be differently expressed in infected C57BL/6 and BALB/c mice.
Methodology: BALB/c and C57BL/6 mice were infected with 1×10^5 bacilli by intra-tracheal route. Thirty (initial phase) and 70 days (late phase) post infection, Colony-Forming Unit number and expression of cytokine receptors were evaluated.
Results: C57BL/6 mice control the infection after 70 days while BALB/c mice did not. Day 70 infected C57BL/6 mice had a higher expression of IFN-gamma receptor (alpha chain) in lung $CD11b^+$ cells compared with day 70 infected BALB/c mice. The expression of IL-10 receptor (alpha chain) was up regulated in lung $CD11b^+$ and $CD11c^+$ cells obtained from day 30 infected BALB/c mice compared with C57BL/6 mice at the same period of infection. In addition, an increase in the expression of IL-12 receptor (beta1 chain) was found only in lung $CD4^+$ cells of day 70 infected C57BL/6 mice.
Conclusion: These data suggest that the differential expression of IFN-gamma, IL-12 and IL-10 receptors in hosts genetically different may represent one of immunological parameters which contribute to coordinate the induction of cellular immune response associated with the distinct capacity to control *M. tuberculosis* infection. Financial support: FAPESP, CNPq, FAEPA.
Disclosure of interest: None declared.

P-085
VIABILITY OF ALVEOLAR MACROPHAGES AFTER IN VITRO INFECTION WITH METHICILLIN-SENSITIVE STAPHYLOCOCCUS AUREUS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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Objective: To compare the viability of alveolar macrophages (AM) after in vitro infection with methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA).
Methodology: Male Wistar rats (n = 12) were anesthetized and tracheotomized in order to obtain AM from the bronchoalveolar fluid. Following the AM isolation procedures, four systems were set up: negative control containing AM (NC), positive control containing AM and 10 μL of lipopolysaccharide (PC), MSSA containing AM and 100 μL of a suspension of ATCC 29213 (OD = 0.15, corresponding to 10^6 CFU mL^{-1}) and MRSA, containing AM and 100 μL of a suspension of ATCC 33591 suspension (OD = 0.15, corresponding to 10^6 CFU mL^{-1}). Systems were then incubated at 37°C in a 5% CO_2 atmosphere for 24 h. AM viability was assessed based on the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] assay. Solubilized formazan concentration was determined using an ELISA reader at 570 nm wavelength. Statistical analysis were performed using Student t test and Mann–Whitney test assuming a significance level of $p < 0.05$.
Results: A significant reduction in the AM viability was observed among systems containing MSSA and MRSA strains when compared to the positive control (MSSA: 18.5 ± 0.22%, $p \leq 0.001$, MRSA: 20.7 ± 0.2%, $p \leq 0.001$, CP: 69.2 ± 0.8%, $p \leq 0.001$). However, none statistically significant differences were observed among MSSA and MRSA systems ($p > 0.05$).
Conclusion: Results suggest that the pathogenic potential of *Staphylococcus aureus* strains may be not affected by the methicillin resistance profile.
Disclosure of interest: None declared.

P-086
IL-17 EXPRESSION IN SKIN OF LEPROSY PATIENTS

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Leprosy is chronic infectious disease caused by *Mycobacterium tuberculosis* with wide clinical spectrum. Although the detection rates has fallen a lot in the last 20 years, Brazil is still considered an endemic country for leprosy by WHO and north region of the country has the largest coefficients. Despite its ancient history, leprosy still presents many aspects that need to be studied, in special regarding the chronic skin inflammation linked to the bacteria-induced lesions. In the last years, a novel lineage of CD4 T helper cells (Th), termed Th17, was characterized by the production of interleukin 17 (IL-17), involved in a plenty of autoimmune and chronic inflammatory reactions. However, its role in the leprosy immunopathology is still unknown. Then, this study aimed to identify whether the expression of IL-17 in leprosy patients. Tissue samples of leprosy patients were obtained from 20 patients at Alfredo da Matta Foundation, in Manaus, Amazonas State, Brazil. Leprosy diagnosis was clinically confirmed and associated with baciloscopy and histopathology. The tissue samples were collected before treatment under topical anesthetic inoculation. The samples were cryopreserved and used for immunohistochemistry analysis using rabbit anti-IL-17 antibody. IL-17 expression was confirmed after immunoperoxidase staining by microscopy analysis. IL-17 expression was consistently higher in leprosy patients than in normal cutaneous tissue from control individuals. IL-17 seemed to be detected specially in the mononuclear cells in the skin cellular infiltration, as visualized in the stained sections. The IL-17 expression was elevated in all clinical forms of leprosy evaluated. The results demonstrated that IL-17^+ cells are present in all clinical forms of the leprosy skin lesions, suggesting that the IL-17 inflammatory response is restricted to the skin lesion site.
Financial support: FAPEAM, CNPq e CAPES.
Disclosure of interest: None declared.

P-087
INHIBITION OF SECRETION OF PRO-INFLAMMATORY CYTOKINES CONTRIBUTES TO THE LONG-LASTING ANTI-INFLAMMATORY PROPERTIES OF CROTOXIN: A NATURAL TOXIN FROM RATTLESNAKE VENOM

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A single dose of *Crotalus durissus terrificus* snake venom induces a long lasting anti-inflammatory effect on paw edema and cell migration induced by carrageenan in mice. Recently it was demonstrated that crotoxin, the main toxin of *Crotalus durissus terrificus* venom, is responsible for this effect. Cytokines are regulators of the acute phase of inflammation, increase the production of chemokines that attract inflammatory cells to the inflammatory site, and also stimulate the expression of adhesion molecules. Cell migration induced by carrageenan has been shown to be mediated by cytokines IL-6 and IL-1β. The aim of this study was to evaluate the action of crotoxin on the secretion of IL-1β and IL-6. A single dose of crotoxin (0.89 μg/50 μL s.c.) or saline (50 μL) was administered 7 days or 1 h before carrageenan injection in males Swiss mice. To evaluate the effect of cytokine secretion, after 4 h of intraperitoneal injection of Cg (300 μg/200 μL) or saline (200 μL), peritoneal exudate was collected and the assay of cytokines, such as IL-1β and IL-6 were determined by ELISA. The peritoneal exudate of animals pretreated with crotoxin showed a significant decrease in the secretion of all pro-inflammatory cytokines evaluated, such as IL-1β and IL-6 when compared to untreated animals. The decrease in cytokine secretion observed in exudates from animals pretreated either 7 days or 1 h with crotoxin was 49 and 71% for IL-1β, respectively, and 12% and 51% for IL-6, respectively. We could suggest that the long-lasting anti-inflammatory effect on cell migration involves the modulatory action of crotoxin on the secretion of important pro-inflammatory cytokines that participate in the inflammatory response induced by carrageenan.
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Disclosure of interest: None declared.

P-088
PRECLINICAL DEVELOPMENT OF ALX-0061, AN ANTI-IL-6R NANOBODY® FOR THERAPEUTIC USE IN RHEUMATOID ARTHRITIS WITH A HIGH IN VITRO AFFINITY AND POTENCY AND A COMPETITIVE IN VIVO PHARMACOLOGICAL PROFILE

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The functions of the pleiotropic cytokine IL-6 are mediated through a receptor system consisting of gp130 and soluble or membrane IL-6 receptor (IL-6R). Deregulation of this system is implicated in a variety of diseases, including rheumatoid arthritis (RA). Blocking IL-6R results in clinical benefit as shown with tocilizumab, an anti-IL-6R monoclonal antibody (mAb). Nanobodies (NB) are therapeutic proteins based on the smallest functional fragments of heavy chain antibodies, naturally occurring in *Camelidae*. They have a high degree of homology to human Ig VH domains but yet have distinct features that favor monovalent high affinity binding. ALX-0061 is a highly specific anti-IL-6R NB engineered to obtain half-life extension (HLE) in vivo, and high target affinity and potency. Monovalent interaction with IL-6R avoids unwanted cross-linking, while the HLE strategy excludes the need for an Fc-region, thus avoiding immune effector functions. These properties were considered important for a potentially improved safety profile compared to full IgG mAbs. ALX-0061 demonstrated primary pharmacology in targeting and neutralizing IL-6R with superior affinity and comparable in vitro potency to tocilizumab. ALX-0061 dose-dependently suppressed inflammation in an induced acute phase response model in cynomolgus monkey. Pharmacokinetic analysis demonstrated a favorable profile which was predicted to translate into convenient dosing in humans. Measurement of circulating target levels supported the calculation of the minimum pharmacological effect level and the first-in-human dose. No unexpected pharmacology has been demonstrated both in vitro and in vivo**.** In conclusion, ALX-0061 combines high potency with favorable PK properties in non-human primates and has the potential to be an effective treatment for multiple inflammatory conditions. A clinical trial application for the treatment of active RA patients has been filed and initiation of a Phase I study is planned for beginning 2011.
Disclosure of interest: None declared.

P-089
BLOCKING IFNA IN LUPUS PATIENTS THROUGH ACTIVE THERAPEUTIC IMMUNIZATION: PHASE I-II CLINICAL STUDY OF IFN-KINOID

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Background: Interferon alpha (IFNα) has been associated with the severity and disease activity of Systemic Lupus Erythematosus (SLE). Active immunization against IFNα with IFNα-Kinoid (IFN-K) has been shown to prevent severe renal lupus disease and to prolong survival in NZW/NZB mice models. Anti-huIFN antibodies neutralize all 13 subtypes of huIFNα and as well as against IFNα in sera of lupus patients.
Objectives: We evaluate IFN-K in lupus patients in terms of safety, immunogenicity, clinical response and impact on IFNα-regulated serum chemokines levels and on IFNα-inducible gene signature.
Material and methods: IFNα-Kinoid (IFN-K, Neovacs SA, Paris, France) is an immunotherapeutic composed of recombinant human IFNα conjugated to KLH as a carrier protein, inactivated and adjuvanted with ISA-51 emulsion. Patients with mild to moderate lupus (SLEDAI 4-10) and positive for anti-dsDNA antibodies are being enrolled in a double-blind, placebo-controlled, phase 1–2, dose escalation study to evaluate four different doses of IFN-K Immune responses are being evaluated through titration of anti-IFNα and anti-KLH antibodies with isotyping, evaluation of neutralizing capacity and of cellular responses. Clinical response is being assessed by regular evaluation of BILAG and SLEDAI scores and titration of serum auto-antibodies. Impact on IFNα regulated chemokines and neutralisation of IFNα-inducible gene signature as assessed by Affymetrix® are evaluated over the study period in comparison to the scores in healthy volunteers who had blood collected to determine baseline IFNα-regulated serum chemokines levels and IFNα-inducible gene signature.
Results: No related serious adverse event has been reported. Few minor and transient local and systemic reactions have been observed following immunization. Complete safety, immunological and clinical results will be presented.
Conclusions: Active immunization with IFNα Kinoid to block IFNα in lupus is an attractive new therapeutic concept.
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P-090
ACTIVITY OF SOLUBLE CXCL12 NEUTRALIGANDS IN VIVO IN A SHORT MODEL OF ASTHMA

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The CXCL12 chemokine plays an important role in inflammation. Our team identified a CXCL12 neutraligand (C05) belonging to the family of chalcones, that inhibits its interaction with CXCR4 and CXCR7 (*JBC 2008*) and the eosinophil infiltration in a model of asthma. Due to its low solubility (C05, 9 μM), we decided to facilitate its administration by adding three types of groups: phosphate (C05-P), L-serine (C05-Ser) and sulfate (C05-S), as prodrugs. The analytical characterization of the three compounds was performed on the Techmed^{ILL} platform (http://www.pcbis.fr): solubility and stability ($t_{1/2}$) were measured in serum, PBS and lung homogenate. The in vitro activity was evaluated by

intracellular calcium assay and in vivo in a 8-day asthma model: Balb/c mice were sensitized (OVA+alum, i.p, D0,1,2) and challenged to OVA or saline (i.n, D5,6,7). Drugs or solvent were administered i.p. or i.n. 2 h before each challenge. Prodrugs are 5–10,000 times more soluble than the chalcone C05. None of these prodrugs modifies the calcium impulse induced by CXCL12. On the other hand, after incubation in mouse serum or lung homogenate, the prodrugs are transformed into C05 (HPLC). In vivo, at 350 μmol/kg i.p, C05-P (PBS) has the same activity as C05 (CMC 1%) with 80% inhibition of eosinophil recruitment in BAL. C05-P versus C05 administered i.n. at a dose of 22 μmol/kg (limit of C05 solubility in PBS) inhibits the eosinophil recruitment by 50 versus 0%, respectively. C05-P i.n. shows an activity 10,000 times higher ($IC_{50} = 10 \pm 7$ nmol/kg) than i.p. administration. A comparison between compounds C05-P, C05-Ser and C05-S (i.n.) at 30 nmol/kg shows similar activity (67, 69 and 64%, respectively). Use of soluble CXCL12 neutraligand prodrugs enable local administration and activity at low doses and offers an interesting strategy for the development of drug candidate for asthma treatment. Furthermore, our short model of asthma is highly efficient to estimate new therapeutic strategies.
Disclosure of interest: None declared.

P-091
STUDY OF PROINFLAMMATORY CYTOKINES IN WOMEN WITH BACTERIAL VAGINOSIS AND CO-INFECTION WITH CHLAMYDIA TRACHOMATIS

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Background: Bacterial vaginosis (BV) is the common type of abnormal vaginal flora that affects women in reproductive age. Changes in the local innate immunity have been reported in this event. Moreover, women with BV can be a *Chlamydia trachomatis* (CT) carrier. The cytokine profile in this co-infection should be better investigated. Objective: To evaluate differences in local proinflammatory cytokines levels in women with bacterial vaginosis with or without co-infection by *Chlamydia trachomatis*.
Material and methods: A total of 156 women attending an outpatient clinic at São Paulo State University, Brazil were enrolled. Vaginal flora was evaluated by Gram stained smears according to Nugent's criteria. Women who were positive for *Candida* sp., *Trichomonas vaginalis* and aerobic vaginitis were excluded. Cervical samples were also collected for the detection of CT by PCR. Additionally, a vaginal wash was performed using sterile saline in the vaginal vault to measure the cytokines, Interleukin (IL)-1β, IL-6 and IL-8 levels by ELISA. Comparison of cytokines levels between the groups was performed by Mann–Whitney test. This study was approved by institutional ethics review board and all women provided a written consent term.
Results: Patients with BV were divided in two groups of 76 women each according to their CT status. Our results showed that IL-1β and IL-6 levels in vaginal washes from women with solely BV had significantly increased levels ($p = 0.04$ and $p < 0.01$, respectively) when compared with the group of women co-infected with CT. In relation to IL-8, no difference between the groups of women with BV accompanied or not by CT infection ($p > 0.68$) was detected.
Conclusion: Bacterial vaginosis itself is responsible for increases in the local proinflammatory cytokines IL-1β and IL6, but not IL-8, regardless the presence of cervicitis by *Chlamydia trachomatis*.
Disclosure of interest: None declared.

P-092
CORRELATION OF BAFF SERUM LEVELS WITH INTRALESIONAL TNF-A ONE'S IN PSORIASIS

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Background: Psoriasis is a chronic inflammatory skin involving Th1 lymphocytes. Recent finding demonstrated that B cell-activating factor of the TNF family (BAFF), one of the most important molecules for B cell development, affected Th1 and Th2 responses and influenced the course of some T cell-mediated inflammatory reactions.
Objective: We have assessed the serum BAFF and the mRNA TNF-α intralesional expression levels. We have also investigated their association with psoriasis severity and looked for the correlation between BAFF titres and the mRNA TNF-α intralesional ones.
Patients and methods: Serum BAFF was measured in 62 patients with psoriasis and 50 healthy controls (HC). Skin biopsy specimens were taken from both lesional and non lesional skin of 24 among the 62 patients and from 4 HC skin. BAFF levels were detected by ELISA. TNF-α mRNA expression levels were analyzed by QRT/PCR. Disease activity was evaluated according to the Psoriasis Area and Severity Index (PASI) score.
Results: The level of serum BAFF was significantly increased in patients with psoriasis compared to HC ($p < 10^{-3}$). In addition, both elevation in serum BAFF and mRNA TNF-α intralesional expression levels were associated with disease activity ($p < 10^{-3}$ in the two cases). In addition, BAFF serum and mRNA TNF-α intralesional expression levels were higher in PsA group when compared to all other psoriatic patients (respectively $p = 0.002$ and $p < 10^{-3}$). More interestingly, the titres of serum BAFF were correlated with mRNA TNF-α intralesional expression levels ($p = 0.008$).
Conclusion: Our results suggest that BAFF may be involved in the pathogenesis of psoriasis. Nevertheless, it is unclear which mechanisms contribute to the high BAFF levels. It is conceivable that an inflammatory process triggers, via the production of inflammatory cytokines notably TNF-α was incriminated.
Disclosure of interest: None declared.

Lipid mediators

P-094
DIVERSE IMPACT OF SECRETORY PHOSPHOLIPASES A2, 11A, V AND X ON PROINFLAMMATORY ACTIVITY OF VASCULAR SMOOTH MUSCLE CELLS (VSMC)

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Diverse Impact of Secretory Phospholipases A_2 IIA, V and X on Pro-inflammatory Activity of Vascular Smooth Muscle Cells (VSMC) Secretory phospholipases A_2 ($sPLA_2$) are lipolytic enzymes, acting mainly on PtdCho of plasma lipoproteins. Their impact on VSMC is considered to be one of the proinflammatory mechanisms leading to atherosclerosis. We investigated the impact of three $sPLA_2$, IIA, V and X on mitogenesis and migration of VSMC and the relationship of physico-chemical state of the milieu on the activity of $sPLA_2$'s and on their interaction with HDL, HDL_3 and LDL. It included the role of the particle size, cholesterol content and sphingomyelin/PtdCho ratio of plasma lipids. The methodology was described in detail in: Pruzanski et al., Lab Invest 81: 757, 2001, BBA 1736: 38, 2005, BBA 1771: 5, 2007 and J. Lipid Res. 49:2161, 2008. The results showed that mitogenic activity of VSMC was most significantly enhanced by gr V $sPLA_2$. $sPLA_2$ hydrolysis of HDL and LDL, enhanced mitogenic activity in order V>X>IIA. Release of PGE_2 from the VSMC was enhanced by gr X and less by V, whereas the release of LTB_4 was enhanced by gr V and less by gr X. Migration of VSMC was enhanced only by gr IIA in a dose/time related manner. All three $sPLA_2$'s showed high sensitivity to the physico-chemical changes in the environment of the digestion medium. Enzymatic activity of $sPLA_2$'s correlated with the lower sphingomyelin/Ptd Cho ratio in HDL as compared to LDL. HDL_3 was hydrolysed more rapidly by all three $sPLA_2$'s and correlated with the higher proportion of the long chains of sphingomyelin in HDL_3. The above studies show that the $sPLA_2$'s, IIA, V and X, exert variable but detrimental effect on VSMC. Their interaction with plasma lipids depend on the physico-chemical state of the milieu and all three participate in the complex proinflammatory mechanism of atherogenesis, especially by reducing HDL level.
Disclosure of interest: None declared.

P-095
THE EFFECT OF DUAL ANTAGONISTS FOR CYSTENYL LEUKOTRIENE RECEPTOR AND THROMBOXANE A2 ON EXPERIMENTAL ALLERGIC ASTHMA IN GUINEA PIGS

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Bronchial asthma is characterized by allergic airway inflammation caused by various mediators. Many recent studies demonstrated the importance of lipid mediators in the onset and development of bronchial asthma. These studies promote our investigation to find out a new inhibitor of lipid mediator as a remedy for allergic bronchial asthma. Our purpose of this study is to evaluate the effects of dual antagonists for cystenyl leukotriene receptor and thromboxane A2 receptor (KP-496 and RS-601) on experimental allergic asthma in guinea pigs. Actively sensitized animals were repeatedly exposed to antigen, and drugs were administered [KP-496 (0.01 and 0.1%); inhalation for 5 min and RS-601 (3 and 10 mg/kg); per orally 2 h] before every antigen exposure. KP-496 (0.1%) and RS-601 (3 and 10 mg/kg) significantly inhibited an antigen-induced increase of airway resistance at 4 h after antigen challenge (late phase response) and airway hyperresponsiveness to acetylcholine. Furthermore KP-496 and RS-601 clearly suppressed the infiltration of inflammatory cells into airway. From above experiments, two dual antagonist, KP-496 and RS-601 showed the inhibitory action on both an asthmatic respiratory disorder and inflammatory changes of airway in guinea pigs. These findings suggest they will be beneficial agents in the treatment of bronchial asthma. (All experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals Science.)
Disclosure of interest: None declared.

P-096
MOLECULAR MECHANISMS OF SPHINGOSINE 1-PHOSPHATE SIGNALING TO ENHANCE OSTEOBLAST DIFFERENTIATION

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Background: Sphingosine 1-phosphate (S1P) is one of the cell-derived lysophospholipid growth factors that signal diverse cellular functions such as proliferation, angiogenesis, and inflammation. We previously demonstrated that S1P signaling induced proliferation and prostaglandin productions by synovial cells from rheumatoid arthritis (RA) patients. FTY720(FTY) is a high-affinity agonist of S1P receptors which induces internalization of S1P receptors, rendering the cells unresponsive to S1P. The present study we investigated the role of S1P signaling for osteoblast differentiation using C2C12, a murine myoid cell line, which differentiate into osteoblasts.
Methods: Osteoblast differentiation was induced by the treatment of bone morphogenic protein (BMP)-2 in the presence or absence either S1P or FTY. Osteoblast differentiation was determined by osteoblast-specific transcription factor Runx2 mRNA expression after 2 days of culture, ALP activity in the cells after 7 days of culture, or osteocalcin production by the cells after 10 days of culture. Smad1,5,8 and ERK1/2 phosphorylation was examined by western blotting.
Results: Runx2 expression, ALP activity and osteocalcin production by BMP-2-stimulated C2C12 was enhanced by addition of either S1P or FTY. This enhancing effect of FTY on osteoblast differentiation was stronger than that of S1P. Both S1P and FTY enhanced BMP-2 stimulated Smad1,5,8 phosphorylation of C2C12. These enhancing effects of S1P/FTY were inhibited by addition of MEK1/2/inhibitor (U0126), indicating that S1P signaling enhanced BMP-2-Smad axis via activation of MEK1/2, ERK1/2 axis.
Conclusions: These results suggest that S1P signaling enhances BMP-2-Smad axis via activation of MEK1/2-ERK1/2 axis, thereby augmenting osteoblast differentiation. FTY may be useful for the treatment of RA by enhancing osteoblast differentiation as well as the inhibition of inflammatory cells infiltration in the synovium.
Disclosure of interest: None declared.

P-097
AIRWAY REMODELING IN MURINE ASTHMA CORRELATES WITH A DEFECT IN PGE2 SYNTHESIS BY LUNG FIBROBLASTS

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Asthma is a chronic inflammatory disease of the airways that can lead to airway fibrosis. One of the key events in this process is the local activation of fibroblasts to myofibroblasts, which expresses α-smooth muscle actin (α-SMA) and actively synthesizes and secretes type I collagen. PGE_2 is a lipid mediator that inhibits fibroblast activation by ligating the EP2 receptor. Idiopathic pulmonary fibrosis is associated with a defect in fibroblast PGE_2 synthesis and/or responsiveness. We investigated if the airway fibrosis in murine asthma is associated with a defect in the synthesis/response axis of PGE_2 by lung fibroblasts. Female BALB/c mice were immunized 2× with ovalbumin (OVA) in alum (ip) and subjected to 3, 7 or 12 airway challenges with OVA in PBS. Lung sections were stained for Collagen-1 and PAS. Fibroblasts were isolated from lung minces after the indicated number of challenges and grown in culture. Cells were incubated with PGE_2, the selective EP2 analog butaprost free acid (BFA), and the direct activator of adenyl cyclase forskolin. Proliferation was measured by [^{3}H]-thymidine incorporation and α-SMA and collagen-I synthesis by western blot. PGE_2 was measured by EIA and EP2 receptor expression by real time RT-PCR. Collagen deposition around airways was absent after 3 challenges, minimal after 7, and intense after 12. This was accompanied by a time-dependent impairment in the ability of the lung fibroblasts to upregulate PGE_2 under IL-1β stimulation, which was maximal after 12 challenges. At this time point we also observed a reduced expression of the PGE_2 synthetic enzymes COX-2 and mPGEs1. The responsiveness of fibroblasts to PGE_2, measured as proliferation, α-SMA expression and collagen-I synthesis, as well as EP2 expression, was not different among the groups. Since PGE_2 inhibits fibroblast activation, a defect in the ability of fibroblasts from animals with chronic asthma to produce this molecule may be implicated in the pathogenesis of airway fibrosis.
Disclosure of interest: None declared.

P-098
PTPN1 AS A MODULATOR OF PLATELET-ACTIVATING FACTOR-INDUCED RESPONSES IN HUMAN DENDRITIC CELLS

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Among pro-inflammatory lipids, platelet-activating factor (PAF) is a key mediator in the induction and progression of many pathophysiologic states such as atherosclerosis, where dendritic cells (DC) can act as regulators of disease progression. As some signalling pathways activated by PAF involve JAK kinases, we investigated the modulation of functional responses of DCs to PAF by one of the regulators these kinases, protein-tyrosine phosphatase non-receptor type 1 (PTPN1). Using DCs, generated from monocytes isolated from healthy volunteers, we down-regulated PTPN1 expression using siRNAs, which decreased PTPN1 protein expression by 25%. Here, we show that even if modest, this down-regulation of PTPN1 is enough to modify the profile of cytokine mRNA expression observed after PAF stimulation, without affecting maturation of DCs. In DCs transfected with PTPN1 siRNAs, as compared to those transfected with control siRNAs, PAF induced significantly higher mRNA levels of IL-6, IL-8 and CCL2 whereas IL-10 level was not significantly affected. On the other hand, PAF-induced mRNA levels of TNF alpha and TGF beta were drastically decreased in presence of PTPN1 siRNAs. Luciferase assay studies in PAF receptor-transfected HEK 293 cells showed that over-expressed PTPN1 down-regulated promoter activity of IL-6 and IL-8 in response to PAF. Our results suggest that the effects of PTPN1 on IL-6 promoter activity are dependent on Tyk2 and Src kinase activity, given that over-expression of mutants of either kinase partially rescues the activity of the IL-6 promoter in response to PAF. Together, our results suggest that PTPN1 is an important modulator of PAF-induced functional responses by influencing cytokine production.
Disclosure of interest: None declared.

P-099
LPA INDUCES LEUKOCYTE RECRUITMENT TO INFLAMMATORY SITES IN VIVO VIA LPA1 AND LPA3

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LPA stimulates fibroblast-like synoviocyte migration in a wound-closing assay and the secretion of IL-8 and IL-6. As IL-8 is a potent chemoattractant involved in inflammatory cell influx into the human synovium and autotaxin-derived LPA has been detected in synovial fluids from rheumatoid arthritis (RA) patients, we assumed that LPA might recruit leukocytes to inflammatory sites either directly or indirectly by stimulating the release of cytokines/chemokines. In this study we used a murine air pouch model to study the influx of immune cells. To assess LPA receptor dependency and the mechanisms of LPA-induced leukocyte recruitment in vivo we used both a pharmacological and a genetic approach. We report that (1) LPA injected into air pouches recruited leukocytes in a concentration- and time-dependent manner; (2) in LPA3$^{-/-}$ mice, LPA-induced leukocyte influx into the air pouch was partially inhibited while LPA3 agonist OMPT-induced leukocyte influx was totally blocked. The LPA1/3 antagonist VPC32183 injected either locally into the air pouch or intravenously completely inhibited LPA-induced leukocyte recruitment in WT and LPA3 deficient mice; and (3) TNFα injected into the air pouch prior to LPA strongly enhanced LPA-mediated leukocyte influx as well as chemokine secretion, such as KC. Interestingly, the CXCR2 chemokine receptor antagonist SB22502 administered i.v. to mice reduced, in a concentration-dependent manner, LPA-mediated recruitment of leukocytes into the air pouch. The ability of LPA to recruit leukocytes and the enhanced production of cytokines/chemokines in TNFα-primed air pouches suggest that LPA, via LPA1 and LPA3 receptors, may contribute to the pathogenesis of RA. The results provide novel insights into the relevance of LPA receptors as potential therapeutic targets for the treatment of autoimmune diseases such as RA.
Disclosure of interest: None declared.

P-100
GENERATION OF FATTY ACID CHLOROHYDRINS BY ADIPOSE TISSUE IN ACUTE PANCREATITIS

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Background: Acute pancreatitis is an inflammatory process of the pancreatic gland that in the severe forms triggers the inflammation in remote organs. An additional characteristic of pancreatitis is the necrosis of peripancreatic adipose tissue due to the release of lipolytic enzymes. These areas of adipose tissue release in turn inflammatory mediators. Here we have evaluated the generation of halogenated free fatty acids by necrotic adipose tissue in a model of acute pancreatitis.
Methods: Pancreatitis was induced in male rats by intraductal administration of 3.5% sodium taurocholate. We obtained samples of adipose tissue and ascitic fluid 3, 6 and 18 h after induction, and the levels of free fatty acids as well as fatty acid chlorohydrins were evaluated by GC–MS. In additional experiments we administered fatty acid chlorohydrins, generated by chlorination of adipose tissue lipid extracts, on the peritoneum of control animals. Three hours later we obtained peritoneal macrophages and the expression of TNFα on these cells was evaluated by RT-PCR.
Results: During pancreatitis, necrotic areas of adipose tissue generate and release free fatty acids as well as its chlorohydrins. We identified oleic acid chlorohydrin and mono- and bis-chlorohydrin of linoleic acid in both adipose tissue and ascitic fluid. Administration of chlorinated lipids in the peritoneal cavity results in an increased expression of TNFα by peritoneal macrophages.
Conclusion: We conclude that during severe acute pancreatitis, the necrotic areas of the peripancreatic adipose tissue generate chlorinated fatty acids. These halogenated lipids could activate macrophages and plays a role in the progression of the systemic inflammation.
Disclosure of interest: None declared.

P-101
THE LRP1-SHCA COMPLEX SHIFTS THE INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) SIGNALING PATHWAY FROM DIFFERENTIATION TO CLONAL EXPANSION DURING ADIPOGENESIS

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The Low-density lipoprotein receptor-related protein (LRP1) is a transmembrane receptor that integrates multiple signaling pathways. Its cytoplasmic domain serves as docking sites for several adaptor proteins such as the Src homology 2/α-collagen (ShcA), which also binds to several tyrosine kinase receptors (TKRs) such as the insulin-like growth factor 1 (IGF-1) receptor. However, the physiological significance of the physical interaction between LRP1 and ShcA, and whether this interaction modifies TKRs signaling, are still unknown. Here we report that LRP1 forms a complex with the IGF-1 receptor, and that LRP1 is required for ShcA to become sensitive to IGF-1 stimulation. Upon IGF-1 treatment, ShcA is tyrosine phosphorylated and translocates to the plasma membrane only in the presence of LRP1. This leads to the recruitment of the Growth factor receptor-bound protein 2 (Grb2) to ShcA, and activation of the Ras/MAP kinase pathway. Conversely, in the absence of ShcA, IGF-1 signaling bifurcates towards the Akt/mTOR pathway and accelerates adipocyte differentiation when cells are stimulated for adipogenesis. These results establish the LRP1/ShcA complex as an essential component in the IGF-1-regulated pathway for MAP kinase and Akt/mTOR activation, and may help to understand how IGF-1 signaling shift from clonal expansion to growth-arrested cells and differentiation during adipogenesis.
Disclosure of interest: None declared.

P-102
SPHINGOSINE-1-PHOSPHATE MODULATES EXPRESSION OF VASCULAR ENDOTHERIAL GROWTH FACTOR (VEGF) IN HUMAN ARTICULAR CHONDROCYTES

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Objective: Although an important role of sphingosine-1-phosphate (S1P) in arthritis has been suggested, its function in chondrocytes is still not fully known. On the other hand, an important involvement of vascular endothelial growth factor (VEGF) in the pathogenesis of osteoarthritis (OA), probably through regulating angiogenesis has been speculated. We here investigated the in vitro effect of S1P on the expression of VEGF in human articular chondrocytes from OA patients.
Methods: Human articular cartilage samples were obtained from patients with OA under informed consent. Chondrocytes were isolated using enzymatic procedure and then cultured in vitro by monolayer, and the cells were stimulated with S1P in the presence or absence of inhibitors of mitogen activated protein kinase (MAPK) signaling molecules and a Gi protein inhibitor pertussis toxin (PTX). VEGF expression and its secretion in culture supernatants were analyzed using quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA).
Results: Although S1P did not enhance basal secretion of MMP-1 and MMP-13, it stimulated VEGF expression in human articular chondrocytes, both at mRNA and protein levels. MAPK inhibitors SB203580 and PD98059 were not effective to suppress the VEGF induction; rather, blocking of the ERK MAPK enhanced the VEGF expression. The Gi protein inhibitor PTX partially attenuated the S1P-induced VEGF secretion.
Conclusions: A potential contribution of S1P in the regulation of VEGF expression in human chondrocytes has been suggested. S1P may thus play a unique role in the pathophysiology of OA through regulating VEGF expression in chondrocytes.
Disclosure of interest: None declared.

P-103
CONTRIBUTION OF COX1 AND COX2 TO PROSTACYCLIN RELEASE BY HEALTHY MOUSE TISSUES

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It is has been suggested that COX2 is the primary source of the protective metabolite, PGI_2 (prostacyclin), because selective COX2 inhibitors lower urinary levels of its metabolites. In most healthy

tissues, however, COX2 protein is rare in comparison to COX1 and we have previously shown that COX1- but not COX2-deficiency prevents PGI_2 release by isolated arteries. Using this bioassay approach, we sought to determine the relative role of COX1 and COX2 in PGI_2 synthesis in other tissues of healthy mice.
Methods: PGI_2 release was determined in 10 mg pieces of heart, liver, kidney and spleen from wild type (WT), $COX1^{-/-}$ and $COX2^{-/-}$ mice ($n = 4$). Within 10 min of death, tissues were dissected and incubated in DMEM containing A23187 Ca^{2+} ionophore (50 μM) with or without the COX1/2 inhibitor diclofenac (100 μM) for 30 min at 37°C. Medium was then removed and PGI_2 measured as its stable breakdown product, 6-keto-$PGF_{1\alpha}$ by ELISA.
Results: In heart pieces, 6-keto-$PGF_{1\alpha}$ accumulation was not altered by COX2-deficiency (WT: 8.2 ± 2.8 ng/ml; $COX2^{-/-}$: 6.0 ± 3.3 ng/ml) but was abolished by COX1-deficiency (<0.3 ng/ml; $p < 0.05$). In liver pieces too, 6-keto-$PGF_{1\alpha}$ accumulation was similar between tissue of WT and $COX2^{-/-}$ mice (WT: 4.0 ± 0.5 ng/ml; $COX2^{-/-}$: 3.5 ± 0.8 ng/ml) but absent in tissue from $COX1^{-/-}$ mice (<0.3 ng/ml; $p < 0.001$). In kidney pieces, 6-keto-$PGF_{1\alpha}$ levels were not different between tissues of WT and $COX2^{-/-}$ mice (WT: 8.2 ± 1.3 ng/ml; $COX2^{-/-}$: 11.1 ± 4.6 ng/ml) but were undetectable in $COX1^{-/-}$ tissues (<0.3 ng/ml; $p < 0.001$). Finally, in spleen pieces, 6-keto-$PGF_{1\alpha}$ accumulation from WT tissues (45.2 ± 8.7 ng/ml) was abolished by COX1-deficiency (<0.3 ng/ml; $p < 0.001$) but not altered by COX2-deficiency (49.1 ± 11.5 ng/ml). In all tissues from WT and $COX2^{-/-}$ mice, diclofenac produced >75% inhibition of 6-keto-$PGF_{1\alpha}$ accumulation.
Conclusion: The primary COX isoform responsible for PGI_2 production in these healthy mouse tissues is COX1 with little contribution from COX2.
Disclosure of interest: None declared.

P-104
LIPOXIN A4 IS A NOVEL ESTROGEN RECEPTOR AGONIST

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Lipoxins (LXs), are endogenously produced eicosanoids, which possess potent antiinflammatory and proresolution bioactivities. The role of LXs in the endometrium is unknown. Our initial observations showed LXA_4 enhanced estrogen receptor (ER)-mediated transcriptional activation in Ishikawa endometrial epithelial cells. Furthermore, we demonstrated that LXA_4 possesses robust estrogenic activity through its capacity to alter cellular proliferation as well as the expression of estrogen-regulated genes implicated in cancer development. Interestingly, LXA_4 also demonstrated anti-estrogenic potential in that it attenuated E2-mediated cellular proliferation, consistent with the effects of a partial ER agonist. Subsequent studies revealed that these actions of LXA_4 were directly mediated by ERα and appear to closely mimic those of the potent estrogen, 17β-Estradiol (E2). Using competitive radioligand binding assays, we confirmed that this lipid binds ER. We additionally demonstrated this estrogenic activity of LXA_4 in mouse uterus in vivo using a uterotrophic assay and the expression of E2-dependent genes as readouts. Taken together our results establish a dual capacity of LXA_4 to modulate estrogenic activity in the endometrium. These findings highlight a previously unappreciated paradigm in LXA_4–mediated activities and reveal novel immuno-endocrine crosstalk mechanisms.
Disclosure of interest: None declared.

P-105
LTB4 IS INVOLVED IN PATTERN OF SUSCEPTIBILTY AND RESISTANCE IN HISTOPLASMA CAPSULATUM INFECTION

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Introduction and objectives: *Histoplasma capsulatum* (*H.capsulatum*) is a dimorphic pathogenic fungus that causes a wide spectrum of diseases. LTs are potent lipid mediators of inflammation and host defense, derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid (AA) metabolism. Recent studies show that susceptibility or resistance of different strains to certain infections, such as *Leishmania amazonensis*, is associated with differential production of LTs. In the present study, we evaluated the LTB_4 production in mice and peritoneal macrophages (PMs) from susceptible and resistant mice after challenge with *H. capsulatum.*
Methods and Results: Mice sv129 (resistant) and C57BL/6 (susceptible) were infected with 5×10^5 yeasts of *H. capsulatum* by intratracheal route. Peritoneal macrophages (PMs) from both strains were infected in vitro for 24 h at a ratio of 1:5 (*H. capsulatum*:macrophage). Lung homogenates and supernatants were collected and the production of LTB_4 was evaluated by ELISA. The phagocytosis was assessed by fluorescence using unopsonized or IgG-opsonized FITC-labeled *H.capsulatum* and MK886, a LTs inhibitor, was added previously to the infection. Susceptible mice showed increased fungal recovery and decreased LTB_4 production when compared with resistant mice. Interestingly, PMs from resistant mice produced higher levels of LTB_4 upon *H. capsulatum* challenge than did those from susceptible mice. As expected, PMs from sv129 phagocytosed 1.9-fold-increased IgG-opsonized-*H.capsulatum* than PMs from C57BL/6. However, phagocytosis of IgG-opsonized-*H.capsulatum* by PMs from C57BL/6 and sv129 was dependent on the endogenous synthesis LTs.
Conclusion: LTB_4 is important mediator involved in the mechanisms of host defense by participating in the patterns of resistance/susceptibility to infection of *H. capsulatum.*
Disclosure of interest: None declared.

P-106
PLGA MICROSPHERES LOADING PROSTANOIDS ARE PHAGOCYTIZED BY ALVEOLAR MACROPHAGE: A POSSIBLE IMMUNE PROTECTION DURING HISTOPLASMOSIS

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Prostaglandins play an important role in inflammatory processes. PGD2 and PGE2 are biological antagonistic, respectively, stimulating or inhibiting the removal of microorganisms in the host. However, these compounds possess poor hydro solubility and chemical instability, difficulty there in vivo administration. It is well known that microspheres (MS) are able to improve stability and sustain the release of substances and target their deliveries. The aim of this work was to prepare and characterize MS containing PGD2 or PGE2; in vitro evaluation of their ability to be phagocyted by alveolars macrophages (AMs); establish the involvement of PGD2 in Histoplasmosis in vivo. The PLGA [poly-(lactic acid-glycolic acid)] MS were prepared by the emulsification-solvent evaporation technique. Size and zeta potential were evaluated in aqueous media by Light Scattering, and morphology analyzed by Scan Electronic Microscopy. The size, zeta potential and shape of MS (MS-PGD2, MS-PGE2 and unload-MS) were proper to be administered through the intranasal route and able to attempt the lung. Phagocytosis were determined after 4 h with 2×10^5 AMs using 1 mg of each MS. Both MS were efficiently phagocytosed by cells. MS-PGD2 had the highest phagocytic index and MS-PGE2 the lowest, when compared with unloaded-MS. MS-PGD2 induced NO_2^- production by AMs. MS-PGD2 or PGD2 soluble decreased cell recruitment to the bronchoalveolar space and decreased the number of yeasts from the lungs and spleens in mice infected with *Histoplasma capsulatum*. Finally, MS obtained in this work could be an alternative approach to development of novel therapeutics based on the lipid mediators.
Disclosure of interest: None declared.

P-107
GAMMA DELTA T LYMPHOCYTES REQUIRE LTB4-BLT1 AXIS TO MIGRATE DURING INFLAMMATION

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Gamma delta T lymphocytes migrate into inflammatory sites and participate in inflammatory and infectious responses. We investigated the involvement of the 5-LO-derived lipid mediator LTB_4 in gamma delta T cell migration. When injected into the intrapleural (i.pl.) space of C57BL/6 mice, LTB_4 triggered gamma delta T lymphocyte mobilization in vivo, a phenomenon also observed in in vitro chemotaxis assays. The i.pl. injection of *Escherichia coli* (LPS) triggered increased levels of LTB_4 in pleural cavities. The in vivo inhibition of LTB_4 biosynthesis by the 5-LO inhibitor zileuton or the FLAP inhibitor MK886 attenuated LPS-induced gamma delta T cell accumulation into pleural cavities. Accordingly, 5-LO KO mice failed to recruit gamma delta T cells into the inflammatory site after i.pl. LPS. Antagonists of the high-affinity LTB_4 receptor BLT1, CP105,696, and LY292476 also attenuated LPS-induced gamma delta T cell accumulation in pleural cavities as well as in vitro chemotaxis toward pleural washes obtained from LPS-simulated mice. LTB_4/BLT1 also accounted for gamma delta T cell migration induced by i.pl. administration of *Mycobacterium bovis* BCG or antigen in sensitized mice. BLT1 was expressed on naïve, resident as well as LPS-recruited gamma delta T cells. Isolated gamma delta T cells were found to undergo F-actin cytoskeleton reorganization when incubated with LTB_4 in vitro, confirming that gamma delta T lymphocytes can respond directly to LTB_4. In addition to its direct effect on gamma delta T cells, LTB_4 triggered their accumulation indirectly, via modulation of CCL2 production in mouse pleural cavities. These data show that gamma delta T cell migration into the pleural cavity of mice during diverse inflammatory responses is dependent on LTB_4/BLT1.
Disclosure of interest: None declared.

P-108
MECHANISMS INVOLVED IN THE INHIBITION OF 5-LIPOXYGENASE PRODUCT BIOSYNTHESIS BY CATALYTICALLY INACTIVE 5-LIPOXYGENASE ISOFORMS

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Leukotrienes play an important role in innate immunity and are active participants in inflammatory diseases such as asthma, atherosclerosis and rheumatoid arthritis. 5-lipoxygenase (5-LO) is the crucial enzyme for leukotriene biosynthesis by catalyzing the transformation of arachidonic acid to leukotriene A_4. We identified several splicing variants of 5-LO in lymphoid and myeloid cell lines and in human neutrophils. All cells that were tested expressed an mRNA encoding for 5-LO that contained the expected 14 exons of the full-length 5-LO. Additionally, we identified several variants of the 5-LO mRNA: one that retained intron 10 (α-10), another that lacked exon 13 (Δ-13), one that lacked exons 10 and 13 (Δ-10,13) and one that lacked the first 96 base pairs of exon 10 (Δ-p10). When the cDNA of these splicing variants were transfected in HEK293 cells, the resulting 5-LO isoforms were expressed but were devoid of catalytic activity. Immunoblot analyses with recombinant 5-LO isoforms demonstrated the expression of the Δ-13 isoform in Raji cells and in human neutrophils. Interestingly, when variant 5-LO isoforms were co-expressed with the active full-length 5-LO, biosynthesis of 5-LO products was inhibited. The focus of ongoing studies is to understand mechanisms regulating the expression 5-LO isoforms and to determine the molecular interactions involved in their inhibition of the biosynthesis of 5-LO products. We are currently investigating the subcellular distribution of the different 5-LO isoforms and their putative interactions with 5-LO-activating protein (FLAP) and coactosin-like-protein (CLP). Overall, these variant 5-LO isoforms likely represent a novel endogenous mechanism for the regulation of the 5-LO pathway and lipid mediator biosynthesis. (*Supported by the Heart and Stoke Foundation of Canada and the Canada Research Chairs (MES); the Natural Sciences and Engineering Research Council of Canada (NF); a scholarship from the Canadian Arthritis Network (LHB)*).
Disclosure of interest: None declared.

P-109
LIPOXINA4 SUPRESSES HEPATOCELLULAR CARCINOMA VIA BLOCKING MACROPHAGES' ACTIVATION

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Aim: To investigate the anti-tumor effect of lipoxin A4 (LXA4), a endogenous pro-resolution lipid mediator, on hepatocellular carcinoma in vitro, in vivo and its mechanism.
Methods: HepG2, U937, THP-1, RAW264.7 and H22 cells were cultured. To assess the effect of tumor-assassinated macrophages (TAM) on HCC cells, in some of the experiments, supernatant from activated macrophages was isolated and applied to stimulate HepG2. Cytotoxicity was first screened by 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Morphological observation, immunofluorescence, flow cytometry analysis, western blot were employed to elucidate the anti-tumor mechanism of lipoxin. Anti-tumor activities against subcutaneoulsy implanted solid tumor induced by H22 cells in mice were evaluated.
Results: Lipoxin could significantly suppress hepatocellular carcinoma, inhibit the proliferation, angiogenesis and invasive ability of HepG2 cells, promote its apoptosis via blocking the activation of macrophages, and in vivo experiment, BML-111, LXA4 receptor (ALX) agonist could inhibit the growth, invasion and angiogenesis in H22 bearing mice.
Conclusion: LXA4 effectively suppressed hepatocarcinoma. It could be a possible candidate for liver cancer therapy, and blocking macrophages' activation will be an effective drug target for liver cancer therapy.
Disclosure of interest: None declared.

P-110
LPS-DIFFERENTIATED MONOCYTIC MONO MAC-1 CELLS ARE ENRICHED IN ARACHIDONIC ACID AND EXPRESS 5-LIPOXYGENASE

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The human monocyte-like Mono Mac-1 (MM1) cell line is a recognized model to study monocyte/macrophage differentiation. In this study, we investigated whether their differentiation with LPS would impact polyunsaturated fatty acid (PUFA) metabolism. Upon treatment of MM1 cells with LPS, the expression of the monocyte marker CD14 was enhanced and this correlated with a significant increase in 5-lipoxygenase expression (assessed by qPCR and immunoblot). Importantly, major changes in the cellular fatty acid profile were also observed. The 16-carbon monounsaturated to saturated fatty acids ratio was greatly decreased in differentiated cells, which would indicate a decrease in stearoyl-CoA desaturase-1 expression during differentiation. Interestingly, we found that differentiated MM1 cells' phospholipids were enriched in 20:4n-6 (arachidonic acid, AA) and 20:5n−3 (eicosapentaenoic acid, EPA) which are the precursors of pro- and anti-inflammatory lipids mediators. Additional experiments were performed to elucidate the putative mechanisms involved in AA and EPA enrichment. When incubated with their 18-carbon precursors, differentiated MM1 cells accumulated twice as much AA and EPA compared to undifferentiated cells. Similarly, the retroconversion to AA and EPA from their 22-carbon counterparts was also more efficient in differentiated cells. Finally and in agreement with the enhanced 5-LO expression and the AA and EPA buildup, we observed that differentiated MM1 cells also acquired the capacity to release AA and to synthesize leukotriene B_4 following ionophore stimulation. In conclusion, MM1 cells represent a good model to investigate changes in fatty acid metabolism and incorporation into phospholipids and in eicosanoid metabolism that accompany human monocyte–macrophage differentiation. (Supported by the Atlantic Innovation Fund (MS), the Canada Research Chairs Program (MS) and the Natural Sciences and Engineering Research Council of Canada (NF)).
Disclosure of interest: None declared.

P-111
CAFFEIC ACID PHENETHYL ESTER (CAPE), A COMPONENT OF PROPOLIS FROM HONEYBEE HIVES, IS A POTENT INHIBITOR OF LEUKOTRIENE BIOSYNTHESIS IN HUMAN NEUTROPHILS

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5-lipoxygenase (5-LO), expressed in a number of myeloid cells such as B cells, monocytes, neutrophils, and mast cells, is the key enzyme in the bioconversion of arachidonic acid into leukotrienes (LTs). LTs are important lipid mediators of inflammation that are also involved in the progression of various inflammatory diseases. A number of naturally occurring compounds have been investigated as potential inhibitors of 5-LO and of LTs biosynthesis. Amongst these are polyhydroxylated compounds such as caffeic acid that are widely distributed in plants and exhibit anti-oxidant and anti-inflammatory properties. In this study, we demonstrate that the naturally occurring caffeic acid phenethyl ester (CAPE), a component of propolis from honeybee hives, is a potent inhibitor of LTs in human neutrophils. The calculated IC_{50} for the inhibition of LTs biosynthesis by CAPE and the known 5-LO inhibitor zileuton were 0.51 and 3.49 μM, respectively. Synthesis of some structural analogues of CAPE was also performed to investigate their structure–activity relationship as free radical scavengers, antioxidants and 5-LO inhibitors. Both ester and amide analogs of CAPE were designed with the rational that esters may be more susceptible to chemical and enzymatic degradation compared to the corresponding amide. Our results show that while these compounds are effective free radical scavengers and antioxidants, certain structural features are required for effective inhibition of LTs biosynthesis in human neutrophils. (Supported by the Heart and Stoke Foundation of Canada and the Canada Research Chairs Program (MES); a scholarship from the Canadian Arthritis Network (LHB) and New-Brunswick Innovation Foundation and Canadian Innovation Foundation (MT)).
Disclosure of interest: None declared.

P-112
CORE AND NS5 PROTEINS OF HEPATITIS C VIRUS INDUCES FORMATION OF LIPID BODIES AND LEUKOTRIENE B4, BUT NOT OF PROSTAGLANDIN E2 IN CELLS OF HEPATITIS C VIRUS PATIENTS INFECTED

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Hepatitis caused by hepatitis C virus is a serious global public health, the main route of transmission is related to contact with contaminated blood or intravenous drug use. Various evidences have suggested that the formation of LBs during some infections. This is a phenomenon that may have implications in the pathogenesis of these infections and is related to the formation of LTB4 and PGE2. The purpose of this study was to characterize the presence of LBs and LTB4 and PGE2 synthesis in peripheral blood mononuclear cells of individuals infected with hepatitis C. The study included 70 candidates for blood donation, which in the period of August/2009 at July/2010 showed reactivity in serological screening tests (ELISA and immunoblot) in the Foundation of Hematology of the Amazon. Peripheral blood mononuclear cells were cultured from under different stimulis for subsequent staining and counting of LBs. The LTB4 and PGE2 in the cell culture supernatant was determinated. We observe that the core and NS5 proteins of HCV stimulates the formation of lipid bodies in cells of HCV positive patients stimulated in vitro, but not induces PGE2 production in this group. However LTB4 is increased in patients infected with HCV compared with HCV negative individuals. HCV positive individuals have increased concentrations of IL-12 and IL-10 in peripheral blood. Our data suggest that in individuals infected with hepatitis C there is a weak signal for the Th1 response and a high number of LBs in PBMC and high concentration of LTB4, IL-12 and IL-10 when compared to HCV patients negative. These results suggest one possible mechanisms of viral escape and may be associated with chronic infection.
Financial support: CNPq and FAPEAM.
Disclosure of interest: None declared.

Reactive intermediates: reactive oxygen species, NO, CO, H2S,…

P-113
BIPHASIC MODULATION OF SKIN BLOOD FLOW BY ENDOGENOUS HYDROGEN SULFIDE IN RATS

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Alteration in skin blood flow has been implicated in a variety of clinical conditions associated with inflammation such as urticaria and diabetes. Hydrogen sulfide (H_2S) has recently been identified as an endogenous gaseous transmitter with ability to induce both vasodilatation and vasoconstriction in a variety of vascular beds. There is little known about the contribution of hydrogen sulfide in modulation of skin blood flow. The present study was designed to investigate the effect of local and systemic administration of hydrogen sulfide on skin blood flow in anesthetized rats. Male Wistar rats were used in this study. Cutaneous blood flow was recorded from soles skin using a laser Doppler flowmeter. Local inhibition of cystathionine-γ-lyase (the enzyme responsible for synthesis of H_2S in vasculature) was associated with a dose-dependent increase in skin blood flow. Cutaneous application of sodium sulfide (a H_2S donor) induced a biphasic response in presence of cystathionine-γ-lyase inhibitor. While low concentration of sodium sulphide made a significant decrease in skin blood flow, higher concertinos induced vasodilatation. Intravenous administration of sodium sulfide induced a significantly less cutaneous vasodilatation in presence of cystathionine-γ-lyase inhibitorThis study is the first study to show a biphasic effect for local H_2S in modulation of skin blood flow in an experimental model.
Disclosure of interest: None declared.

P-114
ROLE OF HYDROGEN SULFIDE AND ITS PRODUCING ENZYMES CYSTATHIONINE-BETA- SYNTHASE (CBS) AND CYSTATHIONINE-GAMMA-LYASE (CSE) IN RAT KNEE

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Objective: Exogenous H_2S delivered to rat knee joint significantly inhibited carrageenan (CGN)-induced synovitis; supporting the role of H_2S donors as therapeutic alternatives for arthritis. We now aimed to identify the existence of H_2S producing enzymes in rat knee and their role in synovitis model.
Methods: Under approval of USP Animal Ethics Committee, anaesthetized rats pretreated with cystathionine-γ-lyase (CSE) inhibitor (DL-propargylglycine; PGly), an H_2S donor (Lawesson's reagent; LR) or saline were intra-articular injected with carrageenan (CGN, 3%) or saline. After 4 h,leukocyte counts were performed in bone marrow (BM), knee samples were collected and, cytokines, protein expression and activity of CSE, cystathionine-b synthase (CBS), caspase-1, NF-kB and AP-1 were measured. The kinetic cycle of H_2S synthesis in synovia was determined based on methylene blue formation method. Data are mean ± SEM for n animals. Statistical analysis was by ANOVA + Bonferroni's test.
Results: Injection of CGN evoked synovitis and increased leukocyte concentration in BM, IL-1b and IL-10 in joint fluid and activity of caspase-1 and NF-kB. Treatment with LR increased IL-10 and attenuated cell infiltration and caspase-1 activity without affecting NF-kB activition. PGLy treatment only augmented AP-1 activity. Quantitative assay shows that CBS and CSE, the key enzymes for H_2S synthesis, were identified in synovial membrane, and H_2S generation augmented in a time-dependent manner. CSE and CBS inhibitors reduce H_2S generation by 50 and 75%, respectively.
Conclusion: The protective effects of H_2S on CGN-induced synovitis might be mediated by increase of IL-10 and down-regulation of caspase-1 activity. This occurs independently of H_2S-producing enzymes found to be constitutively expressed in the rat synovial membrane; reinforcing the importance of exogenous H_2S in arthritis.
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Disclosure of interest: None declared.

P-115
SUPPRESSION OF FIBRONECTIN FRAGMENT-ACTIVATED NF-KB BY HYALURONAN VIA CD44 IN RHEUMATOID ARTHRITIS CHONDROCYTES

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Background: Fibronectin fragments increased in rheumatoid arthritis (RA) joints may contribute to joint destruction because some fibronectin fragments activate catabolic intracellular signals including nuclear factor-κB (NF-κB). Hyaluronan (HA) of high molecular weight inhibits catabolic actions by fibronectin fragments. Although there is evidence that HA works via its cell surface receptors, the involvement of CD44 in HA effect on fibronectin fragment with the NH_2-terminal gelatin-binding domain (FN-f) is still unclear.
Objective: This study was aimed to examine the role of CD44 on HA inhibitory mechanism on NF-κB activation by FN-f in RA chondrocytes.
Methods: Cartilage slices from RA knee joints or chondrocytes in monolayer were cultured with FN-f with or without pretreatment with 2,700 kDa HA. Secreted nitric oxide (NO) levels in conditioned media were determined. Induction of inducible nitric oxide synthase (iNOS) was evaluated with immunoblotting. Activation of NF-κB was assessed with immunoblotting and ELISA. Involvement of CD44 in HA action was evaluated using anti-CD44 antibody.
Results: FN-f-stimulated NO production in a dose-dependent manner in association with iNOS induction. FN-f activated phosphorylation of p65 NF-κB, which resulted in enhanced nuclear translocation of NF-κB. HA suppressed FN-f-stimulated NO production with iNOS down-regulation. Inhibition studies with NF-κB inhibitor indicated the requirement of NF-κB for FN-f-induced NO production. HA suppressed NF-κB activation by FN-f, leading to a decrease in NO production. Anti-CD44 antibody significantly reversed HA effect on FN-f action.
Conclusions: The present study clearly demonstrated that high molecular weight HA suppressed FN-f-activated NF-κB via CD44 in RA chondrocytes. When HA is clinically used for the treatment of RA knee joints by intra-articular injection, it could down-regulate the catabolic action of fibronectin fragments in RA joints through the mechanism demonstrated in this study.
Disclosure of interest: T. Yasuda Grant/Research Support from: Chugai Pharmaceutical Company.

P-116
INHIBITION OF NITRIC OXIDE PRODUCTION AND PROINFLAMMATORY CYTOKINES BY SEVERAL MEDICINAL PLANTS

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A number of medicinal plants have been used to treat various immunological diseases. Nitric oxide (NO) has an important regulatory role in the various types of inflammatory processes. In the present study, extracts of several medicinal plants native to Iran including *Dracocephalum kotschyi*, *Linum persicum*, *Dionysiatermeana, Salvia mirzayanii*, *Ferulago angulata* and *Euphorbia cheiradenia* were investigated for their NO modulatory activity. The level of TNF-α and IL-1β pro-inflammatory cytokines in the macrophage culture were also detected. All the extracts at concentration of 50 μg/ml demonstrated a significant decrease in NO production ($p < 0.001$) at 24 h treatment. This inhibitory effect was also seen after 48 h. Among the extracts, *L. persicum* was the strongest extract in reducing the NO production at 1 μg/ml after both 24 and 48 h (nearly 100% inhibition, $p < 0.001$). *S. mirzayanii* extract showed the mildest effects in 48 h culture. In cytokine release determination, the extract of *L. persicum* significantly inhibited both TNF-α and IL-1β cytokines production by stimulated macrophages ($p < 0.001$). *D. kotschyi*, *D. termeana* and *F. angulata* decreased secretion of IL-1β from the cells. In conclusion, these results indicate the presence of anti-inflammatory and macrophage inhibitory substances in these plants.
Disclosure of interest: None declared.

P-118
PHENOTYPIC DIFFERENCES IN HYDROGEN SULFIDE SYNTHESIS AND SIGNALING IN PRIMARY MACROPHAGES

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Hydrogen sulfide (H_2S) has been shown to be a potent inhibitor of leukocyte infiltration, and appears to contribute to resolution of inflammation in the GI tract. This study investigated both the synthesis and functional characteristics of H_2S in four phenotypically different primary macrophage populations. Bone marrow-derived macrophages are differentiated ex vivo with macrophage-colony stimulating factor, representing a 'classical', inflammatory macrophage. Polyacrylamide-gel beads (BioRad) injected i.p elicited an 'alternative' macrophage phenotype. Finally resident/tissue macrophages were collected from the peritoneal cavity or dissociated from colon lamina propria (LP). Resident peritoneal cells are the first line of host defence, acting to prime the inflammatory response via pattern recognition receptors. LP macrophages, on the other hand, lack many of these receptors due to their proximity to local gut flora. The capacity for macrophages to synthesize H_2S, assessed by methylene blue formation, was shown to be variable across a wide concentration range with LP and resident peritoneal macrophages being the most abundant source. Induction of inflammation, both in vitro and in vivo, reduced the ability of macrophages to synthesize H_2S, paralleled by reduced cystathionine gene and protein expression. The addition of H_2S to macrophage populations modulated cytokine release (TNF-α, IL-10) following inflammatory stimuli, increased phagocytic capacity, and also up-regulated chemotactic responses to *f*MLF in a concentration dependent manner. Mechanisms of H_2S signaling were assessed by intracellular cAMP/cGMP levels and NFκB activation. This study provides the first phenotypic characterisation of H_2S synthesis in different primary macrophage populations. Together, these data suggest an important role for macrophage-derived H_2S in maintenance of tissue homeostasis and resolution of inflammation.
Disclosure of interest: None declared.

P-119
HYDROGEN SULPHIDE REGULATES TRANSIENT RECEPTOR POTENTIAL VANILLOID 1-MEDIATED NEUROGENIC INFLAMMATION IN SEPSIS-ASSOCIATED LUNG INJURY THROUGH ENHANCEMENT OF SUBSTANCE P PRODUCTION

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Hydrogen sulphide (H_2S) is increasingly being recognized as an important pro-inflammatory mediator in various inflammatory conditions including sepsis. The production of H_2S from L-cysteine in the cardiovascular system is catalysed primarily by cystathionine gamma-lyase. H_2S has been shown to induce transient receptor potential vanilloid 1 (TRPV1)-mediated neurogenic inflammation in polymicrobial sepsis. However, endogenous neural factors that modulate this event remain unclear. Here, we examined whether substance P is one important neural element that implicates in H_2S-induced neurogenic inflammation in sepsis-associated lung injury in a TRPV1-dependent manner. Male Swiss mice were subjected to cecal ligation and puncture (CLP)-induced sepsis and treated with TRPV1 antagonist capsazepine 30 min before CLP. DL-propargylglycine, an inhibitor of H_2S formation, was administrated 1 h before or 1 h after CLP, whereas sodium hydrosulfide, an H_2S donor, was given at the same time as CLP. Capsazepine significantly attenuated H_2S-induced substance P production, inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines (MIP-1α, MIP-2) expression, edema formation, and neutrophil sequestration in septic lung. Administration of sodium hydrosulfide further enhanced substance P production and exacerbated lung inflammatory damage but capsazepine reversed these deleterious effects. In the absence of H_2S, capsazepine had no effect on DL-propargylglycine-mediated abrogation of substance P levels, inflammatory cytokine and chemokine production, edema, and myeloperoxidase activity in the lungs of septic mice. Taken together, the present findings reveal that H_2S regulates TRPV1-mediated neurogenic inflammation in sepsis-associated lung injury through enhancement of substance P production. The anti-inflammatory effects of capsazepine may have therapeutic potential for the treatment of sepsis.
Disclosure of interest: None declared.

P-120
STATINS INDUCE HEME-OXYEGNASE-1 IN MOUSE MACROPHAGES : TRANSCRIPTIONAL REGULATION AND ROLE OF NITRIC OXIDE

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Statins have pleiotropic effects in addition to their capacity to lower cholesterol. Heme-oxygenase-1 is an anti-oxidant and anti-inflammatory enzyme responsible for the degradation of heme into biliverdin, carbon monoxide and iron. In this study, we investigated the effect of statins on HO-1 expression in a murine cell line Raw 264.7 and in primary elicited murine peritoneal macrophages (eMPM) obtained from C57BL/6 mice. Induction of HO-1 was obtained in response to 25 mM simvastatin and 10 mM fluvastatin in both Raw 267.4 and peritoneal macrophages. Co-treatment of cells with simvastatin and increasing concentrations of spermine NONOate (SPNO), a nitric oxide donor, or LPS resulted in an additional important expression of HO-1. Statins alone increased NO formation and iNOS expression in Raw 264.7 cells and pre-treatment with inhibitors of NO synthase, *l*-NMMA and 1400 W blocked this effect. This induction was NO independent in primary eMPM cells. In eMPM cells, simvastatin decreased the levels of LPS-induced IL-6 and TNFa. Both fluvastatin and simvastatin increased the promoter activity of the mouse proximal and distal promoter in Raw 264.7 cells which was inhibited by mevalonate and 1400 W. Statins induced phosphorylation of p38 MAP kinase in both cell types and inhibition of these kinases resulted in decrease in HO-1 expression. Gel retardation experiments were performed in both Raw 264.7 cells and primary eMPM for C/EBP, USF and AP-1. Both statins induced nuclear protein-DNA complexes compared to untreated cells after 12 or 24 h incubation with probes for C/EBP or AP-1 but not USF. Protein-DNA complex was supershifted with an antibody specific for C/EBPβ. These data demonstrate that statins has anti-inflammatory effects by decreasing IL-6 and TNFa and increasing HO-1. The role of endogenous NO in statin-induction of HO-1 is variable among different macrophages.
Disclosure of interest: None declared.

P-121
LIPOPOLYSACCHARIDE IN VIVO INCREASEREACTIVE OXYGEN SPECIES GENERATION IN RAT PLATELET MAINLY VIA NADPH-OXIDASE ACTIVATION

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Introduction:The septic responses including thrombocytopenia and increased production of reactive oxygen species (ROS) can be reproduced by LPS in experimental animals.The Src kinase and PI3 K has been involved the ROS production, and may also mediate some effects of LPS. In the present study, we aimed to identify the sources of ROS formation, and to investigate the role of Src and PI3K in modulating ROS formation and aggregation in platelets obtained from LPS-treated rats.
Methods: Wistar rats were injected i.p. with saline or LPS (1 mg/kg), and at 6 or 48 h thereafter arterial blood was collected (Ethical Committee No. 2097-1). ROS production in platelets was measured by flow cytometry DCFH-DA (5 μM). ADP (20 mM)-induced platelet aggregation was evaluated using a two-channel aggregometer.
Results: The ROS production in platelets from LPS-treated rats was 2.5-fold higher ($P < 0.05$) than in saline-injected rats. Incubation of platelets with NADPH oxidase inhibitor DPI (5 μM) significantly attenuated the increased ROS production in LPS group (27 and 60% at 6 and 48 h, respectively). Similarly, the PI3K inhibitor wortmannin (100 nM) and Src kinase inhibitor PP2 (5 nM) significantly attenuated the increased ROS production in ADP (20 μM)-activated platelets in LPS group, whereas acetylsalicylic acid (100 μM) and allopurinol (100 μM) had minimal effects. The ADP-induced platelet

aggregation was significantly reduced in LPS group compared with saline ($P < 0.05$), but incubation with wortmannin or PP2 failed to modify this inhibitory aggregatory response. The incubation of platelets from saline-treated group reduced 60% with wortmannin and 30% with PP2.
Conclusion: Our data indicate that NADPH-oxidase is the main source of ROS formation in platelets isolated from LPS-treated rats, and that activation of Src kinase and PI3 K takes place in the response. On the other hand, Src kinase and PI3 K are not involved in the reduced platelet aggregation seen in LPS-treated rats.
Disclosure of interest: None declared.

P-122
OXIDATIVE STRESS AND MODIFICATION OF RENAL VASCULAR PERMEABILITY ARE ASSOCIATED WITH ACUTE KIDNEY INJURY DURING P. BERGHEI ANKA INFECTION

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Introduction: Some clinical studies speculate that the pathophysiology of malaria associated-acute kidney injury (AKI) may occurs by nephrotoxicity of products of oxidative stress, element present in *Plasmodium* infection, as well as pro-inflammatory response induced by the parasite. This resume briefly summarized our progress in the understanding of mechanisms involved in malaria associated-AKI development during experimental model of severe malaria.
Methods: The parasite load and renal function were monitored in Balb/c mice infected with 10^5 parasitized erythrocytes with *P. berghei* ANKA. To assess renal function, blood urea nitrogen and serum creatinine was evaluated. The presence of parasitized erythrocytes and profile of pro and anti-inflammatory molecules was quantified by real time PCR (kidney tissue) and Bioplex (serum). Products of oxidative stress were estimated by ELISA in plasma of animals described above.
Results: So far, we observed an increase of serum levels of creatinine and urea, as well as a decrease of ratio of creatininuria and proteinuria during acute phase of infection. Furthermore, it was found the adhesion of parasitized erythrocytes in the kidneys of infected mice. These data are consistent with the increased expression of mRNA of iNOS, HIF-1a, IFN-gand ICAM-1, associated with a decrease in the expression of mRNA HO-1 in kidney tissue of infected mice. The measurement of lipoprotein oxidizabilityalso showed a significant increase in plasma and urine of infected animals.
Conclusions: Together, our findings bring some elucidation about mechanisms of acute kidney injury associated with oxidative stress and structural changes during acute phase of *plasmodium* infection.
Supported by CAPES, CNPq and INCT.
Disclosure of interest: None declared.

P-123
HOMOCYSTEINE ENHANCES MMP-9 PRODUCTION THROUGH CYCLOOXYGENASE GENERATION OF ROS MEDIATED ERK AND AKT SIGNAL PATHWAYS

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Homocysteine (Hcy) is a metabolic risk factor for cardiovascular disease. High levels of homocysteine promote oxidant stress in vascular cells and tissue because of the formation of reactive oxygen species (ROS), which have been strongly implicated in development and progression of diabetes mellitus by the activation of matrix metalloproteinase-9 (MMP-9). Thus, we investigated the sources of ROS generation and mechanismsn of MMP-9 production in murine macrophages stimulated with homocysteines. Homocysteine enhanced protein expression and gelatinolytic activity of MMP-9 in murine macrophages. The enhanced MMP-9 expression and activity was significantly attenuated not only by PD98059, a ERK inhibitor but also by SH-5, an Akt inhibitor, respectively. In line with these results, homocysteine exclusively increased the phosphorylation of ERK and AKT, suggesting a role for the ERK and AKT pathway in homocysteine-induced MMP-9 expression and activity. Furthermore, homocysteine stimulated macrophage cells to showed an increased production of ROS, which was significantly attenuated by cyclooxygenase inhibitors such as indomethacin, but not affected by other oxidase inhibitors involving xanthine oxidase, lipoxygenase and mitochondrial, suggesting a potential role of cyclooxygenase in homocysteine-induced ROS generation. Taken together, it is suggested that homocysteine enhances MMP-9 production in macrophages via cyclooxygenase generation of ROS-mediated activation of ERK/Akt signaling pathways, consequently leading to vascular remodeling in diabetes.
Disclosure of interest: None declared.

P-124
PKC AND PI3 K-DEPENDENT SIGNALING PATHWAYS ARE INVOLVED IN LIPOPOLYSACCHARIDES STIMULATION OF CYSTATHIONINE-GAMMA-LYASE GENE EXPRESSION IN RAW264.7 CELLS

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Endogenous H_2S might play an important role in the pathogenesis of inflammatory diseases. Hydrogen sulfide (H_2S) can be endogenously produced in mammalian tissues from cysteine by cystathionine-γ-lyase (CSE). Our previous study has demonstrated lipopolysaccharides (LPS) stimulated the CSE expression and H_2S formation in macrophages. In this study, we investigated the signaling pathways involved in LPS-induced CSE expression in RAW264.7 macrophages. It was found that LPS induced the phosphorylation of protein kinase C (PKC) α. PKCα/β inhibitor GÖ6976 blocked the LPS-induced CSE mRNA expression and CSE-promoter activity. PKC activator phorbol 12-myristate 13-acetate (PMA) significantly stimulated CSE mRNA expression and CSE-promoter activity. LPS treatment also induced the phosphorylation of extracellular signal-regulated kinases (ERK) and p38. However, both p38 inhibitor SB203580 and ERK inhibitor PD98059 could not affect the effect of LPS on CSE mRNA expression and CSE-promoter activity. It is known that phosphoinositide-3-kinase (PI3 K) is the down-stream signaling of LPS. Treatment of cells with PI3K inhibitor LY294002 blocked the LPS-induced CSE mRNA expression and CSE-promoter activity. Our results suggest that PKC and PI3K signaling pathways be involved in LPS regulation of CSE expression in macrophages.

Keywords: Lipopolysaccharides, Cystathionine-γ-lyase, Macrophage, Protein kinase C, Phosphoinositide-3-kinase.

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P-125
NITRIC OXIDE GENERATION IN ENDOTHELIAL CELLS FROM KININ B1 RECEPTOR KNOCKOUT MICE

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Objective: Kinin B_1 receptors are highly induced in the course of inflammation. The present study analyzed the Nitric Oxide (NO) production in primary cultured endothelium obtained from lung explants of B_1 receptor knockout ($B_1^{-/-}$) and wild type (WT) mice.
Methods: Cells (n = 3–4 for each group) were pre-incubated with DAF-2 DA (10 μmol/L), fluorescence was detected in a confocal microscope and quantified by optic densitometry. Images were obtained before and after 5 min of acethylcoline [ACh (1 mmol/L)] stimulation, with 15 s intervals between each image capture. Cells were pre-treated with the NOSynthase (NOS) co-factor tetrahydrobiopterin [BH4 (0.1 mmol/L)] or the substrate L-arginine (1 mmol/L). Control experiments were performed in WT cells in the presence L-NAME (1 mmol/L) or B_1 antagonist [des-arg^9-leu^8-BK (0.1 mmol/L)].
Results: ACh caused a gradual increase in fluorescence that was lower in $B_1^{-/-}$ cells during the whole 5 min of image capture. In all experiments, maximal responses (expressed as *arbitrary units*) were reached 270 s after ACh stimulation, thus, this point of the time curves was chosen to be compared in all treatments. At this point NO release was markedly reduced in $B_1^{-/-}$ (35.8 ± 3.1*) in comparison to WT cells (66.9 ± 3.2). $B_1^{-/-}$ responses were reversed by BH_4 (54.3 ± 1.7), but not by L-arginine (26.2 ± 8.5*). In WT cells, BH_4, L-arginine and des-arg^9-leu^8-BK had no effect (55.0 ± 9.2, 51.1 ± 11.9 and 55.3 ± 4.8, respectively), whereas NO production was blunted by L-NAME (35.8 ± 4.7*) (* $P < 0.05$ vs. WT/ACh).
Conclusions: Targeted B_1 receptor deletion impairs endothelial NO production by mechanisms involved in the reduction of the co-factor BH_4 availability. Since deficient levels of BH_4 induce uncoupling of NOS and production of reactive oxygen species, a relevant role of endothelial B_1 receptors in modulating vascular oxidative stress during inflammation can be suggested.
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P-126
THE EFFECTS OF GARLIC EXTRACT ON THE INFLAMMATORY PATHWAYS IN HEPATIC INJURY

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Background and objectives: Inflammatory responses management such as Reactive Oxygen Species (ROS) formation, lipid peroxidation and reduction in conjugating capacitance of hepatocytes are so important in preventing cell necrosis and death due to some injuries like acetaminophenoverdose which causes severe hepatic necrosis or exposure to some oxidant metal ions.Studies on this field remain a very active area since some of current data are still uncertain. In this study, freshly isolated rat hepatocytes were used to determine the effects of garlic extract on the acetaminophen and metal ion -induced cell cytotoxicity and to compare it with the effect of *N*-Acetyl Cysteine (NAC) as a standard treatment.
Materials and methods: Male Sprague–Dawley rats (200–250 g) wereanaesthetized and after hepatocyte isolation, the cell suspensions were studied and the amount of ROS formation, lipid peroxidation and glutathione depletion were determined using spectrofluorometry, spectrophotometry and analytical HPLC methods respectively, in different groups of cell suspensions.
Results and conclusion: The results of this study indicate that, garlic extract was significantly effective ($P < 0.05$) effective in decreasing ROS formation, lipid peroxidation and glutathione depletion induced by acetaminophen overdose, as well as the first two factors in Ferric ion exposure; but was not significantly effective ($P > 0.05$) in glutathione depletion caused by Ferric ion. NAC was completely effective ($P < 0.01$) in reducing all 3 necrotic factors. In conclusion, we can suggest garlic extract as an anti inflammatory agent in preventing lipid peroxidation and ROS formation. It seems that more studies are necessary on different cell types to confirm this hypothesis.
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P-127
NADPH-OXIDASE INHIBITION THROUGH APOCYNIN MODULATES INFLAMMATORY PROCESS IN RENAL ISCHEMIA AND REPERFUSION INJURY

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Introduction: Acute kidney injury (AKI) is a clinical condition associated with high levels of morbidity and mortality. The ischemia and reperfusion injury (IRI) is one of the most relevant conditions that lead to AKI. The presence of inflammatory cytokines, chemokines, adhesion molecules and the recruitment of leukocytes into the post-ischemic kidneys characterizes the inflammatory process. Apocynin is a pro drug that is converted to a dimer form through the oxidation of mieloperoxidase. It prevents the translocation of the cytosolic oxidase components to the membrane fraction of NADPH-oxidase. The aim of this study is to evaluate the ability of apocynin in preventing the inflammatory process associated to renal IRI.
Methods: C57/BL6 mice were subjected to renal IRI, having their renal pedicles clamped bilaterally for 45 min, and after that, renal blood flow were restored. Sacrifice was performed 24 h after reperfusion. Animals were divided in three groups: sham, IRI and apocynin (4 mg/kg i.p. just before reperfusion). During sacrifice, blood and renal tissue were collected. Renal function was evaluated by serum creatinine and urea

levels. Histological alteration was observed by HE staining. RT-PCR was performed for TNF and IL-6 and IKK expression were assessed by western blotting.

Results: We observed that IRI group presented higher creatinine and urea levels when compared to sham group; whereas in apocynin group it was restored. Histological evaluation showed less inflammatory infiltrates on treated group. Also, IRI showed a significant increase on gene expression of TNF and IL-6, when compared to the other groups. Finally, IKK protein expression was higher on IRI group and apocynin treatment was able to decrease it.

Conclusion: Treatment with apocynin reduced renal dysfunction and preserved histological architecture. Moreover, it also modulated the inflammatory process, leading to renoprotection. Financial Support: FAPESP/CNPq/INCT Complex Fluids.
Disclosure of interest: None declared.

P-128
NEONATAL MALNUTRITION AND PRODUCTION OF NITRIC OXIDE: STUDY OF IN VITRO INFECTION OF ALVEOLAR MACROPHAGES BY STAPHYLOCOCCUS AUREUS

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Objective: To evaluate the production kinetics of nitric oxide (NO) by alveolar macrophages (AM) in well-nourished (N) and malnourished (MN) rats after in vitro infection with *Staphylococcus aureus*.
Methodology: male Wistar rats (n = 12) were breastfed by rats whose diet contained 17% and 8% of protein during lactation (N and NM group, respectively). After weaning, both groups were administered a normoproteic (23% of protein) diet. AM were obtained from the brochoalveolar fluid after tracheotomy. Following the isolation procedures, three systems were set up: negative control containing AM (NC), positive control containing AM and lipopolisaccharide (PC) and SA containing AM and a suspension of ATCC 29213 (OD = 0.15, corresponding to 10^{-6} CFU.mL^{-1}). Systems were then incubated at 37°C in a 5% CO_2 atmosphere for 24 h. During incubation, samples of 100 μL from the supernatant were collected at intervals of 2 h and subsequently nitric oxide was measured with the Greiss reaction. Statistical analysis were performed using Student t test and Mann–Whitney assuming a significance level of $p < 0.05$.
Results: malnutrition led to the decrease of macrophageal production of NO in all systems ($p < 0.005$). Major production of NO was reached at the intervals between 4 and 8 h in both experimental groups, N and MN ($p < 0.001$) in the SA system. In addition, a decrease in nitrite concentration was observed after 10 h with similar values as determined in NC system ($p > 0.005$).
Conclusion: the model of neonatal malnutrition promoted disorders to the function of phagocytic cells, as well as interfered in the macrophageal nitrosative stress. Moreover, *S. aureus* possibly has developed a mechanism for reducing the NO concentration in the extracellular fluid) in order to preserve its cellular integrity.
Disclosure of interest: None declared.

P-129
ENHANCED OXIDATIVE STRESS AS A POTENTIAL MECHANISM INVOLVED IN THE EFFECTS OF FORMALDEHYDE ON LUNG INFLAMMATION

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Introduction: Exposure to air pollutants such as formaldehyde (FA) leads to inflammation, oxidative stress and immune-modulation in airways. FA is emitted by building materials, furniture carpets, wallpaper, plywood, floor coverings and sterilizing agents. We recently demonstrated that FA effects on airways are partially due to an increased oxidative stress. However, the source of reactive oxygen species (ROS) remained to be determined. It is investigated herein the role of antioxidant and oxidant enzymes on the release of ROS after FA exposure.
Material and methods: Male Wistar rats were exposed to FA inhalation (1%, 90 min daily) for three consecutive days. After 24 h of the last FA inhalation, the activities and gene expression of glutathione peroxidase (GPX), glutathione redutase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD) 1 and 2, catalase (CAT), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)- 1 were determined in lung tissue.
Results: FA inhalation did not modify the activity of GPX, GR, GST and CAT, but reduced the activity of SOD when compared to the naïve group. A significant increase in the SOD 1 and 2, CAT, iNOS and COX-1 expression were observed in FA group when compared to the naïve group.
Conclusion: Our data are indicative that FA causes a disruption of the physiological balance of oxidant and antioxidant enzymes in lung tissue, most probably favoring the oxidant pathways and thus positively modulating lung inflammation.
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Disclosure of interest: None declared.

P-130
THE ANTIAPOPTOTIC EFFECT OF LEUKOTRIENE B4 IN NEUTROPHILS: A ROLE FOR NADPH OXIDASE-DERIVED ROS AND NF-KB

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Leukotriene B4 (LTB_4), an arachidonic acid-derived lipid mediator, is a known proinflammatory agent released in many inflammatory situations and it is able to activate biological responses in human neutrophils (PMN) as well as reactive oxygen species (ROS) generation by the NADPH oxidase complex. LTB_4 delays neutrophils spontaneous apoptosis through the activation of classical pro-survival signaling, which in turn may corroborate to the onset of a chronic inflammatory condition. Recently, ROS have emerged as second-messengers, coordinating intracellular signaling cascades, and thus modulating several biological phenomena, including apoptosis. In this study, we aim to elucidate the putative role of NADPH oxidase-derived ROS in LTB_4 antiapoptotic effect. PMN were isolated from whole blood of healthy volunteers by Ficoll-Paque^{TM} density gradient. ROS production was evaluated by cytochrome *c* reduction as well as lucigenin and luminol enhanced-chemiluminescence. Apoptosis was determined by cell morphology and annexin V-phosphatidylserine binding. Mitochondrial membrane potential was assessed by flow cytometry of JC-1-stained cells. Protein expression was evaluated by western blot analysis of total or nuclear extracts. Our data show that NADPH oxidase-derived ROS are critical to LTB_4 pro-survival effect on neutrophils. This event depends on redox modulation of NF-κB translocation and IκB-α phosphorylation/degradation. We have also observed that LTB_4-induced Bad degradation and mitochondrial stability requires NADPH oxidase activity. Our results strongly indicate that LTB_4-induced antiapoptotic effect in neutrophils occurs via ROS-dependent signaling routes and we do believe that a better knowledge of molecular mechanisms underlying neutrophils spontaneous apoptosis may contribute to design better strategies to control chronic inflammation.

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Disclosure of interest: None declared.

Proteases and anti-proteases

P-131
EFFECTS OF RELAXIN ON THE EXPRESSION OF MMP-1 AND MMP-8 IN HUMAN PERIODONTAL LIGAMENT CELLS

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Objectives: Relaxin (RLX), a member of the insulin/relaxin family of structurally related hormones, has an influence on many physiologic processes, such as collagen turnover, angiogenesis, and antifibrosis. The periodontal ligament (PDL) contains fibroblasts, blood vessels, nerves, and a large amount of collagen, primarily of type I. The homeostasis of the PDL implicates towards intensive and subtle transcriptional and translational regulation of the collagens and the matrix metalloproteinases (MMPs) genes. In response to mechanical stress during orthodontic tooth movement, the PDL produces inflammatory cytokines and MMPs in vivo. Therefore, it is considered that thishormone may have an influence on orthodontic tooth movement through alterations of PDL. In the present study,we investigated the effects of RLX on the release and gene expression of MMP-1 and 8 by hPDL cells in vitro.

Methods: hPDL cells were cultured with 100 ng/ml RLX for 0–72 h. The mRNA levels of the MMP-1 and 8 were determined by using real-time PCR and the protein levels were determined by using ELISA. Effects of specific inhibitors of p38 mitogen-activated protein kinase (MAPK) activity, extracellular signal-related kinase (ERK) activity and c-Jun N-terminal kinase (JNK) activity on RLX-induced MMP-1 and 8 expression in hPDL cells were also investigated by real-time PCR.

Results: Real-time PCR analysis revealed that RLX increased MMP-1 and 8 mRNA expression in a time-dependent manner. The release of MMP-1 and 8 also increased in a time-dependent manner. ERK inhibitor and JNK inhibitor considerably decreased the RLX-induced MMP-1 expression, whereas p38MAPK inhibitor markedly inhibited MMP-8 expression.

Conclusion: These results indicate that RLX stimulated the expression of MMP-1 and 8 in hPDL cells via MAPK signaling pathways. Therefore, RLX may contribute to periodontal connective tissue and bone matrix remodeling during orthodontic tooth movement via MMP-1 and 8.

Disclosure of interest: None declared.

P-132
COOPERATION OF MEDULLASIN AND AZUROCIDIN IN ELASTINOLYSIS BY GRANULOCYTES

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We clarified the mechanism of elastinolysis by granulocytes. Proteases in granulocytes that have been reported to have elastinolytic activity, such as neutrophil elastase, proteinase-3 (p29b, AGP7, proteinase-4), showed negligible elastinolytic activity when orcein-elastin was used as a substrate. Although medullasin alone showed negligible elastinolytic activity, it expressed significant elastinolytic activity in the presence of the granulocyte protein, azurocidin, in the incubation mixture. The mechanism by which the product of partial digestion of azurocidin by medullasin induced the elastinolytic activity of medullasin was studied. Neither the low nor high-molecular weight fractions produced by the digestion of azurocidin by medulllasin showed elastinolytic activity by themselves. However, medullasin expressed significant elastinolytic activity in the presence of high-molecular-weight fraction produced by the digestion of azurocidin by medullasin. The product of partial digestion of azurocidin by medullasin forced the protease to adsorb to elastin fibers, thereby, inducing elastinolytic activity. These results indicate that elastinolytic activity of granulocytes is not derived from single proteases such as medullasin, neutrophil elastase, proteinase-3, but from the cooperation between medullasin and azurocidin in granulocytes.

Disclosure of interest: None declared.

P-133
STIMULATORY EFFECTS OF A SNAKE VENOM METALLOPROTEINASE (BAP1) ON ISOLATED SYNOVIOCYTES

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Matrix metalloproteinases (MMPs), a family of Zn-dependent neutral proteolytic enzymes, are known to play a critical role in erosion of cartilage during arthritis. Moreover, levels of these enzymes are increased in inflamed articular joints. MMPs and snake venom metalloproteinases (SVMPs) share common catalytic domain organization and exhibit identical Zn-binding motif. Therefore, studies on SVMPs may provide insights into the functions of MMPs. In this study we investigated the effects of BaP1 on isolated synoviocytes, the main articular cells involved in production of inflammatory mediators, evaluating: (a) release of prostaglandin E_2 (PGE_2); (b) gene and protein expression of ciclooxigenase-2(COX-2); (c) participation of NF-kB in COX-2 protein expression, and (d) expression of EP4 receptor. B type synoviocytes isolated from rat knee joint synovial membranes were used. Levels of PGE_2 were measured by EIA, and gene and protein expression of both COX-2 and EP4 receptor determined by real time RT-PCR and W.blot, respectively. Results showed that BaP1 induced release of PGE_2 from B type synoviocytes after 1, 3 and 6 h, but not after 30 min incubation, in comparison with control cells. BaP1 induced COX-2 gene and protein expression by synoviocytes (30 min–3 h) without modification on constitutive COX-1. Inhibition of NF-kB by TPCK or SN50 compounds significantly decreased BaP1-induced COX-2 protein expression. Increased levels of EP4 receptor expression (52 and 65 kDa) were also detected in synoviocytes stimulated by BaP1 (3 h). In conclusion, BaP1 can directly stimulate synoviocytes to synthesize PGE_2 and express COX-2. This effect is mediated by NF-kB. In addition, BaP1 is able to increase expression of EP4, a PGE_2 receptor.This may contribute for amplification of PGE_2-induced effects. These findings suggest novel regulatory mechanisms for metalloproteinases in B type synoviocytes, which may be relevant for inflammation and pain in the joints.
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Disclosure of interest: None declared.

P-134
PROTEINASE-ACTIVATED RECEPTOR 2 STIMULATES COLONIC EPITHELIAL CYCLOOXYGENASE-2 EXPRESSION VIA METALLOPROTEINASE-DEPENDENT EGF RECEPTOR TRANSACTIVATION

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Proteinase-activated receptor (PAR) 2, a G-protein-coupled receptor activated through N-terminal cleavage by serine proteinases, has been implicated in both intestinal inflammation and epithelial proliferation. Cyclooxygenase (COX)-2 is over-expressed in the gut during inflammation as well as in colon cancer. We hypothesized that PAR_2 drives COX-2 expression in intestinal epithelial cells. Treatment of Caco-2 colon cancer cells with the PAR_2-activating peptide 2-furoyl-LIGRLO-NH_2 (2fLI) for 3 h led to an increase in intracellular COX-2 protein expression that was not observed following treatment with a reverse-sequence PAR_2-inactive peptide. COX-2 expression was also increased in Caco-2 cells following a 3 h treatment with either trypsin, cathepsin G or proteinase 3, all serine proteinases known to cleave the N-terminus of PAR_2. Treatment of Caco-2 cells with 2fLI for 30 min significantly increased metalloproteinase activity in the cell culture supernatant. Increased epidermal growth factor receptor (EGFR) phosphorylation was observed in cell lysates following 40 min of treatment with 2fLI. The broad-spectrum metalloproteinase inhibitor marimastat inhibited both COX-2 expression and EGFR phosphorylation. The EGFR tyrosine kinase inhibitor PD153035 also abolished 2fLI-induced COX-2 expression. Although PAR_2 activation increased ERK MAPK phosphorylation, ERK pathway inhibitors failed to affect 2fLI-induced COX-2 expression. However, inhibition of Rho kinase signalling by Y27632 and inhibition of PI3 kinase signalling by LY294002 each prevented 2fLI-induced COX-2 expression. In conclusion, PAR_2 activation drives COX-2 expression in Caco-2 cells via metalloproteinase-dependent EGFR transactivation and activation of Rho kinase and PI3 kinase signalling. Our findings provide a mechanism whereby PAR_2 participates in the progression from chronic inflammation to cancer in the intestine.
Disclosure of interest: None declared.

P-135
CROSS TALK BETWEEN TOLL-LIKE RECEPTOR 4 AND PROTEINASE ACTIVATED RECEPTOR 2 IS INVOLVED IN VASCULAR FUNCTION

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PARs and TLRs receptors are involved in innate immune response. Aim of this study was to evaluate the possible cross-talk between PAR-2 and TLR4 in vascular district in physiological condition by using in vivo and ex vivo models. In this study we used thoracic aortas harvested from both naïve and endotoxemic rats for in vitro studies. Arterial blood pressure was monitored in anesthetized rats in vivo. In aortic homogenates immunoprecipitation of TLR4 receptor followed by PAR-2 immunoblot and western blot analysis for TLR4 and PAR-2 were performed. PAR-2, but not TLR4, expression was enhanced in aorta of endotoxemic rats. PAR2-AP-induced vasorelaxation was increased in LPS-treated rats aortic rings. TLR4 inhibitors, curcumine and resveratrol, reduced PAR-2-AP-induced vasorelaxation and PAR-2-AP-induced hypotension in both naïve and endotoxemic rats. Immunoblotting with anti-PAR-2 of anti-TLR4 immunoprecipitates revealed PAR-2/TLR4 association in both naïve and endotoxemic rats. Western blot performed on surnatants of anti-TLR4 immunoprecipitates showed the presence of PAR-2 exclusively in aorta of endotoxemic rats. Finally, in $TLR4^{-/-}$ mouse aortic rings, PAR2-AP-induced vasodilatation was impaired compared to wild type. PAR-2 and TLR4 are constitutively associated and contribute to vascular homeostasis.
Disclosure of interest: None declared.

P-136
INTRACELLULAR PATHWAYS RESPONSIBLE FOR THE TRYPSIN-INDUCED INCREASE OF TRANSEPITHELIAL RESISTANCE IN INTESTINAL EPITHELIAL CELLS

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Patients with clinically active Crohn's Disease exhibit increased intestinal permeability. However, apical addition of serine proteases

like trypsin to intestinal epithelial cell monolayers reduces permeability as shown by increased transepithelial resistance (R_{TE}). This effect of trypsin is independent of activation of protease-activated receptors, but the underlying mechanism is unknown. Given the central role of epidermal growth factor receptor (EGFR) in epithelial cell biology and homeostasis, we hypothesized that trypsin increases R_{TE} by activating EGFR.
Methods: R_{TE} of confluent SCBN monolayers and mouse colonic tissue was measured in Ussing chambers. To determine a role for EGFR-associated signaling, cells were pretreated with inhibitors of EGFR (PD153035), ErBb2 (AG879), ERK-1/2 (PD98059), PI3 K (LY294002) and MMPs (marimastat, MMT) andthen stimulated apically by trypsin or EGF. Flux of FITC-dextran across the monolayer was measured following treatment with PD153035 or AG879 and trypsin.
Results: Apical trypsin increased R_{TE} by 300% ($p < 0.05$) within 60 min. PD153035 or AG879 caused a significant dose-dependent decrease in trypsin-induced ΔR_{TE}, with IC_{50}s of 0.38 and 2.73 μM, respectively. PD98059, LY294002 and MMT also reduced trypsin-induced ΔR_{TE} by 66, 63 and 25% respectively. Pretreatment of cells with PD153035 or AG879 prevented trypsin-induced reduction of dextran flux. Apical EGF increased R_{TE} by 80%. PD153035 and AG879 reduced this increase by 99 and 84% respectively. Also, soybean trypsin inhibitor decreased R_{TE} in mouse colon in vitro, suggesting that luminal serine proteases maintain barrier function.
Conclusions: Our data suggest that trypsin increases R_{TE} via activation of EGFR and ErBb2 and partially via activation of ERK-1/2, PI3K and MMPs. Serine proteases may strengthen epithelial barrier to enhance the efficiency of electrolyte and nutrient transport, and may represent a method to counter barrier defects caused by intestinal inflammation.
Disclosure of interest: None declared.

P-137
ANTI-INFLAMMATORY EFFECT OF BLACK TEA. EFFECT ON THE ACTION OF NEUTRAL SERINE PROTEASES IN GRANULOCYTES

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Tea has various beneficial effect on health. Green tea contains catechins, theanine, and vitamins such as folic acid and ascorbic acid which exert healthy effect. In contrast to green tea, black tea contains polymers of catechins such as theaflavin or thearubigin other than catechins. In this study we examined the effect of black tea extract, theaflavin, and thearubigin on the activity of neutral serine proteases in granulocytes. Neutral serine proteases in granulocytes such as medullasin and cathepsin G were shown to play important roles in biophylaxis. Both proteases stimulate lymphocyte function and enhance NK cell activity. Medullasin causes inflammation leading to the development of atherosclerosis. Mice fed with diet containing black tea components such as theaflavin or thearubigin revealed decreased medullasin activity dose-dependently, but cathpsin G activity decreased only slightly. In contrast to black tea components, addition of green tea components such as epicatechin, epigallocatechin, and epigallocatechin gallate to the diet of mice caused an increment of both medullasin and cathepsin G activity. From these results described above drinking of black tea is considered to be useful for the prevention of atherosclerosis by decreasing medullasin activity in granulocytes.
Disclosure of interest: None declared.

P-138
ELASTASE–PROTEOLYTIC STIMULUS LEADS TO REDOX IMBALANCE IN THE EARLY STAGE OF PULMONARY INFLAMMATORY RESPONSE

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Pulmonary emphysema has become epidemic worldwide. The mechanisms by which extracellular matrix degradation occurs are not yet fully understood. However, literature described both inflammatory and oxidative processes as critical to parenchyma destruction. Thus, this study focused to investigate the partipation of oxidative stress during the emphysema establishment process induced by elastase in mice. C57BL/6 mice were submitted at a protocol with pancreatic porcine elastase (PPE) instillation (0.05 U, 0.5 U (i.t.) or 3 U (i.n.) of PPE per animal), to induce pulmonary emphysema in vivo ($n = 6$). Lung tissue was collected on days 1, 7, 14 and 21 after PPE instilation. Vehicle treated mice were used as controls. Emphysema typical morphological alterations were seen just at 21 days after PPE 0.5 U. TNF-α was elevated at 7 and 14 days after PPE 0.5 U, and was accompanied by a reduction in IL-10 levels at the same time-points. MPO was elevated at the three time-points with PPE 0.5 U. The levels of Nitrite, TBARS and the SOD activity were also increased at 7 days after PPE 0.5 U, while CAT activity was decreased. When the dose was enhanced to 3 U of PPE and a group with just 1 day after stimulus was performed, CAT activity presented reduced at 1 day, while SOD activity was increased at the same time-point. These results are corroborated with mRNA expression, with an increase in Nrf2, Sirt-1, CAT and GPx. However, there was no difference in mRNA SOD expression between any groups (control, 1, 7, 14 and 21 days). ELISA for TGF-β1 showed a reduction just at 21 days after 3U of PPE. These results indicate that the proteolytic pathway has a link with the oxidative pathway. These data suggest that redox imbalance generated by proteolytic stimulus may be crucial in the early stage of the injury, and has no effect in later stages, when in fact, emphysema is installed. But the stimulus for the antioxidant response remains enhanced, as seen by the RT-PCR.
Disclosure of interest: None declared.

P-139
HEPARANASE AS A SIGNALING MOLECULE IN CANCER AND INFLAMMATION

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Heparanase is a β-D-endoglucuronidase which degrades heparan sulfate, a key component of the extracellular matrix and basement membrane. The degradation of the ECM is essential for both

physiological and pathological processes, including inflammation, wound healing, tumour angiogenesis and metastasis. Heparanase is also known to have non-enzymatic functions by regulating cell adhesion, cell signalling and differentiation. Although heparanase has been proposed to facilitate leukocyte migration through degradation of the ECM and basement membrane, its role in generating an inflammatory response by mediating the release of chemokines, cytokines and growth factors have not been determined. In this study, the role of heparanase in regulating the expression of these factors in innate immune cells has been studied. Peripheral blood monocyte cells were stimulated in vitro with endotoxin-free heparanase and cytokine release was examined. Heparanase treatment of cells resulted in the release of a range of pro-inflammatory cytokines including IL-8, IL-10, TNF, IL-6 and IL-1β. A similar pattern of cytokine release was observed after addition of soluble heparan sulfate, implying that the role of heparanase in induction of inflammation may be due to the enzymatic role of cleaving of its substrate. This result was validated via an abolishment of cytokine release following the treatment of MyD88$^{-/-}$ mouse spleen cells with heparanase, suggesting that cytokine release occurs via the toll-like receptor pathway, which heparan sulfate fragments are known to signal through. These data suggests that heparanase can promote inflammation via the release of pro-inflammatory cytokines.
Disclosure of interest: None declared.

P-140
ELAFIN ANTIPROTEASE EXPRESSION IS DECREASED WHILE PROTEOLYTIC ACTIVITY WAS INCREASED AT MUCOSAL SURFACE IN IBD PATIENTS

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Animal models suggest that proteolytic balance at the surface of inflamed mucosa is broken towards an increased proteolytic activity. This increase could be explained by an important release of proteases, a decreased expression of endogenous inhibitors, or both. We have investigated here the proteolytic activity released by fresh colonic tissue biopsies of IBD patients (Crohn's disease: CD, and Ulcerative Colitis: UC) and non-IBD patients. The expression of elafin, a serine protease inhibitor naturally expressed at mucosal surfaces was investigated.
Methods: Non-IBD, CD and UC patient colonic biopsies were harvested and incubated (1 h, 37°) in culture media (HBSS). Arginin cleavage specific activity was measured in biopsy supernatants using a chromogenic substrate (tosyl-GPR-*p*Na). Other biopsies were paraffin-embedded and used for the detection of elafin mRNA by a fluorescently labeled riboprobe.
Results: A significant increase in proteolytic activity was detected in supernatants from CD, and UC biopsies compared to control patients. In non-inflamed biopsies, we detected a strong expression of elafin mRNA in the epithelium, while only few cells in the sub-mucosa expressed this mRNA. In both UC and CD biopsies, elafin mRNA was detected in the sub-mucosa in a zone that correlated with infiltrated immune cells. However in the intestinal epithelium itself, elafin mRNA expression was clearly reduced.
Conclusions: Our results showed that in IBD patients, proteolytic balance of the mucosa is disrupted towards an increased proteolytic activity. Elafin mRNA was constitutively expressed by epithelial cells in non-IBD patient tissues, but in CD and UC patient tissues, a down-regulation of elafin was observed in the epithelium. This study showed that the intestinal protease-antiprotease balance is altered in IBD and proposes that deficient expression of elafin by the epithelium could participate to explain this unbalance.
Disclosure of interest: None declared.

P-141
RELEASED OF TRYPSIN IV BY HUMAN INTESTINAL EPITHELIA IN RESPONSE TO PATHOGENIC OR COMMENSAL FORMS OF ESCHERICHIA COLI

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We have previously reported that human intestinal epithelial cells (IECs) released from their basolateral side significant proteolytic activity upon stimulation by LPS. Further, we have observed that LPS-induced increased permeability of intestinal epithelium could be prevented in vitro by treatment with a large spectrum serine protease inhibitor. Here, we investigated the effects of pathogenic (EPEC) or commensal (HB101) forms of *Escherichia. coli* (*E. coli*) on the expression of the trypsinIV by IECs. We also investigated the role of proteolytic activity in *E. coli*-induced increased permeability.
Methods: Caco-2 cells (human IECs) were polarized for 15 days and apically stimulated for 5 h with HB101 and EPEC at 2×10^8 CFU/well, in the presence or not of the serine protease inhibitor FUT-175 (50 mg/ml). Epithelial permeability was evaluated by dextran-FITC apical to basal passage method. We have evaluated the expression of the trypsin IV mRNA by qRT-PCR and trypsinogen IV by western-blot.
Results: We have observed that EPEC, but not HB101 was able to break the barrier function of IECs, increasing the passage of dextran-FITC. The serine protease inhibitor FUT-175 was able to completely prevent this increased permeability in response to EPEC. Trypsin IV mRNA was expressed in Caco-2 cells infected with EPEC, while it was significantly down-regulated when cells were exposed to commensal HB101. The trypsinogen IV protein was also detected in supernatants of IECs exposed to EPEC, but not in supernatants of IECs exposed to HB101.
Conclusions: The intestinal epithelium infected by a pathogenic *E. coli* (EPEC), but not by a commensal form (HB101) becomes leaky and the release of proteases seems to play a major role in this event. Epithelial trypsin IV, which is differentially regulated by pathogenic or commensal forms of *E. coli*, could play an important role in this permeability changes and overall in alerting the immune system to pathogen exposure.
Disclosure of interest: None declared.

P-142
NEUTROPHIL EXTRACELLULAR TRAPS (NETS) CONTRIBUTE TO THE DYSREGULATION OF PROTEOLYSIS IN CYSTIC FIBROSIS (CF) SPUTUM

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Introduction: In CF, the protease/antiprotease imbalance results in the degradation of lung tissue but therapies aiming at reestablishing this balance by the administration of inhibitors gave limited results. The different distribution of neutrophil serine proteases (NSPs) in CF secretions may explain that they are differently regulated by their natural inhibitors. The aim of this work was to investigate the contribution of neutrophil extracellular traps (NETs) to the regulation of NSPs activity.
Methods: Proteases activities and DNA were quantified in CF sputum and in suspensions of neutrophils isolated from sputum or control blood. We analysed the inhibition of proteases by natural inhibitors in total sputum before and after DNase treatment. NETs secretion was triggered by incubation of isolated neutrophils with *S. aureus* and *P. aeruginosa* and NETs were observed by scanning electron microscopy and confocal microscopy.
Results: Elastase (HNE), protease 3 (Pr3) and cathepsin G (CG) are active in CF sputum and resist to inhibition because of their binding to extracellular DNA and other macromolecular compounds. Part of sputum DNA belongs to NETs as deduced from the presence of NETotic neutrophils in sputum. HNE activity in whole sputum increases dramatically after DNase treatment and can be fully inhibited whereas Pr3 and CG activity increase only slightly and still resist inhibition. CF pathogens induce a higher secretion of NETs by sputum neutrophils than by control blood neutrophils but the activity of the 3 NETs-bound proteases can be measured only after their solubilization from NETs by DNase.
Conclusion: We conclude that NETs contribute to DNA load in CF sputum and constitute a reservoir of active proteases that can be mobilized by DNase. The improvement of anti-inflammatory therapies by protease inhibitors requires a solubilization of the three major NSPs in lung secretions in parallel to the administration of inhibitors.
Disclosure of interest: None declared.

P-143
CONVERSION OF THE POLYVALENT SERINE PROTEASE INHIBITOR SERPIN B1 INTO A SPECIFIC INHIBITOR OF THE WEGENER GRANULOMATOSIS AUTOANTIGEN, PROTEINASE 3

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The pathophysiological function of proteinase 3 (PR3) is not well understood mainly because of its close structural and functional resemblance with neutrophil elastase and the absence of a specific inhibitor able to target its active site in vivo. Based on the structural analysis of the active sites of human neutrophil elastase (HNE) and PR3, we have raised recombinant serpins derived from the polyvalent inhibitor serpinB1 (monocyte, neutrophil elastase inhibitor), that specifically inhibit PR3 and differ from wild-type serpinB1 by only few residues in their reactive center loop. The rate constant of association between the best serpinB1 mutant and purified PR3 was 1.4×10^7 M^{-1} s^{-1} which is about 100-fold higher than that observed with wild-type serpinB1, and compares with that of alpha1-PI towards HNE. Proteolytic cleavage of the mutant serpinB1 by HNE does not impair the formation of the SDS stable, irreversible complex with PR3 when a molar excess of HNE is present in the reactional mixture. Mutant serpinB1inhibits soluble PR3 as well as membrane-bound PR3 at the surface of activated neutrophils and clears induced PR3, but not constitutive PR3, from the cell surface. Such a specific inhibitor should help investigating the biological function of this protease and possibly serve as a therapeutic agent in PR3-related inflammatory diseases such as Wegener granulomatosis.
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P-144
RELEASE OF CYTOKINES INDUCED BY A SNAKE VENOM METALLOPROTEINASE IN ARTICULAR JOINT: IN VIVO AND IN VITRO STUDIES

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Snake venom metalloproteinases are structurally related to matrix metalloproteinases (MMPs) with regard catalytic domain. MMPs levels are increased in inflamed articular joints and have been implicated in arthritis. During joint inflammation, articular synovial fibroblasts (B type) are the main cells involved in the release of inflammatory mediators. In this study we investigated the capacity of a metalloproteinase isolated from *Bothrops asper* snake venom (BaP1) to induce release of interleukins (ILs) -1β, -6 and 18 in the articular joints of rats and the effects of this protease on synovial fibroblasts in culture, evaluating cytokines production and TNF-α gene expression. Male Wistar rats were injected with BaP1 (5 mg/joint) or bovine serum albumin (BSA-control). At selected periods of time after this injection concentrations of ILs were determined by EIA. B type synoviocytes were isolated from rat knee joints synovial membranes and incubated with BaP1 (12.5 mg/mL) or medium alone (control). TNF-α gene expression was evaluated by real time PCR. Results showed that in vivo BaP1 induced release of IL-1β, IL-6 (1–3 h) and IL-18 (12–48 h). Stimulation of B type synoviocytes with BaP1 caused release of IL-1β (12–24 h), IL-6 (30 min–24 h), IL-18 (1 and 24 h) and TNF-α (1–6 h) in the supernatant of cultures. In addition, BaP1 induced gene expression of TNF-α (5 min–3 h) in synoviocytes. Together, obtained data showed the capacity of BaP1 to induce the release of ILs in the articular cavity of rats and the ability of this protease to directly stimulate B type synoviocytes to produce and release of inflammatory cytokines. Up regulation of gene expression of TNF-α may be the primary mechanism for production of this mediator induced by BaP1. Moreover, data suggest that B type synoviocytes are target cells for MMPs during inflammatory processes in the articular joints.
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Disclosure of interest: None declared.

P-145
INVOLVEMENT OF INFLAMMASOME PATHWAY AND MATRIX METALLOPROTEINASES IN THE DEVELOPMENT OF LIVER FIBROSIS IN MICE

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Introduction: Liver fibrosis is the result of a complex interaction between different cell types and is characterized by an inflammatory process in response to chronic injury and by the activation of hepatic stellate cells (HSCs), leading to the accumulation of extracellular matrix. We investigated the involvement of inflammasome pathway, the role of P2XR7 and of matrix metalloproteinases (MMP and TIMP-1) in the CCL4-induced inflammatory process and liver fibrosis in mice. Methods: CCL4-induced liver injury was obtained by intraperitoneal injection of 0.35 ml/kg CCL4 dissolved in a 1:12 ratio with mineral oil in C57BL/6 males. Animals were injected once with CCL4 and liver were harvested. In another set of experiments, mice were treated twice a week for 3 weeks and were sacrificed 2 days after the last injection. Livers were harvested either into 10% buffered formalin for histological evaluation or snap-frozen into liquid nitrogen for mRNA or cellular lysate preparations. Collagen deposition was analysed by red Sirius staining. Components of Inflammasome, MMPs and TIMP-1 were analysed by ELISA and/or immunostaining and/or QPCR Results: CCL4 induced a significant collagen deposition in liver after 3 weeks but not at 24 h, as determined by red sirus staining and increased alpha1-collagen expression. Pro-MMP-9 and TIMP-1 were increased both at 24 h and 3 weeks, but MMP-2 only at 3 weeks. An increase in Nlrp3 inflammasome transcription was observed at 24 h and 3 weeks but purinergic receptor P2rx7 and Pannexin 1 were only significantly elevated at 3 weeks. In control mice, immunostaining showed a low expression of P2RX7 and PANX1 in the portal area, NLRP3 was not detectable, whereas CCL4 induced a significant increase expression of NLRP3, PANX1 and P2RX7 at 24 h but more markedly at 3 weeks. Conclusions: These results showed that CCL4-induced an early activation of inflammasome pathway inducing an imbalance between MMP and TIMP-1 and leading to collagen deposition and fibrosis.
Disclosure of interest: None declared.

Sensors and cell signalling

P-146
ROLE OF MYD88, NOD1 AND NOD2 ON P38 MAPK AND NF-KB ACTIVATION AND RANKL/OPG EXPRESSION INDUCED BY TLR AND IL-1R SIGNALING IN BONE MARROW STROMAL CELLS

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Nucleotide-binding oligomerization domain (Nod)-like receptors (NLRs) were initially described as cytosolic receptors for bacterial components while toll-like receptors mediated bacterial recognition at the cell surface. Mutations in *NOD1* and *NOD2* genes are associated with hyperinflammatory responses and stimulation of Nod2 in osteoblasts synergistically enhances osteoclast formation induced by LPS, IL-1α, and TNF-α; suggesting that Nod proteins play an important role modulating signaling pathways associated with the immune response. Since NF-kB and p38 MAPK are signaling pathways involved in the expression of inflammatory mediators, we examined the role of Nod1, Nod2 and MyD88 proteins on the activation of these signaling pathways by western-blot. The involvement of Nod1, Nod2 and MyD88 in the regulation of bone turnover was assessed by determining their role on the modulation of receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) mRNA expression by RT-PCR. Bone marrow stromal cells (BMSCs) were obtained by flushing the marrows of long bones of MyD88, Nod1 and Nod2 KO mice. BMSCs obtained from wild-type C57/Bl6 mice were used as controls. Cells were stimulated with *Escherichia coli* (TLR4 agonist) and *Porphyromonas gingivalis* (TLR2 and -4 agonist) lipopolysaccharide and to interleukin-1beta. MyD88 −/− cells showed decreased activation of NF-κB, which was completely abrogated in cells lacking either Nod1 or Nod2. Activation of p38 MAPK in MyD88 −/− BMSC was preserved, but was impaired in Nod1 −/− and Nod2−/− cells. MyD88, Nod1 and Nod2 were all required for LPS-induced RANKL expression. Inhibition of OPG mRNA induced by LPS and IL-1 required MyD88 and Nod1, but not Nod2. Our results suggest that MyD88, Nod1 and Nod2 modulate activation of NF-κB and p38 MAPK and may play important roles in regulation of bone turnover in inflammatory and infectious conditions.
Disclosure of interest: None declared.

P-147
NOTCH -AND TRANSDUCIN-LIKE ENHANCER OF SPLIT (TLE)-DEPENDENT HISTONE DEACETYLATION EXPLAIN IL-12 P70 INHIBITION BY ZYMOSAN IN HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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IL-12 p70/IL-23 balance is central to the development of the Th1 and Th17 responses. IL-12 p70 and IL-23 share a common chain and differ by another chain, IL-12 p35 (*il12a*) in IL-12 p70 and IL-12 p19 (*il23a*) in IL-23. The molecular mechanism explaining the IL-12 p70/IL-23 balance relies on the regulation of *il12a* and *il23a*. Human monocyte-derived dendritic cells were obtained by differentiation with GM-CSF and IL-4. Phosphorylation of c-Rel and histone H3 by mitogen- and stress-activated kinase (MSK) and protein kinase A were studied in cells and on recombinant proteins. Chromatin immunoprecipitation was used to study the transcriptional regulation of *il12a* and *il23a*. Coimmunoprecipitation assays were used to address the association of the Notch family proteins with acetylated-K14-histone H3. The transcriptional regulation *of il23a* depended on the activation of c-Rel and histone H3 phosphorylation, as judged from the association of c-Rel with the *il23a* promoter and the correlation between IL-23 production and S10-histone H3 phosphorylation. Zymosan blocked the transcription of

il12a induced by other stimuli and triggered the nuclear translocation of the transcriptional repressors Hes1, Hes5, Hey1, and TLE. In addition, zymosan induced the interaction of Hes1 and TLE with histone H3 phosphorylated on S10 and deacetylated on K14. Inhibition of class III histone deacetylases increased the production of IL-12 p70 and partially blunted the inhibitory effect of zymosan on the production of IL-12 p70.
The selective induction of IL-23 by β-glucans is explained by the activation of c-Rel associated with S10-histone H3 phosphorylation in the *il23a* promoter and inhibition of *il12a* transcription by a mechanism involving corepressors with ability to bind TLE and to promote histone deacetylation.
Disclosure of interest: None declared.

P-148
CROTOXIN, A RATTLESNAKE TOXIN, INHIBITS INTRACELLULAR SIGNALING INVOLVED IN PHAGOCYTOSIS BY NEUTROPHILS

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During phagocytosis, the engulfment of the particle begins with the nascent phagosome formation, which occurs at 5 min. At nascent phagosomes an increase on tyrosine phosphorylation and actin polymerization are observed, this polymerization leads to phagosome maturation. Previous studies showed that *Crotalus durissus terrificus* snake venom inhibits the phagocytic activity of macrophages and neutrophils and that crotoxin, the main component of the venom, is responsible for this effect. In macrophages, crotoxin cause reorganization of the actin cytoskeleton and inhibition of phosphotyrosine. The aim of this study was to investigate the effect of crotoxin on tyrosine phosphorylation and actin polymerization on nascent phagosome of neutrophils. Neutrophils were obtained from peritoneal cavity of rats 4 h after the intraperitoneal administration of carrageenan (4.5 mg/kg) and then incubated (1 h) with crotoxin (0.08 μg/mL) and submitted to phagocytosis of opsonized zymosan for 5 min. For in vivo assays, neutrophils obtained from rats treated with crotoxin (0.1 mg/kg), 2 h before carrageenan, were also submitted to phagocytosis. Tyrosine phosphorylation and actin polymerization were analyzed by immunocytochemical after incubation with anti-phosphotyrosine and staining with phalloidin. The results showed that crotoxin induced a marked reduction in staining of phosphotyrosine and F-actin on neutrophils during phagocytosis, when compared to the controls. Unlike what occurs in macrophages, our results demonstrate that crotoxin inhibits tyrosine phosphorylation and consequently actin polymerization which may contribute to explain the inhibitory effect of crotoxin on phagocytosis by neutrophils. Furthermore, taking into account the importance of these phagocytes on inflammatory response, these results contribute to the elucidation of the mechanisms involved in the anti-inflammatory effect of crotoxin. Supported by FAPESP, CAPES and INCTTOX.
Disclosure of interest: None declared.

P-149
LIVE RSV FAILS TO INDUCE AN INFLAMMATORY RESPONSE VIA HUMAN TOLL-LIKE RECEPTORS 3, 4 AND 7

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Respiratory syncytial virus (RSV) is the most important respiratory pathogen in infants and young children. 2–3% of primary RSV infections result in severe cases of bronchiolitis and pneumonia requiring hospitalization and supportive care. Treatment options are very limited. Excessive inflammation triggered by the host is believed to play a major role in the development of the clinical manifestations. The underlying molecular mechanisms are not well defined but mainly involve the activation of the transcription factor NF-κB. Hence, elucidating the mechanisms of NF-κB activation after RSV infection will likely help to develop new anti-inflammatory therapies. Here, we used transfected HEK 293-derived cell lines that overexpress selected human Toll-like receptors (TLRs) and harbour a NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene, and measured SEAP reporter activity upon infection with live RSV or stimulation with purified TLR ligands (controls). In addition, parental cells that harbour the SEAP reporter gene but generally lack TLR expression were used to control for endogenous responses. We demonstrate that HEK 293 cells are permissive for RSV; however the live virus fails to activate human TLRs 3, 4 and 7. Moreover, live RSV is unable to interfere with TLR3, -4 and -7 activation in HEK 293-derived cells mediated by their specific, purified agonists poly(I:C), *E. coli* LPS, and R848, respectively. This suggests that RSV can evade recognition by these innate immune receptors and may explain why in some children the immune system fails to control primary infection with this virus. Studies are ongoing to determine which pattern recognition receptors are essential for the human innate immune system to sense RSV infection.
Disclosure of interest: None declared.

P-150
INHIBITION OF CLATHRIN/DYNAMIN-DEPENDENT ENDOCYTOSIS INTERFERES WITH TRAM -TRIF SIGNALING PATHWAY

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TLR4 plays fundamental roles in provoking a robust anti infectious inflammatory reaction. Recognition of LPS by TLR4 could trigger generally two signaling pathways, the MyD88- dependent and -independent pathways. Additionally, this recognition could also induce the internalization of LPS/TLR4 complex, which is considered as an interacted process with signaling events. To deeply investigate the role of LPS/TLR4 complex internalization in macrophage activation, we did the following experiments. The localization of LPS and TLR4 as well as the changes of major proinflammatory cytokines and signaling molecules were observed or detected in macrophages treated with or without internalization inhibitors such as clathrin inhibitor (MDC), dynamin inhibitor (dynasore) and endosome acidifying maturation inhibitor (chloroquine). These results showed LPS down-regulated cell surface TLR4 level and both LPS and TLR4 were endocytosed into cytoplasm and colocalized at same local site. On the other hand, inhibitors of clathrin/dynamin, MDC and dynasore, could maintain higher TLR4 on the cell surface and less LPS/TLR4 within the cells because of blocking LPS/TLR4 internalization. However, CQ, an inhibitor of endosome acidification, markedly decreased TLR4 amount on the cell surface and increased LPS and TLR4 amounts within the cells. Furthermore, the data from ELISA, cytokine antibody array and western blot assays

demonstrated that only MyD88-independent pathway was involved in early phase of LPS internalization; but in later phase, TLR4 back to cell surface could mediate the MyD88-dependent pathways again, and start to the next round of internalization. Taken together, this study clarifies that internalization of the LPS/TLR4 complex not only maintained the TLR4 recycle, but also took part in the macrophage activation by the MyD88-independent pathway. This finding might provide a new approach to treat the over-inflammatory diseases.
Disclosure of interest: None declared.

P-151
GILZ AND THE NEW ISOFORM L-GILZ MEDIATE THE ANTI-INFLAMMATORY EFFECTS OF GLUCOCORTICOIDS

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Glucocorticoids (GC) are of extraordinary therapeutic value in a wide range of inflammatory, autoimmune and inflammatory diseases. Their therapeutic activity is due to regulatory effects on activation, cell growth and differentiation in a number of cells and tissues, including cells of the immune/inflammatory system. With the aim to deeply analyze the molecular mechanisms of GC action, we have identified a number of GC-induced genes including glucocorticoid-induced leucine zipper (GILZ), a protein rapidly induced by GC treatment. Using different experimental models of inflammatory diseases, we show GILZ is an important mediator of the anti-inflammatory and immunosuppressive effects of GC. Moreover, we identified a new GILZ isoform, L-GILZ, involved in mediating the effects of GC on inflammation and on cell differentiation. Furthermore, our study demonstrates that both GILZ and L-GILZ are crucial mediators of GC-induced effects in that mediate the anti-inflammatory/immunosuppressive activity. In particular, GILZ regulates T cell activation and differentiation, cytokines, including pro-inflammatory cytokines, production and inflammatory process development. Results could provide new means to predict sensitivity of normal and tumor cells to treatment with GC and to outline new therapeutic approaches.
Disclosure of interest: None declared.

P-152
MECHANISM OF FOXO FACTORS NUCLEAR EXCLUSION BY THE ANTI-INFLAMMATORY GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) PROTEIN

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GILZ is an ubiquitous protein whose expression is induced by glucocorticoids in haematopoietic cells. GILZ regulates signal transduction pathways central to inflammation, such as AP-1, NF- κB and FOXO. Indeed, Forkhead transcription factors have been increasingly recognized to play a role in immune homeostasis, especially in chronic inflammation. Nuclear import of FOXO factors follows stress signals such as oxidative stress, leading to up-regulation of target genes, whereas nuclear export is favoured by phosphorylations, mainly by Protein Kinase B, and subsequent 14-3-3 recruitment. We recently showed that GILZ inhibits FOXO factors transcriptional activities in the acute promyelocytic leukaemia HL60 cells independently of PKB phosphorylation but through a Crm-1 dependent nuclear exclusion of FOXO3 leading to its relocalization to the cytoplasm. The aim of this work was to elucidate the mechanism of FOXO factors relocalization by GILZ. First, we showed that the cytoplasmic localization of FOXO3 in the presence of GILZ was not due to an association of FOXO3 with shuttling GILZ. Our hypothesis is that GILZ may promote phosphorylation of FOXO factors on regulatory sites affecting their nucleo-cytoplasmic localization, possibly through new 14-3-3 binding sites creation. ERK, JNK or p38 Mitogen Activated Protein kinases activities were inhibited by GILZ, as assessed in GILZ-expressing HL-60 clones. We invalidated MAPK phosphorylation sites (S284, S294, S425) on FOXO3-WT and FOXO3-TM (triple mutant with mutated PKB sites) and observed that these mutants were still significantly inhibited by GILZ. Interestingly, mutation of Ser 413, a target of AMP-activated protein kinase (AMPK) located in a consensus 14-3-3 recognition motif, along with substitution of Ile 394 to Ala in the NES of FOXO3, strongly impaired the inhibitory effect of GILZ, suggesting that GILZ could promote the association of an export complex on FOXO factors independently of PKB sites.
Disclosure of interest: None declared.

P-153
MODULATION OF ENDOPLASMIC RETICULUM STRESS IN RENAL ISCHEMIA AND REPERFUSION INJURY BY HEME OXYGENASE-1 INDUCTION

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Introduction: Acute kidney injury (AKI) remains an independent risk factor for mortality and morbidity and is well established that inflammation plays an important role in the pathophysiology of the disease. An organelle that suffers cellular stress is the endoplasmic reticulum (ER), leading to altered protein folding. The accumulation of unfolded proteins generates a cell response, which tends to return to homeostasis, but may cause the production of inflammatory factors and even cell death. The Heme oxygenase-1 (HO-1) is an enzyme with cytoprotective and anti-inflammatory properties. Thus, its induction may lead to a modulation of endoplasmic reticulum stress (ERS). The aim of this study is to evaluate if ERS generated by AKI can be modulate by HO-1 induction. Also, we tested if ERS can be induced in ox-LDL stimulated macrophages.
Methods: C57/BL6 mice were subjected to bilateral ischemia and reperfusion injury (I/R) for 45 min and sacrificed after 0, 15 and 30 min, and 6, 12 and 24 h of reperfusion. A group of animals was also treated with Hemin, an HO-1 inducer. We collected blood samples for renal function assessment and renal tissue to determine the gene expression of HO-1, TNF-a, HIF-1a, BiP and CHOP. Moreover, it was also done immunoblotting for ERS markers. Further, macrophages were stimulated with ox-

LDL (50 μg/mL) for 2, 6 and 24 h, and RT-PCR was performed for inflammatory molecules
Results: Animals treated with Hemin had lower serum urea and creatinine levels when compared with I/R group. Moreover, treated group had higher gene expression of HO-1 and lower expression of TNF-αand HIF-1a. Also, ERS molecules had lower gene and protein expression in treated animals. Finally, ox-LDL stimulated macrophages had a significantly increase on ERS, with concomitant higher levels of IL-6.
Conclusion: Induction of HO-1 promotes an attenuation of ERS, reducing inflammation and increasing cell survival.
Financial support: FAPESP/INCT Complex Fluids/CNPq.
Disclosure of interest: None declared

P-154
DELPHINIDIN INDUCES THE IL-2 PRODUCTION THROUGH CALCIUM RELEASE ACTIVATED CALCIUM (CRAC) AND THE NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT) ACTIVATION IN T CELLS

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Delphinidin is the major anthocyanidin present in many pigmented fruits and vegetables, which has antioxidant, anti-inflammatory, and antiangiogenic properties. Their properties have been studies in number cell types, but is unknown their effect on T cell. In this study, delphinidin was found to increase the interleukin-2 (IL-2) production and expression in Jurkat cells via Calcium Release Activated Calcium (CRAC) and activation of the Nuclear Factor of Activated T cells (NFAT). By spectrofluorimetric experiments, we tested the efficacy of putative CRAC inhibitors, BTP-2 and gadolinium (Gd^{3+}) on Ca^{2+} entry induced by delphinidin. BTP2 and Gd^{3+} reduced the calcium entry stimulated by delphinidin in a dose-dependent manner. In experiments of patch clamp, we observed that delphinidin was able to stimulate a calcium release-activated Ca^{2+} current (I_{CRAC}), which was inhibited in part by BTP-2 and Gd^{3+}. Moreover, in assays of bioluminiscence imaging of NFAT activity in single living Jurkat F6 cells stably expressing the luciferase reporter gene under control of three NFAT response elements, we showed that delphinidin induced the NFAT activity at 2 h post stimulation. Meanwhile, we observed that delphinidin induced the IL-2 production and expression in Jurkat cells after 24 h. To asses if CRAC and NFAT contributes to the IL-2 production and expression induced by delphinidin, we treated the Jurkat cells with BTP-2 or an inhibitor of calcineurin, Cyclosporine A (CsA). Both, significantly decreased the IL-2 production and expression induced by delphinidin. Finally, we observed that delphinidin induced ERK1/2 phosphorylation at 120 min and that inhibitors of MAPK/ERK kinase, PD98059 and U0126, reduced the IL-2 production induced by delphinidin, not so the inhibitor of JAK2, AG490. With this results, we concluded that delphinidin is able to induces IL-2 through of CRAC, NFAT and MAPK/ERK activation in T cells. Supported by DID-UACh, CONICYT 21090900 and AT-24100037.
Disclosure of interest: None declared.

P-155
INVOLVEMENT OF JNK/AP-1 PATHWAY IN ENDOTHELIN-1-INDUCED CONNECTIVE TISSUE GROWTH FACTOR EXPRESSION IN HUMAN LUNG FIBROBLASTS

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Endothelin-1 (ET-1) is a vasoconstrictive peptide, as a key mediator of vasoconstriction and tissue repair. It is found that patients with asthma have significantly higher ET-1 levels as compared with normal subjects. Connective tissue growth factor (CTGF) is an immediate early gene, and overproduction of CTGF underlies the development of lung fibrosis. Previous studies have shown that ET-1 induces expression of matrix-associated genes in lung fibroblast through MEK/ERK pathway. However, little information is known about the signaling pathway of CTGF expression in lung fibroblasts caused by ET-1. In this study, we found that ET-1-induced CTGF expression was inhibited by BQ123 (ET_AR antagonist), but not BQ788 (ET_BR antagonist). Pretreatment of SP600125 (a JNK inhibitor) and transfection with JNK1/2 DN significantly reduced ET-1-induced CTGF expression. ET-1-induced CTGF luciferase activity was predominately controlled by the sequence −747 to −408 bp upstream of the transcription start site on the human CTGF promoter, suggesting ET-1-induced CTGF expression was most controlled by the AP-1 binding region of CTGF promoter. Pretreatment of curcumin (AP-1 inhibitor) inhibited ET-1-induced CTGF expression. Furthermore, ET-1 caused increases in c-Jun phosphorylation, the formation of AP-1-specific DNA–protein complex, and the recruitment of c-Jun to the CTGF promoter. These results suggest that ET-1 acting through ET_AR, activates the JNK/AP-1 signaling pathway, which in turn initiates AP-1 activation and recruitment of AP-1 to the CTGF promoter, and ultimately induces CTGF expression in human lung fibroblasts.
Disclosure of interest: None declared.

P-156
HUMAN PLATELETS USE MEMBRANE EXPRESSED TLR2 MOLECULES TO SECRETE A PANEL OF CYTOKINES IN A NF-κB/MYD88-DEPENDANT MECHANISM

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Blood platelets are central to haemostasis and exert important effects on the regulation of certain elements of the immune system, as they interplay in innate (including inflammation) and adaptive immunity. The purpose of this study was to further investigate TLR2 stimulation and NF-κB/MyD88-dependent signalling machinery. Platelet-rich plasma (PRP), from healthy donors, was stimulated with the TLR2 ligand Pam3CSK4 (w or w/o human TLR2 blocking MoAbs and w or

w/o Bay 11-7082, a non-reversible inhibitor of IκB-a phosphorylation to reduce NF-kappaB activity). Expression of TLR2 on the platelet surface was determined by flow cytometric analysis. Western blot was performed to detect platelet intracellular protein. The levels of soluble cytokines sCD62p, RANTES, PDGF-AB, PF4, and sCD40L were measured using specific ELISA. TLR2, MyD88 and NF-κB p65 subunit is expressed on human platelets as demonstrated by Western blot and flow cytometry. The engagement of TLR2 with Pam3CSK4 significantly increased the level of sCD62p, RANTES and sCD40L and significantly attenuated, when platelets were pre-incubated with an anti-TLR2 MoAb. Finally, modulation of platelet soluble factors release was observed if platelets were pre-treated by NF-κB p65 inhibitors prior to engagement of platelet TLR2 Pam3CSK4 or thrombin receptor activating peptide. Unavailable reagents/techniques suitable for anucleated cells permit to explore MYD88 pathway. Our observations support the concept that NF-κB proteins are central in platelet inflammatory functions. We recently proposed that platelets adapt the subsequent responses, with polarized cytokine secretion; after signal danger receptor involvement and these resulted complete this, with a potential role of NF-κB. Platelet expression of TLR and transcription factors must be, now, apprehended for therapeutics focus on transcription factors TLR dependant, as NF-κB inhibitory drugs.
Disclosure of interest: None declared.

P-157
CYCLOPHILIN B INHIBITS TNF-ALPHA EXPRESSION IN LPS-STIMULATED MACROPHAGES: IMPLICATION OF BCL-3

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Initially identified as cyclosporin A-binding proteins, cyclophilins A and B (CyPB) are inflammatory mediators that induce migration of T lymphocytes and monocytes/macrophages. Cyclophilin A was also reported to induce expression of pro-inflammatory mediators as TNF-α, IL-1β or IL-8 in monocytes/macrophages. However, these last findings are controversial and no data are available concerning the responses of macrophages to a long-term exposure to cyclophilins. We recently demonstrated that a pre-treatment of macrophages with CyPB reduced the secretion of TNF-α induced by LPS, suggesting that CyPB can regulate inflammatory responses in macrophages. Based on this hypothesis, we examined the molecular mechanisms responsible for desensitization of LPS-induced TNF-α expression. So we used macrophages derived from human peripheral blood monocytes and THP-1 cells, which can be efficiently differentiated into macrophage-like cells. Quantitative real time PCR analysis allowed us to demonstrate that CyPB strongly inhibits the expression of TNF-α mRNA. We also found that CyPB induced in macrophages expression of Bcl-3, a protein known to enhance binding of p50/p50 homodimer, a nuclear factor devoid of transcriptional activity, to the promoter of TNF-α gene. We therefore analyzed the molecular events that down-regulate transcriptional activation of TNF-α gene expression in CyPB-treated cells. By using chromatin immunoprecipitation, we confirmed that pre-treatment of cells with CyPB induced the accumulation of p50/p50 NF-κB, which bound to TNF-α gene promoter in place of p65/p50, thus reducing transcriptional activation. Finally, silencing the expression of Bcl-3 by RNA interference efficiently restored LPS-induced TNF-α expression in CyPB-treated cells, thus confirming the involvement of Bcl-3 in the inhibitory activity of CyPB. Altogether, our results support the hypothesis that CyPB is a regulatory factor, which may take part in the modulation of the inflammatory response.
Disclosure of interest: None declared.

P-158
THE ROLE OF INTRACELLULAR RECEPTORS NOD-1 AND NOD-2, AND ADAPTADOR MOLECULE OF TLRS, MYD88 DURING ACUTE RENAL ISCHEMIC INJURY

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Introduction: Ischemia and reperfusion injury (IRI) is the main etiological factor of acute kidney injury (AKI). Recent data showed the importance of Toll-like receptors on the IRI. Even more recently, it has been discovered another family of innate immunity receptors, present within the intracellular compartments, the NOD-like receptors (NLR). The role of NLR in the kidney is less clear, but there is evidence of its involvement in renal diseases. The objective of this study was to evaluate the involvement of TLR by studying its adaptador protein MyD88, and intracellular Nod-1 and Nod-2 in the AKI triggered by IRI.
Methods: C57BL6 WT animals and knockout (KO) to MyD88, Nod-1, Nod-2, RIP-2 and double KO Nod1/2 were subjected to 45 min of ischemia. Blood samples and kidney tissue were collected for biochemical analysis, gene expression, protein and histological features at different times of reperfusion.
Results: Initially, we observed higher gene expression of MyD88, Nod-1 and Nod-2 in renal tissue after different periods of IRI. After 24 h of reperfusion we observed that WT and Nod-1KO animals showed a significant renal dysfunction assessed by serum creatinine and indices of acute tubular necrosis while knockout animals for MyD88, Nod-2, RIP-2 and Nod1/2 showed a significant protection of renal function when compared to WT. Also, WT animals showed extensive areas of hypoxia compared to different KO, and mRNA expression of HIF-1α was expressed 2.5× more than Nod-2, RIP-2, Nod1/2 and MyD88 KO animals. Interestingly, the gene expression of Bcl-2 was reduced in WT mice while in KO animals it was up regulated. We also verified that KO mice presented significantly less serum levels of pro-inflammatory molecules, compared to WT animals.
Conclusion: Besides the TLR, the intracellular sensors Nod-1 and Nod-2 play a role in the pathogenesis of AKI by different mechanisms that result in an exacerbation of the inflammatory process, leading to a renal dysfunction. Fapesp/CNPq/INCT
Disclosure of interest: None declared.

P-159
METABOTROPIC GLUTAMATE RECEPTOR STIMULATION ACTIVATES THE NF-KB PATHWAY AND IS REGULATED BY A20 AND OPTINEURIN

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High amounts of glutamate are found in the brains of people with multiple sclerosis. Glutamate might affect neuroinflammation via effects on cell types within and peripheral to the central nervous system. Metabotropic glutamate receptors (GRM) comprise a unique family of G-protein coupled receptors that are promising targets for therapeutic interference. The binding of glutamate to GRMs induces signal transduction cascades that lead to gene-specific transcription. However, the molecular mechanisms that mediate and regulate GRM-induced gene expression are still largely unclear. We are studying intracellular signal transduction in response to type I GRMs (GRM1 and GRM5). Group I agonists are known to activate phospholipase C (PLC), leading to release of calcium and activation of protein kinase C (PKC). We could show that overexpression of GRM1 and GRM5 in HEK293T cells induces the activation of an NF-κB dependent reporter gene. Co-expression of the ubiquitin-editing protein A20 or the GRM1 and ubiquitin-binding protein optineurin (OPTN) significantly prevented GRM1- and GRM5-induced NF-κB activation in a synergistic manner. Interestingly, mGluR1 triggered the generation of a shorter OPTN fragment, whose formation could be inhibited by A20. In conclusion, our results indicate an important role for NF-κB activation and its regulation by A20 and OPTN in type I GRM signaling.
Disclosure of interest: None declared.

Cell death mechanisms

P-160
THE SURVIVAL OF HUMAN DENDRITIC CELLS IS CONTROLLED BY THE TRANSCRIPTIONAL FACTOR NRF2 IN RESPONSE TO CHEMICAL SENSITIZERS

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Dendritic cells (DC) are professional antigen presenting cells playing a major role in the induction of primary immune response. After the exposure of DC to skin contact sensitizers, such as nickel ($NiSO_4$) or dinitrochlorobenzene (DNCB) an up-regulation of phenotypic markers, cytokine secretion and activation of signaling pathways such as Mitogen Activated Protein Kinases (MAPK) are observed. These compounds generate a chemical stress that can be perceived as a danger signal by DC, leading to their maturation. Among signaling pathways known to be redox-sensitive, the Nrf2/Keap1 pathway is central for the detection of electrophilic molecules. In the absence of a chemical stress, Keap1 associates with Nrf2 and targets it to degradation. In the presence of an electrophilic compound, Keap1's conformation is modified leading to Nrf2 translocation to the nucleus and transcription of its target genes. To study the role of the transcriptional factor Nrf2 in response to chemical sensitizers, DC derived from monocytes (Mo-DC) were treated with $NiSO_4$, DNCB or cinnamaldehyde (CinA) for different times and Nrf2 expression was measured by Western blot. Accumulation of Nrf2 protein was observed after 4 h of stimulation.

To evaluate the role of Nrf2 in cell survival, Mo-DC have been transfected with small interfering RNA (siRNA) to invalidate in vitro *nrf2* transcripts and were then treated with $NiSO_4$ or DNCB or CinA for 18 h. AnV and 7-AAD staining were used to measure apoptotic and necrotic cells respectively. Results showed an increase of secondary necrotic cells ($AnV^+/7\text{-}AAD^+$) and total apoptotic cells ($AnV^+/7\text{-}AAD^-$) when Nrf2 is invalided. Otherwise, living cells decreased in absence of Nrf2. These results suggest that the transcriptional factor Nrf2 would play a role in cell survival and would protect DC from contact sensitizers. Now, we are focusing our work on caspase signaling pathway in order to know if Nrf2 controls caspase activation, known to be involved in DC maturation.
Disclosure of interest: None declared.

P-161
THE ROLES OF RIP1 IN OXIDATIVE STRESS-INDUCED PARTHANATOS CELL DEATH

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RIP1 (receptor interacting protein 1) has been regarded as an important regulator at the switch point of cell's fate between life and death. It was originally believed to be important for NF-κB activation and its protein kinase activity is dispensable. Previous studies also reported that RIP1 and subsequent c-Jun N-terminal kinase (JNK) activation may mediate DNA damager N-methyl-N′-nitro-N′-nitrosoguanidine (MNNG)-induced necrotic cell death. However, the detail mechanism was not well-investigated. MNNG can cause excess DNA strand breaks that lead to poly (ADP-ribose) polymerase-1 (PARP-1) overactivation and cell death (parthanatos). In this study, we used MNNG-treated mouse embryonic fibroblasts (MEFs) to elucidate the regulatory roles of RIP1 in MNNG-induced parthanatos. We found that MNNG-induced cell death can be abrogated in RIP1 deficient cells or by using silence RNA technique to knockdown RIP1 expression. MNNG can also induce rapid PARP-1 and JNK activation, biphasic reactive oxygen species (ROS) production and intracellular calcium increase. The early ROS production occurring at 1 min and peaking at 5–15 min after MNNG treatment partially resulted from NADPH oxidase. In contrast, the late phase of ROS production occurring at 30 min and time-dependently increasing up to 6 h after MNNG treatment was generated by mitochondria. Results further indicated the involvement of RIP1 in sustained late phase ROS production and calcium increase. The interactive roles of RIP1 with ROS, JNK and calcium elevation in MNNG-induced parthanatos were characterized. Moreover, we found that RIP1 is also involved in oxidative stress-induced NF-κB and AMPK activation. Taken together, RIP1 is a key mediator for oxidative stress-induced cellular signaling and death.
Disclosure of interest: None declared.

P-162
DOUBLE DEALINGS OF APOPTOTIC CASPASES IN EPIDERMAL KERATINOCYTE COMMITMENT TO APOPTOSIS AND DIFFERENTIATION INDUCED BY TRAIL

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Terminal differentiation of epidermal keratinocytes to corneocytes is a unique model of cell death, which is indispensable for the homeostasis of skin. Despite earlier disputation, cornification has been distinguished from other types of programmed cell death, especially apoptosis. The previous report revealed that differentiating keratinocytes induced by cell confluence can be resistant to tumor necrosis factor related apoptosis-inducing ligand (TRAIL)-induced apoptosis and indicated differentiation is distinct from death receptor-mediated apoptosis. In our study, we report during confluence-induced differentiation, TRAIL mRNA is up-regulated in HaCaT and normal human epidermal keratinocytes (NHEKs). After treating dividing NHEKs with TRAIL, markers of keratinocyte differentiation such as involucrin and type 1 transglutaminase can be induced in addition to the induction of apoptosis. However, under the pretreatment of calcium in NHEKs, expression of differentiation markers cannot be further enhanced by TRAIL but cell death-induced by TRAIL is diminished. When blocking the activity of caspase 3 and 8, both differentiation and death of NHEKs triggered by TRAIL are abolished but differentiation induced by calcium and phorbol 12-myristate 13-acetate are barely affected. Moreover, TRAIL-evoked signaling of ERK and p38 and degradation of p63 are also attenuated by inhibiting the activation of caspase 3 and 8. Comparing floating cells with adherent NHEKs after the treatment of TRAIL, we find much more abundant expression of differentiation markers including involucrin, keratin 10 and loricrin is detected in floating cells than adherent NHEKs. These findings provide a new insight into the complicated relationship between apoptosis and cornification, and also unravel the novel role of apoptotic caspases in keratinocyte differentiation.
Disclosure of interest: None declared.

Allergy and inflammation

P-163
FEMALE SEX HORMONES MEDIATES BRONCHIAL REMODELING AND RESPONSIVENESS IN CHRONIC ALLERGIC LUNG INFLAMMATION

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Introduction: Asthma is a chronic lung inflammatory disease that modifies bronchial structure, causing remodeling and increased responsiveness. Remodeling is recognized by fibrosis of sub-epithelial basement membrane, hypertrophy of bronchialglands, goblet cell hyperplasia, thickening of airway epithelium. In this study, we investigated the role of female sex hormones in airway remodeling and responsiveness in a model of chronic lung inflammation.
Material and methods: Female C57B/6 mice were subjected to ovaries removal (OVx) and were OVA-sensitized (OVA 1 mg/ml SC 7 and 14 days later). Control group consisted of sham OVx-OVA. After day 21, OVA-challenge (aerosol, 15 min, OVA 1%) was performed three times a week during three weeks (OVx-OVA). After 5 days of the last OVA-challenge, part of the animals (OVx-OVA and sham OVx-OVA) was submitted to in vitro tracheal maximal contractile response (E_{max}) to methacholine (MCh) evaluation and histological analysis (*picrusirius red* and PAS staining) to identify collagen deposition and mucus production on bronchial compartment. The other part of the animals (OVx-OVA and sham OVx-OVA) was anesthetized, mechanically ventilated and respiratory mechanics assessment (total lung resistance, *R*, and elastance, E) was performed.
Results: Measurements of bronchial mucus and collagen were significantly lower (6 and 2 times lower respectively) after chronic antigen exposure of OVx-OVA compared with their sham OVx-OVA counterparts. OVx-OVA showed reduced tracheal responsiveness in vitro to MCh (E_{max}: 3.65 g) in comparison to sham OVx-OVA (E_{max} 1.6 g). Total lung resistance (*R*) and elastance (E) were higher and lower respectively in sham OVx-OVA.
Conclusions: Removal of ovaries affected the magnitude of lung inflammatory response after chronic exposure to antigen suggesting that female sex hormones could mediate the airway responsiveness and remodeling in chronic allergic lung inflammation.
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Disclosure of interest: None declared.

P-164
EFFECTS OF ADENOSINE ON TOLL-LIKE RECEPTORS INDUCED CYTOKINE PRODUCTION IN HUMAN MAST CELLS

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The mast cell is an important inflammatory effector in both innate and adaptive immunity. Adenosine, an endogenous nucleoside, modulates a large number of cellular systems in inflammation. While activation of mast cells through IgE receptors (initiation of allergic reaction in adaptive immunity) is known to be modulated by adenosine, the effects of adenosine on toll like receptors (TLRs) induced mast cells activation (initiation of inflammation in innate immunity) has not been thoroughly studied. We pharmacologically characterized the actions of adenosine on TLR induced IL-8 release from human cultured mast cells (HCMC). Treatment of bacterial peptidoglycan (PGN) and lipopolysaccharides (LPS), specific ligands of TLR2 and TLR4 respectively, significantly increased IL-8 production from HCMC. Adenosine and NECA at subnanomolar concentrations (10^{-8}–10^{-7} M) potentiated PGN-mediated IL-8 production which was not observed in LPS treated cells. A_1 receptor agonists, CCPA and 2′MeCCPA, both markedly potentiated the IL-8 production by PGN dose-dependently. Moreover, a similar effect was also found with A_3 receptor selective agonists 2Cl-IB-MECA and HEMADO. A_2 receptor was not involved in the enhancing effect as A_2 receptor specific CGS21680 and CV1808 were found not to affect the PGN effect. The potency of adenosine agonists was found in rank order of 2′MeCCPA > NECA > CCPA > HEMADO > 2-Cl-IB-MECA » CGS21680/CV1808. When the effects of specific adenosine receptor antagonists on the potentiating action of NECA on PGN induced IL-8 release were investigated, significant inhibition of the potentiating action of NECA was observed with antagonists of A_1 receptor, CGS15943 and PSB36, and A_3 receptor, MRS3777, but not that of A_{2B} receptor, PSB1115. These results suggest that A_1/A_3 receptors are responsible for the potentiating effect of adenosine on mast cell activation by PGN in human innate immune reactions.
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Disclosure of interest: None declared.

P-165
OCCURRENCE OF BIPHASIC LATE AIRWAY HYPERRESPONSIVENESS IN A MURINE MODEL OF ARTHMA

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Nonspecific airway hyperresponsiveness (AHR) is one of cardinal features of bronchial asthma. We have described TNF-α-induced late AHR in a murine model of asthma, in which protocol, the mice received twice intraperitoneal immunization, followed by broncho-provocation twice with aerosolized ovalbumin with a 1-week interval. The AHR occurs at 10–14 after the second airway allergen challenge. In this study, we examined the kinetics of late AHR up to 60 h in a 6 h intervals We found that the specific airway resistance increased 4-fivefold at 12 h after airway challenge of allergen, followed by the second phase response being larger and broader in resistance at 24–48 h, peaking at 36 h with 3.5 to 4.5-fold increase, indicating that biphasic late AHR occurred in this murine model of asthma. The first phase of late AHR was abrogated by pretreatment of the sensitized animals with anti-TNF-α. Intratracheal administration of IgE as well as IgG immune complexes (ICs) were able to induce first phase of late AHR via producing TNF-α in the airways in normal animals. The second phase of late AHR was abrogated by pretreatment of the sensitized animals with CpG-ODN, which inhibited Th2 asthmatic phenotypes, such as airway levels of IL-4 and IL-5 and eosinophils, but it affected neither airway levels of TNF-α, nor the magnitude of the first phase of AHR. These data indicate that biphasic late AHR occurs in a murine model of asthma, in which TNF-α and Th2 response are responsible for the development of the first and second phases of late AHR, respectively.
Disclosure of interest: None declared.

P-166
A SYNTHETIC CHRYSIN ANALOGUE, 5,7-DIHYDROXY-8-(PYRIDINE-4YL) FLAVONES, POSSESSES POTENT ANTI-INFLAMMATORY ACTIVITY

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In order to find the anti-inflammatory flavonoid derivatives having optimum chemical structures, various 8-heterocyclic-substituted chrysin derivatives were previously synthesized and their effects on prostaglandin E_2 (PGE_2) production from lipopolysaccharide (LPS)-treated mouse macrophage cell line, RAW 264.7 cells were evaluated. Through this screening procedure, 5,7-dihydroxy-8-(pyridine-4yl)flavone (C-721) among the derivatives was selected for further pharmacological study. Contrary to the parent molecule, chrysin, C-721 was found to potently inhibit PGE_2 and NO production from LPS-treated RAW cells. The IC_{50} values of C-721 were 6.2 and 22.6 μM, respectively for COX-2 mediated PGE_2 and iNOS-mediated NO production. Western blotting and reverse transcriptase-polymerase chain reaction analysis proved that this compound inhibited PGE_2 production at least in part by COX-2 down-regulation and COX-2 inhibition, while C-721 inhibited NO production mainly by down-regulation of iNOS expression. In addition, C-721 inhibited TNF-α and IL-6 production at 10–50 μM. In vivo study has revealed that oral and intraperitoneal administration of C-721 showed 25.2–44.3% inhibition against λ-carrageenan-induced paw edema in mice. Furthermore, this compound inhibited collagen-induced arthritis in mice by intraperitoneal injection. All these results suggest that C-721 may have a potential for a synthetic lead compound as new anti-inflammatory agent.
Disclosure of interest: None declared.

P-167
ULTRASTRUCTURAL CHANGES OF AIRWAY IN CHRONIC OVALBUMIN MOUSE MODEL OF ASTHMA

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The mechanisms underlying airway remodeling in asthma remain unknown and ultrastructural changes of asthmatic airway might reveal novel mechanisms. To investigate the histopathological and ultrastructural features of the airway in a chronic ovalbumin mouse model of asthma, two groups of BALB/c mice [Sham and ovalbumin (OVA) n = 6 each] were sensitized and challenged with 1.5% OVA in PBS [OVA group (30 min per day, alternative days)] or PBS (Sham group) for 4 weeks. After determining airway hyperresponsiveness (AHR) to methacholine, mice were sacrificed and lungs were taken for both histopathological and electron microscopic studies. AHR in response to methacholine was enhanced in OVA mice. Histopathological studies revealed the presence of denuded bronchial epithelial cells, goblet cell metaplasia and mucus hypersecretion and cellular infiltration in perivascular and peribronchial area. The infiltrated cells were composed mainly of eosinophils, lymphocytes and macrophages. Transmission electron microscopy (TEM) revealed that there were inactive granules in chronic compared to partial active granules in acute asthma. There were signs of epithelial apoptosis such as shrinkage of bronchial epithelia, widening of intercellular space between adjacent bronchial epithelial cells, and subepithelial fibrosis. Basement membrane were thickened and composed mainly of fibroblast and collagen. Interestingly, we found mucus granules in both ciliated and Clara cells. In addition, there is increase in the number of mitochondria in smooth muscle as well as evidence of mitochondrial injury in asthmatic mice. In conclusion, TEM revealed the interesting and crucial ultrastructural changes of asthmatic airway in relation to airway remodeling and thus it appears to be a useful tool in understanding its pathogenesis.
Disclosure of interest: None declared.

P-168
EVALUATION OF THE SENSITIZING POTENTIAL OF CHEMICALS BASED ON THEIR CHEMICAL REACTIVITY

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Allergic contact dermatitis (ACD) resulting from skin sensitization is a common occupational and environmental health problem, in which dendritic cells (DCs) play an essential role. Indeed, after hapten binding, internalization and processing, DCs acquire a mature phenotype and migrate from the skin to the draining lymph nodes where they present hapten-peptide complexes to naive T cells. Haptens need to link with proteins to form a stable antigenic complex, able to induce an immune response. Our hypothesis is that chemical reactivity is necessary for a chemical to be a sensitizer allowing both covalent binding to proteins and stimulation of DC maturation by mimicking "danger signals" signalling. To date there is no evidence that a positive correlation exist between the capacity of a chemical to bind to a protein and the amount of stress it induces in DC allowing its maturation. As it is now well accepted that chemical stress will induce the up-regulation of detoxification systems, allowing DC to handle the perturbation of cellular redox homeostasis, the aim of this study is to evaluate sensitizer potency based on their chemical reactivity using the activation of the Nrf-2 pathway. For this purpose, dendritic (Mo-DCs) and dendritic-like cells (THP-1) were exposed to six "true" haptens with different reactivity (two haptens with high cysteine affinity, two haptens with high lysine affinity and two haptens with mixed cysteine/lysine affinity) and two pro-haptens and pre-haptens. Activation of the Nrf-2 pathway was assessed by measuring the mRNA expression of 4 Nrf-2-dependent genes: *nqo1*, *hmox*, *il-8* and *gpx* by qPCR. Our results show that chemical sensitizers were able to induce the transcription of Nfr-2 target genes in both models, in correlation with CD86 and/or CD54 surexpression. These studies will allow developing a new approach for detecting chemical sensitizers in integrating data coming from peptide reactivity tests and data on activation of dendritic cells.
Disclosure of interest: None declared.

P-169
SUSTAINED IMMUNE SUPPRESSION BY CTLA4-IG IN TWO HAPTEN-INDUCED INFLAMMATION MODELS

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The aim of this study was to investigate the effect of CTLA4-Ig on the induction of contact hypersensitivity (CHS). CHS is a T cell mediated immune reaction in response to epicutaneous application of a hapten and consists of two phases; sensitization- and elicitation phase. Mice were sensitized with DNFB or oxazolone and 5 or 6 days later, a challenge dose was applied. The inflammatory response is quantified by measuring the increase in ear-thickness at various time-points after challenge and the treatment groups are compared to isotype-treated control groups. Firstly, CTLA4-Ig was given in the doses 25, 5 and 1 mg/kg a day prior to sensitization. This study revealed a dose-dependent suppression of the ear-swelling response in both DNFB- and oxazolone-induced CHS, and the effect was most marked in the DNFB model. To obtain full inhibition in the oxazolone model, the doses were increased up to 125 mg/kg which reduced the response very close to background level. Mice treated with CTLA4-Ig were re-sensitized and re-challenged 3 weeks after the first sensitization without any further treatment with CTLA4-Ig. In both models, CTLA4-Ig still had a suppressive effect 3 weeks after administration although no CTLA4-Ig could be detected in serum. The effect was again most significant in the DNFB model. Based on these data, we can conclude that CTLA4-Ig induce a long-term immunosuppression of both DNFB- and oxazolone-induced CHS. The effect was most marked in the DNFB model, which could indicate quantitative difference between the two models. Although the effects of CTLA4-Ig on CHS have been studied previously, this study is the first to compare the effect of this compound in both DNFB- and oxazolone-induced CHS and to show a long-term effect of CTLA4-Ig in the oxazolone model.
Disclosure of interest: None declared.

P-170
ACUTE RENAL INJURY MODULATES PHAGOCYTIC AND MICROBICIDE ACTIVITIES MACROPHAGE MODEL OF EXPERIMENTAL ALLERGIC LUNG INFLAMMATION IN RATS

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Introduction: The renal ischemia-reperfusion injury (IRI) is a systemic inflammatory reaction with predominance of a Th1 response, which affects other organs, modulating a local inflammatory response, including the lung. The lung inflammation is a complex disease that involves the participation of inflammatory mediators and Th2 lymphocytes.
Objectives: To evaluate the phagocytic and microbicide capacity of macrophages in rats with lung inflammation that underwent IRI.
Materials and methods: The alveolar macrophages of rats sensitized to OVA were evaluated for testing phagocytosis and killing, 24 h after antigen challenge the animals were submitted or not to 60 min of renal ischemia. The reperfusion injury were analyzed 24 h after the re-establishment of renal blood flow. Serum creatinine, urea, renal histology and lung tissue were evaluated.
Results: Bronchoalveolar lavage has a high cellular infiltrate in immunized animals (420%) compared with control animals, IRI significantly reduced this infiltration (52%). Macrophages from animals immunized and challenged with OVA presented a 10× increased phagocytic capacity of red cells compared to control group, whereas immunized animals subjected to IRI showed a reduction in phagocytic index of 68%. Killing of *K. pneumoniae* by macrophages from immunized animals is (56%) higher compared with control group, being reduced in animals submitted to IRI (45%). The immunized and challenged group showed an increase in gene expression of IL-10 (450%), HO-1 (259%), IFN-γ (460%) and MCP-1 (370%), when compared to the immunized group subjected to IRI. The levels of urea and creatinine were only altered in animals that underwent IRI showing increased urea (239%) and creatinine (324%) levels in serum compared to control.
Conclusions: Renal ischemia and reperfusion injury alters the phagocytic and microbicide capacity of macrophages, reducing lung inflammation to OVA. FAPESP, CNPq, complex fluids INCT.
Disclosure of interest: None declared.

P-171
DISTRIBUTION OF IMMUNOGLOBULINS, EOSINOPHILIC CATIONIC PROTEIN AND ANTI-NUCLEAR ANTIBODIES IN PATIENTS WITH ALLERGY TO WHEAT FLOUR, RICE AND POLLEN ALLERGENS

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The aim of this study was to identify proteins which can be responsible for cross-reactivity in patient's sera being positive for wheat and reacting also with rice and pollen allergens and explore the effect of thermal processing on the IgE reactivity of rice proteins. We stained also levels of total IgG, IgA, IgE, ECP and presence of autoantibodies. Total IgG, IgA were measured by nephelometry (Dade-Behring BNII™). The total IgE, specific IgE and ECP were measured by IMMULITE 2000®. Antinuclear (Anab) antibodies were detected by indirect immunofluorescence (The Binding Site Ltd.) Sera were then processed by immunoblotting using proteins from wheat flour, raw and cooked rice. We have analyzed sera of 138 patients which were divided into three groups according positivity to specific IgE. Thirty-three patients were positive only for wheat and were marked as a group I. Patients positive for both wheat and pollen allergens were marked as a group II (68 patients) and group III (37 patients) were patients positive only for pollen allergens. We have found a strong cross-reactivity with broad spectrum of rice components (116–7 kDa) in patients (group II) having both, wheat and pollen specific IgE Abs. Patients with specific IgE against wheat or pollen allergens only, (group I and III) reacted with limited number of rice components. We have found out the same level of total IgE and ECP in group I and II but the levels of IgA and IgG were higher in group II. The most common clinical diagnose in group I was atopic eczema (17 patients). In group III we found the lowest level of total IgE. On the other hand the level of total IgA, IgG and ECP were the highest as a presence of autoantibodies.
Conclusion: Interindividual variation in the IgE antibody response may involve the clinical expression and reactivity of the each patient. There is also evidence that patients from group I and II differ at the level of total IgG, IgA and ECP form patients allergic only to pollen allergens.
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P-172
RENAL ISCHEMIA AND REPERFUSION INJURY MODULATE LUNG ALLERGIC INFLAMMATION PROBABLY ALTERING TH1/TH2 BALANCE BY CHANGING CELLULAR SIGNAL TRANSDUCTION

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Introduction: The Th1/Th2 balance represents an important factor in the pathogenesis of the renal ischemia-reperfusion injury (IRI). Besides, IRI causes a systemic inflammation that can affect other tissues, such as the lungs.
Objectives: Investigate the ability of renal IRI in modulating the pulmonary function in a specific model of allergic lung inflammation.
Materials and methods: C57Bl/6 mice were immunized with ovalbumin/alumen on days 0 and 7 and challenged with ovalbumin (OA) aerosol on days 14 and 21. After 24 h of the second antigen challenge, the animals were subjected to 45 min of ischemia. After 24 h of reperfusion, the bronchoalveolar lavage (BAL) fluid, blood and lung tissue were collected for analyses.
Results: Serum creatinine levels increased in similar extension in allergic and non-immunized animals subjected to IRI. However, BAL presented reduction in total cells (46%) and neutrophils (58%) when compared with control allergic animals not submitted to IRI. In addition, OA challenge induced phosphorylation of ERK and Akt, and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lung homogenates. After renal IRI, phosphorylation of ERK, and expression of COX-2 and iNOS were markedly reduced, however there was no difference for phosphorylation of Akt between sham and ischemic OA challenged animals. Mucus production was also reduced in allergic mice after renal IRI. IL-4, IL-5 and IL-13 were markedly down regulated in immunized/challenged mice subjected to IR.
Conclusions: These results suggested that renal IRI modulate lung allergic inflammation probably by altering the Th1/Th2 balance, at least in part, by changing cellular signal transduction factors. FAPESP, CNPq, Complex Fluids INCT.
Disclosure of interest: None declared.

P-173
THE KUNITZ-TYPE INHIBITOR DMTI-II, ISOLATED FROM DIMORPHANDRA MOLLIS SEEDS, EXACERBATES THE PULMONARY ALLERGIC GRANULOCYTE RECRUITMENT IN RATS

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DMTI-II (23-kDa trypsin inhibitor purified from *Dimorphandra mollis* seeds) promotes acute inflammation accompanied by an early infiltration of eosinophils, a critical cell type involved in allergic diseases. We have evaluated here the capacity of DMTI-II to enhance the allergic pulmonary inflammation, looking over time to the leukocyte trafficking from bone marrow to peripheral blood, and their recruitment into the allergic airways. Male Wistar rats were sensitized and challenged with ovalbumin (OVA). At 2–16 h prior to OVA challenge, animals were exposed to DMTI-II (10 μg). Bronchoalveolar lavage fluid (BAL), circulating blood and bone marrow were examined at 24 h post-OVA challenge. Challenge with OVA significantly increased the influx of total inflammatory cells, neutrophils

and eosinophils in BAL and lung tissue. Pre-exposure to DMTI-II potentiated the total inflammatory cells and neutrophil recruitment ($p < 0.05$). Neutropoiesis and neutrophilia accompanied the pulmonary cell influx. Pre-exposure to DMTI-II also significantly increased the eosinophil recruitment to BAL, an effect starting at 4 h, remaining markedly elevated at 16 h ($p < 0.05$). Eosinopoiesis and eosinophilia (seen within 2–4 h) were also observed. Exposure to DMTI-II alone increased the IL-4 levels, and further increased the IL-4 levels in OVA-challenged rats. The levels of IgE, LTB_4 and eotaxin in OVA-challenged rats were greater compared with non-sensitized rats, but DMTI-II exposure failed to further enhance such levels. In summary, our study shows that DMTI-II itself presents granulocytopoietic activity, and enhances allergen-induced neutrophil and eosinophil mobilization from bone marrow to lung tissues that is accompanied by enhanced IL-4 production.
Disclosure of interest: None declared.

P-174
PRIOR EXPOSURE TO STAPHYLOCOCCAL ENTEROTOXIN TYPES A AND B (SEA AND SEB) POTENTIATES THE ALLERGIC PULMONARY EOSINOPHIL INFILTRATION IN MICE

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Clinical evidences have shown a strong association between *Staphylococcus aureus* and pathogenesis and/or exacerbation of bronchial asthma. However, few studies have attempted to evaluate whether SEA and SEB exposure enhances the allergic cell pulmonary infiltration. In the present study, we evaluated the effects of pulmonary SEA and SEB pre-exposure on the eosinophil (EO) recruitment of allergic mice. At 4–48 h prior to OVA challenge, mice were intranasally instilled with SEA or SEB (1 μg). Pre-exposure to SEA significantly increased the eosinophil recruitment to BAL (at 48 h) and pulmonary tissues (12 and 48 h). SEB also potentiated the EO influx to BAL (at 4 h pre-exposure) and pulmonary tissues (at 12 and 24 h pre-exposure). The eotaxin levels in lung homogenates of OVA-challenged mice pre-exposed to SEA (4 h: 1,593.59 ± 261.57 pg/g) or SEB (4 h: 1,598.86 ± 156.45 pg/g) were greater compared with OVA-challenged mice instilled with saline (1,067 ± 68.26 pg/g; $P < 0.05$). No significant differences were detected for IL-4 and IL-6 in lung tissue. The lung EO infiltration in OVA-challenged mice pre-exposed to SEA was accompanied by marked increases in the EO forms in bone marrow (24 h: $0.33 \pm 0.07 \times 10^6$ cells/ml) and circulating blood (24 h: $0.45 \pm 0.07 \times 10^6$ cells/ml) compared with saline-instilled mice (0.15 ± 0.04 and $0.14 \pm 0.03 \times 10^6$ cells/m, respectively). The levels of IL-5 and eotaxin in bone marrow were 180 and 125% higher ($P < 0.05$) in OVA-challenged mice pre-exposed to SEA compared with saline-instilled mice. Mice pre-exposed to SEB also exhibited eosinopoiesis and blood eosinophilia, along with increased levels of IL-5 and eotaxin in bone marrow. In conclusion, airways pre-exposure to SEA and SEB exacerbates the pulmonary eosinophilic inflammation and promotes eosinopoiesis in OVA-challenged mice by mechanisms involving eotaxin and IL-5 production.
Disclosure of interest: None declared.

P-175
FEMALE SEX HORMONES HAVE A DUAL EFFECT ON PULMONARY ALLERGIC INFLAMMATION BY MODULATING LUNG CYTOKINE RELEASE

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Introduction: In previous studies we have shown that antigen sensitization 7 days after ovariectomy (OVx) reduces pulmonary inflammation in an acute model of experimental asthma, suggesting that the course of lung inflammation (IAP) depends on female sex hormones (FSH) circulating levels. By measuring the levels of IL-1β, TNF-α, IL-4, IL-13 and IL-10 in pulmonary explants from a murine model of asthma, in this study we investigated how FSH could possibly influence the mechanisms triggering the allergic lung inflammation.
Methods: Female Balb/c mice were ovariectomized (OVx) or falsely operated (Sham-OVx) on day 0, sensitized with ovalbumin (grade V OVA/alum) on day 7 and challenged with OVA (grade V) once a day, for 15 min. on the days 14, 15 and 16. Twenty-four hours after the last challenge, mice were killed in excess of ketamine and xylazine. Upon lungs removal, the cytokines IL-1β, IL-4, IL-10 and IL-13 were measured in samples of pulmonary explants using Duo Set (R&D System^{O}) kits for ELISA.
Results: There was a lung tissue increase of IL-1β, IL-4, TNF-α and IL-13 in Sham-OVx and OVx group (with or without lipopolysaccharide or OVA stimuli) with regard to their corresponding basal controls. Overall, the rise in these cytokines levels were significantly less in OVx than in Sham-OVx mice. Explants from Sham OVx animals had significantly higher levels of IL-10 regarding to basals, and these were even higher in the OVx group.
Conclusion: It is presumable that FSH mediate inflammatory cytokines release by the lungs and can thereby modulate the development of pulmonary allergic inflammation.
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Disclosure of interest: None declared.

P-176
FEMALE SEX HORMONES AS PROMOTERS OF FORMALDEHYDE-INDUCED LUNG INFLAMMATION

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Introduction: Formaldehyde (FA) is a common indoor and outdoor pollutant that is found in many products including particle board, plywood, floor coverings and office furniture. FA causes lung inflammation and may impair lung cell recruitment after an allergic stimulus. Considering the positive relationship between female sex hormones (FSH) and asthma, we evaluated the role of FSH on allergic lung inflammation after FA inhalation.

Material and methods: Female Wistar rats were ovariectomized (OVx) or not (Sham-OVx) and after 7 days subjected or not to FA exposure (1%, 3 days, 90 min/day). Subsequently the rats were sensitized and challenged with ovalbumin (OVA). After 24 h the bronchoalveolar lavage (BAL) and the myeloperoxidase enzyme activity (MPO) were determined. In a parallel set of experiments, groups of rats 7 days after OVx were treated with estrogen (280 mg/kg, sc) or progesterone (200 mg/kg, sc) 4 h before each FA inhalation; after this the rats were sensitized and challenge with OVA. After 24 h the BAL and MPO were evaluated.
Results: OVx in FA/OVA group did not alter the total cells and the MPO activity in BAL when compared to Sham-OVx group, but in theOVA/OVA group there were reductions of both total cells and MPO activity. The treatment with estrogen or progesterone increased the neutrophils and eosinophils in BAL whereas reduced the MPO activity of FA/OVA OVx group when compared to the untreated group. Treatment with estrogen, but not with progesterone increased the neutrophils and eosinophils in BAL of OVA/OVA OVx group when compared to the untreated group.
Conclusion: Our data are indicative that estrogen and progesterone are positive modulators in an animal model of FA-induced lung inflammation.
Financial support: FAPESP (2008/58108-3).
Disclosure of interest: None declared.

P-177
CONNECTIVE TISSUE DISORDERS AS THE FACTOR THAT CHANGES CHILDHOOD ASTHMA SYMPTOMS AND EXACERBATIONS

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Objective: High rate of connective tissue disorders (CTD) has changed childhood asthma (A) symptoms and exacerbations that leads to diagnosis and therapeutic mistakes.
Aim: To study symptoms and exacerbations of A in children with CTD manifestations.
Methods: 80 patients aged 3–18 with partly-controlled A, diagnosed according to GINA guidelines (Updated 2009) were studied during 2 years. 33 (41.3%) children had mild persistent A, 37 (46.2%)—moderate persistent A, 10 (12.5%)—severe persistent A. Community-acquired pneumonia (CAP) was diagnosed in 55 (8.8%) children. All patients had manifestations of CTD.
Results: Mycoplasma pneumoniae (Mp) was detected in 44 of 55 (80%) patients with A and CAP, Cytomegalovirus (Cmv)—in 20 of 55 (36.3%), Chlamydia pneumoniae (Cp)—in 16 of 55 (29.1%) children. 41.4% of patients had evidence of multiple co-infections—Mp, Cp and Cmv. Cough variant of A was in 22 (27.5%) patients. Recurrent episodes of CAP caused A exacerbation in 47.3% of children with CAP. Pulmonary hypertension (PH) was diagnosed in 23 (28.8%) patients, 18 (22.5%) of them had CAP and pulmonary fibrosis (PF). In 33.3% of patients with A, PH, and PF, pneumatocele formation was detected on chest X-ray and CT. Treatment of the patients with A exacerbation due to CAP included controller medications [inhaled corticosteroids (ICS)] with macrolide antibiotic therapy (MAT).
Conclusions:

1. Close relationship between A and CTD in studied patients is revealed.
2. 27.5% children with mild persistent A had cough variant of asthma.
3. A exacerbation was due to CAP caused by Mp in 4/5 of patients.
4. Recurrent CAP was diagnosed in 32.7% of asthmatic patients with CTD.
5. One third of children with A and CTD had signs of PH, 77.5% of them had A with CAP
6. 22.5% of children with A, CAP and CTD had PH and PF, 33.3% of them had pneumatocele formation on chest X-ray and CT scans.
7. A treatment included ICS and MAT to achieve A control.

Disclosure of interest: None declared.

P-178
IMPLICATION OF SYK KINASE IN THE INTERACTION BETWEEN NEUROKININ-1 RECEPTOR (NK1R) AND FCεRI FOR THE REGULATION OF BASOPHIL FUNCTION

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A more targeted therapy to control allergy symptoms requires a better understanding of its molecular mechanisms. The RBL-2H3 basophilic cell line (RBL) is the reference model for the study of FcεRI, the high-affinity receptor for IgE. We have recently shown that (1) RBL cells express the substance P (SP) and its receptor, NK1R, (2) binding of IgE to FcεRI increases the expression of NK1R and (3) aggregation of IgE-FcεRI complex induces autocrine activation of the NK1R, which in turn amplifies the response of FcεRI (e.g. degranulation). However, molecular mechanisms regulating the interaction between the tachykinergic system SP/NK1R and FcεRI remains unknown.
Hypothesis: Syk, a proximal element of the FcεRI signaling pathway, is involved in IgE binding-increased NK1R expression, and the autocrine activation of NK1R resulting in the amplification loop responsible of FcεRI-induced basophil activation.
Objective: To demonstrate the role of Syk in the molecular processes described.
Methodology: A stable cell line RBL expressing the NK1R conjugated to the fluorescent protein eGFP (NK1R-eGFP) was established. The expression of the NK1R-eGFP after incubation with IgE, in the presence or absence of a specific Syk inhibitor (BAY61-3606, 250 nM) was measured by flow cytometry. The level of internalization of NK1R-eGFP after IgE–FcεRI complex aggregation was determined by confocal microscopy.
Results: Cytometric analyses indicated that the increased expression of NK1R resulting from the binding of IgE to FcεRI is reduced when Syk activity is inhibited. Moreover, confocal microscopic analyses showed that the internalization of NK1R-eGFP in response to IgE-FcεRI complex clustering is Syk dependent.
Conclusion: Our study demonstrates a novel role for Syk as a regulator of the interaction between the tachykinergic system SP/NK1R and FcεRI to control both the expression of NK1R and an autocrine pathway responsible of the amplification of basophil-mediated allergic responses.
Disclosure of interest: None declared.

P-179
A CASE OF DRUG-INDUCED HYPERSENSITIVITY SYNDROME TREATED WITH GANCICLOVIR

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Introduction: We report a case of drug-induced hypersensitivity syndrome (DIHS) following phenytoin treatment. Symptoms worsened after stopping phenytoin. Treatment with ganciclovir and corticosteroid permitted a rapid improvement without relapse.
Case-report: A 46-year-old alcoholic man with hypovitaminosis D presented with fever, confusion and epilepsy. Cerebrospinal fluid (CSF) was consistent with meningitis due to *Streptococcus suis*. Dexamethasone, antibiotics and phenytoin were administrated. After 3 weeks, he developed new fever (40°C) with hypereosinophilia, hepatitis, lymphadenopathy and a CD8 T cells lymphocytosis. New bacterial sepsis was excluded. Diagnosis of phenytoin-associated DIHS was made. Four days after stopping phenytoin and starting methylprednisolone, a generalized rash developed. Skin biopsies showed CD8 T cells inflammation. Serologic testing for hepatitis B, C, AIDS, CMV, EBV, HHV6 and autoimmune antibodies showed IgG antibodies for EBV and HHV6. Polymerase chain reaction (PCR) did not detect EBV, CMV or HHV6 in peripheral blood, but was positive for HHV6 in bronchial lavage. The reactivation of HHV6 was serologically demonstrated by increased IgG titer. A 2-week treatment with ganciclovir was added to the corticotherapy. This combination permitted a rapid resolution of DIHS without relapse.
Discussion: DIHS involves cross-reactive lymphocytes, especially to phenytoin and HHV6. Symptoms include temperature, hypereosinophilia, rash, lymphadenopathy and hepatitis. DIHS is a drug reaction but the viral component has to be taken into account, especially in immunodepression like here (alcoholism, meningitis necessitating dexamethasone, hypovitaminosis D). In our patient, the association of corticosteroids and ganciclovir may have shortened the course of DIHS, without relapse or subsequent reactivation of CMV which can be seen later.
Conclusion: This case emphasizes the importance to consider DIHS and its treatment with antiviral drugs.
Disclosure of interest: None declared.

P-180
CLINICAL AND EPIDEMIOLOGICAL ANALYSIS OF 8639 PATIENTS WITH EOSINOPHILIA

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Rationale.
Differential diagnosis of eosinophilia is broad, including allergic, rheumatological, infectious and neoplastic diseases. Absolute count of peripheral eosinophil count, age and gender of the patients might be helpful in the process of reaching the origin.
Methods: We extracted all the 8,639 patients with peripheral eosinophilia from our electronic medical record from 2003 to 2008, and confirmed maximum eosinophil count and highest Eo. rate individually. Then we reviewed the patients' medical record to analyze the final diagnosis and divided into three groups based on count of peripheral eosinophil i.e. 500–1,499/mm^3, 1,500–2,999/mm^3 and over 3,000/mm^3. We grouped into diagnosed cases and undiagnosed cases, and divided secondary eosinophilia cases into 14 types of diseases.
Results: The majority of patients with high eosinophil count revealed high eosinophil rate. Cases that improved after transient eosinophilia without any intervention were higher in 500–1,499 group. About a third of patients with eosinophilia was diagnosed as having allergic diseases. Some specific diseases (i.e. episodic angioedema with eosinophilia, DRSS and Churg–Strauss syndrome) displayed distinctive patterns that may be useful for correct diagnosis. Very high eosinophil count over 3,000/mm^3 or over 5,000/mm^3 was more common in female, children with age of less than 10, and over 80.
Conclusions: Understanding epidemiology of eosinophilia is helpful to diagnose clinically important etiologies such as allergic diseases.
Disclosure of interest: None declared.

P-181
EFFECTS OF HYDROGEN SULFIDE (H_2S) ON ANTIOXIDANT ENZYMES AND OXIDATIVE PROTEIN MODIFICATIONS IN ALLERGIC AIRWAY INFLAMMATION

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Recent studies have shown that endogenous hydrogen sulfide (H_2S) may have an anti-inflammatory role in the pathogenesis of airway inflammation. Weinvestigated the effect of H_2S on leukocyte infiltration, antioxidant enzyme activities and protein nitrotyrosine (NT) residues in the airways of allergic mice at 24–96 h after ovalbumin (OVA) challenge. Results demonstrate that the peak of eosinophil infiltration into the lungs of OVA-sensitised control (non-treated) mice, seen at 48 h after antigen challenge, was significantly reduced by treatment of allergic mice with the H_2S donor, sodium hydrosulfide (NaHS; 14 μmol/kg, i.p.). Treatment with NaHS also resulted in a significant reduction of neutrophils in the bronchoalvelar lavage (BAL) at 24 and 48 h. A significant increase in NT residues was seen in control at 48 and 96 h after OVA-challenge, but no modifications were induced by NaHS. Superoxide dismutase activity was significantly increased in the lungs of control until 96 h after OVA challenge, whereas a decrease in their activity was produced by NaHS at 24 h. In addition, NaHS also prevented the augmentation in catalase activity at 48 h, as seen in control. Although no modifications were shown by the control, NaHS treatment significantly decreased glutathione peroxidase activity in the lungs at 48 h after OVA challenge. These results suggest that administration of H_2S-donor to mice significantly attenuated OVA-induced allergic airway inflammation. H_2S may act as an antioxidant agent in the lungs of allergic mice by decreasing the activity of antioxidant enzymes. On the other hand, the beneficial effect of H_2S on eosinophil and/or neutrophil migration to the lungs of mice during the allergic response is independent on oxidative protein modifications.
Financial support: FAPESP and CNPq.
Disclosure of interest: None declared.

P-182
REDUCTION OF CYTOKINES/CHEMOKINES EXPRESSION ATTENUATES THE ALLERGIC LUNG INFLAMMATION IN LEPTIN-DEFICIENT MICE

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Introduction: Allergic lung inflammation is characterized by onset airway constriction, increased vascular permeability, mucus secretion, and recruitment of inflammatory cells, contributing to pulmonary dysfunction Leptin, an adipokine of the obesity (ob) gene, is synthesised and secreted by adipocytes in proportion to their mass; besides suppressing satiety and increasing metabolism, leptin is considered a proinflammatory cytokine.
Objective: To investigate the role of cytokines/chemokines in the development of the allergic lung inflammation in leptin-deficient mice (ob/ob).
Materials and methods: C57Bl/6 and ob/ob mice were immunized with ovalbumin/alumen on days 0 and 7 and challenged with ovalbumin (OVA) aerosol on days 14, 16, 18, 20 and 22. After 24 h of the last antigen challenge, the bronchoalveolar lavage (BAL) was performed, blood was collected and lungs were harvested for analyses.
Results: Significantly fewer cells were recovered from OVA-challenged ob/ob versus OVA-challenged C57BL/6; mucus and collagen production were also reduced in OVA-challenged ob/ob versus OVA-challenged C57BL/6 (83 and 28%, respectively). In OVA-challenged ob/ob, the decreased BAL cytokines/chemokine levels were observed, when compared to OVA-challenged C57BL/6 (73% IL-4; 39% IL-12; 36% RANTES). Compared with OVA-challenged C57BL/6, OVA-challenged ob/ob also presented a reduced expression of IL-13 (63%) and TGF-β (66%) in lung tissue.
Conclusions: These results demonstrate that attenuation of the allergic lung inflammation in leptin-deficient mice is strongly influenced by down-regulation of proinflammatory cytokines/chemokines expression.
Financial support: FAPESP, CNPq, and INCT Complex Fluids.
Disclosure of interest: None declared.

P-183
TNF-ALPHA AND IL-5 MEDIATES THE EOSINOPHIL ACCUMULATION IN BONE MARROW OF OBESE SENSITIZED MICE

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Eosinophils (EOS) in bone marrow are derived from myeloid precursors in response to cytokine activation. Following antigen challenge, EOS are released into the circulation and recruited to tissues. Recently, we showed that a 10-week diet-induced obesity in mice stimulates eosinophilopoesis and enhances EOS trafficking from BM to lung tissues (Calixto et al. in Br J Pharmacol 159:617–625, 2010). The present study aimed to investigate the expression of adhesion molecules and chemokine receptor, as well as the influence of cytokines on bone marrow eosinophilopoesis of obese mice. In obese mice, ovalbumin (OVA) challenge largely increased the bone marrow EOS counts at 48 h post-challenge ($2.2 \pm 0.4 \times 10^6$/ml; $P < 0.05$) compared with lean group ($0.6 \pm 0.2 \times 10^6$/ml). Expression of VLA-4 in bone marrow EOS from obese mice was significantly lower ($P < 0.05$) compared with lean animals, as assessed by flow cytometry assays. In addition, the adhesion of bone marrow EOS to ICAM-1 and VCAM-1-coated plates in vitro was significantly lower in obese compared with lean mice. The levels of eotaxin in bone marrow supernatant did not differ between obese and lean groups. No differences in the CCR-3 expression in bone marrow EOS surface were observed. Treatment of obese mice with antibodies anti-IL-5 (2 mg/kg) and anti-TNF-α (2 mg/kg) normalized the number of immature and mature form of bone marrow EOS, as compared with lean mice treated with these antibodies. In conclusion, the increased bone marrow EOS in OVA-challenged obese mice is likely to reflect a down-regulation of VLA-4 in EOS surface, and hence a decreased interaction with the ligands ICAM-1 and VCAM-1. The bone marrow EOS differentiation in obese mice may involve TNF-α and IL-5 production, with no involvement for eotaxin or its receptor CCR-3 (experimental protocols were approved by the Ethics Committee of UNICAMP; No. 1496-1).
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Disclosure of interest: None declared.

P-184
CAN DIETARY EXTENSIVELY HYDROLYZED SOY PROTEIN OR AMINOACID FREE FORMULA INDUCE CYTOKINES PRODUCTION BALANCE IN COW'S MILK ALLERGY?

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Introduction and objective: Considering the importance of cytokines on allergy symptoms exacerbation and oral tolerance development, this study aimed to verify whether dietary treatment may induce a balance in the production of cytokines pro (IL-4, IL-13) and anti-inflammatory (IL-10, TGF-β).
Methods: Fourteen infants fed hydrolyzed soy protein (HSF) and 5 fed amino acid free (AA) formula (Alergomed, ComidaMed from Germany) during 120 days were evaluated. The CMA diagnosis was made with IgE levels, clinical parameters, elimination diet, challenge test and skin prick test. Afterward, the children were treated with cow's milk and derivatives of elimination diet and their replacement by HSF or AA. Serum cytokines were determined before the dietary treatment, in the presence of symptoms (T0) and after 120 days without allergy symptoms (T1). Data were presented as mean ± SE. Statistical analysis was performed by paired t test with $p < 0.05$ adopted as significant level.
Results: 54% of infants with CMA were IgE-mediated and 46% were non IgE-mediated. The pro-inflammatory cytokine, IL-13 was significantly reduced by both treatments 2.02 ± 1.45–0.33 ± 0.06) ($p < 0.05$). Regarding IL-4, a significant decrease was also observed from T0 to T1 (4.093 ± 1.73–0.34 ± 0.13) ($p = 0.01$). By the other hand, the anti-inflammatory cytokines, IL-10 and TGF-β, were significantly increased by both treatments (IL-10 from 13.56 ± 3.77–24.08 ± 5.08 ($p = 0.05$), (TGF-β from 44.98 ± 4.89–57.03 ± 3.17) ($p = 0.05$).
Conclusion: These data show that HSF and AA increase the anti-inflammatory cytokines, IL-10 and TGF-β and decrease the pro-inflammatory cytokines, IL-13 and IL-10. As the imbalance between these cytokines play an important role in the symptoms exacerbation hindering the development of oral tolerance, this study shows that proper dietary treatment is able to alter inflammatory mediators leading to reduction of clinical symptoms.
Disclosure of interest: None declared.

P-186
DIFFERENTIAL REQUIREMENTS FOR INTERLEUKIN (IL)-4, IL-12 AND IFN-GAMMA IN ANAPHYLAXIS INDUCED BY THALASSOPHRYNE NATTERERI FISH VENOM

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Introduction: Allergies are a significant and widespread public health problem. We demonstrated recently that *Thalassophryne nattereri* fish venom is able to induce a memory immune response with a high production of IL-5 and specific IgE. Then, the development of murine models of venom-induced anaphylaxis that mimic physiologic and immunologic features of human disorder has facilitated both understanding of the underlying mechanisms and evaluation of potential therapeutic approaches.
Objective: To investigate the roles of interleukin (IL)-4, IL-12 and IFN-γ in both systemic anaphylaxis and late phase eosinophic reaction following exposure to fish venom.
Methods and Results: Balb/c, C57BL/6 (*WT*) and IL-4, IL-12 and IFN-γ deficient mice were immunized i.p. with venom and challenged by i.p. or intranasal instillation. Immediate hypersensitive responses, inflammatory cells in BALF, lung or peritoneal cavity, cytokine in the supernatant of splenocyte cultures and venom-specific antibodies were analyzed. Our data show that i.p. challenge in BALB/c mice experienced anaphylactic reaction, produced Th2 cytokines and venom-specific anaphylactic IgE and IgG1. Mice challenge by nasal instillation presented reduced anaphylactic score, but high levels of IgE with pulmonary eosinophilic inflammation. Also, we demonstrated that IL-4 *KO* mice failed to develop anaphylactic shock or local Th2 inflammation, produced low levels of IgG1 and increased levels of IgG2a. IL-12 and IFN-γ *KO* mice showed no inhibition of anaphylaxis. IFN-γ *KO* mice presented elevated number of inflammatory cells in the peritoneum that was decreased in IL-12 *KO* mice. Both deficient mice produced elevated levels of IgG1 and diminished levels of IgG2a.
Conclusion: Together, the data indicate that IL-4 contributes to systemic Th2 sensitization and anaphylaxis after i.p or intranasal sensitization and IL-12, as opposed to IFN-γ, drives inflammatory cell recruitment.
Support: FAPESP and CNPq
Disclosure of interest: None declared.

P-187
COCKROACH ALLERGEN CONTAINS MULTIPLE SERINE PROTEINASES THAT ENHANCE PULMONARY INFLAMMATION VIA PROTEINASE-ACTIVATED RECEPTOR-2 (PAR2) AND CAN ALSO TARGET PARS 1 AND 4

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Background: Sensitization to cockroach allergen ranks among the most significant world-wide risk factors for asthma in inner city communities. Cockroach allergen extract (CE) is known to contain proteolytic activity, and we find that sensitization to cockroach allergens in mice is dependent on the activation of PAR_2 in the airway by CE serine proteinases. Our aim was to identify, isolate and characterize proteolytic allergens in the cockroach allergen extract.
Methods: Trypsin-like activity in the CE (Greer, Lenoir NC) was quantified with a fluorogenic trypsin substrate (Gln-Ala-Arg [QAR]-aminomethyl coumarin [AMC]) and visualized with an activity-based probe (ABP). Three ABP-labeled CE enzymes were detected and purified using ion-exchange chromatography. Each isolated enzyme was distinguished by (a) its Km for different fluorogenic peptides, and (b) its relative K_i for inhibition by different trypsin inhibitors. Additionally, each enzyme was tested for (1) activating PAR_2–dependent calcium signaling in PAR_2-expressing rat KNRK cells and (2) cleaving synthetic peptides representing the tethered ligand (TL) sequences of PARs 1, 2 and 4.
Results: The CE contained $\sim$25 units/mg protein of trypsin-like activity (QAR-AMC) and three ABP labeled serine proteinases (E1, E2, E3) with MWs of about 20–26 kDa. Each enzyme had distinct substrate K_m and inhibitor K_i values for different substrates and inhibitors. The enzymes activated PAR_2 in a calcium signaling assay and cleaved PAR-derived TL peptides to unmask the receptor-activating sequences of PARs 1, 2 and 4.
Conclusions: Cockroach allergen contains three biochemically distinct trypsin-like proteinases that are able to target PARs 1, 2 and 4. Thus PARs in addition to PAR_2 may be able to enhance allergenicity and airway inflammation in vivo.
Disclosure of interest: None declared.

P-188
EXPRESSION AND FUNCTIONAL ROLES OF THE PURINERGIC RECEPTOR P2Y12 IN HUMAN EOSINOPHILS

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Identification of new target molecules through which eosinophils activate and secrete their stored proteins may be highly significant for our understanding about the pathobiology of host immune responses to parasites and allergic inflammation, as well as reveal new therapeutic targets for the control of the eosinophilic disorders. We have recently reported the expression of the purinergic P2Y12 receptor (P2Y12R) in human eosinophils (Neves et al. in JACI, 2010), however its contributions to eosinophilic inflammation remains to be elucidated. The P2Y12R was recently described as crucial in mediating the proinflammatory actions of the leukotriene E4 (LTE4) and appointed as a new therapeutic target for pulmonary allergic diseases (Paruchuri et al. in JEM, 2010). A better understanding of the components of the immune response that mediate response to helminthes and allergens is still needed. In this work we evaluated the expression and functional roles of the purinergic P2Y12R in isolated human eosinophils. We isolated eosinophils from blood of normal and allergic donors by negative selection. By flow cytometry we confirmed the expression of the P2Y12R on isolated human eosinophils. After ADP stimulation, eosinophils secrete eosinophil peroxidase (EPO) as measured in the supernatants by a colorimetric assay. Eosinophil EPO secretion in response to ADP was differentially inhibited by the blockage of P2Y12 and P2Y1 receptors, both known to be responsive to ADP and expressed

in eosinophils. Eosinophils treatment with ADP or LTE4 did not induce or prevent eosinophil apoptosis as assed by flow cytometry after labeling with annexin V. In conclusion we are showing that human eosinophils respond to ADP stimulation via membrane-expressed P2Y12R that elicit eosinophil EPO secretion. Our studies provide innovative insights that relate the P2Y12R expression to its functional roles in human eosinophil activation and survival. Supported by FAPERJ (RJ, Brazil) and CNPq-PIBIC (Brazil).
Disclosure of interest: None declared.

P-189
THE ACTIVITY OF ANACARDIACEAE FRACTION AND PHENOLIC ACID DERIVATES FROM METHANOL EXTRACT OF ANACARDIACEAE FAMILY IN LYMPHOCYTE PRESENT IN PLEURAL CAVITY AND IN LYMPH NODES

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It is well established the involvement of T cells subsets in the pathogenesis of allergic airway inflammation. *Schinus Terebinthifolius* Raddi is a native plant from America widely used in folk medicine. We previously described the anti-allergic effect of acetate fraction obtained from their leaves in mouse pleurisy model inhibiting eosinophil influx. In this study we aimed to determinate the effect of Anacardiaceae fraction (ST fraction) in lymphocyte influx to the pleural cavity, its action in subcutaneous lymph nodes cells and in cytokines modulation. Intrathoracic OVA challenge (12.5 mg/cavity) triggered a $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells accumulation at 24 h, concomitant with an increase of $CD3^+CD25^+$ and $CD3^+CD4^+CD25^+$ cells as well as of $CD3^+CD4^+CD44^+$ cells into pleural cavity of sensitized mice. Oral pre-treatment (ST fraction, 100 mg/kg) significantly inhibited the increase of $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocyte and the expression of CD44. The treatment failed to inhibit the expression of CD25 but was able to attenuate these cell numbers. The IL-2 and IL-4 production by T cells from subcutaneous lymph nodes was inhibited after in vitro treatment of ST fraction (100 μg/mL) or phenolic acid (1–100 μg/mL). We can suggest that the diminished eosinophils observed previously, seems to be also directly related to the lower number and activation of lymphocytes reaching the inflammation site suggesting an anti allergic and an immunoregulatory effect to Anacardiaceae fraction, observed after the mouse antigen challenge.
Disclosure of interest: None declared.

P-190
THE ROLE OF CD11D INTEGRIN IN EOSINOPHILIC INFLAMMATION

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The prevalence of asthma worldwide is increasing for reasons that are unclear. Eosinophilic inflammation is a characteristic feature of asthma. Members of leukocyte integrin family (CD18) are present in eosinophils and are involved in allergic response. The CD11d integrin is expressed on most human leukocytes, including eosinophils. The surface expression of CD11d in eosinophils can be regulated by various stimuli and can function as ligand for VCAM-1. However the role of CD11d in asthma and in other allergic diseases is largely undefined. In this study, we analyzed the role of CD11d in in vivo models of eosinophilic inflammation. In this study, CD11d knockout mice (KO) and wild type (WT) mice were sensitized intraperitoneal injection of ovalbumin (OVA) and $Al(OH)_3$ in saline on days 1 and 10. From days 19–24 after sensitization, mice were challenged daily with either OVA (5%) or PBS by aerosol. Six hour after the last aerolization, we observed that KO mice shown lower infiltration of eosinophils in bronchoalveolar lavage (BAL) and less intense pulmonary inflammatory process than WT mice, after allergen challenge. Moreover, the airway responsiveness (AHR) analyses in a plethysmography suggest a decrease in AHR in KO mice. The measured of IL-5 in BAL was increased in KO than WT mice. Nevertheless, there was no increase in the number of eosinophils of allergic bone marrow KO mice. Intravital microscopy analyses of the mesentery showed that allergic KO mice have less leukocyte rolling and adhesion compared with allergic WT mice. Moreover, our results showed problems in the migratory ability of granulocytes from KO mice toward chemotactic factor eotaxin, in a transwell system. Our results suggest the important role of CD11d integrin on migration process, airway responsiveness and in control of inflammatory mediators in vivo during allergic inflammatory response in a mice model. This date provide new insights about the new candidate of anti-allergic therapies.
Disclosure of interest: None declared.

P-191
FERMENTED PRODUCT FROM BIFIDOBACTERIUM BREVE C50 HAS AN ANTI-INFLAMMATORY EFFECT VIA DENDRITIC CELLS

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Several bowel diseases, such as colitis and Crohn's disease, affect the equilibrium of intestine tissue by promoting a high inflammatory context. The control of the inflammation by immune cells in the gut is crucial in the prevention of these disorders. In particular, dendritic cells (DC) play a key role both in innate and in adaptative immunity. Depending on the state of maturation and on cytokine secretion, DC can induce either specific immune response or immune tolerance. During the maturation process, for example, in the presence of zymosan (TLR2 agonist), LPS (TLR4 agonist) or fermented product, DC change their morphology and the expression pattern of their maturation markers. Here, we investigated the effect of a bacteria-free supernatant fermented by *Bifidobacterium breve* C 50 (BbC50sn) on the maturation markers of human DC (hDC). BbC50sn was able to mature hDC after 48 h of stimulation by inducing the expression of the same profile of maturation markers such as CD25 and CD83 compared to other maturation agents. In addition, hDC stimulated by BbC50sn secreted more IL-10 pro-tolerogenic cytokine than IL12, a pro-inflammatory cytokine. The

maturation pathway activated by BbC50sn was shown to be through at least Toll like receptor 2 (TLR2). Nevertheless hDC stimulated by BbC50sn demonstrated a specific kinetics of maturation markers expression compared to hDC stimulated by other TLR2 agonist such as zymosan. The precise mechanism of the anti-inflammatory pathway induced by TLR2 engagement by BbC50sn remains currently unknown. One possibility could be that TLR2 pathway could modulate innate immunity receptors. These data show that BbC50sn was able to induce the maturation of DC and to skew towards a pro-tolerogenic profile by inducing IL10 secretion. These properties of BbC50sn are thought to act via TLR2. The BbC50sn could have anti-inflammatory and anti-allergic effects in the gut environment and might therefore protect against inflammatory bowel disease.
Disclosure of interest: None declared.

Infection, sepsis and inflammation

P-192
HEART RATE DYNAMICS DURING SYSTEMIC INFLAMMATION

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Under healthy condition cardiac rhythm exhibits a complex dynamic which is due to a non-linear interplay between autonomic nervous system and cardiac pacemaker cells. Systemic inflammation is associated with decreased heart rate variability which is a strong predictor of survival in patients with sepsis. However, underlying mechanism of this phenomenon is not understood. We hypothesized that cardiac pacemaker cells may act as a target for inflammatory cytokines leading to alteration in heart rate dynamics during systemic inflammation. Systemic inflammation was induced by intraperitoneal injection of endotoxin (1 mg/kg) in rats. Cardiovascular signals were recorded in conscious animals using telemetric system. Spontaneously beating atria were isolated and beat-to-beat variation in isolated atria was studied using an organ bath system. Sample entropy was calculated to assess the degree of cardiac cycle regularity. Short-term fractal scaling component of the cardiac cycle were calculated by using detrended fluctuation analysis (DFA). Endotoxemia was associated with a biphasic increase in heart rate in conscious rats. A significant increase in cardiac cycle regularity was also observed in endotoxemic animals starting 3 h after endotoxin administration. Beat-to-beat variation of isolated spontaneously beating atria exhibited a scale invariant (fractal-like) dynamics in both healthy and endotoxemic rats. However, short-term fractal scaling component in control atria was compatible with white noise ($\alpha_1 = 0.53 \pm 0.07$) which was significantly altered to a different (anti-correlate) type of fractal dynamics ($\alpha_1 = 0.19 \pm 0.02$, $p < 0.05$) in endotoxemic atria. These data propose that altered cardiac cycle dynamics during systemic inflammation does not necessarily reflect the altered input from the autonomic nervous system, but rather a remodeling of the cardiac pacemaker dynamics by inflammatory mediators.
Disclosure of interest: None declared.

P-193
ENDOTOXEMIA IMPAIRS CHRONOTROPIC RESPONSIVENESS TO CHOLINERGIC STIMULATION AND DECREASES HEART RATE VARIABILITY IN CONSCIOUS RATS

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Cardiac cycle is regulated by a complex interplay between autonomic nervous system and cardiac pacemaker cells. Decreased heart rate variability (HRV) and increased cardiac rhythm regularity is associated with poor prognosis in patients with sepsis. However, the underlying mechanism of decreased HRV in sepsis is unknown. In complex systems, uncoupling of the regulatory centers is known to be related to greater regularity and less variability. The present study was aimed to test the hypothesis that impaired responsiveness of cardiac pacemaker to autonomic nervous system may lead to uncoupling of the cardiovascular regulatory mechanisms during systemic inflammation. Systemic inflammation was induced by intraperitoneal injection of endotoxin (1 mg/kg) in rats. Cardiovascular signals were recorded in conscious animals using telemetric system. Heart rate dynamics was analysis using Poincaré plot. Spontaneously beating atria were isolated and chronotropic responsiveness to adrenergic and cholinergic stimulation was assessed using standard organ bath. Vagal modulation of cardiac cycle (as assessed by SD1 in Poincaré analysis of HRV) exhibited a significant reduction in endotoxemic rats. Endotoxemia was also associated with a significant hypo-responsiveness of isolated atria to cholinergic stimulation (IC50 of charbacholine changed from $5.8 \pm 0.3 \times 10^{-7}$ M in controls to $9.3 \pm 0.9 \times 10^{-7}$ M in endotoxemic rats, $P < 0.05$). The chronotropic responsiveness to adrenergic stimulation was identical in controls in comparison with endotoxemic rats. These data propose that altered cardiac responsiveness to cholinergic stimulation may lead to uncoupling of cardiac pacemaker cells from autonomic neural control. This may explain decreased HRV during systemic inflammation.
Disclosure of interest: None declared.

P-194
TOXICITY STUDY OF ORAL AND INTRAVENOUS ARISTOLOCHIC ACID IN WISTAR RATS

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A dried root of *Aristolochia tagala* Cham. is often used in Thai traditional medicine as antipyretics, anti-inflammatory agent, muscle relaxant, appetite-enhancing agent, and analeptics (Sathornviriyapong et al. in 41:420–432, 2007). However, some *Aristolochia* species have

been reported to cause nephrotoxicity due to aristolochic acid (AA) and its derivatives (Tran et al. in 2008; 6). Here, we have investigated whether AA administered intravenously or intragastrically causes nephrotoxicity in vivo. Male Wistar rats (200–210 g) were randomly divided into three groups in which the rats were intragastrically administered water or 100 mg/kg AA, and another group was intravenously administered 100 mg/kg AA. Serum urea and creatinine were measured before and 7, 14 and 21 days after a single treatment. There was no significant difference in serum urea and creatinine between groups either at baseline, 14 or 21 days after the treatment. A great rise in serum urea and creatinine was observed at day 7 after the treatment. In addition, the intravenous AA significantly increased lipopolysaccharide induced-liver damage measured with serum alanine transaminase (ALT) on day 22 after the treatment. These results highlight that (i) the intravenous aristolochic acid significantly caused nephrotoxicity and precipitated liver damage induced by lipopolysaccharide, and that (ii) these effects were not observed in rats received AA through intragastric gavage.
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PHAGOCYTIC ACTIVITY AND ROS PRODUCTION OF LPS TOLERANT MACROPHAGES

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Sepsis induces a systemic inflammatory response leading to tissue damage and cell death. LPS tolerance changes inflammatory response and many studies have shown that tolerant mice have higher survival rates after a LPS challenge. To study potentially new mechanisms of immune regulation in endotoxemia, we examined macrophage function in experimental tolerance. LPS tolerance was induced in Balb/C mice exposed to 1 mg/kg of LPS/day during 5 days. The injections were subcutaneous to avoid direct effect on peritoneal cells. Naïve mice received distilled water. Peritoneal macrophages were isolated and we observed that LPS treated mice presented a larger number of peritoneal macrophages ($1.6 \pm 0.4 \times 10^6$ vs. $3.7 \pm 0.6 \times 10^6$). We then used opsonized sheep erythrocytes, opsonized zymosan and non-opsonized particles of *Candida albicans* respectively, for Fc-, C3b- or manose-receptor mediated phagocytosis evaluation. We found a reduction in the phagocytosis by Fc (41% in control vs 23% in tolerant group) and manose receptors (23% in control vs 16% in tolerant group). On the other hand, ROS production was increased in macrophages from tolerant mice, both in basal (0.1 ± 0.08 vs. 1.3 ± 0.6 μM H_2O_2) and after PMA stimulation (0.25 ± 0.07 vs. 5.9 ± 1.5 μM H_2O_2). These differences can be due to a macrophage (reprogramming) activation as demonstrated by the expression of CD80 and CD86. We observed an increase of CD80 and decrease of CD86 expression in tolerant mice of 9 and 7%, respectively. These data suggest that although phagocytic activity of macrophages is suppressed in tolerant mice, bacterial clearance may be more effective due to increased ROS production and antigen presentation to other leukocytes.
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P-196
LIPOPOLYSACCHARIDE-INDUCED CYTOKINE SECRETION DIFFERS BETWEEN THP-1 CELLS, PERIPHERAL BLOOD MONONUCLEAR CELLS, AND MONOCYTES

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Introduction: A cell culture model to monitor endothelial activation in the early phase of Gram-negative sepsis has been established and used to test the effect of mediator modulation with various adsorbent polymers for extracorporeal blood purification. Human umbilical vein endothelial cells (HUVEC) were activated with a conditioned medium (CM) which was obtained by a 4 h-stimulation of monocytic THP-1 cells with 10 ng/ml of lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* in a medium containing 10% human plasma. The aim of this work was to determine possible differences of THP-1 cells, PBMC, or monocyte activation and resulting differences in HUVEC stimulation.
Methods: Density gradient centrifugation with Ficoll-Paque yielded PBMC and further negative selection with the MyPure Monocyte Isolation Kits (Dynal, Invitrogen) resulted in 95% pure monocytes. After an overnight resting period, THP-1 cells, PBMC, or monocytes were stimulated with LPS for 1, 2, 4, 6, 8, and 24 h (1×10^6 cells/ml) and the CM were applied to HUVEC for 16 h. Protein secretion of tumour necrosis factor α (TNF-α), interleukin (IL)-6, IL-8 and IL-10 was determined with a multiplex cytokine array (Luminex, Bio-Rad).
Results: In general, cytokine secretion was comparable for THP-1 cells, PBMC, and monocytes; however, THP-1 cells did not secrete IL-6 and IL-10. After stimulation of the HUVEC with the various CM for 16 h, it was seen that concentrations of TNF-α decreased, IL-10 remained stable, and IL-6 increased. Only the IL-8 secretion from HUVEC was different for conditioned media obtained from THP-1 cells, PBMC, or monocytes. CM from THP-1 cells lead to secretion of IL-8 from HUVEC, while CM from PBMC or monocytes resulted in a decrease of IL-8 concentrations after 16 h of HUVEC stimulation.
Conclusion: Our data demonstrate that expression of cytokines is different for LPS-stimulated THP-1 cells, PBMC, and monocytes, which also influences the subsequent activation of endothelial cells.
Disclosure of interest: None declared.

P-197
STUDY OF PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM BLOOD CULTURES

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Background: Bloodstream infections cause significant morbidity and mortality worldwide and are among the most common healthcare associated infections. Rapid detection and identification of clinically relevant microorganisms in blood cultures is very essential and

determination of antimicrobial susceptibility pattern for management of antimicrobial therapy.
Objectives: The aim of present study was to investigate the type of bacteria isolated from blood cultures and determination of their antibiotic susceptibility pattern.
Methods: During 18 months, 2,790 blood culture samples were screened. The positive blood cultures were examined and the organisms were identified as per standard procedures. Antimicrobial susceptibility testing was performed for all isolates by use of disk diffusion technique, according to CLSI guidelines.
Results: From total blood culture samples, 155 (5.6%) were positive. The most common isolated gram negative bacilli were *Klebsiella pneumoniae* 52 (33.5%), *Eschericia coli* 32 (20.6%) and *Enterobacter* sp. 15 (9.7%) and coagulase negative staphylococci (CONS) as predominant gram positive cocci, all the isolated bacteria showed the highest degree of resistance to ampicillin (98.7%), cefalexin (70.3%) and trimethoprim- sulfamethoxazole (69.7%). Gram positive cocci were also fully resistant to penicillin.
Conclusion: Present study revealed that both gram positive and gram negative bacteria were responsible for bloodstream infections and most of the strains were multi-drug resistant. The most common isolated bacteria from blood cultures were *Klebsiella pneumoniae* and *E. coli*. Ciproflexacine was the most effective antibiotic against gram negative bacilli, while vancomysin was mostly effective against gram positive cocci.
Disclosure of interest: None declared.

P-199
TNF-ALPHA INCREASES CILIA-DRIVEN PARTICLE TRANSPORT IN THE MOUSE TRACHEA

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Background: The tracheal cilia epithelium prevents via its highly effective clearance functions the contamination of the lower airways by pathogens. These specialist epithelium cells are not only in contact with the gas phase, in addition they can also be influenced by inflammatory mediators carried in the blood stream. These mediators alter the protective function of the cilia epithelium (Jain et al. 1995). Since TNF-α plays a key role within the inflammatory cascade, we investigated its effect onto the cilia-driven particle transport (CPT).
Material and methods: Adult male mice (15 weeks old) were sacrificed by an overdose isoflurane, subsequently the trachea was rapidly removed under sterile conditions and transferred into an incubation chamber of an Olympus upright brightfield microscope. CPT and cilia-beat-frequency (CF) were continuously recorded over a period of 130 min as described previously (König et al. 2009) while exposed to different doses of TNF-α. RT-PCR was implemented to demonstrate TNF-α-receptor expression in tracheal epithelium.
Results: RT-PCR experiments provided evidence of the constitutive expression of TNF-α-receptor type 1. Exposure to TNF-α induced a concentration dependent increase of CF and CPT that occurred approximately with a latency of 45 min. CPT and CF were significantly higher after 51 min of incubation when compared to control ($p < 0.05$ for 360 ng, $p < 0.001$ for 1,000 ng, $p > 0.05$ for 100 ng). The increase of CF showed a strong correlation with the velocity of CPT ($r = 0.86$).
Conclusion: Exposure to TNF-α induces an increase in CPT and CF within 45 min, hence it improves the cilia clearance function of the trachea. This may prevent further contamination of the lower airways and may also contribute to reduced inflammation periods. Future studies will investigate the cellular mechanisms of the observed TNF-α response.

References
Jain B, et al. Am J Phys. 1995;268:911–917.
König P, et al. PLoS One, 2009;4:e4938.

Disclosure of interest: None declared.

P-200
LERCANIDIPINE ATTENUATES PROINFLAMMATORY CYTOKINES AND VASCULAR INFLAMMATION INDUCED BY LIOPPOLYSACCHARIDE/INTERFERON-GAMMA VIA THE INHIBITION OF NF-KB ACTIVATION

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Accumulating evidence suggests that inflammation as well as oxidative stress play essential roles in atherogenesis, progression of atherosclerosis, and plaque instability and rupture. Clinical studies have shown that calcium channel blockers (CCBs) inhibit the progression of atherosclerosis. Recent studies have attributed additional anti-oxidative characteristics to the anti-hypertensive drug lercanidipine, a third generation CCB. An anti-inflammatory effect of lercanidipine has, however, yet to be established and therefore we carried out a series of in vitro studies to investigate anti-inflammatory effect and possible mechanism of action of lercanidipine in rat aortic smooth muscle cells (RASMCs) induced by lipopolysaccharide/interferon-γ (LPS/IFN-γ). Pretreatment with lercanidipine (1 or 10 μM) suppressed the release of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) caused by LPS/INF-γ. Lercanidipine also had potent inhibitory effect on LPS/INF-γ-induced upregulation of nitrite level, iNOS protein and iNOS mRNA. To investigate the mechanism of the action of lercanidipine, the extent of NF-κB activation will be monitored by electrophoretic mobility shift assay. Lercanidipine concentration-dependently attenuated the LPS/INF-γ-induced DNA binding activity of NF-κB and the phosphorylation of inhibitory κBα (IκBα), resulting enhanced nuclear translocation of p65 protein. Furthermore, we demonstrated that lercanidipine suppressed the phosphorylation of ERK1/2, JNK and p38. Taken together, these results suggest that that lercanidipine suppress LPS/INF-γ-induced iNOS expression by down-regulating NF-κB through inhibition of MAPKs and IκBα signaling pathways. These findings imply that the inhibition of NF-κB may be involved in the modulation of vascular inflammation response by lercanidipine.
Disclosure of interest: None declared.

P-201
INHIBITORY ACTIVITY OF ASPIRIN AND DICLOFENAC AGAINST CANDIDA ALBICANS STRAINS

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Candida albicans is an opportunistic dimorphic fungus that exists as a commensally and colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract. It is the most common pathogen of human fungal infection and can cause both superficial and serious systemic disease. The prophylactic and curative treatments with antifungal drugs can cause the appearance of *Candida* resistant-strains. The aim of our study was to evaluate the antifungal activity of two anti-inflammatory drugs, aspirin and diclofenac sodium, and investigate the effect of combination of these drugs with ketoconazole. Two strains were isolated from pharyngeal secretions. Strains were identified by biochemical and molecular methods (RFLP of ARN ribosomal 5.8S gene) as *Candida albicans* using *Candida albicans* ATTC 10231 as reference strain. Viability cells tests were done in the presence of 2 mg/ml aspirin and diclofenac sodium and 0.32 μg/ml ketoconazole. Results showed that aspirin alone and aspirin-ketoconazole combination have the same effect on C. *albicans* strains and cell viability decrease with 70–74%. Unlike aspirin, diclofenac sodium–ketoconazole combination induced higher antimicrobial activity (76.5–80% inhibition) than diclofenac sodium alone (40–73% inhibition). The effect of aspirin and diclofenac was similar with activity of ketoconazole at MIC breakpoint. Antifungal activity of non-steroidal anti-inflammatory drugs was strain depending.

According to our results aspirin and diclofenac sodium alone or in combination with ketoconazole have a remarkable antifungal activity against *Candida albicans* strains. Association of anti-inflammatory drugs with antifungal compounds could be more efficient for treatment of candidiasis.

Keywords: Candida, aspirin, anti-inflammatory drugs.
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Disclosure of interest: None declared.

P-202
SCREENING OF THE ANTI-INFLAMMATORY PEPTIDES AGAINST MD-2 AND ITS DEPRESSION ON TLR4 ACTIVATION STIMULATED BY LPS

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Many documents suggested that the synergic effect of MD-2 to LPS stimulating on TLR4 might be critical step during LPS-TLR4 response signaling pathway. MD-2 has been considered as one of the most important and potential targets to interfere the activation of excessive inflammation caused by LPS. Varied efforts are being made to disable MD-2. In this research, we combined bioinformatics and the technique of phage peptide display to try to obtain peptides that would antagonize against MD-2. Based on our bioinformatics analysis of MD-2 protein, NH2-FSKGKYKCV-COOH (K128-132) might be the key sequence for MD-2 binding LPS, thus random phage display peptide library Ph.D.-C7C was used for selecting antagonistic peptide against the sequence. After three round selections, three positive clones binding to the sequence were picked out. Further results showed that all three peptides can bind directly to the MD-2 protein identified by Sulfo-SBED biotin Label transfer. In addition, all three peptides can inhibit the productions of TNF-alpha and IL-6 in U937, THP-1 macrophage cell lines and health human peripheral blood monocytes stimulated by LPS in dose dependent manner. Furthermore, BALB/C mouse challenged by LPS (O111:B4) were used to estimated the protection of these peptides from sepsis, but only two of the peptides can reduce mortality of the infected mice from 100 to 55.6% (5/9), and 54.5% (6/11), meanwhile the levels of TNF-alpha and IL-6 in serum and homogenized lung were profound reduced in peptides treated group, moreover the pathological examination showed that the pulmonary inflammatory responses were alleviated in peptides treated group. These studies demonstrated that interference focused at binding site between MD-2 and LPS might be one potential therapeutic strategy for LPS induced sepsis, and the two peptides might be considerable agents against sepsis.

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P-203
A SELECTIVE CYCLOOXYGENASE-1 INHIBITOR, PIROXICAM, REVERSES ENDOTOXIN-INDUCED HYPOTENSION IN RATS: CONTRIBUTION OF VASOACTIVE EICOSANOIDS AND NITRIC OXIDE

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NO produced by iNOS is responsible for endotoxin-induced vascular hyporeactivity and hypotension resulting in multiple organ failure. Endotoxic shock is also characterized by decreased expression of COX-1, CYP4A, and eNOS. Our previous studies demonstrated that dual inhibition of iNOS and COX with a nonselective inhibitor indomethacin restores blood pressure presumably due to increased production of 20-hydroxyeicosatetraenoic acid (20-HETE) derived from arachidonic acid by CYP4A in endotoxemic rats. The aim of this study was to investigate the effects of a selective COX-1 inhibitor, piroxicam, on the endotoxin-induced changes in blood pressure, expression of COX-1, COX-2, CYP4A1, eNOS, iNOS, and hsp90, and production of PGI_2, PGE_2, 20-HETE, and NO. Injection of endotoxin (10 mg/kg, i.p.) to male Wistar rats caused a fall in blood pressure and an increase in heart rate associated with elevated renal 6-keto-PGF_{1a} and PGE_2 levels as well as an increase in COX-2 protein expression. Endotoxin also caused an elevation in systemic and renal nitrite levels associated with increased renal iNOS protein expression. In contrast, systemic and renal 20-HETE levels and renal expression of eNOS, COX-1, and CYP4A1 were decreased in endotoxemic rats. The effects of endotoxin, except for renal COX-1 and eNOS protein expression, were prevented by piroxicam (10 mg/kg, i.p.), given 1 h after injection of endotoxin. Endotoxin did not change renal hsp90 protein expression. These data suggest that a decrease in

the expression and activity of COX-2 and iNOS associated with an increase in CYP4A1 expression and 20-HETE synthesis contributes to the effect of piroxicam to prevent the hypotension during rat endotoxemia. [This work was supported by the Research Foundation of Mersin University (BAP ECZF EMB (BT) 2006-3 and BAP SBE EMB (TC) 2008-6 DR), Novartis Turkey, USPHS NIH Grant HLBI-19134-34, and USPHS NIH Grant HLBI-19134-33A1.]
Disclosure of interest: None declared.

P-204
MOLECULAR EPIDEMIOLOGY AND PRESENCE OF VIRULENCE-ASSOCIATED GENES AMONG STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS EPIDERMIDIS STRAINS FROM PATIENTS WITH DIABETIC FOOT ULCER: FOCUS ON MRSA AND MRSE STRAINS FROM NEUROPATHIC VS. ISCHEMIC FOOT ULCERS

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The purpose of present study was: (1) to evaluate frequency of virulence genes among *Staphylococcus aureus* (*eta*, *fnb*A, *cna*, *etd, edin*) and *Staphylococcus epidermidis* (*atl*A, *ica*AB and IS*256* insertion element) strains yielded from diabetic foot ulcer (DFU) patients; (2) to compare gene frequency among strains originating from patients with neuropathic (NFU) versus ischemic (IFU) foot ulcers and among MRSA vs. MSSA strains; (3) to assess distribution of multi-virulence gene presenting isolates among *S. aureus* strains. Gene analysis showed that *eta* was more often associated with MRSA than MSSA isolates from NFU and with MSSA than MRSA strains from IFU patients ($p < 0.05$). The presence of *eta, fnbA* and *edin* genes was more often seen in *S. aureus* strains yielded from ulcer tissue compared to toe web swabs of DFU patients and in strains yielded from ulcer curettage of NFU vs. IFU patients ($p < 0.05$). *S. aureus* isolates originating from NFU were frequently characterized by simultaneous presence of 4–5 studied virulence genes. *S. epidermidis.* IS*256* insertion factor was significantly more often associated with MRSE than MSSE isolates and its frequency was higher in isolates yielded from ulcer tissue than toe web of DFU patients. It suggests that detection of *eta*, *fnbA* and *edin* genes can be useful to discriminate between invasive and skin colonizing *S. aureus* strains and that IS*256* insertion element might be a molecular marker of *S. epidermidis* invasiveness in DFU patients.
Disclosure of interest: None declared.

P-205
TLR4 AND 5 DIFFERENTIAL INVOLVEMENT IN BURKHOLDERIA CENOCEPACIA-INDUCED DEADLY PNEUMONIA

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The aim of the present study was to determine which toll-like receptor(s) (TLRs) were involved in triggering the inflammatory response responsible for the death due to *B. cenocepacia* pneumonia. Indeed, we previously showed that MyD88, a key downstream adapter for most the TLRs, was involved the pathogenesis. We specifically focus on the TLRs 4 and 5, as these two receptors are the main ones involved in the recognition of *P. aeruginosa*, a flagellated Gram- bacterium like *B. cenocepacia.* Mice deficient for TLR4, TLR5 or both were infected intratracheally with a suspension of *B. cenocepacia*. Animals were then observed daily for signs of morbidity. Alternatively, bronchoalveolar lavages (BAL) were collected at different time points to further determine cytokine concentrations and the number of CFU of *B. cenocepacia.* We observed that the innate immune response of the host to *B. cenocepacia* lung infection was due to TLR4 and not to TLR5. As for the MyD88$^{-/-}$ strain, TLR4$^{-/-}$ mice were protected from death and cytokine and chemokine synthesis to infection were reduced. By contrast, we observed a reduced pathogen burden in the case of TLR4$^{-/-}$ mice compared to the enhanced (but transient) pathogen burden observed with MyD88$^{-/-}$ mice, suggesting that another TLR was involved in bacterial clearance. The data clearly demonstrate a deleterious implication of TLR4 in the host to *B. cenocepacia* lung infection.
Disclosure of interest: None declared.

P-206
CLOSED FRACTURE CALLUS MAY CONTAIN MICROBES RESPONSIBLE FOR NON-UNION

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Objectives: More than 1% of closed fractures of lower limbs and 6% of implanted materials are complicated by inflammation despite all efforts to avoid infection.
Aim: The question arises whether this clinical complication is not caused by bacteria dwelling in limb tissues.
Materials and methods: Skin, subcutaneous fat, muscle and fracture gap callus were obtained from 155 adult patients operated on due to closed comminuted fractures of tibia or femur, 75 because of non-alignment of bone axis and 80 due to delayed fracture healing.
Results: Aerobic bacteria were isolated from gap callus of 12% healing and 31% non-healing fractures. In subcutaneous tissue and muscles isolates were found only sporadically. No anaerobic bacteria were detected. PCR amplifications of 16 s rRNA were found positive in 40% of callus specimens proving presence of bacterial DNA even when no isolates were found. The 95% similarity of the genetic pattern of some strains from foot skin and callus, estimated with RAPD technique, suggested their foot skin origin.

Conclusions: The colonizing bacterial cells and their DNA were detected in fracture callus but not other deep tissues. Contamination was precluded by lack of isolates in disinfected cutis, subcutis, muscles and materials used for sampling cultured after surgery. We suggest that certain strains of bacteria dwell in normal tissues of lower limbs and may cause inflammation upon stimulation by trauma. Their source may be tissue fluid, superficial and deep lymphatics, and

lymph serving the physiological transport to the regional lymph nodes of microorganisms penetrating foot skin during microinjuries.
Disclosure of interest: None declared.

P-207
MOLECULAR AND PHENOTYPIC HETEROGENEITY OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM PATIENTS WITH ISCHEMIC VS. NEUROPATHIC DIABETIC FOOT ULCERS

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Adhesion proteins, degradative enzymes and other proteins ensure bacterial parasitism as well as virulence. The aim of our study was to compare characteristics of *S. aureus* strains isolated from patients with ischemic (IFU) and neuropathic (NFU) diabetic foot ulcers.
Methods: Study was done on 36 *S. aureus* isolates obtained from ulcer base curettage (UC) and skin biopsy (SB) of patients divided into IFU and NFU group. For detection of *fnbA* and *cna* genes (encoding binding proteins for fibronectin and collagen) bacterial DNA was extracted and PCR with specific primers was done. For detection of gene expression bacterial RNA was extracted and mRNA production was estimated in RT-PCR. Ability of biofilm formation was determined using Congo red agar (CRA) method and for production of polysaccharide intercellular adhesin (PIA) the staining with antiserum was used. For testing of in vitro enzyme production Difco spirit blue agar with lipase reagent and Difco DNase test agar were used. Gelatinase activity was detected using 3% gelatin medium.
Results: The prevalence rate of *fnbA* gene and its expression were more often associated with strains originating from UC and SB of NFU than IFU. Expression of *cna* gene was higher in strains originating from NFU than IFU. The frequency of strains producing biofilm was higher among isolates originating from NFU than IFU when tested by CRA method. Activities of gelatinase, DNase and lipase were found in higher rate of strains yielded from UC of IFU than NFU.
Conclusions: It can be suggested that the high rate of PIA producing strains with hydrolytic enzyme activity present in IFU can be responsible for the worse outcome of this type of ulcers in contrast to NFU.
Disclosure of interest: None declared.

P-208
POSTOPERATIVE IMMUNE SUPPRESSION IN VISCERAL SURGERY: AN INTESTINAL MOUSE MODEL OF THE INFLAMMATORY REACTION TO A SURGICAL TRAUMA

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Background: Post-operatively acquired immune dysfunction is associated with a higher mortality rate in case of septic complications. As details of this severe clinical problem are still unknown and intervention strategies are lacking, animal models are essential to characterise the mechanisms involved.
Materials and methods: Isolated splenocytes were stimulated ex vivo with LPS and the release of cytokines was determined. The degree of surgical trauma was analysed by detection of high-mobility group box 1 (HMGB1) and IL-6 in serum.
Results: We have adapted the previously described animal model of intestinal manipulation (IMM) so as to provide a model of surgically induced immune dysfunction (SID). Following intestinal manipulation mice showed elevated serum levels of HMGB1 and IL-6. Ex vivo cytokine release by splenocytes was suppressed at early and late time points in the post-operative period and the degree of suppression correlated with the extent of surgical trauma.
Conclusions: In this study we describe a surgically-induced immune dysfunction (SID) animal model in which a significant surgical trauma is followed by an immune dysfunction syndrome. This model may be qualified for the characterisation of the post-operative immune dysfunction syndrome and to analyze its inflammatory components.
Disclosure of interest: None declared.

P-209
GLUCOCORTICOID RECEPTOR DIMERIZATION IN MACROPHAGES SUPPRESS INFLAMMATORY RESPONSES

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Endogenous glucocorticoids (GC) have a protective role in septic shock. GCs exert their effects via the glucocorticoid receptor (GR) that alters gene expression by binding to GC responsible elements (GRE) as a dimer in the promoter region of target genes or by interacting with and thus interfering with other transcription factors as a monomer.
We show that the GR in Macrophages (Mphs) is important for the repression systemic inflammation. Mice lacking the GR in myeloid cells ($GR^{LysMCre}$) are highly susceptible to LPS induced septic shock. Also binding as a dimer to DNA is important for protection from septic shock. Mice with impaired GR dimerization (GR^{dim}) display a strong lethality in septic shock models. Mphs from GR^{dim} mice are resistant to suppression of some inflammatory mediators (IL1b, NO). We identified GR dimer controlled genes, possibly essential in inhibition of inflammation in a micro array analysis from Mph mRNA isolated from wt and GR^{dim} mice. One of the genes encoded Sphingosine kinase 1 (Sphk1). Sphk1 mRNA and activity of the enzyme was found to be up regulated synergistically in wt Mphs co-treated by lipopolysaccharide (LPS) and the GC analogue dexamethasone (Dex). In GR^{dim} Mphs the up regulation was significantly attenuated, indicating that GR dimerization was required for this process. The mechanism for the Sphk 1 mRNA regulation by LPS and Dex is protein synthesis and p38 dependent. Sphk 1 and Sphk 2 are key enzymes for production of sphingosine-1-phosphate (S1P). In primary LPS activated Mphs S1P is important for the repression inflammatory cytokines and seems to function as anti-inflammatory mediator of the dimerized GR. The impact of Sphk1 in anti-inflammatory actions of the GR is currently exploited in vivo. We demonstrate that GR dimerization in Mphs is required for immunosuppression in systemic inflammation. Mphs of GR dimer deficient mice serve as an

excellent tool to identify novel GC regulated genes important for inhibition of inflammation.
Disclosure of interest: None declared.

P-210
SERUM AMYLOID A1 IS ELEVATED IN E. COLI-INFECTED MICE UROTHELIA

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Serum amyloid A (SAA) is an acute phase protein involved in homeostasis of inflammation. SAA is necessary in host defense and has beneficial properties in protection against viral and bacterial infections. Urothelial infections have been especially difficult to treat since latent intracellular pools of bacteria remain in urothelial cells, also after treatment with antibiotics, and cause episodes of recurrent cystitis without evident re-infections. To date it has been unclear whether SAA is present in infected urothelium and whether it could be protective against bacterial infections. The major aim of this study was to better understand the role of SAA in murine infections of the urothelium with E. coli and determine the differential expression of SAA isoforms in the urothelium and in the serum (and whether SAA could act in a protective manner). Urothelial SAA protein was detected by immunofluorescence in mouse tissue. SAA1 and SAA3 mRNA expression in the liver, urothelium and urinary bladder were quantitated using real-time PCR from infected and noninfected mice. Serum SAA1/2 and SAA3 were detected by ELISA. The biofilm inhibition assay was performed using crystal violet to stain adherent/biofilm-associated bacteria adhered to plastic. Our experiments revealed that SAA is highly expressed in urothelial cells of E. coli infected mice versus non-infected mice. Especially, there is a high increase in cytoplasmic SAA during infection as compared to the nuclear SAA in urothelial cells. We also have evidence that following E. coli inoculation into bladders, SAA1 mRNA expression is higher than that of SAA3 in the urothelium. SAA1/2 can act protectively, by inhibiting E. coli biofilm formation. In conclusion, urothelial SAA is elevated significantly following E. coli infection. SAA could be a good serological marker for E. coli urothelial infection, where it might also perform bactericidal functions.
Disclosure of interest: None declared.

P-211
HIGH PREVALANCE OF ANTI-DENGUE VIRUS IGG IN PATIENTS WITH VENOUS THROMBOEMBOLISM AND POSSIBLE ROLE OF DENGUE VIRUS IN THROMBOSIS

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Venous thromboembolism is a lethal disorder includes deep vein thrombosis and pulmonary embolism. Hereditary and acquired factors are accused in the etiology. Existence of large vWF multimers in plasma due to absence of ADAMTS13 is the major factor in the pathogenesis of thrombotic thrombocytopenic purpura. ADAMTS13 is a metalloprotease that splits vWF multimers. The absence of ADAMTS13 is also shown in certain diseases where there is increased tendency to thrombosis. Dengue is a viral disease with an increasing incidence worldwide. Recently, dengue virus (DV) infections have been associated with a deficiency of ADAMTS13. Thrombocytopenia is also another finding in DV infection. However, the underlying mechanisms remain unknown. **Aims:** In an earlier study, we have demonstrated significantly lower levels of ADAMTS13 and significantly higher levels of vWF in the plasma of 30 patients with venous thrombosis and pulmonary embolia vs 30 healthy individuals ($p < 0.001$). In this study, we aimed to determine the presence of anti-DV IgG in the same patient and healthy control groups, retrospectively. Methods: Thirty plasma samples from patients and 30 plasma samples from healthy individuals (totally 60) were participated in this study. Plasma samples were tested by anti-DV IgG ELISA.
Results: Anti-DV IgG was detected in 13 of 30 (43%) and 3 of 30 (10%) in patient and healthy control groups, respectively ($p = 0.006$). Conclusions: The presence of anti-DV IgG in 43% of the patients versus 10% of controls imply a possible association with DV infection, deficiency of ADAMTS13 and the etiology of thrombosis ($p < 0.05$). However, the underlying mechanism should be clarified. Roles of TNF-α IL-1β and IL-6 in the development of disseminated intravascular coagulation thus leading to microvascular thrombosis were well characterized. Persistent agents such as DV could be playing a key role in triggering the proinflammatory cytokines in the same manner. Our further studies focused on this way.
Disclosure of interest: None declared.

P-212
DETECTION OF PARVOVIRUS B19 AND IL-6 IN SYNOVIAL FLUIDS OF PATIENTS WITH OSTEOARTHRITIS

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Pathophysiology of osteoarthritis was associated with the accumulation of inflammatory mediators in the joint space. Accumulation of immunoglobulins secondary to chronic inflammation or exposure of antigen in the joint space has been well described. Parvovirus B19 (PVB19) is a ubiquitous pathogen normally causes a mild short-lived illness. However, this virus is potentially capable of causing significant pathology and of long-term persistence. Certain inflammatory disorders have been linked to PVB19 infection. Enhanced IL-6 production in hematopoietic cell lines transfected with NS1 protein of PVB19, providing evidence that NS1 protein may be involved with transactivation of the IL-6 gene promoter. IL-6 is a ubiquitous and pleomorphic cytokine, multicellular in origin that acts on numerous cells and tissues. Hence, NS1 protein expression could play a role in the ongoing inflammation and cell damage with persistent PVB19 infections in nonpermissive cells such as synovial membrane cells.
Aims: To provide more insight into the possible role of this virus in the etiology of osteoarthritis.

Methods: Synovial fluid samples from 42 patients with osteoarthritis and 10 healthy control (totally 52) were participated to this study. Anti-PVB19 IgG, anti-PVB19 IgM, IL-6 levels and PVB19 DNA were assayed by ELISA and PCR.
Results: PVB19 DNA was detected in 28 of 42 (66.66%) and in 3 of 10 (30%) in patients and controls. Anti-PVB19 IgG and IgM response were detected in 21 of 42 (50.00%) and in 2 of 42 (4.76%) patients. Anti-PVB19 IgG and IgM were also detected in 1 of 10 (10.00%) and in 0 of 10 (0.00%) controls. IL-6 was positive in 15 of 42 (36%) and in 3 of 10 (30%) patients and controls. All IgG (+) samples had PVB19 DNA (100%, $p < 0.001$). 11 of 15 IL-6 (+) samples had PVB19 DNA (+) (73.33%, $p < 0.05$).
Conclusions: These findings support the presence of PVB19 acting as a transactivator of IL-6 as reported earlier and ongoing inflammation contributing cell damage in osteoarthritis.
Disclosure of interest: None declared.

P-213
EVALUATION OF THE ROLE OF CARBENOXOLONE DISODIUM SALT IN REDUCTION OF SYSTEMIC INFLAMMATION INDUCED BY S. TYPHIMURIUM LPS IN A MOUSE MODEL

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The strategies for treatment of systemic inflammation are based on neutralization of LPS or cytokines induced by it. Heat shock proteins (HSPs) inhibit production of pro- inflammatory cytokines. Carbenoxolone induces the production of HSPs. The aim of this study is the evaluation of the effect of Carbenoxolone in reduction of the severity of systemic inflammation induced by LPS in a murine model. 80% lethal dose of *Salmonella typhimurium* LPS was determined in balb/C mice, also appropriate dose of drug for efficient reduction in mortality was determined. LPS with drug were injected to different groups, in different times. LPSCarb group (injected with LPS + D-GalN and Carbenoxolone simultaneously) and LPS1Carbi.p. (Received Carbenoxolone by i.p. injection 1 h after LPS + D-GalN), after 24 h the mortality rate was calculated and the severity of necrosis measured in the livers of survived mice. Then mice were injected with LPS + D-GalN and drug in different times, after 3 h serum samples were collected and the concentrations of TNF-α and IL-10 were measured. Administration of carbenoxolone with LPS + D-GalN reduced the lethality rate from 80 to 20%. The severity of necrosis in the liver of LPSCarb and LPS1Carbi.p. groups were significantly decreased in compare with LPS group (injected by LPS + D-Gal) ($P = 0.01$ and $P = 0.04$ respectively). The concentration of TNF-α in mice received LPS were significantly increased in compare with control group ($P = 0.032$). The changes in the IL-10 concentrations were accompanied with changes in TNF-α concentration. Carbenoxolone significantly reduced TNF-α concentration, inflammation and damage due to LPS, however changes observed in IL-10 concentration was in response to TNF-α concentration changes.
Disclosure of interest: None declared.

P-214
SCD163 IN CRITICALLY ILL PATIENTS

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CD163 is a transmembrane scavenger receptor for haptoglobin–hemoglobin complexes expressed by monocytes and macrophages with a role in the resolution of inflammation. Inflammatory stimuli induce ectodomain shedding of soluble CD163 (sCD163). High serum levels of sCD163 predicted mortality in some inflammatory diseases. We investigated sCD163 release and predictive power towards outcome in critical illness. This is a secondary analysis of 2 randomized clinical studies. Critically ill patients ($n = 1657$) received conventional (insulin administered only for blood glucoses >215 mg/dL) or intensive insulin therapy (glucose maintained at 80–110 mg/dL). Matched healthy volunteers ($n = 24$) were included as controls. We measured serum levels of sCD163 as well as gene expression levels in liver biopsies. On day 1, critically ill patients had twofold higher sCD163 levels than controls ($p < 0.001$). Day 1 sCD163 levels were higher in patients with an ICU stay >5 days (long-stay) versus short-stay patients ($p < 0.001$). sCD163 further increased by day 5, 10 and 15 in long-stayers and remained elevated until the last day, the latter irrespective of short or long stay. Non-survivors and patients who developed new kidney injury, liver dysfunction, polyneuropathy or bacteremia had higher sCD163 levels from day 1 onwards. Moreover, even after correction for baseline risk factors, elevated day 1 sCD163 levels remained independently predictive for organ damage, ICU stay, and mortality. Avoiding hyperglycemia with insulin reduced sCD163 levels. Gene expression of CD163 in liver was strongly elevated in critically ill patients as compared with controls. Circulating sCD163 levels were elevated during critical illness, with the liver as a potential source. This study hallmarks elevated sCD163 as marker of disease severity and predictor of outcome in critical illness. Reduced sCD163 with intensive insulin therapy supports reduced inflammation and may contribute to improved outcome with this therapy.
Disclosure of interest: None declared.

P-215
SYNERGISTIC EFFECT OF GLUTAMINE AND CIPROFLOXACIN IN REDUCTION OF PSEUDOMONAS AERUGINOSA-INDUCED SEPTIC SHOCK

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Systemic inflammatory response induced by over expressing inflammatory mediators is the main pathogenic mechanism of septic shock. Glutamine (Gln), a non-essential amino acid, has been demonstrated to inhibit pro-inflammatory cytokine release through enhanced heat shock protein (HSP) expression. The aim of this study was the evaluation of the effect of co-administration of Gln and ciprofloxacin in reduction of septic shock severity caused by *Pseudomonas aeruginosa* in mice. Balb/c mice were divided to five groups. Mice of the control negative group injected only with PBS and those of the positive control group received 75% lethal dose (LD75) of *Pseudomonas aeruginosa*. Mice of the remaining three groups were administered by ciprofloxacin, glutamine or both 30 min after injection of LD75 of the bacteria. Five hours after injection of the bacteria, serum samples were collected and concentrations of TNF-α, IL-10 and HSP-70 were measured. Eleven hours after injection of the bacteria, livers of the mice were removed and the severity of necrosis was determined. The survival rate was considered for 10 days. The results showed that injection of glutamine in combination with ciprofloxacin significantly increased the survival rate and the serum HSP-70 and IL-10 concentration and significantly decreased the serum TNF-α concentration and the liver necrosis in comparison with the positive control group. The findings indicated that Gln has a synergistic effect with ciprofloxacin in reduction of *Pseudomonas aeruginosa*-induced septic shock.
Disclosure of interest: None declared.

P-216
FBP62, FIBRONECTIN BINDING PROTEIN OF STREPTOCOCCUS ANGINOSUS INVOLVES THE VIRULENCE IN MOUSE INFECTION

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Background: *Streptococcus anginosus* is a part of the normal flora found in human dental plaque. Although it has emerged that *S. anginosus* infection could be associated with oral, esophagus and gastric cancers, its pathogenic factors are still unclear. We previously identified fibronectin binding protein gene, *fbp62,* which could associate the adherence of *S. anginosus* to mucosal epithelial cells. In this study, by using the *fbp62* knockout mutant we investigated the pathogenic involvement of Fbp62 in an *S. anginosus*-infection model.
Materials and methods: An *fbp62* knockout mutant (Δ*fbp62*) was prepared by homologous recombination from *S. anginosus* NCTC 10713. The adhesive ability of Δ*fbp62* to the immobilized fibronectin and HEp-2 cells was analyzed by using [^{3}H]-labeled bacteria. The survival rate and the abscess formation were investigated in an *S. anginosus*-infection mouse model with ip injection of Δ*fbp62*. The mRNA expression of inflammatory cytokines (TNF-α, IL-1β, IL-6) of mouse macrophages was analyzed by real-time PCR.
Results: The adhesive ability of Δ*fbp62* to HEp-2 cells as well as the immobilized fibronectin was considerably reduced. In the mice infected with Δ*fbp62*, the mortality and the abscess formation were substantially lower than those in the mice infected with the wild type of bacteria. However, no difference was observed in the mRNA expression of inflammatory cytokines between the macrophages from Δ*fbp62*- and wild type of *S. anginosus*-infected mice.
Conclusions: The present findings indicated that the fibronectin binding proteins, Fbp62, of *S. anginosus* could play an important role in the adherence to mucosal epithelial cells, and suggested that the adhesive ability could be involved in the pathogenicity of *S. anginosus*.
Disclosure of interest: None declared.

P-217
NATURAL KILLER CELLS DIRECTLY RESPOND AND KILL MYCOBACTERIA

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Natural killer (NK) cells play a pivotal role in the effector arm of host innate immune defense system. The major functions of NK cells in killing tumor transformed or virus-infected cells were well established. However, whether NK cells can directly kill bacteria remains poorly understood. In this study, using the pathogenic mycobacterium *Mycobacterium kansasii* as the study model, our results indicated that human NK cells directly kill mycobacteria in a contact dependent manner, using the cytolytic proteins, perforin and granulysin as the weapons. These results indicate that the antibacterial activity is constitutively expressed in NK cells, in which it requires prior activation, granulysin and perforin, plays the dominant role in NK cell antibacterial activity. Moreover, the expression of NKG2D, NKp30, NKp44 and NKp46 upstream signaling molecule of the MAP kinases pathway, are also enhanced. *M. kansasii*-enhanced granulysin and perforin expression and antibacterial activity are effectively inhibited by pretreatment with the inhibitors of JNK (SP600125), ERK-1/2 (PD98059), p38 (SB203580), or by siRNAs against JNK-1, ERK-2 and p38. A similar killing phenomenon by perforin and granulysin was also observed in *Mycobacterium tuberculosis*, suggesting a conserved killing mechanism. In conclusion, results from this study showed NK cells directly kill mycobacteria.
Disclosure of interest: None declared.

P-218
SYK PLAYS DUAL ROLES IN TLR-MEDIATED SIGNALING PATHWAY

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Toll-like receptor (TLR) is a major family of pattern recognition receptors (PRRs) and plays a crucial role in innate immunity. Microbial pathogens can activate TLRs signaling and trigger dramatic immune responses. The Spleen tyrosine kinase (Syk) presents in innate immune cells and plays a key initiation of ITAM-containing immunoreceptors signaling and TLRs signaling. However, the precise role of Syk in TLRs signaling is still controversial. Here we show the dual role of Syk in TLR4-mediated signaling cascade and inflammatory gene expression in primary murinemacrophages. We found that Syk-deficient macrophages after LPS stimulation can induce higher expression levels of inflammatory genes and proteins. Loss of Syk also increases TAK1 downstream signaling cascades activation after LPS stimulation. In contrast, less LPS-induced TBK1 activation and type I IFN (IFNb) gene expression in Syk-deficient cells than wild type cells were observed. In HEK293T overexpression system, we also found Syk interacts with

TLR signaling molecules, such as TLR4, MyD88, TRAF6 and TRAF3. Taken together, our results demonstrate the role of Syk in TLR4-mediated TAK1 and TBK1 signaling pathways and the opposite regulatory effect on inflammatory responses. We anticipate our finding to be a starting point forth dual role of Syk in immune responses and the delicate regulation of Sykcan tune the innate immune response to less inflammation in late infection phase.
Disclosure of interest: None declared.

P-219
THE EXPRESSION OF NEUROPEPTIDE GALANIN IS SIGNIFICANTLY DOWN REGULATED IN POLYMICROBIAL SEPSIS

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The neuropeptide substance P (SP) has been shown to be an important mediator in lung injury during polymicrobial sepsis. SP exhibits its proinflammatory effects via increased capillary permeability and neutrophil extravasation. In a mouse model of polymicrobial sepsis, animals were protected against lung injury after administration of a specific SP receptor antagonist. For the neuropeptide galanin no data concerning septic conditions are available so far. However, in murine skin, galanin has been shown to inhibit plasma extravasation induced by SP and calcitonin-gene related peptide (CGRP). This suggests galanin as an opponent of SP and CGRP in neurogenic inflammation. To study a possible role of galanin during systemic inflammation we investigated the expression of the galanin system, SP and TNF-α mRNA in organs of septic mice. Sepsis was induced in C57/BL6 mice by colon ascendens stent peritonitis (CASP), because this animal model mimics closely human septic conditions. Lungs were removed 12 h after surgery. Quantitative real time PCR was performed to determine the relative expression of galanin, TNF-α and SP mRNA. We were able to detect a significant up regulation of SP (60-fold) and TNF-α (110-fold) expression and a 50% down regulation of galanin in lungs of septic mice compared to sham operated animals. The expression of the galanin receptors R1, R2 and R3 was not changed. These results implicate that galanin is down regulated in the initial immune response to favour pro-inflammatory mediators. In further course galanin might be increased as an opponent of SP to avoid an overshooting immune response, including vasodilatation, neutrophil accumulation and organ dysfunction.
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Disclosure of interest: None declared.

P-220
ROLES OF PROTEIN KINASE C AND SRC FAMILY TYROSINE KINASE IN RHOA ACTIVITY IN THORACIC AORTAE FROM ENDOTOXEMIC RATS

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Septic shock is characterized by severe hypotension and reduced response to vasopressor agents, called vascular hyporeactivity. The hypotension and vascular hyporeactivity are associated with the development of multiple organs dysfunction which causes death eventually. Thus, it is important to investigate the mechanism of vascular hyporeactivity in septic shock. Among the cell signaling pathways that are crucial to control vascular tone, Ca^{2+}-sensitization contraction has become more and more important. The small GTP-binding protein, RhoA, plays a crucial role in mediating smooth muscle contraction. Activation of RhoA leads to inactivation of myosin light chain phosphatase via activation of Rho-kinase. It has been shown that Src family tyrosine kinase (SFK) can activate RhoA via phosphorylation of guanine nucleotide exchange factor. In addition, it is reported that activation of protein kinase C (PKC) can lead to Ca^{2+}-independent vasocontraction through activation of RhoA. The purpose of this study was to investigate the role of PKC and SFK in RhoA activity in thoracic aortae from endotoxemic rats. Rats received an intravenous injection of lipopolysaccharide (LPS, 10 mg/kg) for 4 h. After then, rats were sacrificed and the thoracic aortae were excised and immediately incubated in GF-109203× (a PKC inhibitor) and PP2 (a SFK inhibitor). We found that RhoA activity was decreased significantly in aortae from endotoxic rats. GF-109203× and PP2 had inhibitory effect in aortae from endotoxic rats only. In addition, the phosphorylation of SFK was decreased by GF-109203× in endotoxemic rats. In conclusion, PKC and SFK might play a more crucial role in RhoA activation and PKC might play a more important role in activation of SFK endotoxemic rats. These results suggest that RhoA is related to vascular hyporeactivity caused by LPS-induced septic shock. In addition, PKC and SFK might be targets for improving vascular hyporeactivity.
Disclosure of interest: None declared.

P-221
THERAPEUTIC EFFECTS OF LEVOSIMENDAN ON PERITONITIS-INDUCED SEPTIC SHOCK WITH MULTIPLE ORGAN DYSFUNCTION SYNDROME IN RATS

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Sepsis/septic shock and its sequelae, multiple organ dysfunction syndrome (MODS), are major contributors of mortality in critical ill patients. Levosimendan (LS) is a fairly new calcium sensitizer with positive inotropic properties without increasing myocardial oxygen consumption. Therefore, we evaluated the effects of levosimendan in Wistar rats with MODS induced by cecal ligation and puncture (CLP). Rats were divided into six groups: (1) sham-operation (SOP), (2) SOP+ vehicle [infusion dose (5% glucose) 30 μl/kg/min for 6 h, loading dose 120 μl/kg for 10 min i.v. at 3 h after SOP], (3) SOP + LS (infusion dose 0.3 μg/kg/min for 6 h, loading dose 12 μg/kg for 10 min i.v. at 3 h after SOP) (4) CLP, (5) CLP+ vehicle [infusion dose (5% glucose) 30 μl/kg/min for 6 h, loading dose 120 μl/kg for 10 min i.v. at 3 h after CLP] and (6) CLP + LS (infusion dose 0.3 μg/kg/min for 6 h, loading dose 12 μg/kg for 10 min i.v. at 3 h after CLP). Our results showed that LS (1) improved hypotension, hypoglycemia, and vascular hyporeactivity caused by CLP, (2) reduced ALT, AST, creatinine, BUN and LDH in plasma, (3) reduced plasma NO and IL-1β level, superoxide anion

levels and lung inducible NO synthase expression, (4) decreased lung, liver, intestine polymorphonuclear neutrophils (PMN) infiltration, (5) attenuated apoptosis of spleen, (6) improved metabolic acidosis, (7) improved the survival rate compared to the CLP + vehicle group. Thus, the beneficial effect of LS may be attributed to reducing the plasma concentration of NO and IL-1β as well as organ superoxide anion levels and decreasing lung, liver, intestine PMN infiltration and spleen apoptosis, thereby decreasing the mortality rate in peritonitis-induced septic animals.
Disclosure of interest: None declared.

P-222
MODULATION OF INFLAMMATORY RESPONSE DURING SEPSIS: ROLE OF LIPOXIN A4

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Introduction: Pneumonia is the major cause of sepsis in intensive care units. Sepsis is the result of the host response to microbial infection, and can be considered as an excessive systemic inflammatory response. Lipoxins (LX) are lipid mediators with potent anti-inflammatory actions. Previous works show that pathogens may take advantage from the anti-inflammatory environment provided by LXA_4. The aim of this work is to study the role of LXA_4 in sepsis.
Methods: Pneumonia-induced sepsis was induced by intra-traqueal inoculation of *Klebsiella pneumoniae* (4×10^8 CFU/animal) in Swiss mice. Sham-operated mice were used as control. Mice received lipoxin receptor (FPR2/ALX) antagonist BOC-1 (1 μg/kg; i.p.) soon after surgery and every 12 h in survival experiment. Six hours after sepsis induction, animals were sacrificed and bronchoalveolar lavage (BAL) and tissues were obtained for analysis.
Results and discussion: Neutrophils (PMN) migration to lungs from septic animals increased when compared to sham group. Animals exhibited a high degree of bacterial load in BAL, spleen and heart. Compared to septic mice, animals treated with BOC-1 exhibited increases of PMN number to the infectious focus and consequently a reduced bacterial load and dissemination. Interestingly, LXA_4 levels in BAL from septic mice were higher compared to sham animals. Treatment with BOC-1 also increased survival rate. Therefore, LXA_4 levels increased in sepsis and FPR2/ALX antagonist BOC-1 reduced bacterial load and improved survival. These data suggest for the first time that a reduction in anti-inflammatory mechanisms in the beginning of the sepsis process could be an interesting therapeutic alternative.
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P-223
TLR7 REQUIRES AEP FOR PROCESSING AND SIGNALLING BUT NOT TLR3

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The innate immune system provides the first barrier against pathogens. It has evolved to recognise a broad range of pathogens and discriminate between self and non-self. Toll-like receptors (TLRs) are the crucial sensors of pathogen associated molecular pattern or PAMPS. Intracellular Toll-like receptors (TLR3, 7 and 9) localise in endosomes and recognize single stranded RNA and nucleotides from viruses and bacteria. This interaction induces their conformational changes resulting in the production of proinflammatory cytokines and up regulation of cell surface molecules in dendritic cells (DCs). In the absence of stimulation, TLRs are retained in the endoplasmic reticulum (ER) together with another ER resident protein, UNC93B. Upon stimulation, they relocate to the endo-lysosomal compartment, allowing the recruitment of the adaptor molecule, MyD88, and thereafter, signal. It has been shown that mouse TLR9 requires proteolytic cleavage in the endosomes to be functional. This is an important step, as it is the activation of the MyD88 signaling pathway that leads to the production of pro-inflammatory cytokines and maturation in DCs. Indeed, upon stimulation, full length TLR9 is cleaved into a C-terminal-fragment and this processing is highly dependent on a cysteine protease named asparagine endopeptidase (AEP). We have now evidence that TLR7 is also cleaved by AEP into a C-terminal fragment competent for signaling. Like TLR9, a boost in AEP activity, induced shortly after TLR7 stimulation, promotes TLR7 cleavage and correlates with an increased acidification in endosomes. Moreover, mutating a putative AEP cleavage site in TLR7 strongly decreases its signaling in DCs. In contrast, TLR3 processing and signaling seems to require a different set of proteases. As endocytic TLRs recognize DNA/RNA from various parasites, bacteria, viruses and are likely to play an important role in many inflammatory diseases, we are now developing infection models in AEP deficient mice.
Disclosure of interest: None declared

P-224
DIC, NEW 5-(4-PYRIDIL)-4,5-DIHYDROISOXAZOL DERIVATE INHIBITS NF-KB/MAP KINASE PATHWAY AND EXERTS ANTI-INFLAMMATORY EFFECT IN EXPERIMENTAL SEPSIS

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Introduction: The functioning of the immune system is finely balanced by the activities of pro-inflammatory and anti-inflammatory mediators or cytokines. Unregulated activities of these mediators can lead to development serious inflammatory diseases. Therefore, anti-cytokine agents that target biosynthetic pathways of pro-inflammatory cytokines would offer an attractive alternative to the treatment of inflammatory diseases. Recent data showed the capacity of VGX-1027 [(S,R)-3-phenyl-4,5-dihydro-5-isoxasole acetic acid] to inhibit the increase of circulating levels of tumor necrosis factor α and interleukin-1β in lipopolysaccharide (LPS)-challenged mice. In this study, we evaluated the role of DIC, a 5-(4-pyrildil)-4,5-dihydroisoxasole derivate, as a potential anti-cytokine compound.
Results: DIC significantly decreased the levels of inducible TNF-α, IL-1β and interleukin-6 (IL-6) in RAW 264.7 macrophages stimulated with LPS. DIC also inhibits the activity of cyclooxygenase-2 (COX-2) in LPS-stimulated RAW 264.7 macrophages with consequently decrease of prostaglandin-2 (PGE2) levels. Electrophoretic

mobility shift assay revealed that DIC attenuated the LPS-induced DNA binding of NF-kB. In addition, it was found that treatment with DIC significantly inhibited the activation of p38 and ERK MAP kinase. Taken together, these results suggest that anti-inflammatory effect of DIC in LPS-treated RAW 264.7 macrophages is associated with the inhibition of NF-kB transcriptional activity, possibly via the MAP kinase pathway. Furthermore, in vivo, DIC conferred increase in the survival of mice that have been subjects to cecal ligation and puncture, an experimental sepsis model.
Conclusions: We believe that the anti-cytokine activity of DIC could be due to the NF-kB/MAPK pathway inhibition. In addition, our data suggest that DIC may potentially play a role in the therapy of inflammatory disease as sepsis.
Keywords: Pro-inflammatory cytokines, Sepsis, 4,5-dihydroisoxasole, NF-kB.
Disclosure of interest: None declared.

P-225
PREVENTIVE EFFECTS OF CHLORELLA ON LPS-INDUCED INFLAMMATION AND SEPTIC DEATH IN MICE

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Inflammation is a host response to infection and is characterized by elevated inflammatory mediators. Two of the major mediators during inflammation, i.e. nitric oxide (NO) and prostaglandin E2 (PGE2), are released by macrophages and neutrophils. Overt inflammation can lead to detrimental consequence, such as septic shock, which is known due to excessive NO and proinflammatory cytokines production. Chlorella has been shown to have various remarkable biological effects, including anti-inflammatory effect through inhibition of iNOS pathway and proinflammatory cytokines (e.g. IL-6 and TNF-α production), however its protection against LPS-induced sepsis has not been studied. In this study, we investigate protective effects of *Chlorella* extract against LPS-induced sepsis in mice. Endotoxin-induced septic shock was initiated by injecting 20 mg/kg of lipopolysaccharide (LPS) in mice. Mice were given different dosages of *Chlorella* extract (10, 20, 30 mg/kg, orally) 30 min after the treatment of LPS. Serum NO production was prepared and measured as nitrite (using Griess reagent), IL-6 and TNF-α were concurrently monitored by using commercial ELISA kits. The mortality rate was observed 12, 24, and 36 h after the LPS treatment.
Chlorella extract administration decreased LPS-induced mortality as evidenced by increased survival rate in a dose dependent manor. The survival rate remained as high as 95% in those mice treated with 30 mg/kg of *Chlorella* extract compared to 40% of survival rate in the control (vesicle treatment) 36 h after LPS injection. *Chlorella* extract treatment also significantly suppressed LPS-induced serum NO, IL-6 and TNF-α levels. In conclusion, *Chlorella* extract effectively prevents LPS-induced septic mortality by reducing inflammatory mediator and proinflammatory cytokines production.
Disclosure of interest: None declared.

P-226
INFLUENZA A VIRUS INDUCES MUC5AC, A MAJOR SECRETED PULMONARY MUCIN, IN A PROTEASE-EGFR-ERK-SP1-DEPENDENT PATHWAY

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Introduction: The airways mucus gel performs a critical function in the defense of the respiratory tract against pathogenic and environmental challenges. In respiratory infections, mucins such as Muc5AC could be protective and able to sequester pathogens [1].
Aims: (1) To determine whether IAV (seasonal and pandemic Influenza A strains) is able to modulate Muc5AC production in airway epithelial cells. (2) To dissect the molecular pathways involved in IAV modulation of Muc5AC synthesis.
Results: We demonstrate here that seasonal and pandemic strains infection of NCI-H292 cells (MOI = 1) induced significantly MUC5AC gene expression. Influenza A/Scotland/20/74 (H3N2), the most virulent strain, lead to a significant Muc5AC increase compared to mock-treated cells, and this was dependent on viral replication. Moreover, using the same strain, we showed that in vivo infection of C57Bl/6 mice with 150 or 300 pfu lead to a threefold increase of Muc5ac RNA expression at day 3 and 4 post-infection compared to PBS-treated mice. We demonstrated that the Sp1 transcription factor is involved in IAV-induced MUC5AC gene expression and that this is mediated by the MEK/ERK signaling pathway. Next, we showed that this pathway was initiated upstream by a matrix metalloproteinase (TACE)-mediated epidermal growth factor receptor (EGFR) activation and that TGF-a is one of the ligands implicated in EGFR activation [2, 3].
Conclusion: We show for the first time that IAV up-regulates Muc5AC through a TACE/EGFR/MAPK pathway and that this induction is IAV-replication dependent. We are studying now if up-regulation protects against IAV infection or whether Muc5AC over-expression is deleterious in IAV-induced deleterious exacerbations in chronic lung diseases, such as asthma or cystic fibrosis.

(1) Puchelle E, et al. Eur Respir J. 1992;5:3–4.
(2) Kohri K, et al. Am J Physiol Lung Cell Mol Physiol. 2002;283:L531–L540.
(3) Chokki M, et al. Am J Physiol Lung Cell Mol Physiol. 2004;30:470–478.

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P-227
THE SURGEON'S EXPERIENCE DOES NOT AFFECT THE INFECTION COMPLICATIONS AFTER LOWER THIRD MOLAR SURGERY

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It remains controversial whether prophylactic antibiotics are necessary after lower third molar removal (LTMR) and whether a surgeon's clinical experience impacts this possible need. This study evaluated and compared postoperative complications of patients who were operated on by surgeons ranging in clinical experiences. These surgeons included an oral surgeon specialist, a PhD student in Oral Biology, and a senior dental student. In the preoperatory period and on the second and seventh postoperative days, the following parameters were analyzed: pain, infection, swelling, trismus, body temperature, C-reactive protein levels and salivary neutrophil counts. During surgery the following parameters were analyzed: systolic, diastolic and mean arterial pressure, oximetry, heart rate, quality of anesthesia, amount of local anesthetic, bleeding, surgery difficulty and duration of surgery. There were some differences in the surgery duration, local anesthetic amount, anesthesia quality, bleeding, pain experienced, trismus, C-reactive protein levels and salivary neutrophil counts. However there were no changes in hemodynamic parameters, rescue analgesic medication, wound healing, swelling, body temperature, confirmed case of dry socket or any other type of local infection in the patients. Taken together, the results of the present study suggest that antibiotic prescriptions were unnecessary after LTMR no matter what the surgeons' clinical experiences were.
Disclosure of interest: None declared.

P-228
TRPV1 PROTECTS AGAINST SEPSIS BY REGULATING MACROPHAGE RESPONSES

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TRPV1 has a protective role on lipopolysaccharide (LPS)-induced sepsis (Clark et al. 2007). We have now evaluated the role of TRPV1 in sepsis by using a murine model of polymicrobial sepsis caused by cecal ligation and puncture (CLP). Male C57/BL6 WT and TRPV1KO mice were utilised. CLP triggered leukocyte migration into the peritoneal cavity of both WT and TRPV1KO mice when compared to sham animals. No difference was observed in the total cell number between CLP WT and TRPV1KO mice but a reduced number of intact mononuclear cells were found in TRPV1KO peritoneal cell samples. Analysis of apoptotic genes in the peritoneal cells by Q-PCR showed that CLP TRPV1KO mice presented increased expression of FAS, p53 and caspase-3 when compared to CLP WT mice. Also, peritoneal TNFα levels were higher in CLP TRPV1KO when compared to CLP WT mice. CLP increased the peritoneal nitric oxide (NO^x) and ROS (superoxide and H_2O_2) levels in WT but not in TRPV1 KO mice. In vitro phagocytosis was assessed in cultured peritoneal macrophages obtained from WT and TRPV1KO mice pre-treated with oyster glycogen. WT and TRPV1KO unstimulated macrophages exhibited similar phagocytosis. However, under LPS stimulation, TRPV1KO macrophages showed impaired phagocytosis. Q-PCR analysis of PI3 Kα and β, essential for phagolysosome formation, were reduced in CLP TRPV1KO in comparison to CLP WT peritoneal cells. Our results provide novel evidence that TRPV1 can regulate immune cell functioning and survival in sepsis. We suggest that TRPV1 protection in sepsis is related to its role in regulating immunity.

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Clark et al. FASEB J. 2007;2:3747–55

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P-230
DIFFERENTIAL INFLAMMATORY RESPONSES IN MACROPHAGES BY VANCOMYCIN-SUSCEPTIBLE STAPHYLOCOCCUS AUREUS AND VANCOMYCIN-INTERMEDIATE STAPHYLOCOCCUS AUREUS

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Introduction: This study is designed to explore the differential inflammatory responses in macrophages by the different resistant strains of vancomycin-susceptible *Staphylococcus aureus* (VSSA) and vancomycin-intermediate *Staphylococcus aureus* (VISA).
Materials and methods: Macrophages (RAW264.7 cell line) were infected using two strains of VSSA and VISA isolated from a patient with septic arthritis. The time course was 0, 6, and 12 h and the doses were 1×10^3, 1×10^4, and 1×10^5 CFU/ml, respectively. Gene regulation including cyclooxygenase 2 (COX-2), 5-lipoxygenase, IL-1beta, IL-6, and TNF-alpha was investigated by reverse transcriptase-polymerase chain reaction (RT-PCR). COX-2 protein expression and signal transduction were evaluated by western blotting and electrophoretic mobility shift assay (EMSA), respectively.
Results: COX-2 and 5-lipoxygenase gene expression did not influenced by starvation within 12 h. COX-2 gene induction by VSSA and VISA infection in macrophages was in a dose-dependent and time course-dependent manner. VSSA yielded an intense, significant response compared to VISA in early infection (6 h) and also produced similar effects by dosage experiments ($p < 0.05$). The 5-lipoxygenase pathway did not demonstrated similar responses. VSSA also produced a higher level for IL-1beta and IL-6 gene expression than VISA did. TNF-alpha showed an increase tendency but no significant difference. COX-2 protein expression showed significant difference at a concentration of 1×10^5 CFU/ml VSSA and VISA. Signal transduction involved the phosphorylation of extracellular regulated kinase 1/2 (p-ERK1/2) and NF-kB activation.
Conclusion: Our data may suggest that changes of *Staphylococcus aureus* strains to the antibiotic sensitivity may induce differential inflammatory responses in macrophages and play an important role in the host inflammatory adaptation.
Disclosure of interest: None declared.

P-231
TRAUMATIC TISSUE DAMAGE: THE SEARCH FOR TRAUMA ALARMINS

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Introduction: Traumatic injury generates an inflammatory response which is proportional to injury severity. Candidates for trauma Alarmins include HMGB1, Mitochondrial DNA (MtDNA) and eukaryotic nucleosomes. We wished to investigate the role of these substances in the sterile inflammatory response to tissue damage when isolated from other activators of inflammation.
Methods: Blood was drawn from human trauma patients on arrival to a major trauma centre. Injury severity score (ISS) and admission base deficit (BD) were used to identify a "Trauma" cohort (T) with tissue injury and no hypoperfusion (ISS > 4, BD ≤ 2 mmol/l) and a "Trauma-Shock" cohort (TS) (ISS > 4, BD > 2 mmol/l). The "Control" (C) group was defined as ISS 0-4, BD ≤ 2 mmol/l. Concentrations of HMGB1, MtDNA and nucleosomes were measured in plasma using ELISA or qPCR.
Results: 300 patients were recruited. Levels of MtDNA (27.18 qPCR cycles, range 24.7–28.1, $p = 0.002$) were higher in the T cohort compared to C (27.6 cycles, range 26.9—28.4). Cycles correlated with ISS ($p < 0.001$, $r^2 = 0.1$) and were not influenced by shock (BD). HMGB1 demonstrated a similar trend but not a dose-dependent increase. Nucleosome levels were not raised in the T cohort. HMGB1 was elevated in the TS cohort (17.0 ng/ml range 0.0–160.0, $p < 0.001$) compared to C (8.4 ng/ml range 0.0–68.3) and levels correlated with base deficit ($p < 0.001$, $r^2 = 0.1$) but not ISS. In the TS cohort, nucleosome levels (19.6 Au range 0.0–114.2, $p < 0.001$ vs. C: 4.7 Au range 0.0–66.4) correlated with ISS ($p = 0.01$) and BD ($p = 0.02$, $r^2 = 0.07$).
Conclusions: After traumatic injury, in the absence of shock and other activators of inflammation, circulating MtDNA levels reflected injury severity. These molecules may be trauma alarmins. Nucleosomes were not released by tissue injury alone. HMGB1 levels increased in proportion to the base deficit and not injury severity. Our study findings suggest that HMGB1 may not be passively released by pure tissue damage in human trauma.
Disclosure of interest: None declared.

P-232
TOLL-LIKE RECEPTORS (TLR) 2 E 4 AND THE ADAPTER MOLECULE MYD88 PLAY A ROLE IN SEPSIS-INDUCED ACUTE KIDNEY INJURY

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Introduction: Sepsis is considered one of the main causes of acute kidney injury (AKI) in ICU patients, mainly due to bacteria infection. TLR2 e TLR4 are important innate immunity receptors responsible for pathogen-associated molecular pattern structure recognition that when activated, they trigger, with the adaptor molecule MyD88, a signaling cascade that results in the expression of pro-inflammatory cytokines/chemokines.
Objective: To study TLR2, TLR4 and adaptor molecule MyD88 role in sepsis-induced AKI.
Methodology: C57BL/6, TLR2 KO, TLR4 KO and MyD88 KO male mice were subjected to sepsis by cecal ligation and puncture (CLP) with two perforations using 23G needle. Blood and kidney tissue samples were collected after 24 h for analysis.
Results: After 24 h, TLR2 KO, TLR4 KO e MyD88 KO animals subjected to CLP presented preserved kidney morphology, with lower acute tubule necrosis scores. We observed less areas of hypoxia when compared with WT animals (WT 3.07 ± 1.2; TLR2 KO 1.89 ± 0.9; TLR4 KO 1.76 ± 1.02; MyD88 KO 0.01 ± 0.02) and less areas of apoptosis (WT 0.01 ± 0.003; TLR2 KO 0.006 ± 0.004; TLR4 KO 0.008 ± 0.003; MyD88 KO 0.001 ± 0.0006). TLR2 KO and TLR4 KO animals presented a tendency towards less injury, while MyD88 KO animals were completely protected compared to WT animals (*Urea* WT 237.57 ± 28.9; MyD88 KO 91.71 ± 4.9) (*Serum Cr* WT 1.86 ± 0.54; MyD88 KO 0.44 ± 0.02). We also observed a lower expression of proinflammatory cytokine mRNA (IL1-β, TNF-α, IL-6, IL17 e KC), in TLR2 KO, TLR4 KO and MyD88 KO kidneys when compared to WT. We observed less neutrophil infiltration in MyD88 KO kidneys 24 h after sepsis. MyD88 KO animals also presented higher survival scores when compared to WT animals.
Conclusion: Here, we concluded that the innate immunity participates in sepsis-induced polymicrobial AKI, mainly through MyD88 pathway.
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Disclosure of interest: None declared.

P-233
ROLE OF ESTRADIOL ON MODULATION OF INFLAMMATORY MEDIATORS AND RESISTANCE TO INFECTION INDUCED BY ENTEROCOCCUS FAECALIS

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Background: It has been demonstrated that ovarian hormones modulate immune response during inflammatory conditions, acting in production of cytokines, as well as nitric oxide (NO) synthesis. The aim of this study was to evaluate the participation of estradiol in the resistance to infection and also, in nitrate and tumor necrosis factor alpha (TNF-α) production in rats submitted to *Enterococcus faecalis* infection.
Materials and methods: Experiments were performed in female Wistar rats ovariectomized and treated for 3 consecutive days with two doses of estradiol (50 or 100 μg/kg) or vehicle. On the third day, 2 h after the last injection, each rat received an intraperitoneal injection of bacterial suspension of *Enterococcus faecalis*. Blood was collected by cardiac puncture at 6 and 24 h after bacterial inoculum administration, for blood culture, plasma nitrate and TNF-α measurement. Nitrate was detected by chemiluminescence, while TNF-α were determined by ELISA sandwich kit. Furthermore, the microbiological

tests were determined in blood and liver at the same experimental periods.
Results: Estradiol at the higher dose was effective in decreasing bacterial dissemination in the liver ($p < 0.05$), but not in blood at 6 and 24 h after bacterial challenge. Moreover, estradiol also increased significantly plasma nitrate concentration only at 24 h ($p < 0.001$), while TNF was increased at both time evaluated ($p < 0.05$). The febrile response was observed 3 h after *Enterococcus faecalis* administration, but it did not demonstrate statistical difference among the experimental groups.
Conclusion: These data suggest that estradiol does not have effect in body temperature of ovariectomized rats submitted to *Enterococcus faecalis* infection. However, it seems to be effective to oppose the bacterial spread in liver, and also modulate the production of inflammatory mediators, such as NO and TNF-α.
Disclosure of interest: None declared.

P-234
S-NITROSYLATION AND EPIGENETIC REGULATION DURING ENDOTOXIN TOLERANCE

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Administration of lipopolysaccharide (LPS) in low levels induces a resistance state called tolerance, with a silencing of inflammatory genes. Endotoxin-tolerant cells and leukocytes from septic patients exhibit alterations of the cellular signaling that can protect against a lethal challenge of LPS. This condition is regulated by several mechanisms, included changes in chromatin structure, an epigenetic process of gene reprogramming. Nitric oxide (NO) participates of the tolerance conditions. It is known that the interaction of NO with cysteines residues, the S-nitrosylation, could alter the structure of enzymes and consequently its functions. We investigated whether the tolerance could modulate the S-nitrosylation of enzymes involved in the epigenetic regulation such as the Histone Deacetylases (HDAC), responsible by a repression in the gene expression, and consequently modulate the production of the proinflammatory mediators. THP-1 human promonocytes were cultivated in RPMI (C group), submitted to tolerance to LPS (T group 500 ng/mL during 24 h) or challenge (D group 1 μg/mL during 24 h). We used tricostatin (TSA 30 nM) to inhibit the HDAC activity, LNAME (100 μM) to inhibit the NO release and SGNO (0.5 mM) as NO donor. The HDAC activity and cytokine levels (IL-6, IL-10 and TNF) were measured with ELISA kits. S-nitrosylation was assessed by biotin switch and the NO levels by Griess Reaction. Tolerance increased the total amount of S-nitrosylated proteins. We found reduced HDAC activity in the T group that was associated to increasing of S-nitrosylated HDAC-2. Endotoxin tolerance cells demonstrate a reduced levels of IL-10 ($p < 0.05$) and IL-6 ($p < 0.05$). However, the administration of HDAC or NO inhibitors repressed the effects of the tolerance in HDAC activity as well as cytokines production.Our results demonstrate an important role of endotoxin tolerance in the epigenetic regulation and suggest that the S-nitrosylation may have implication for inflammatory repression.
Disclosure of interest: None declared.

P-235
CHOLECYSTOKININ MODULATES INFLAMMATORY MEDIATORS AND RESTORES LIPOPOLYSACCHARIDE-INDUCED HYPOTENSION

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Background: Nitric oxide (NO), tumor necrosis factor-α (TNF-α) and interleukin (IL)-10 play a key role in innate immune system controlling microbial infection. However, during septic shock their exacerbated formation is associated with several deleterious complications, as hypotension. Cholecystokinin (CCK) was firstly described as a gastrointestinal hormone, but immune cells express their receptors, suggesting a possible involvement of this hormone in modulation of inflammatory response. Our aim was to evaluate the role of CCK on NO, TNF-α and IL-10 production during endotoxemia in rats as well as lipopolysaccharide (LPS)-induced hypotension.
Materials and methods: Male Wistar rats received an intravenous injection of CCK (0.4 or 40 μg/kg) 10 min before LPS (1.5 mg/kg) administration. The mean arterial pressure and cardiac output were monitored continuously during 6 h after endotoxin injection. Blood was collected for plasma nitrate, TNF-α and IL-10 determinations at 2, 4 and 6 h after LPS. Nitrate was detected by chemiluminescence, while cytokines were determined by ELISA sandwich kits.
Results: The LPS induced-hypotension was reverted by the pretreatment with CCK only at the lower dose tested ($p < 0.01$). Moreover, CCK reduced plasma nitrate levels at 2 and 6 h after LPS administration ($p < 0.05$), but did not alter TNF-α production. The plasma IL-10 concentration was increased ($p < 0.05$) by CCK administration at 2 and 4 h after endotoxemia.
Conclusion: These data suggest that CCK improves hypotension and reduces NO formation during endotoxemia in rats. Furthermore, CCK regulates positively anti-inflammatory cytokine IL-10 synthesis, however do not modify TNF-α.
Disclosure of interest: None declared.

P-236
WNT5A EXPRESSION BY PORPHYROMONAS GINGIVALIS LPS VIA NF-KB AND STAT1

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Background: Periodontitis is an inflammatory disease caused by gram-negative periodontopathic bacteria which can lead to loss of tooth-supporting structures and alveolar bone resorption. Wnt signaling molecules play important roles in bone biology, apoptosis and chronic inflammation. Recent studies have suggested an association of these molecules with various disorders including cardiovascular diseases, rheumatoid arthritis, and osteoarthritis. We previously demonstrated that Wnt5a mRNA expression was up-regulated in chronic periodontitis tissue when compared to non-periodontitis tissue. In this study, we investigated the modulation of Wnt5a mRNA expression by periodontopathic bacteria.
Methods: Human monocytic cell line THP-1 were stimulated with *Porphyromonas gingivalis* (*P. gingivalis*) LPS, *Escherichia coli* (*E. coli*) LPS, IL-6, IFN-β, or IFN-γ. To investigate the involvement of NF-κB and JAK/STAT pathways in the modulation of Wnt5a expression, techniques were used including inhibition assay, transfection, western blotting, luciferase assay and EMSA. The levels of Wnt5a mRNA were determined by real-time RT-PCR.
Results: In THP-1 cells, Wnt5a expression was significantly up-regulated by *P. gingivalis* LPS than *E. coli* LPS. *P. gingivalis* LPS induced Wnt5a mRNA expression and NF-κB activity. *P. gingivalis* LPS markedly reduced the cytosolic level of IκBα than *E. coli* LPS. The induction of Wnt5a mRNA expression was augmented by co-stimulation with IFN-γ and overexpression of STAT1, but it was suppressed by STAT1 siRNA.
Conclusion: IFN-γ synergistically enhanced the *P. gingivalis* LPS induced Wnt5a expression through STAT1 in parallel with the activation of NF-κB. These findings will help reveal the mechanism of molecular pathogenesis of periodontal disease.
Disclosure of interest: None declared.

P-237
NEONATAL LIPOPOLYSACCHARIDE EXPOSURE CHANGES THERMOREGULATORY RESPONSE TO ESTRADIOL DURING ENDOTOXEMIA IN ADULT RATS

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Background: Early life immune challenge has been considered an adaptive defense strategy against pathogens when the innate immune system is not completely developed. Our aims were to evaluate whether neonatal lipopolysaccharide (LPS) challenge alters body temperature response in adult female rats during endotoxemic shock and whether ovarian hormones may participate in this response.
Methods: Female Wistar rats were intraperitoneally injected with LPS (100 μg/kg) (nLPS) or saline (nSal) at post-natal day 14. Then, experiments were performed in adults submitted to endotoxemic shock (10 mg/kg), surgically implanted with biotelemetry probes to body temperature monitoring. Blood were collected to measure tumor necrosis factor (TNF)-α and interleukin (IL)-10 levels at 1 and/or 2 h after LPS administration.
Results: The LPS injection in adult nSal rats caused an initial hypothermia, followed by a febrile response. However, nLPS showed an increased hypothermia and an attenuation of fever ($p < 0.05$). The bilateral ovariectomy abolished the difference in body temperature between the nLPS and nSal rats. Ovariectomized rats treated with estradiol cypionate (ECP) restored hypothermia and the suppressed febrile response ($p < 0.01$). Moreover, the same results were not obtained when the animals were supplemented with ECP and medroxyprogesterone acetate (MPA). The nLPS rats displayed a significant reduction in TNF-α levels and an increase in IL-10 levels when compared to nSal animals ($p < 0.01$). The ECP injection significantly enhanced IL-10 and suppressed TNF-α in nLPS ($p < 0.001$), but it did not change the inflammatory response in the nSal rats. The ECP + MPA regiment in the nLPS rats reduced TNF-α, but eliminated IL-10 stimulation in comparison to the nSal group.
Conclusion: These data suggest that neonatal LPS challenge alters the thermoregulatory response during endotoxemic shock in adulthood and estradiol could mediate this difference.
Disclosure of interest: None declared.

P-238
PKC-Θ IS REQUIRED FOR THE DEVELOPMENT OF EXPERIMENTAL CEREBRAL MALARIA BUT DISPENSABLE FOR ASSOCIATED LUNG PATHOLOGY

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PKCΘ is required for the development of experimental cerebral malaria but dispensable for associated lung pathology Cerebral malaria is the most severe neurological complication in children and young adults infected with *Plasmodium falciparum*. In mice T-cell activation is required for the development of *Plasmodium berghei* ANKA (*Pb*A)-induced experimental cerebral malaria (ECM). In order to characterize the T cell activation pathway involved, we addressed the role of protein kinase C-Theta (PKC-θ) in ECM development. Here we report that PKC-θ deficient mice are resistant to ECM development. In the absence of PKC-θ no neurological sign of CM developed after blood stage *Pb*A infection. Resistance of PKC-θ deficient mice correlated with unaltered cerebral microcirculation and absence of ischemia, as documented by magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA), while wild-type mice developed distinct microvascular pathology. Recruitment and activation of CD8+ T cells, and ICAM-1 expression were reduced in the brain of resistant mice while the pulmonary inflammation and edema associated with *Pb*A infection were still present in the absence of functional PKC-θ. PKC-θ deficient mice however developed high parasitemia and succumb at 3 weeks with a severe anemia. Therefore, PKC-θ signaling is crucial for the development of brain microvascular pathology resulting in fatal ECM but not for pulmonary inflammation and edema.
Disclosure of interest: None declared.

P-239
THE BACTERICIDAL EFFECT OF THE BACTERIOCIN-LIKE SUBSTANCE FROM BACILLUS SUBTILIS ISOLATED FROM KOREAN FERMENTED SOYBEAN PASTE ON E. COLI O157:H7 AND S. TYPHI

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Some bacterial species present in fermented foods are regarded as probiotics because of their ability to modulate the prevention of some intestinal infections and the modulation of the inflammatory immune response. Spore-forming bacteria such as the genus *Bacillus* make convenient probiotics as they can survive transit through the stomach intact and in the gut. The present study was carried out for the isolation of bacteriocin-like substance (BLS) producing *Bacillus subtilis* from a traditional Korean fermented soybean paste which is consumed commonly as a low cost source of high protein food and safe food. All *B. subtilis* and *B. licheniformis* isolates demonstrated inhibitory activity against *S. aureus* using agar spot inhibition assay. Interestingly, one strain *B. subtilis* (BS1800) displays a wide range of antibacterial activity against *S. aureus, E. coli, E. coli O157:H7* (EHEC) and *S. typhi*, but not *K. pneumoniae, P. aeruginosa* and *S. marcescens*. Furthermore, BS1800 completely killed *E. coli,* EHEC and *S. typhi* after 3 days coculture in LB broth using viable cell count on MacConkey agar. The bactericidal effect on EHEC and *S. typhi* was strain specific. BLS of BS1800 was purified using ammonium sulfate precipitation and was shown to have a molecular mass of 8 kDa as determined by SDS-PAGE analysis. We therefore propose strain BS1800 as a suitable candidate probiotics for use in the prevention and treatment of infections caused by EHEC and *S. typhi*.
Disclosure of interest: None declared.

P-240
MYCOBACTERIUM TUBERCULOSIS HUMAN CLINICAL ISOLATES INDUCES EXACERBATE PULMONARY INFLAMMATION IN MICE

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Background: *Mycobacterium tuberculosis (Mtb)* is a virulent intracellular pathogen that infects and persists in host macrophages, resulting in granuloma formation and collagen deposition in the lung. The mechanisms that confer resistance to *Mtb* or results in establishment of disease are poor understood. Data from the literature suggest that differences in *Mtb* virulence contribute to setting up of the disease.
Objective: Our purpose was investigated the immune response and lung pathology in mice infected with isolates recovered from patients with noncavitary (SV009), cavitary (SV038) and extrapulmonary (SV068) active tuberculosis and compared with H37Ra (avirulent) and H37Rv (virulent) *Mtb* strains.
Methods: Female Balb/c mice were infected intratracheally (i.t) with $1x10^5$ CFU/100 μL of *Mtb* strains or PBS. Neutrophils and mononuclear cells recruitment to the lung were accessed by bronchoalveolar lavage (BALF). Colony formating units (CFU), lung histology, nitric oxide (NO) and cytokines were evaluated 15, 30, 60 and 90 days post infection (p.i.).
Results: Mice infected with H37Ra and H37Rv survival until the end of 60 days p.i. when compared with clinical isolates. Mice infected with SV009, SV038 and SV068 induced more cellular recruitment (neutrophils and mononuclear cells) into BALF at 30 and 60 days p.i. Spleen CFU recovered 90 days p.i. was higher in mice infected with SV009 strain. Histology of lung tissue, demonstrated that animals infected with SV068 present greater number of foamy macrophages containing *Mtb* and intense infiltrate of neutrophils in perivascular and perialveolar spaces mainly at 60 days p.i.; and this animals produced more NO than the other strains at 30 days p.i. Cytokines production were variable, especially IFN-γ, TNF-α, IL-6 and IL-10, and may be modulating the exacerbation of inflammatory response.
Conclusion: Our findings suggest that SV009, SV038 and SV068 can be more virulent than the other *Mtb* strains.
Disclosure of interest: None declared.

P-241
DIFFERENTIAL PLATELET TLR4 ENGAGEMENT AND SUBSEQUENT MONONUCLEAR CELLS MOBILIZATION AFTER LPS EXPOSURE

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Platelets are anucleated cells that play a major role in primary haemostasis but their role in inflammation has now been recognized. Platelets contain and secrete various immunomodulatory factors, which behave as ligands for immune receptors. Platelets express several transcription factors that exert non-genomic functions. We tested for the relevance and the finality of platelets TLR4 pathways. Expression of TLR4 on the platelet surface was determined by flow cytometry analysis. Western blot was performed to detect platelet intracellular protein: MyD88, TBK-1, IRAK-1, JNKs, MAPk, TRAF3, TRAF6, IRF-3, IKK-i, IkB-a, NF-κB p65 subunit or phospho-tyrosine antibody 4G10 and α-tubulin. TLR4 is expressed on human platelets and the percentage of $CD41^+$ $TLR4^+$ platelets increase after TRAP stimulation compared to unstimulated control. Following the addition of *E. coli* or *S. minnesota* LPS there was no significant alteration of CD62P expression. There were striking differences in the levels of the sCD40L, sCD62P, PF4, RANTES and PDGF-AB induced by two different LPS stimuli. Western blot analyses using antibodies to MyD88 and TRIF subunits showed that both proteins were consistently present in purified human platelet lysates. We indeed observed that *E. coli* or *S. minnesota* LPS platelet stimulation modulated phosphorylation protein profiles by using a phosphotyrosine specific antibody 4G10. Finally, supernatants from short term cultured platelets stimulated with two different LPS stimulate differentially blood mononuclear cells with different patterns of cytokine secretion. These data demonstrate a different panel of these chosen secreted cytokines that may reflect consistent variation in the composition of platelet supernatants secreted after TLR4 engagement. This data question the sentinel role of platelets in their environment, their capacity of sensing differentially even through one given PRR, and their subsequent immunoregulatory function.
Disclosure of interest: None declared.

P-242
EFFECTS OF THE XENOGENEIC IMMUNE RESPONSES IN A RAT MODEL OF SEPSIS

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Host immune responses to xenografts show similarities to those of fighting infection. In this study we investigated the effect of xenogeneic stimulation and xenoantibody induction on bacterial infection. Lewis rats were immunized with different protocols of hamster blood and assessed how these procedures affected the development of sepsis induced by cecal ligation and puncture (CLP). Rats that received three injections of hamster blood at 2-week intervals (IgG Group) elicited very high titers of anti-hamster IgG and relatively low specific IgM antibodies at day 40 after initiating the immunizations. Day 40 was thus selected to conduct CLP in this rat cohort. Another set of rats received three injections within a week inducing high titers of anti-hamster IgM and relatively low IgG levels at day 5 after the first injection (IgM Group). They were assigned for CLP at day 5. Multiple hematological, biochemical and general-health parameters were monitored during the study. IgM Group showed a significant increase in blood neutrophils (two, sixfold; $p < 0.05$) and monocytes (one, fivefold; $p < 0.05$) just prior to CLP, whilst IgG Group did not show changes. A CLP procedure that produced a mild septic state was not associated with any mortality in IgG Group, whereas in IgM Group resulted in higher mortality [8/17 (47%), $p < 0.05$]. In a more severe CLP model IgG Group showed an improvement of rat general-health parameters that did not reduce mortality (68.7 vs. 76.5% controls). We also determined xenoantibody reactivity to bacteria isolated from blood of septic rats. Interestingly, we observed a threefold increase in IgM and IgG reactivity to *Enterococcus faecalis* using sera from Group IgM rats collected just prior to CLP, but not to *Escherichia coli*. In conclusion, the generalized immune response induced by a short xenogeneic stimulation had a detrimental effect on the septic process, despite the induction of antibodies that cross-react with some of the microorganisms isolated in the blood.
Disclosure of interest: None declared.

P-244
HYPERTONIC SALINE SOLUTION DECREASE PULMONARY DAMAGE IN A RODENT MODEL OF ENDOTOXEMIA

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Hypertonic saline solution (HS solution, NaCl 7.5%) has shown to modulates immune function favorably and decrease organ injury triggered by endotoxic shock. Therefore, our objective was to investigate the effects of HS solution on the mechanism involved in acute respiratory injury, in an experimental model of endotoxemic shock. Wistar rats (220–260 g) received lipopolysaccharide—LPS (10 mg/kg, intraperitoneal, ip) and volume after 15 min. The animals were assigned in four groups ($n = 6$ per group): control group (not subjected to LPS); LPS group (injected with LPS 10 mg/kg i.p); HS group (injected with LPS and treated with hypertonic saline, 4 mL/kg) and NS group (injected with LPS and treated with normal saline, 34 mL/kg). At 24 h after treatment, pulmonary mechanics, pulmonary edema, and lung histology to evaluate collagen deposition were measured. Hypertonic solution improved pulmonary resistance, compared to LPS and NS groups. HS presented a reduction of 52 and 65% in the resistance. Collagen deposition showed reduced on HS group compared to LPS and NS groups, 61.5 and 82.2% respectively. There were no differences in pulmonary elastance and pulmonary edema with HS. Therefore, we concluded that treatment of endotoxemic shock with HS solution can decrease pulmonary damage and improve the respiratory function.
Disclosure of interest: None declared.

P-245
PERIPHERAL INFLAMMATION AND OXIDATIVE STRESS IN LIPOPOLYSACCHARIDE-TREATED RATS: INVOLVEMENT IN THE INITIAL STEPS OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) physiopathology is recognized as multifactorial and remains unclear. This disease which presents two characteristic lesions, senile plaques and neurofibrillary degeneration is specific to humans. Until now, there is no relevant animal model at our disposal. Recent evidence suggesting that inflammation may be a third important component which may contribute actively to disease progression, we hypothesized that repeated infections during a lifetime facilitate Alzheimer's disease via neuroinflammation and oxidative stress.
Aim: The aim of this study was to mimic an infectious state in rats using acute or repeated LPS administration (1 mg/kg; i.p.).
Methods: After LPS administration, reactive oxygen species (ROS), nitrites/nitrates (NOx), malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) were used as inflammatory and oxidative stress markers in different brain regions and/or blood. Moreover cognitive, cholinergic system and memory function have been assessed on animals after peripheral inflammation (LPS).
Results: We have shown memory impairment and cholinergic system disorders after LPS treatment. LPS also induced a disruption of the blood–brain-barrier (BBB). We also observed that LPS exposure significantly increased ROS and NO cortex production (26 and 63%, respectively) associated with a significant decrease in blood vitamin E (40%).
Conclusion: These results indicate a possible relationship between peripheral infection/inflammation, neuroinflammation and disturbances of the cholinergic system which could initiate AD in patients.
Disclosure of interest: None declared.

P-246
EFFECT OF HYPERTONIC SALINE SOLUTION (NACL 7.5%) TREATMENT ON SYSTEMIC INFLAMMATION AND HEART DAMAGE IN A RODENT MODEL OF ENDOTOXEMIA

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Hypertonic saline solution (HS, NaCl 7.5%) promote immediate blood volume expansion, restore cardiac output, improve microcirculation and modulate immune responses. Therefore, our objective was to investigate the effects of HS solution on the

systemic inflammation and heart damage in a rodent model of endotoxemic shock. Wistar rats (220–260 g) received lipopolysaccharide—LPS (10 mg/kg, intraperitoneal, ip) and volume after 15 min. The animals were assigned in four groups (n = 6 per group): control group (not subjected to LPS); LPS group (injected with LPS 10 mg/kg i.p); HS group (injected with LPS and treated with hypertonic saline, 4 mL/kg) and NS group (injected with LPS and treated with normal saline, 34 mL/kg). At 1,5; 4 and 24 h after treatment, serum concentration of cytokines and at 24 h after treatment heart fibrosis were measured. Collagen deposition in heart decreased in HS and NS compared with LPS group, 38.5 and 49%, respectively. Serum concentration of cytokines showed decreased level of IL-10 in HS (22.3 ± 3.77) and NS (24.15 ± 1.6) treatment compared to LPS (43.84 ± 7.36) at 24 h; decreased level of IL-6 in HS (1,839.7 ± 914.96) and NS (1,330.38 ± 455.46) level compared to LPS (11,583.7 ± 1065.7) at 1,5 h and decreased level of IL-6 in HS (8,333.46 ± 1,631.6) and NS (4,026.6 ± 1,933) compared to LPS (14,040.4 ± 3,142.5) at 4 h after treatment, $p < 0,05$. Therefore, we concluded that treatment of endotoxemic shock with both HS and NS solution can decrease systemic inflammation and heart damage.
Disclosure of interest: None declared.

P-247
INVOLVEMENT OF CCL2 AND NITRIC OXIDE ON BACTERIAL CLEARANCE IN SEPTIC MICE

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Survival during sepsis requires both swift control of infectious organisms and tight regulation of the associated inflammatory response. The aim of this study was investigate the role of CCL2 and NO in clearance of bacteria after CLP model. For this, mice were subjected to CLP model. After 6 h, the numbers of CFU counts and measured of mediators were determined. To investigate the role of CCL2 in clearance of bacteria, the animals were treated with anti-CCL2 and 6 h after CLP the peritoneal fluid were collected. The numbers of CFU were significant increased in anti-CCL2 group. Peritoneal macrophages treated with CCL2 or anti-CCL2 and stimulated with *E. coli* presented the significant increased in numbers of CFU after anti-CCL2 treatment and decreased in numbers of bacteria after CCL2 treatment. These results suggest an important role in the elimination of CCL2 in models of bacterial sepsis. To investigate the involvement of NO in more bacterial elimination, we submitted the deficient mice of iNOS (iNOS−/−) to CLP. We observed the significant increased in numbers of CFU in iNOS−/− group. In order to examine the relationship between CCL2 and NO in clearance of bacteria, peritoneal macrophages of deficient mice to CCL2 (CCL2−/−) and WT mice were stimulated with LPS, IFN-g or LPS + IFN-g, and the NO production was determined. We observed the significant decreased in NO levels into CCL2−/− group after all treatments when compared to the control group. In order to investigate the signaling pathway involved in NO production by macrophages treated with CCL2, we analyze the ERK1/2 pathway by immunolocalization technique. After CCL2 treatment we observed the increase in fluorescence of pERK and iNOS. We conclude that the CCL2 and NO are important mediators involved in better elimination of bacteria in models of sepsis. We observed that CCL2 promotes the release of NO by a mechanism associated with ERK1/2 pathway signaling.
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Disclosure of interest: None declared.

P-248
INDUCTIVE AND SUPPRESSIVE NETWORKS REGULATE TLR9-DEPENDENT GENE EXPRESSION IN VIVO

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Background and objectives: Synthetic oligodeoxynucleotides (ODN) expressing "CpG motifs" mimic the immunostimulatory activity of bacterial DNA. CpG ODN interact with TLR9 to stimulate an innate immune response characterized by the production of Th1 and pro-inflammatory cytokines. We predicted that microarrays could be used to identify reproducible changes in gene expression induced by CpG ODN activation in mice treated in vivo over time, and that network analysis would allow us to identify regulators of gene expression.
Methods: cDNA was generated from total RNA isolated from spleen cells of mice 30 min to 3 days after in vivo treatment with 400 μg of CpG or control ODN. Hybridization was performed on murine genome microarrays (NCI). Analyses were conducted on four independently derived RNA samples for each time point. R2 was 0.90 ± 0.04 for all matched samples. Network analysis was performed using ingenuity pathway analysis.
Results: Gene activation ($p < 0.00001$) was observed within 30 min of CpG ODN treatment, peaked at 3 h, and fell to near background levels after 72 h. TNFa, IL-1b, NF-kB and IFNg played central roles in up-regulating the early expression of immune related genes. Two distinct patterns of gene expression were observed: One subset of genes was activated shortly after CpG ODN administration and remained up-regulated for a prolonged period, while unique subsets of additional genes were activated at specific time points, and were rapidly down-regulated. Several genes responsible for this down-regulation (MYC, IL1RN, SOCS1) were identified.
Conclusions: This analysis identifies two distinct patterns of gene regulation associated with CpG induced activation of the innate immune system of mice. A small number of regulatory genes triggers the patterned up-regulation of immune related genes from 30 min through 72 h. A separate set of down-regulatory genes subsequently dampens what would otherwise be a continuous positive feedback loop.
Disclosure of interest: None declared.

P-249
INDUCTION OF HUMAN CYTOMEGALOVIRUS PROTEINS IN LATENTLY INFECTED DENDRITIC CELLS BY CYSTEINYL LEUCOTRIENES

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Background: Human Cytomegalovirus (HCMV) is a ubiquitous betaherpesvirus. Primary infection with HCMV causes several health problems and once resolved, infection is followed by persistence of the virus in a latent form. The virus can be reactivated, resulting in a reemergence of the virus and development of complications in several diseases. HCMV is carried by monocytic or pre-monocytic cells and can be reactivated when these cells are recruited to inflammatory sites. Cysteinyl leukotrienes (cysLTs) are bioactive lipid mediators implicated in inflammatory processes and associated diseases. CysLTs mediate their activity via, at least, two major receptors CysLT1R and CysLT2R, both expressed on peripheral blood monocytes.
Objective: We investigated the potential for cysLTs to modulate HCMV latency and/or replication.
Methods: HCMV mRNA and proteins (IE1/2 and PP65) were quantified using peripheral blood monocytes (PBM), PBM-derived dendritic cells and PBM-derived macrophages from healthy HCMV-positive donors. HCMV major immediate promoter (MIEP) activation was assessed using luciferase gene-tagged promoter constructs.
Results: In macrophages and mature DCs, we found an increase of HCMV mRNA and protein expression after 48 h of stimulation with cysLTs. HCMV mRNA and protein expression in immature DCs was induced by cysLTs during and after differentiation. These cells are normally non-permissive to viral replication. CysLT1R antagonist did not prevent cysLT-induced HCMV mRNA and protein. In addition, we found a 28-fold increase of MIEP enhancer transcription in HEK293 cells stably transfected with the CysLT2 receptor. This increase was reduced by NFkB and PKC inhibitors by 100 and 34% respectively.
Conclusion: Our data indicate that cysLTs, via CysLT2R stimulation, activate latent HCMV and may increase HCMV replication.
Disclosure of interest: None declared.

P-250
ACTIVATION AND INHIBITION OF THE HEAT SHOCK RESPONSE—EFFECTS ON INFLAMMATION AND CANCER

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Heat shock proteins (HSPs) are key to cell survival. Our previous work used an adenoviral vector (AdHSP) to enhance Hsp70 expression in a model of sepsis that induces acute respiratory distress syndrome (ARDS). We have shown that AdHSP attenuates activation of the pro-inflammatory NF-κB pathway and limits cell division in pulmonary epithelial cells. We hypothesized that AdHSP attenuated NF-kB activation at multiple points in the IκBα degradative pathway. Other sets of preliminary studies revealed that AdHSP treatment decreased the expression of several cell division markers by limiting over proliferation of alveolar type II cells, by inhibiting hyper-phosphorylation of the regulatory retinoblastoma protein (Rb). However, AdHSP-induced HSP70 expression, is only one technique for protecting the lungs. We propose that the heat shock response (HSR) is activated, in part by the membrane dependent calcium channel receptor transient receptor potential vanilloid type-1 (TRPV1). We showed that Capsazepine, a selective antagonist of the TRPV1, and TRPV1 siRNA can abolish the heat shock and Capsaicin-induced activation of HSR and the consequent accumulation of Hsp70, 90 and 25 chaperones in various mammalian epithelial cells. Capsazepine treatment prevented Capsaicin-induced stabilization of IκBα and cell to cell adhesion and induced apoptosis. The Capsazepine-mediated blockage of the heat shock response was confirmed with EGTA, a calcium chelator, suggesting that HSR-sensing and signaling in mammalian cells depends on the transient entry of calcium by way of Ca channels, as in the case of the TRPV1 vanilloid receptors. Treatment of breast carcinoma cells with Etoposide and Capsazepine showed a reduction in the expression of HSPs, and activation of pro-apoptotic pathway.
Disclosure of interest: None declared.

P-251
MODULATORY ROLE OF FEMALE SEX HORMONES ON THE CONTROL OF CELL TRAFFICKING INTO LUNG DUE TO LPS EXPOSURE

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Introduction: Acute respiratory distress syndrome is a severe complication of acute lung injury (ALI) characterized by hypoxemia, lung neutrophil infiltration and edema. Lipopolysaccharide (LPS) exposure is a major cause of ALI induction. Female sex hormones (FSH) may exert protective or deleterious effects on Th1 and Th2-mediated lung inflammation. We investigated herein the modulatory role of FSH on Th1 lung inflammation induced by LPS notably on cell recruitment.
Material and methods: Seven days after ovariectomy (OVx), female mice (C57BL/6, n = 5–8/group) were subjected to intranasal instillation of LPS (*Escherichia coli*, 100 μg/ml, 1 μl/g) or saline (0.9% NaCl, 1 μl/g). The animals were euthanized 24 h thereafter, and the cells present in bronchoalveolar lavages (BAL), peripheral blood and bone marrow lavages were quantified. Furthermore, lung myeloperoxidase (MPO) activity was also analyzed. Otherwise intact animals were subjected to the same procedures and served as controls (Sham-OVx group).
Results: LPS increased the number of cells in BAL from both OVx and Sham-OVx groups regarding their respective controls and the basal groups. There was a marked increase in total BAL cells in the OVx + LPS group over Sham-OVx + LPS. Neutrophils and lymphocytes in BAL of OVx + LPS group significantly increased relative to Sham-OVx mice. Interestingly, after instillation of LPS, OVx induced a reduction in the total number of bone marrow cells regarding Sham-OVx + LPS. No changes in the circulating leukocyte countings were detected among all groups. Finally, LPS increased lung MPO activity in OVx group when compared with Sham-OVx + LPS.
Conclusions: FSH exerts an endogenous control of inflammation by downregulating the traffic and the lung accumulation of inflammatory cells induced by LPS exposure. Thus, it is conceivable that FSH-depriving conditions could blunt endogenous control of inflammatory process.
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Disclosure of interest: None declared.

P-252
ROLE OF PSEUDOMONAS AERUGINOSA FLAGELLIN IN AIRWAY MUCIN PRODUCTION: RELEVANCE FOR CYSTIC FIBROSIS DISEASE

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Purpose: *Pseudomonas aeruginosa* is an opportunist flagellated Gram-negative bacterium present in the lung of up to 80% of cystic fibrosis (CF) patients, causing hypersecretion of airway mucus, chronic inflammation and airway obstruction. The mechanisms by which *P. aeruginosa* induces mucus hypersecretion are incompletely understood. In the present study we aimed to determine whether and how *P. aeruginosa* flagellin leads to mucus hypersecretion.

Methods: (i) Human airway epithelial cells (NCI-H292) were infected in vitro for 24 h with a wild-type (WT) *P. aeruginosa* (PAK strain), with its flagellin-deficient mutant (DFliC). The amounts of MUC5AC, MUC5B and MUC2 mucins (both mRNA and protein), as well as of the pro-inflammatory interleukin-8 (IL-8), produced by NCI-H292 were quantified in vitro. (ii) WT B6, TLR4$^{-/-}$, TLR5$^{-/-}$, MyD88$^{-/-}$ and TLR4/5$^{-/-}$ mice were infected intranasally with either the WT PAK strain or with ΔFliC mutant and the amount of mucins expressed in the lung was determined by RT-PCR and Alcian blue staining.

Results: (i) Infection with WT PAK, but not with the Δ*FliC*, lead to: (a) production of a significantly high level of MUC5AC both in vitro and in vivo in lung of WT mice; and (b) an increase in the level of IL-8 cells production; (ii) treatment of cells with *P. aeruginosa*-flagellin induced *MUC5AC*expression through ERK1/2 dependent pathway; (iii) similar levels of mucins mRNAs were detected in the lung of WT, TLR4$^{-/-}$ and TLR5$^{-/-}$ mice infected with WT PAK. However, significantly lower levels of mucins were detected in the lung of MyD88$^{-/-}$ and TLR4/5$^{-/-}$ mice compared to WT mice.

Conclusion: Our results demonstrate that *P. aeruginosa* flagellin induces airway mucin hypersecretion through TLR5-dependent and independent mechanisms.

Disclosure of interest: None declared.

P-253
THE ROLE OF CD11D INTEGRIN IN THE PHYSIOPATHOLOGY OF SEPSIS INDUCED BY SALMONELLA TYPHIMURIUM

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Integrins are adhesion molecules that play a pivotal role during inflammatory process. The most relevant integrins for leukocyte are members of the CD18 subfamily. The leucointegrins subfamily includes CD11a, CD11b, CD11c and CD11d molecules. CD11d integrin is expressed on most human leukocytes, including neutrophils and macrophages. CD11d integrin has been associated with pathological events in some inflammatory diseases, as rheumatoid arthritis. Despite the existing data, the pathophysiological role of this molecule remains unknown. In this study, we aimed to investigate the central role of CD11d integrin in the inflammatory processes in model of *Salmonella* infection. In this study, we used CD11d Knockout mice (CD11dKO) and wild type (WT) mice infected by an intraperitoneal injection of *Salmonella* Typhimurium and *S. aureus*. Our results showed that CD11dKO mice were more susceptible to infection. In CD11dKO mice we observed higher bacteremia, decreased migration of leukocytes to the peritoneum and increased levels of TNF-α, IL-6 and MIP-1α, 24 and 72 h after infection, when compared to the WT. Macrophages seem to be important cells in the onset of the observed responses, which are mediated by specific factors of Gram negative and independent of the CD11b and TLR4 expression. Moreover, our results showed problems in the migratory ability of granulocytes from Cd11dKO mice toward chemotactic stimuli. We did not observe any correlation between the expression of CD11d integrin and nitric oxide production and presence of apoptotic cell death, after infection. However, we have shown correlation between CD11d integrin and IL-1β and IL-18 production. Our results indicate an important role of CD11d molecule in the inflammatory response after *Salmonella* infection and suggest a different role of CD11d integrin in Gram negative and Gram positive bacterial infections. Moreover, our results suggest for the first time the involvement of CD11d integrin in inflamossoma activation.

Disclosure of interest: None declared.

P-254
INTERLEUKIN (IL)-18 PROMOTES GROWTH OF PARACOCCIDIOIDES BRASILIENSIS WITHIN HUMAN MONOCYTES VIA MANNOSE RECEPTOR MODULATION

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Introduction and objectives: IL-18 is a proinflammatory cytokine and its role in paracoccidioidomycosis, a deep mycosis, is not fully understood. The effect of IL-18 on monocyte activity against *P. brasiliensis* (Pb) was studied by evaluating the fungicidal activity, H_2O_2 production, the modulation of TLR2, TLR4 and mannose receptor (MR) expression, and IL-18, IL-10, TNF-α, IL-12 and IL-15 production after challenge in vitro with fungus.

Methods: Monocytes were stimulated with IL-18 and treated with anti-TLR2, anti-TLR4, anti-CD14 and/or anti-MR before challenge with Pb18 (virulent) and Pb265 (avirulent). Fungal recovery (FR) and H_2O_2 production were assessed. TLR2, TLR4, MR and CD14 expression were analyzed by flow cytometry and IL-18, IL-10, TNF-α, IL-12 and IL-15 production by ELISA.

Results: The results showed that IL-18 increased FR from infected monocytes and the blockage of receptors with specific neutralizing antibodies showed that this effect of IL-18 was mediated by MR. The challenge with Pb18 also increased MR, TLR2 and TLR4 expression. No alterations on H_2O_2 levels were observed. The challenge with Pb18 and Pb265 induced monocytes to produce IL-18, TNF-α, IL-10, IL-12 and IL-15. IL-18 production was associated with MR increase, while TNF-α and IL-10 production was associated with TLR4 and TLR2 respectively. The high IL-12 production was not related with none of receptors studied.

Conclusions: Although IL-18 was able to induce high levels of pro-inflammatory cytokines, the effect of IL-18 on the increase of FR from monocytes would be due to modulation of MR that in addition to facilitate the entry of the fungus in the cell, will activate a new molecular pathway that links engagement of MR with peroxisome proliferator-activated receptor (PPARγ) activation, which regulates the macrophage inflammatory response, thereby playing a role in paracoccidioidomycosis pathogenesis. *Financial support:* FAPESP (2007/04042-9 and 2007/00755-0) and CNPq (151565/2009-0).
Disclosure of interest: None declared.

P-255
A NOVEL PEPTIDE FOR TREATMENT OF MIDDLE EAR FLUID AND ASSOCIATED HEARING IMPAIRMENT IN OTITS MEDIA

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Objective: To reduce inflammation and subsequent hearing loss in otitis media (OM)
Background: OM is characterized by inflammation and fluid in the middle ear, which can impact hearing. No current therapeutic addresses this fluid and accompanied hearing impairment. We have identified a peptide, P13, derived from the vaccinia virus A52R protein. Pathogens such as viruses produce proteins during the infectious process which have been demonstrated to modulate the human immune response. P13 demonstrates potent in vitro cytokine inhibition in response to toll-like receptor signaling.
Methods: Murine middle ear inflammation was induced by transtympanic injection of heat-killed *S. pneumonia.* Twenty-four hours post-bacterial injection, animals with documented middle ear inflammation were treated with either P13 or control administered systemically, or topically as ear drops. Inflammation was documented by middle ear histology, and auditory brainstem responses (ABRs) used to monitor hearing.
Results: P13, administered after inflammation was established, decreased middle ear fluid area, number of cells in middle ear fluid, and tympanic membrane thickness. Most importantly, P13 decreased both the severity and duration of hearing impairment. Five days after bacterial injection, P13 reduced hearing loss approximately 50% as compared to controls. By day 13, hearing thresholds in P13 treated animals returned to normal, while control groups continued to demonstrate significant hearing impairment.
Conclusions: P13, administered topically as ear drops, reduced inflammation and reduced hearing impairment in a murine model of middle ear inflammation. Preclinical data suggests that P13 may be useful as a treatment for the fluid retention and hearing impairment seen in patients with otitis media.
Disclosure of interest: S. Hefeneider Stock ownership or royalties of: stock ownership in 13therapeutics, Inc., S. McCoy Stock ownership or royalties of: stock ownership in 13therapeutics, Inc., D. Trune Consultant/Speaker's bureau/Advisory activities with: Advisory board member of 13therapeutics, Inc.

P-256
CHARACTERIZATION OF LUNG INFLAMMATORY RESPONSE DURING MURINE SEVERE MALARIA

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Severe malaria, or cerebral malaria (CM), is responsible for 2 millions deaths each year, and is characterized by fever, cerebral commitment and respiratory distress. Even though pulmonary edema and acute respiratory distress syndrome are described during CM, the inflammatory response in the lungs has not been characterized yet. The aim of this study was to characterize lung inflammation during experimental severe malaria. One day after the infection, *P. berghei* (5×10^6 infected RBC)-infected C57BL/6 mice presented increased lung elastance, transpulmonary pressure and viscoelastic pressure when compared to non-infected mice. Morphometric and histological analysis revealed edema formation and leukocyte accumulation in the interstitial tissue (but not in the alveolus) 5 days after the infection. A significant increase in the numbers of total leukocytes 3 days (mainly due to neutrophils) and 5 days (mainly due to gd and $\alpha\beta$ T cells) after infection was observed. Lung tissues of *P. berghei*-infected mice presented increased levels of the cytokines TNF, IFN-γ and IL-6, at day 3 and 5 post-infection. The 5-lipoxygenase (5-LO)-derived lipid mediator leukotriene (LT)B_4 was detected at day 3 post-infection. Our results demonstrate that *P. berghei* infection triggers an important lung inflammation, which is characterized by mechanical changes at the early phase of malaria infection, followed by edema formation, production of inflammatory mediators and leukocyte infiltrates.
Financial support:FAPERJ/CNPq
Disclosure of interest: None declared.

P-257
B LYMPHOCYTE SECRETES PRO-INFLAMMATORY CYTOKINES PRODUCED BY TLR AND NLR PATHWAY IN RESPONSE TO SALMONELLA INFECTION

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It has been demonstrated that *Salmonella* are able to infect and survives within B-lymphocytes but there is no information regarding B-lymphocyte ability to produce pro-inflammatory cytokines in response to *Salmonella* infection via TLR or NLR pathways.

Objectives: To analyze the ability of *Salmonella* infected B lymphocyte to produce pro-inflammatory cytokines by TLR or NLR pathway.
Results: TNFa and IL-6 was secreted in response to magnitude of infection: TNFa (pg/mL), non- infected {NI} 0 ± 0, MOI 1/10, 28 ± 2, MOI = 50 84 ± 5, LPS 54 ± 2, CpG, 0 ± 0; IL-6 (pg/mL), NI, 50 ± 6, MOI = 10, 53 ± 8, MOI = 50, 100 ± 11, LPS, 78 ± 9 and CpG, 48 ± 8. RANTES secretion (pg/mL) was not different among different magnitude of infection: NI, 165 ± 0, MOI = 10, 300 ± 10, MOI = 50, 295 ± 4, LPS, 297 ± 14 and CpG, 97 ± 20. In regards of NLR pathway, B lymphocyte were able to secrete IL-1b (pg/mL) in response to *Salmonella* infection (NI 0 ± 0 and MOI = 10, 88 ± 20).
Conclusions: These data show that B-cells are not impaired to produce pro-inflammatory mediators in response to *Salmonella* infection. These mediators are produced by TLR pathway (TNFa and IL-6) as well as by NLR pathway (IL-1b), however in contrast to macrophages B-cells are able to resist the cytotoxic effect of these cytokines. The ability of B-lymphocytes to resist this event could make them a good "niche" for *Salmonella* favoring disseminated infection.
Disclosure of interest: None declared.

P-258
NITRIC OXIDE-GUANYLATE CYCLASE-PROTEIN KINASE G AXIS ACTIVATION CONTRIBUTE TO NEUTROPHIL DYSFUNCTION AND MORTALITY IN SEPSIS

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In severe sepsis neutrophils present a state of unresponsiveness to chemotactic stimuli. This correlates with a poor prognosis and may contribute to worsen the clinical outcome in sepsis by precluding an efficient bacterial clearance. Experimental data have implicated nitric oxide (NO) in this phenomena. However NO synthesis inhibition in sepsis leads to increased mortality despite the restored neutrophil migratory function. This could be at least in part due to an inhibition of the microbicidal effects of NO, suggesting that a better understanding of the roles of NO in sepsis is required. Here we investigated the molecular mechanisms triggered by NO that lead to decreased neutrophil chemotactic responses in sepsis. Our results show that stimulation of human neutrophils with lipopolysaccharide, a Toll-like receptor (TLR) 4 ligand, as well as lipotheichoic acid, a TLR2 ligand, decreased in vitro neutrophil responsiveness to classic chemoattractants as interleukin-8, leukotriene B_4 and fMLP. These effects involved the activation of the NO/guanylate cyclase (GC)/protein kinase G (PKG) signaling pathway. Further we found that NO-GC-PKG signaling induces both CXCR2 and BLT1 internalization which was paralleled by increased expression of the GPCR kinase, GRK-2. Based on these findings we reasoned that GC inhibition could restore neutrophil migration without impairing NO microbicidal activity. In fact, we found that pharmacological inhibition of GC activity in an experimental model of sepsis resulted in increased survival, restored neutrophil migration and increased bacterial clearance, while inhibition of NO synthesis rendered mice susceptible to infection and increased bacterial counts. Finally we show that GC inhibition increased survival even after the onset of sepsis, suggesting its potential clinical application. Together our results suggest a mechanism by which NO interferes with neutrophil chemotaxis and point to GC as a potential therapeutical target in sepsis.
Disclosure of interest: None declared.

P-259
IN VITRO INFECTION CAUSED BY METHICILLIN-SENSITIVE STAPHYLOCOCCUS AUREUS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS: IFN-Γ, IL-12 AND IL-10 PRODUCTION IN LYMPHOCYTES OF RATS WITH NEONATAL MALNUTRITION

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Objective: To study the production of IFN-γ, IL-12 and IL-10 in lymphocytes of rats with neonatal malnutrition (NM) and a group of well-nourished rats (N), in vitro infected with Methicillin-sensitive *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA).
Methodology: Male Wistar rats (n = 12) were breastfed by rats whose diet contained 17 and 8% of protein during lactation (N and NM group). After weaning, both groups were administered a normoproteic diet. Following the isolation of lymphocytes from, four systems were formed: negative control composed by lymphocytes (C−), positive control added with lipopolysaccharide (C+) and two systems, MSSA and MRSA, stimulated by the strains. Cytokine measurements were performed using ELISA kits (Quantikineâ m, R&D Systems) from samples collected in the supernatant after cultured for 24 h incubation. Statistical analysis were run using Student t test and Mann–Whitney assuming a significance level of $p < 0.05$.
Results: malnutrition led to the decrease of ponderal growth as well as reduction in the production of IFN-γ in the systems C+ ($p = 0.001$), C− ($p = 0.001$), MSSA ($p = 0.006$) and MRSA ($p = 0.002$). However, there was no statistic difference concerning the production of IL-12 in all systems: C+ ($p = 0.485$), C− ($p = 0.614$), MSSA ($p = 0.818$) and MRSA ($p = 0.699$). Similarly, there was no statistically significance for the production of IL-10: C+ ($p = 0.781$), C− ($p = 0.394$), MSSA ($p = 0.937$) and MRSA ($p = 0.124$). In addition, none statistically significant differences were observed among methicillin-sensitive and methicillin-resistant strains for all tested cytokines in all experimental groups.
Conclusion: The model of neonatal malnutrition revealed disorders in body mass. Moreover, the production of IFN-γ was also compromised. MRSA and MSSA strains have produced similar cytokine concentrations in all groups.
Disclosure of interest: None declared.

P-260
THE ROLE OF CPLA2-ALPHA IN PSEUDOMONAS AERUGINOSA-INDUCED LUNG INJURY

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Pseudomonas aeruginosa (P.a.) induces lung injury in pulmonary diseases including cystic fibrosis (C.F.). P.a. is known to activate the host cytosolic phospholipase A2α (cPLA2α), which catalyzes the release of arachidonic acid (AA) and its metabolite prostaglandin E2 (PGE2). However, the mechanisms and role of cPLA2a in P.a.-induced lung injury have not been investigated. Here, we investigated lung inflammation and mortality in mouse models of pulmonary infection by a laboratory (PAK) and a clinical strain (CHA) of P.a. CHA revealed to be more potent than PAK in inducing lung inflammation and animal mortality. cPLA2α-null mutation attenuated animal mortality caused by both PAK and CHA but failed to reduce lung inflammation or bacterial proliferation. CHA infection led to increased mortality in CF compared to non-CF mice. Pharmacological inhibition of cPLA2a attenuated CHA-induced CF mice mortality without interfering with lung inflammation. CHA triggered AA and PGE2 release and cPLA2α phosphorylation in lung epithelial cell line A549 at higher levels than PAK. This was accompanied by increased p38 MAPK phosphorylation. Inhibition of cPLA2α and p38 attenuated both PAK- and CHA-induced AA and PGE2 release by A549 cells. However, inhibition of cPLA2a had partial effect on IL-8 secretion. CHA caused LDH release by A549 cells at higher extent than PAK both of which were reduced by cPLA2a inhibitors. Together these finding suggest that cPLA2α plays a role in P.a.-induced lethality in pulmonary infection models including CF. This effect seems to occur via a process related to cPLA2a-induced toxicity independently from lung inflammation and bacterial proliferation.
Disclosure of interest: None declared.

P-261
C5A IS PRODUCED DURING INFLUENZA A INFECTION AND CONTRIBUTES TO NEUTROPHIL RECRUITMENT AND LUNG PATHOLOGY

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Influenza A viruses infect millions of people worldwide causing annual epidemics and pandemics. Potent inflammatory responses are commonly associated with severe cases of Influenza A infection. Complement system is an important mechanism of innate and humoral responses to infections that is activated during Influenza A infection and mediates viral neutralization. C5a is an anaphylatoxin that was reported to be produced in patients infected with the last H1N1 pandemic virus. We hypothesized that C5a contributes to lung pathology following Influenza A infection and that the reduction of C5a levels could decrease inflammation. To address this question C57Bl/6j mice were infected intranasally with 10^4 PFU of the mouse adapted Influenza A virus WSN/33 H1N1 or inoculated with PBS (Mock). After 1, 4, 7 and 10 days mice were killed and submitted to bronchoalveolar lavage (BAL) in order to determine C5a levels using ELISA. In another experiment, we used a salivary derived protein from blood-sucking ticks with potent C5a scavenger action—OmCI—to treat a group of Influenza A infected mice prior to infection and daily until day 5; another infected group received PBS. After 6 days of infection mice were killed, BAL performed and lungs collected. We found increased levels of C5a in BAL from the fourth to the 10th day of infection. Treatment with the C5a scavenger protein reduced C5a levels in BAL and also reduced neutrophil infiltration to the airways, lung epithelial injury and overall pathology induced by the infection, without affecting viral clearance. This suggests that C5a targeting during Influenza A infection could be a promising approach to reduce excessive inflammatory reactions associated with the severe forms of the flu.
Disclosure of interest: None declared.

P-262
ENDOTHELINS ARE KEY FACTORS IN ARTICULAR INFLAMMATION

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Endothelins (ETs) are pro-inflammatory peptides involved in pain, fever, edema and cell migration. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by joint inflammation, as well as local and systemic increased ET-1 levels. The present study demonstrates the effect of ET antagonism in articular inflammation on zymosan- and collagen-induced arthritis and also show the modulation of ET-1 levels by lipoxin $(LX)A_4$, an endogenous anti-inflammatory mediator. Two hours after induction of zymosan (500 mg/cav, i.a)-induced arthritis, ET-1 mRNA expression levels were increased in synovial extracts, which was followed, at 6 and 24 h, by massive edema formation and neutrophil influx into inflamed joint. Pre-treatment with bosentan (10 mg/kg; i.v.), a dual pharmacological ET receptor antagonist, significantly impaired 6 h zymosan-induced leukocyte accumulation and edema formation. ET receptor blockade also decreased zymosan-induced production of TNF-a, CXCL1 and leukotriene (LT) B_4 within 6–24 h. Bosentan (10 mg/kg; i.p.) post-treatment effectively attenuated clinical score of established collagen-induced arthritis in mice and caused a significant decrease on paw edema. LXA_4 in vivo pre-treatment (20 ng/cav, i.a.) significantly impaired zymosan-induced preproET-1 mRNA expression (80% of inhibition) with concomitant suppression of zymosan-induced edema formation and neutrophil influx. In addition, in vitro pre-treatment of neutrophils with LXA_4 (1–100 nM) inhibited ET-1(100 nM)-induced activation and chemotaxis, evaluated by shape change and boyden chamber assay, respectively. Our results suggest that pharmacological blockade of ET receptors, or the modulation of ET-1 expression, may serve as a possible novel therapeutic tool for the treatment of inflammatory and autoimmune articular diseases.
Disclosure of interest: None declared.

P-264
ROLE OF TOLL LIKE 9 RECEPTOR IN SEPSIS-INDUCED NEUTROPHIL PARALYSIS

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Sepsis is the mainly cause of mortality in intensive therapy units. Recently, we demonstrated that severity of the sepsis associates with a failure of neutrophil migration to the infection focus. The event depends on CXCR2 desensitization by a mechanism mediates by GRK-2 induction. In the present study, it was used CLP (cecal ligation and punction) model in TLR9 deficient mice ($TLR9^{-/}$) mice to determine the role of this receptor on the process. Compared with WT, the $TLR9^{-/-}$ mice submitted to severe CLP did not present failure of neutrophil migration to infection focus, resulting in low bacteria, systemic inflammatory response and high survival rate. Investigating the mechanism by which the deficiency of TLR9 could prevent the failure of neutrophil migration, it was observed that neutrophils derived from septic $TLR9^{-/-}$ mice expressed high levels of CXCR2 and reduced GRK2 induction. Therefore, the poor outcome of severe sepsis correlates with TLR9 activation in neutrophils which induces CXCR2 desensitization with consequent neutrophil paralysis.
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Disclosure of interest: None declared.

P-265
A NOVEL PEPTIDE TREATMENT FOR SYSTEMIC INFLAMMATION

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Objective: To reduce inflammation and decrease mortality in murine models of systemic inflammation
Background: Sepsis is a prevalent and life-threatening medical condition characterized by development of a dysregulated inflammatory immune response and associated tissue damage. This dysregulated inflammatory response is initiated by activation of Toll-like receptor (TLR) signaling in response to bacteria and/or bacterial products such as LPS. We have identified a peptide, P13, derived from the vaccinia virus A52R protein. Pathogens such as viruses produce proteins during the infectious process which have been demonstrated to modulate the human immune response. P13 demonstrates potent in vitro cytokine inhibition in response to toll-like receptor signaling.
Methods: P13 was tested in two murine models of systemic inflammation, both for inhibition of cytokines, and for prevention of mortality. Animals were treated with P13 administered subcutaneously, followed immediately by administration of LPS, and serum examined 2 h later for TNF-α. In separate experiments, mice were injected i.p. with LPS and 2 and 6 h later treated subcutaneously with P13 or scramble control peptide. The mice were monitored every 3–4 h for lethality and the experiment was terminated after 96 h. In the second model, mice underwent cecal ligation and puncture (CLP). At 1.5 h post-CLP, one group of mice was treated i.v. with P13 and a second group treated subcutaneously. The control group was left untreated and mice monitored over a 156 h time period for survival.
Results: P13 demonstrated significant inhibition of serum TNF-α (approximately 50%) following LPS injection. In both the LPS and CLP mortality models, P13 improved survival approximately 40–50%.
Conclusions: P13 reduced cytokines and improved survival in murine models of systemic inflammation. This has implications in treating human systemic inflammatory diseases.
Disclosure of interest: S. Hefeneider Stock ownership or royalties of: Stock ownership in 13therapeutics, Inc., J. Gold Consultant/Speaker's bureau/Advisory activities with: Advisory board of 13therapeutics, Inc., S. McCoy Stock ownership or royalties of: Stock ownership in 13therapeutics, Inc.

P-266
EPIDEMIOLOGY OF THE STREPTOCOCCAL INFECTIONS IN THE INFECTIOUS DISEASES HOSPITAL OF DURRES FOR THE PERIOD 2000–2005

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Background: The streptococcal infections present one of the greatest problems of the infectious diseases. These are the commonest bacterial infections in humans. They cause tonsillitis, pyogenic skin infections, scarlatina, acute rheumatoid arthritis etc.
Objectives: (1) Studying epidemiologic features of streptococcal infections in Durres for 2000–2005. (2) Demographic social description of population being studied. (3) Making evident the determining, etiologic factors of diseases in population. (4) Identifying the groups with high risk to develop diseases. (5) Dynamic description of the infections according the respective years.
Materials and methods: Study was done at Durres hospital for the period 2000–2005, was retrospective. Were included 603 patients for 6 years, 359 females, 244 males. 393 patients lived in city and 210 patients in rural area. Study was divided in 4 age groups: 19–30 years, 31–40 years, 41–50 years and over 50 years. Population was classified according to profession: workers, officials and unemployed. All data were taken from registry of the in-patients, in which there included specific examinations and bacteriological assay.
Results: Majority of cases were from city 65%, towards 35% from rural area. 60% of subjects were males. Angina folicularis made up 49% of cases, pharyngitis 35%, 3% were arthritis and nephritis. The most affected groups according to age were: 19–30 years (48%), and 31–40 years (23%). The majority of the cases belonged to the year group 2003–2004, and the minority to the year 2000.
Conclusion: Related to our results we concluded that sex plays a role in the epidemiological results. Anginas were the most frequent disease. The greatest number of the patients belonged to the active age group, related that to the life style which induces eating outside and making a collective life. So we concluded that age was an important factor in the distribution of the diseases.
Disclosure of interest: None declared.

P-267
THE EFFECT OF MAJOR INFLAMMATORY BURN INJURY ON IMMUNOPHENOTYPE OF PERIPHERAL BLOOD T LYMPHOCYTES

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Background: Lymphocytes immunephenotyping is a reflection of the functional level of immune system. It was found that serum soluble β2-microglobulin (β2 M) may modulate the function of T lymphocytes. Our aim in this work is to determine the activation state of T cells after major burn by detection of CD25 as an early activating marker of T-lymphocytes and HLA-Dr expression as a late one and their relation to β2-microglobulin.
Methods: T lymphocytes of 26 major burn patients complicated with infection were analyzed in 24 h, 1 and 2 weeks after burn injury, using monoclonal antibodies of CD3, CD4, CD8, CD25 and HLA-Dr by flowcytometry and comparing them with those of 26 apparently healthy donors. Serum level of β2 M was also tested using ELISA technique.
Results: There was a statistically significant reduction in $CD3^+$ T lymphocytes ($p < 0.000$), CD4/CD8 ratio ($p = 0.01$) in the first 24 h compared to controls. The $CD3^+$ cells increase after 2 weeks ($p = 0.03$). HLA-Dr was markedly increased after 2 weeks ($p = 0.001$), while absolute number of $CD25^+$ T lymphocytes was lower than controls. The β2 M of patients samples of the study were significantly higher than that of the controls (P values: <0.000, <0.001 and <0.000 respectively). Significant negative correlations were detected between mean values of β2-microglobulin in 24 h and 1 week samples and both values of CD3 and CD4 positive cells. Moreover, there was a significant negative correlation between mean values of β2 M in the 24 h samples and values of CD25 expression.
Conclusions: The data obtained suggested persistent activation of T lymphocytes at 2 weeks after major burn inflammation. HLA-Dr expression can reflect post burn lymphocyte activation. Extensive destruction of tissues by the mechanisms of necrosis may be a basic reason for the increase of β2 M serum level and the significant reduction in number of activated lymphocytes which explained by negative correlations observed in this study.
Disclosure of interest: None declared.

P-268
THE NLRC4 INFLAMMASOME CONTRIBUTES TO RESTRICTION OF PULMONARY INFECTION BY FLAGELLATED SPECIES OF LEGIONELLA

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The Nlrc4 inflammasome is triggered in response to contamination of the host cell cytoplasm with bacterial flagellin, which induces pyroptosis, a form of cell death that accounts for restriction of bacterial infections. Although induction of pyroptosis has been extensively investigated in response to *Salmonella typhimurium* and *Legionella pneumophila*, little is known regarding the role of the inflammasome for restriction of non-pneumophila *Legionella* species. Here, we used five species of the *Legionella* genus to investigate the importance of the inflammasome for restriction of bacterial infection in vivo. By infecting mice deficient for inflammasome components, we demonstrated that caspase-1 and Nlrc4 contribute to restriction of pulmonary infection with *L. micdadei*, *L. bozemanii, L. gratiana*, and *L. rubrilucens*. *Legionella longbeachae*, a non-flagellated bacterium that fails to trigger pyroptosis, was not restricted by the inflammasome and induced death in the infected mice. In contrast to *L. longbeachae*, flagellin mutants of *L. pneumophila* did not induce mice death; therefore, besides bypassing the Nlrc4 inflammasome, *L. longbeachae* may employ additional virulence strategies to replicate in mammalian hosts. Collectively, our data indicate that the Nlrc4 inflammasome plays an important role in host protection against opportunistic pathogenic bacteria that express flagellin.
Disclosure of interest: None declared.

P-269
ENDOTOXIN TOLERANCE REPRESENTS A DISTINCTIVE STATE OF ALTERNATIVE POLARIZATION IN HUMAN MONONUCLEAR CELLS

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Classical (M1) and alternative (M2) polarization of mononuclear cells such as monocyte and macrophages, is known to occur in response to challenges within a micro-environment, like the encounter of a pathogen. Lipopolysaccharide, also known as endotoxin (LPS), is a potent inducer of inflammation and M1 polarization. LPS can also generate an effect in mononuclear cells known as endotoxin tolerance, defined as the reduced capacity of a cell to respond to LPS activation following an initial exposure to this stimulus. Using systems biology approaches in peripheral blood mononuclear cells and monocyte derived macrophages involving microarrays and advanced bioinformatic analysis, we determined that gene responses during endotoxin tolerance were similar to those found during alternative polarization, featuring gene and protein expression critical for the development of key M2 mononuclear cell functions, including reduced production of pro-inflammatory mediators, expression of genes involved in phagocytosis as well as tissue remodeling. Moreover, expression of different metallothionein gene isoforms, known for their role in the control of oxidative stress and in immunomodulation were also found to be consistently up-regulated during endotoxin tolerance. These results demonstrate that after an initial inflammatory stimulus, mononuclear cells undergo an alternative polarization probably to control hyper-inflammation and heal the affected tissue.
Disclosure of interest: None declared.

P-270
PLATELETS PARTICIPATE TO INFLAMMATION IN HIV-1 INFECTED PATIENTS BY RELEASING SOLUBLE CD154

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A major immunomodulatory molecule is soluble CD40L (sCD154) and activated platelets are estimated to produce 95% of the plasma sCD154 pool. It has been recently shown in mice that HIV-1 Tat induced activation of platelets resulting in sCD154 release. In this work we investigated platelet activation state and their ability to release sCD154 upon stimulation in HIV-1 infected patients under HAART. We noted that membrane expression of the platelet activation marker CD62P did not differ significantly between HIV-1 infected-patients and healthy donors (respectively 14.1 ± 9.2 and 10.9 ± 2.7%) suggesting that platelets were not activated in HIV patients. However, we evidenced a significant difference in plasma soluble CD62P (sCD62P) levels between HIV-1 patients (82.7 ± 27 ng/ml) and healthy donors (43.9 ± 20.4 ng/ml) that could result from CD62P shedding from the membrane of activated platelets. Then we examined sCD154 plasma levels and we noted that they were significantly higher in HIV-1 patients compared to healthy donors ($p < 0.01$), i.e. 2.4 ± 1 and 1 ± 0.9 ng/ml, respectively. Next, we tested whether thrombin was able to induce the release of sCD154 by platelets from HIV patients. We observed that platelet isolated from HIV patients respond as well as platelets from healthy donors to thrombin stimulation by releasing comparable amounts of sCD154 (respectively 2.6 ± 1.5 and 2.3 ± 1.3 pg/ml for 3×10^8 platelet) whereas only healthy donor platelets could release a significant amount of sCD62P upon stimulation (55.4 ± 24 vs. 25.6 ± 9.9 ng/ml for 3×10^8 platelet for HIV patients). This suggests that while activated platelets of HIV patients are not able anymore to release sCD62P, they remain able to release sCD154 and could therefore boost an inflammatory response in case of treatment failure.
Disclosure of interest: None declared.

P-271
BIOMARKERS OF INFLAMMATION IN YOUNG HIV SEROPOSITIVE PATIENTS WITH UNDETECTABLE VIRAL LOAD

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Objective: Biomarkers of inflammation are related to cardiovascular-diseases, insulin resistance and premature aging. Our aim is to evaluate the level of inflammatory biomarkers in young adult HIV seropositive patients with undetectable viral load under antiretroviral therapy. In Romania, HIV-infected population is made mainly of young adults, parenterally infected in their childhood, before 1990 and multiexperienced afterwards.
Methods: We present the preliminary results of an ongoing prospective Romanian research grant (PNCDI2 no.62077/2008) on HIV infected patients undergoing HAART, recruited in a tertiary care hospital, during 12 months. Tumor necrosis factor-alpha (TNF alpha), interleukin-6 (IL6), monocyte chemotactic protein 1 (MCP1), high-sensitivity C-reactive protein (hsPCR) were monitored every 6 months.
Results: Our study included 106 patients, characterized by: M:F ratio 1.3; median age of 31 years, mode age of 20 years; median CD4 cell count 492/mmc (IQ 334;738). Subjects with undetectable HIV viremia had median values for TNF alpha, IL6, MCP1, hsPCR as follows: 9.5 pg/ml (IQ 6.4;21.2), 27.4 pg/ml (IQ 23.4;34.2), 278.4 pg/ml (IQ 183;397), 1.9 ng/ml (IQ 0.6;4.6), respectively. There was no important difference of median values of these markers between the patients with undetectable HIV viral load and those with detectable viremia. Even though the majority of patients had levels within the normal range, we found significant increases of TNF alpha, IL-6, MCP-1 between the first 2 follow up visits.
Conclusions: Despite controlling HIV replication, it seems that levels of inflammatory markers are slightly increasing in our HIV seropositive young adults. Except for antiretroviral therapy, specific anti-inflammatory medication might improve the inflammatory status of these patients in order to prevent cardio-vascular and metabolic diseases at early age or premature aging.
Disclosure of interest: None declared.

P-272
MAPK PHOSPHATASE-1 PLAYS A CRITICAL ROLE IN PROTECTION AGAINST TNF-INDUCED LETHAL SHOCK

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The mitogen-activated protein (MAP) kinase phosphatase (MKP) family of proteins plays an important role in regulating inflammation by inactivating MAP kinases. MKP-1/DUSP-1 is a nuclear, dual specificity phosphatase known to dephosphorylate MAP kinases. We are interested in TNF-induced systemic inflammation, because we believe that a tight control of this inflammation might increase the therapeutic value of TNF in cancer therapy. Since glucocorticoids protect very well against TNF-induced inflammation, and since MKP-1 is strongly induced by glucocorticoids, we hypothesize that MKP-1 can also regulate TNF-induced inflammation. In general, our results demonstrate that MKP-1% mice are more sensitive to TNF-induced inflammation in the liver, and show high levels of apoptosis in intestinal cells. These results can be mechanistically explained by a prolonged JNK activation and consequently stronger induction of AP-1 dependent genes and induction of apoptosis. To go deeper into the mechanism behind this sensitivity, the sensitivity of JNK1% and JNK2% mice to the in vivo effects of TNF was studied. In contrast to JNK1%, JNK2% mice are protected against TNF-induced lethal shock. Furthermore, the sensitivity of MKP-1% mice could be reversed by knocking-down JNK2, since double knock-out MKP-1/JNK2% mice showed enhanced resistance to TNF.
Disclosure of interest: None declared.

P-273
CIRCULATING COX-DERIVED EICOSANOID(S) INHIBITS MACROPHAGE FUNCTION IN MICE AND HUMANS WITH LIVER DISEASE

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Introduction: We hypothesised that a circulating plasma factor caused leukocyte immunoparesis in liver cirrhosis.
Methods: Mice peritoneal macrophages were cultured in media supplemented with 30% plasma from naïve & bile duct ligated (BDL)

mice ± LPS and supernatants analysed for cytokines. Complementary experiments were performed on human monocyte-derived macrophages treated with LPS in media supplemented with 25% plasma from healthy volunteers or inpatients with advanced liver cirrhosis. ESI/LC–MS/MS lipidomic plasma analysis was performed.
Results: Supplementing culture media with murine BDL plasma reduced LPS-induced IL-1/6/10 & TNF-α production, which was completely reversed by Indomethacin, with no effect of naive plasma ($p < 0.01$). Lipidomics revealed rises in PGF2α/E2. Human macrophage LPS-induced TNF-α secretion was significantly increased by supplementation of media with healthy volunteers' plasma ($n = 8$) compared to culture media alone ($p < 0.001$). In contrast TNF-α secretion from macrophages co-incubated with cirrhosis plasma showed a bimodal distribution with profound immune suppression observed with 4/10 of the cirrhosis samples. The immune suppressive effect was partially reversed by the PGE2/D2 receptor antagonist AH6809 ($p < 0.05$). Lipidomics ($n = 5$/group) demonstrated significant upregulation of PGF2α ($p < 0.001$) and 15HETE ($p < 0.05$) with trends in PGE2/12HETE.
Conclusion: This is the first description of immune inhibitory properties of COX-1/2 derived lipids within BDL mice and cirrhosis patients'. Whilst immune suppressive properties of PGE2 have been previously demonstrated, PGF2α may have an unexpected role.
Acknowledgments: Wellcome Trust.
Disclosure of interest: None declared.

Environment and inflammation

P-275
DENDRITIC CELLS ACTIVATED BY THE PARTICULATE MATTER ROFA EXACERBATE THE PULMONARY ALLERGIC INFLAMMATION

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Background: Studies suggest that pollutants are important cofactors in the development and exacerbation of lung diseases. Dendritic cells (DC) are crucial in regulating immune responses and play an important role in both the induction and maintenance of asthma. However, the mechanism of action of DC on the exacerbation of lung inflammation induced by particulate matter is unclear.
Objectives: We evaluated in vivo the role of residual oil fly ash (ROFA) in the exacerbation of inflammation in a murine model of asthma and in in vitro assay the effect of ROFA in the maturation of BMDC in response to allergen (OVA); and using a cell transfer assay the capacity of ROFA-pulsed BMDC to modulate the allergic inflammation.
Methods and Results: In vivo: We evaluated in lung, lymph nodes and spleen of the OVA-sensitized BALB/c mice challenged with OVA and ROFA the subsets of DCs, the number of Treg, IFN-g^+ and IL-5^+ T lymphocytes and the expression of Ly6C and CD40 in $CD11b^+F4/80^+$ macrophages. ROFA exposition exacerbated lung inflammation induced by OVA, increasing eosinophilia and goblet cells as well a decreasing the number of Treg, IFN-g^+ and IL-5^+ T lymphocytes in lung tissue. In spleen, ROFA maintains elevated the number of mDC, IFN-g^+ and IL-5^+ T lymphocytes and activated macrophages. Using an adoptive transfer model, we also showed that ROFA-pulsed BMDC transferred to allergic mice after challenge exacerbated eosinophilic airway inflammation. In vitro: BMDC were pulsed with OVA and/or ROFA. The pollutant by itself induces the maturation of BMDC increasing the expression of costimulatory molecules and the production of cytokines and MMPs. The activated status of BMDC induced by OVA was sustained after ROFA stimulation.
Conclusion: ROFA promotes the maturation of mDC, which lead to an enhanced effector Th2 cell activation that could be one of the mechanisms of pollutant-enhanced allergic inflammation.
Support: FAPESP and CNPq
Disclosure of interest: None declared.

P-276
SIGNALING THROUGH TOLL-LIKE RECEPTORS ENHANCES SUSCEPTIBILITY TO ASTHMA IN MICE EXPOSED TO ENVIRONMENTAL POLLUTANT DURING NEONATAL PERIOD

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Objective: Neonatal mice exposure to ambient pollutant 1,2-naphthoquinone (1,2-NQ) enhances animal susceptibility to inflammation at adulthood. Since Toll-like receptors (TLRs) in mammals function as crucial sentinels of infectious and non-infectious antigens throughout the respiratory tract, this study was undertaken to examine TLRs as a potential signaling pathway of this response.
Methods: Under approval of USP Animal Ethics Committee, $TLR4^{-/-}$ and $MyD88^{-/-}$ knockout (KO) neonate mice and their wild type (WT) were exposed three times via nebulization to 1,2-NQ or its vehicle. Eight weeks later, animals were sensitized and challenged with ovalbumin (OVA). Bronchial hyperresponsiveness (BHR) was assessed and samples collected 24 h after the last challenge. Eosinophils in bronchoalveolar lavage (BAL) and bone marrow (BM), cytokines in BAL, IgE in serum and mRNA expression of TLRs and adaptors molecules in lung were measured. Results are mean ± SEM for $n = 6$ animals. Stats were by ANOVA plus Bonferroni's test.
Results: Increased number of eosinophils was seen in BAL and BM of WT mice exposed to 1,2-NQ and OVA compared to allergic WT mice exposed to vehicle. The lung mRNA expression of TLR2, TLR4 and TLR7 or TRIF molecules was higher in WT mice exposed to 1,2-NQ, with or without OVA, compared to WT mice exposed to vehicle. $TLR4^{-/-}$ and $MyD88^{-/-}$ KO mice exposed to 1,2-NQ and OVA exhibited a significant decrease in BHR, cytokines concentration (but not IgE) and eosinophils counts in BAL (2.5 ± 0.5 and $1 \pm 0.1 \times 10^4$ cells/BAL) compared to $TLR4^{-/-}$ and $MyD88^{-/-}$ KO mice exposed to vehicle and OVA (39 ± 8 and $9 \pm 1 \times 10^4$ cells/BAL, respectively).
Conclusion: TLRs may serve as a signal transduction pathway increasing susceptibility to allergic response in mice exposed to ambient pollutant during neonatal period. This might be one mechanism that contributes to common pulmonary illnesses in children living in heavy traffic areas.
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Disclosure of interest: None declared.

P-277
ACTIVATION OF IMMUNE DENDRITIC CELLS BY SIO2 NANOPARTICLES

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Due to their unique physico-chemical characteristics, nanoparticles (NPs) are now one of the leading technologies. As a non-metal oxide, silica (SiO_2) NPs have found extensive applications in industry and biomedicine, hence leading to possible occupational, medical and/or environmental exposures. Aiming at evaluating their toxicological impact, we asked the question of SiO_2 NPs acting as immune adjuvants. Experiments were carried out on dendritic cells (DCs) which immunological role is to initiate an adaptative immune response when antigens and "danger signals" are concomitantly present within their surrounding environment. Under these conditions, DCs undergo a maturation process leading to phenotypical modifications. We therefore investigated whether SiO_2 NPs may alter this process. Primary cultures of both human monocyte derived DCs (MoDCs) and murine bone-marrow DCs (BMDCs) were exposed to 100 nm SiO_2 particles. Particle size and zeta potential were characterized using photon correlation spectroscopy (PCS) and zetametry, respectively. After 24 h, NPs internalization, cell viability and markers of cell maturation were studied. Using fluorescent NPs, microscopic observations revealed that NPs were found within the DCs after 24 h of exposure. Cytotoxicity evaluation indicated that both DCs types showed about 20% cell death after 24 h of exposure to 100 μg/mL SiO_2 NPs. Phenotypic analyses, carried out at this subtoxic concentration, showed that both MoDCs and BMDCs undergo a maturation process after SiO_2 NPs exposure as evidenced by significant upregulation of maturation markers at their surface (CD40, CD86, and CCR7 or CD83) as well as by a significant release of cytokines (IL-6, IL-8, IL-10, TNF, CCL5, CXCL10). Our results suggest that SiO_2 NPs may have an impact on the immune system function through the maturation of MoDCs and BMDCs. Further experiments will be dedicated to the understanding of signaling pathways involved in such a maturation process.
Disclosure of interest: None declared.

P-278
EARLY-LIFETIME EXPOSURE TO POLLUTANT INDUCES DIFFERENTIAL SUSCEPTIBILITY TO LUNG INFLAMMATION IN C57BL/6 MOUSE GENDER

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Objective: Whereas prior to puberty cases of asthma is higher in boys than girls, post puberty there are similar numbers in both genders. Moreover, it is known that children living close to high density traffic areas have enhanced susceptibility to respiratory diseases. The purpose of this study was to evaluate whether early-lifetime exposure to 1,2-naphthoquinone (1,2-NQ), a diesel exhaust contaminant, induces differential lung inflammation in juvenile male and female mice.
Methods: Under approval of USP Animal Ethics Committee, neonate mice were exposed three times via nebulization to 1,2-NQ or its vehicle. Sixteen days later, animals were sensitized and challenged with ovalbumin (OVA). Bronchial hyperresponsiveness (BHR) was assessed and samples collected 24 h after the last challenge. Leukocytes in bronchoalveolar lavage (BAL) and bone marrow (BM) or cytokines in BAL were measured. Results are mean ± SEM for *n* animals. Stats were by ANOVA plus Bonferroni's test.
Results: The OVA response in prior exposed 1,2-NQ male mice induced a significant increase of leukocytes, characterized by eosinophils and neutrophils, in BAL ($71 \pm 18 \times 10^4$ cells/BAL) and BM ($38 \pm 3 \times 10^6$ cells/BM) compared to BAL ($19 \pm 4 \times 10^4$ cells/BAL) and BM ($14 \pm 2 \times 10^6$ cells/BM) of female groups. In the BAL male group, the Th1/Th2 cytokine concentration was significantly higher compared to the female group, but the BHR was similar in both groups prior to exposure to 1,2-NQ.
Conclusion: Our results show, for the first time, that upon neonatal exposure of male and female mice to 1,2-NQ pollutant, the allergic response to OVA during puberty was still greater in male mice compared to female, thus suggesting that males are at greater risk than females. This may be related to increased differences of cytokine concentration in males compared to females.
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Disclosure of interest: None declared.

P-279
EFFECTS OF CARBON NANOTUBES ON CYTOKINE SECRETION BY PERIPHERAL BLOOD MONONUCLEAR CELLS FROM HEALTHY SUBJECTS AND ALLERGIC PATIENTS

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Carbon nanotubes (CNT) are promising engineered nanomaterials. However, nanosized particles are likely to exhibit pro-inflammatory activity and increase immune response to allergens. Thus, in the present study, we evaluated the effects of CNT on the secretion of cytokines by peripheral blood mononuclear cells (PBMC) from healthy subjects or mite-allergic patients. Freshly prepared PBMC, stimulated or not with innate [Toll-like receptor (TLR) 1–9 agonists], polyclonal T-cell (phytohemagglutinine A, PHA) or adaptative (mite allergen extract) stimulus were cultured for 48, 72 h or 5 days, respectively, in the presence or absence of multi-walled CNT (0–100 μg/mL). The cytokine secretion was quantified in the culture supernatants by ELISA. Basal secretion of all the cytokines assessed (TNF-α, IL-2, IL-5, IL-6, IL-12/23p40 or IFN-γ) was not altered by CNT in PBMC from both healthy subjects or allergic patients. In PBMC from healthy subjects, secretion of TNF-α, IL-6 and IL-12/23p40 evoked by the TLR-4 agonist, lipopolysaccharide (LPS), was however increased in a dose-dependent manner by the nanomaterials. Significant increases in the release of these cytokines were also observed in PBMC from healthy subjects stimulated with a TLR-2 or TLR-3 agonist, suggesting a pro-inflammatory activity of CNT. In PBMC from mite-allergic patients, CNT inhibited allergen-induced

IL-5 production, although they increased TNF-α secretion evoked by LPS. However, IL-2 and IFN-γ secretion induced by PHA in PBMC from both healthy and allergic subjects was increased by the presence of CNT. These data demonstrate that multi-walled CNT interfere differently with innate and adaptative immune responses in human PBMC and suggest differences in sensitivity to multi-walled CNT among cells of the immune system.
Disclosure of interest: None declared.

P-280
IN VIVO HYDROQUINONE EXPOSURE ALTERS CIRCULATING NEUTROPHILS ACTIVITIES AND IMPAIRS LPS-INDUCED LUNG INFLAMMATION

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Introduction: Hydroquinone (HQ) is an environmental contaminant, present in the cigarette smoke and an important benzene metabolite, which cause detrimental effects on immune cells. The toxicity of in vivo HQ exposure on neutrophil mobilization into the *E. coli* lipopolysaccharide (LPS)-inflamed lung was investigated.
Methods: Male Swiss mice were exposed to aerosolized vehicle (control) or 12.5, 25 or 50 ppm HQ (1 h/day/5 days). One hour after last exposures, animals inhaled LPS (0.1 mg/ml/10 min). Three hours later, number of circulating leukocytes and in the bronchoalveolar lavage fluid (BALF) were quantified using Neubauer chamber and stained smears; adhesion molecules on circulating neutrophil and lung microvessels endothelial cells expressions were quantified by flow cytometry and immunohistochemistry, respectively; myeloperoxidase activity was measured in the pulmonary tissue by colorimetry; cytokines in the BALF were determined by enzyme immunoassay; neutrophil oxidative burst was quantified by flow cytometer; plasma malonaldehyde (MDA) levels were measured by high performance liquid chromatography.
Results: In vivo HQ exposure increased plasma MDA levels and oxidative burst in circulating neutrophils, and reduced LPS-induced neutrophil migration into the BALF, as these cells were maintained in the pulmonary tissue. The reduced number of cells in the BALF was not dependent on alterations on circulating leukocytes number, cytokines secretions in the BALF and lung endothelial adhesion molecules expressions, but elevated β2 and β3 integrins and platelet-endothelial cell adhesion molecule-1 (PECAM-1) expressions in circulating neutrophils, which were not further enhanced by fMLP in vitro stimulation.
Conclusions: Low levels of HQ exposures affect circulating neutrophils, which may contribute to the impaired host defense during intoxications.
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Disclosure of interest: None declared.

P-281
TOBACCO SMOKING AND THE HLA–DR SHARED EPITOPE (SE) COMBINE TO INFLUENCE MATRIX METALLOPROTEINASE (MMP)-9 EXPRESSION IN RHEUMATOID INFLAMMATION

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Aims: To assess the effect of smoking and the HLA-DR shared epitope (SE) copy number on aryl hydrocarbon receptor (AHR) activation and the expression of inflammation-related genes in rheumatoid synovium. To consider the effects of the polycyclic aromatic hydrocarbon (PAH), benzo(*a*)pyrene (B*a*P), on MMP9 gene expression in human U937 cells.
Methods: Synovial membrane tissues were obtained at joint replacement surgery from patients fulfilling ACR criteria for RA. Patient smoking status was established retrospectively. Patients were typed for HLA-DR SE allotypes using sequence specific-primer-PCR. AHR, CYP1A1, AHRR, IL17A, CXCL12 and MMP9 gene expression in synovial tissue was assessed using qRT-PCR. U937 cells were treated with phorbol 12-myristate 13-acetate (PMA) and/or B*a*P. Western blotting was used to assess AHR knockdown by AHR-targeted siRNA. Statistics utilised paired or the multirange Dunnett's *t* tests and variance of ANOVA regression analysis.
Results: Smoking was responsible for AHR activation in synovia "reported" by significantly increased expression of both CYP1A1 ($P < 0.001$) and AHRR ($P < 0.01$) genes. Although not significant, there was a trend towards more IL17A expression in synovia from non-smoking RA patients ($P = 0.06$) with double copies of the SE ($P = 0.08$). Synovial MMP9 expression was affected by both smoking ($P = 0.02$) and SE copy number ($P = 0.007$). In vitro, PMA-activated U937 cells responded to B*a*P treatment with increased expression of AHR and MMP9 genes. AHR knock-down through RNA interference reduced MMP9 expression consistent with a role for AHR in transcriptional regulation of this gene.
Conclusions: Smoking impacts directly on joint synovial tissue in RA. MMP9 is the first identified gene that is influenced by the combined effect of smoking and the SE copy number in RA. PAHs, such as B*a*P have a role in regulating MMP9 gene expression in U937 cells. The data suggest reciprocal and synergistic roles for smoking and the SE in rheumatoid inflammation.
Disclosure of interest: None declared.

P-282
DOUBLE WALLED CARBON NANOTUBES INDUCE IL-1B SECRETION IN HUMAN MONOCYTES THROUGH THE NLRP3 INFLAMMASOME ACTIVATION

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With the increased use of carbon nanotubes (CNTs), public concern about their potential risk to human health has also risen. Although recent studies showed the in vitro and in vivo pro-inflammatory potential of CNTs, the inflammatory signaling pathways triggered by these particles in human monocytes remain unknown. Here, we showed that CNTs-free LPS did not induce synthesis and secretion of TNFα, IL-1β and IL-6 pro-inflammatory cytokines by human monocytes from healthy donors. However, CNTs increased IL-1β secretion in a dose dependent manner when monocytes were primed with LPS Toll-like receptors ligands. Moreover, we demonstrated that CNTs-increased IL-1β secretion was specifically caspase-1 dependent. Furthermore, by a siRNA approach targeting Nrlp3 receptor in human monocytes, we highlighted a decrease of IL-1β secretion in response to CNTs. Altogether these data demonstrate for the first time the involvement of the Nlrp3 inflammasome pathway in the pro-inflammatory effect of CNTs. Then, we explored the signaling pathway leading to the Nlrp3 inflammasome activation in response to CNTs. We showed that CNTs-induced IL-1β secretion was dependent on potassium efflux. Moreover, using pharmacological approaches, we demonstrated that the increase of IL-1β secretion after stimulation with CNTs was linked to their phagocytosis associated to cathpesin-B protease activation and phagosomal vacuolar acidification. These findings provide real evidence that CNTs trigger inflammasome signaling pathway and hence their use could present a potent health risk.
Disclosure of interest: None declared.

P-283
ANTI-INFLAMMATORY ACTIVITY OF NEOROGIOLTRIOL A NEW BROMINATED DITERPENOID ISOLATED FROM THE RED ALGAE LAURENCIA GLANDULIFERA

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Species of the red algae genus *Laurencia* elaborate an astonishing variety of structurally unusual secondary metabolites frequently presenting anti-inflammatory properties. From *Laurencia grandulifera,* we have isolated and characterised, by means of spectral data analysesneorogiotriol, a new tricyclic brominated diterpene. The aim of this study was to evaluate the anti-inflammatory properties of this compound.
Methods: The anti-inflammatory effect of Neorogiotriol was evaluated both in vivo using carrageenan-injected rats in the paw and in vitro on lipopolysaccharide (LPS)-treated RAW 264.7 macrophages stably transfected or not with the NF-kappaB-dependent luciferase reporter.
Results: The in vivo study demonstrated that intra-peritoneal injection of neorogiotriol (1 mg/kg) resulted in the significant reduction of carregeenan-induced rat oedema in the paw. In vitro, our results showed that neorogiotriol treatment decreased the luciferase activity in LPS-
stimulated RAW264.7 cells independently of MAPK pathways. Neorogiotrioltreatment significantly decreased LPS-induced tumor necrosis factor-alpha (TNF-a) secretion in RAW 264.7 cells. Further study indicated that this compound also significantly inhibited the release of nitric oxide (NO) and the expression of cyclooxygenase-2 (COX-2) in LPS-stimulated RAW264.7 cells.
Conclusions: The results demonstrated that neorogiotriol could play a significant role in the inhibition of inflammatory processes.
Disclosure of interest: None declared.

P-284
ACTIVATION OF G-PROTEIN COUPLED RECEPTOR 43 BY SHORT CHAIN FATTY ACIDS REGULATES INFLAMMATION ON ACUTE GOUT: ROLE OF DIET AND COMMENSAL MICROBIOTA

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Short-chain fatty acids (SCFAs) are produced by bacterial fermentation of dietary fiber and bind to GPR43 modulating inflammatory responses. Gout is an inflammatory disease characterized by release of uric acid crystals into the joint cavity and neutrophil infiltration that leads to tissue damage. Our aims are understand the mechanism of SCFAs as well as, the role of GPR43 in gout. Treatment with SCFA protected mice from injury by intra-articular injection of Monossodium Uric Acid crystal showing reduction of pro-inflammatory cytokines and increasing anti-inflammatory mediators, such as, IL-10 and TGFbeta inducing neutrophils's apoptosis. Interestingly, we observed that GPR43ko mice presented reduction in inflammatory parameters after gout induction due deficiency on recruitment of cells, when compared to MSU-injected WT mice. Germ-free mice, which no express SCFAs, showed similar "hiporresponsive" and inhibited recruitment of cells. However if we treated GF with acetate before challenged with MSU, this "phenotype" was reversed. Thus, microbiota-induced SCFA and GPR43 activation are necessary for proper neutrophil recruitment. However, SCFA treatment during an established inflammatory response promoted resolution of the inflammation. We suggest that endogenous microbiota shapes the host's ability to respond to inflammatory stimuli. The presence of SCFAs provides a link between diet, gastrointestinal bacterial metabolism and inflammation.
Disclosure of interest: None declared.

P-285
EVALUATION OF THERAPEUTIC INTERVENTION WITH NATURAL PRODUCT IN CUTANEOUS WOUND HEALING: THE USE OF CAPYBARA OIL

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In the interaction culture and nature is remarkable use of wildlife for medicinal purposes in different human societies. The oil extracted from subcutaneous fat of capybaras (*Hydrochaeris hydrochaeris*) has certain properties that help in curing respiratory diseases, arthritis, hypercholesterolemia, and also acts as a powerful healing for external wound (although not proven in clinical and experimental research). The aim of this work is assess cutaneous wound healing in Swiss mice with the topical application of capybara oil. Forty-two Swiss mice, male, adults were allocated into two groups (N = 42): capybara oil group (N = 21, divided into three subgroups with N = 7) oil treated; and control group (N = 21, divided into three subgroups with N = 7) who received no treatment. The daily application of oil was about standardized circular wound of 0.6 mm diameter on the dorsum of each animal. At days proposed (7, 14 e 21 days after injury), mice were deeply anesthetized with Thiopental; were then fixed to the surgical table to collect morphological data and surgical specimens. Wound excised with 1 cm margin of healthy skin around the lesion, deep to the fascia muscle, were fixed and prepared accordingly the histological routine to be analyzed on light microscopy. Statistical analyses were realized (ANOVA test, $p < 0.0001$). In Swiss mice, 7 days after wounding, the capybara oil group presented a thicker neoepidermis than control group. In all groups mast cells were present, but the capybara oil group showed a higher amount of mast cells than the control group in 7 days after wounding. Wound contraction was evaluated showed a progressive reduction in wound area during the experiment, but 7 days after injury, the wound area was less in the capybara oil group. The capybara oil seems to exert a beneficial effect on the cutaneous wound healing.
Disclosure of interest: None declared.

Autoimmunity

P-286
A STUDY OF PATIENTS WITH RHEUMATOID ARTHRITIS: RELATIONSHIP BETWEEN THE FUNCTION OF NEUTROPHILS WITH ACTIVATION OF THE COMPLEMENT SYSTEM AT THE SYSTEMIC LEVEL

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Evaluation of the respiratory burst induced by receptors FcγR (for the Fc portion of IgG) and CR (for complement) was carried out in rheumatoid arthritis (RA) patients presenting the active disease or not. Simultaneously, the cooperation between these receptors and their expression, the chemotaxis of peripheral blood neutrophils, and the systemic activity of the complement system were also investigated. Neutrophils were stimulated with IC-IgG (immune complex of IgG) opsonized with normal human serum (NHS) or not, or with IC-IgG opsonized with RA human serum (RAHS). ROS (reactive oxygen species) production by RA neutrophils of patients with active or inactive disease responded to IC-IgG opsonized with NHS. However, these RA neutrophils presented poor FcγR/CR cooperation, as indicated by decreased ROS production upon stimulation with IC-IgG opsonized with RASH. Neutrophils of active RA patients presented significantly augmented CR1 and CR3 expression, as well as slight elevation in FcγRII and FcγRIII expression. Only patients with active disease exhibited positive correlations between FcγR and CR. The complement alternative pathway was activated and the chemotaxic activity of RA serum was increased only in the case of patients with active disease. Taken together, these results indicate that several abnormalities of the complement system exist at the systemic level, as reflected by the neutrophil function, particularly in RA patients with active disease. Namely, these abnormalities are impaired membrane receptor cooperation, activation of the alternative pathway, and presence of pre-existing chemoattractant factors in the serum, which together may contribute to RA pathogenesis.
Disclosure of interest: None declared.

P-287
MODELING HUMAN RHEUMATOID ARTHRITIS IN NHP: TYPE II COLLAGEN INDUCED ARTHRITIS IN CYNOMOLGUS MACAQUES

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Collagen induced arthritis (CIA) rodent models have been extensively used in rheumatoid arthritis (RA) research. An RA model in non-human primate (NHP) is particularly demanded because of the close phylogenesis that provides the cross-reactivity to human for different development compounds using most modern drug technologies. However, NHP RA model has been reported extremely difficult because of the low and inconsistent disease incidence. We studied type II collagen induced arthritis in Cynomolgus monkeys. Following immunization with collagen, the disease progression was monitored for 8 weeks. Overall the arthritic incidence reached 87% and the average arthritic incidence of proximal interphalangeal (PIP) joint reached near 90%, significantly higher than what was previously reported. The average swelling of PIP joint increased approximately by 45%. Radiography, histopathology and histomorphometry analysis of the joint bones well supported the arthritic disease with the similar characteristics of human RA joints. The average arthritic score was significantly reduced with the single agent treatment of methotrexate or dexamethasone. Our results demonstrated the successful establishment of an reliable CIA in Cynomolgus monkeys, providing a valuable tool for studies of RA disease in pathogenesis, biomarker, translational research, and most importantly, anti-arthritic therapeutics as well as other relevant diseases, such as anemia of chronic disease and arthritic pain.
Disclosure of interest: None declared.

P-288
ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN THE GENERATION OF REGULATORY T CELLS IN TUMOR-BEARING MICE

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Macrophage migration inhibitory factor (MIF) involves tumorigenesis by facilitating tumor proliferation, evasion of apoptosis, and

angiogenesis; however, its role in tumor immunity of host remains unclear. In this study, we investigated effect of MIF on tumor progression and generation of tumor regulatory T cells (Treg). The results showed that tumor growth rate was significantly lower in MIF knockout (KO) mice than in wild type (WT) mice when syngenic colon cancer cells (CT26 cells) were injected. The infiltration of CD4 (+) and CD8 (+) T cells in tumor mass was significantly higher in MIF KO mice than in WT mice. Flow cytometric analysis of both spleen and tumor cells revealed that MIF KO mice had significant lower levels of tumor-associated Treg than WT mice. In parallel, the expression of GITR and CTLA-4 was also reduced in MIF KO mice. Interestingly, there were three cases of tumor rejection among twenty MIF KO mice, but not in WT mice. The number of CD4 (+) and CD8 (+) T cells in these mice with rejection was further increased as comparable to tumor-naïve mice. Moreover, the frequency of Treg was much lower in spleen of tumor-rejected mice than in those of tumor-bearing MIF KO mice. In contrast, when induced by anti-CD3 + IL-2 + TGF-b, the number of the Treg in the spleen was significantly higher in MIF KO mice than in WT mice, suggesting that these stimuli may be associated with the decreased Treg in MIF KO mice. As expected, MIF KO mice showed significant lower levels of IL-2 production by spleen cells stimulated with anti-CD3 than WT mice. In the mean time, both IFNg production by spleen cells and the frequency of CD8 (+) and IFNg (+) cells were not different between the two groups. Taken together, our data suggest that MIF promotes tumor growth in mice by increasing tumor Treg through the regulation of IL-2 production. Thus, anti-MIF treatment might be useful to enhance adaptive immunity of host to colon cancers.
Disclosure of interest: None declared.

P-289
LONG-TERM ANTIBODY PRODUCTION AND MAINTENANCE OF LONG-LIVED ANTIBODY-SECRETING CELLS (ASC) ARE SUSTAINED BY TWO DISTINCT SUBTYPES OF MEMORY CD4 + T CELLS

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Introduction: The rising awareness of the importance of long-lived antibody-secreting cells (ASC) for the maintenance of protective and autoimmune humoral memory generates research efforts to gain more insight into the biology, migratory behavior and mainly survival mechanisms. We previously demonstrated that *Thalassophryne nattereri* fish venom induces a humoral memory immune response in mice with the differentiation of ASC $B220^{neg}$ dependent on IL-5 and IL-17A secreted by T cells. However, how and when CD4+ T cells participate of the generation of ASC still not understood.
Objective: Evaluate the importance of effector/memory CD4+ T cells in the maintenance of ASC.
Methods and Results: To investigate the phenotypic features of effector/memory T cells, BALB/c mice were immunized three times with 10 mg of venom in alum and killed at days 15, 49 and 121. Our results show an early formation of effector CD4 + T cells in the peritoneum and bone-marrow (BM) and memory CD4 + CD44 + T cells expressing both CD40L and Ly6C in spleen and peritoneum and expressing Ly6C only in BM. In the memory phase, the effector CD4 + T cells migrate to spleen and the CD4 + CD44 + CD40L + Ly6C + memory T cells exit from the spleen remaining in the peritoneum. Bone-marrow is the appropriated niche for CD4 + CD44 +
Ly6C + central memory T cells. To confirm the importance of memory CD4 T cells in memory Ab production, adoptive transfer of memory cells from spleen and BM was conducted. Bone-marrow cells from long-lasting venom immunized mice, but not control or splenic cells, induced high and persistent levels (80 days after transfer) of specific antibody production (IgG1 and IgG2a) in naïve recipients that was impaired in CD4 or CD28 *KO* recipients.
Conclusions: Thus, in the memory phase of immunity, central memory helper T cells are maintained in BM as resting but rapidly expressed CD40L and efficiently induced the production of high-affinity antibodies by B lymphocytes.
Support: FAPESP and CNPq.
Disclosure of interest: None declared.

P-290
INTRAVENOUS IMMUNOGLOBULIN INHIBITS AUTOANTIBODY PRODUCTION BY B-1 CELLS

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Many autoimmune diseases (ADs) are characterized by the production of autoantibodies (autoAbs) specific for self-antigens. However, the precise mechanism for the pathogenesis of autoimmunity is unknown. On the other hand, Intravenous Immunoglobulin (IVIg) exhibits therapeutic effects in the treatment of variety of ADs in clinical practice, while the precise mechanisms have also been left unclear. Innate B cells or B-1 cells, which produce mainly natural antibodies including weakly autoreactive antibodies, have a distinct lineage different from conventional B cells or (B-2 cells). Recently, B-1 cells are shown to play a role in development of ADs when they are activated, and the class-switch is induced upon stimulation of their toll-like receptor (TLR)9 with unmethylated CpG oligonucleotide. Here we show that IVIg treatment in mice injected with CpG inhibits the proliferation and activation of B-1 cells. Upon stimulation of B-1 cells with CpG in vitro, IVIg attenuated the autoantibody production as well as the IL-10 production that is necessary to induce CSR, while it did not reduced the IL-6 production. The inhibitory effect of IVIg was dependent on the $F(ab')_2$ but independent on the Fc. In addition, IVIg upregulated the expression of CD22, a B cell inhibitory receptor, on B-1 cells. However, IVIg treatment exhibited a comparable inhibitory effect on B-1 cells from wild-type and CD22-knockout mouse. Inspections of IVIg-induced modulation of intracellular signaling revealed that the IVIg attenuated several TLR9-initiated signaling pathways, such as phosphorylation of TAK1, NF-kB and ERK, but not IRAK-1 and p38 MAPK. We propose a novel inhibitory mechanism of IVIg in ADs, in which IVIg inhibits TLR9 signaling that leads to production of autoreactive antibody in a $F(ab')_2$-dependent manner.
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P-291
DISTINCT PATHOLOGICAL PATTERNS IN RELAPSING-REMITTING AND CHRONIC MODELS OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND THE BENEFICIAL EFFECT OF GLATIRAMER ACETATE

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The roles of inflammatory versus neurodegenerative processes in the pathology of multiple sclerosis (MS) and in its animal model experimental autoimmune encephalomyelitis (EAE) are controversial. Novel treatment strategies aim to act within the CNS to reduce inflammation and induce neuroprotection and repair processes. In this study we analyzed and compared the in situ pathological manifestations of relapsing-remitting PLP- and chronic MOG- induced EAE models, using transmission electron microscopy (TEM) and immunohistochemistry. The effect of immunomodulatory treatment by glatiramer acetate (GA, Copaxone) on myelin damage/repair and on motor neuron loss/preservation was studied in both models. TEM analysis revealed multiple spinal white matter damages, demyelination, axonal degeneration and axonal loss, with different occurrence in the two EAE models. Hence, in the relapsing-remitting model most of the axons manifested myelin aberration, whereas in the chronic model axonal degeneration was more widespread. Remyelinating fibers with thin compact myelin sheathes were detected mainly in the PLP model and their number increased with time. Loss of motor neurons was manifested only in the chronic model. In GA-treated mice, smaller lesions and higher amounts of normal appearing axons were found, with preservation of motor neuron number and formation. Furthermore, for each time point, significantly more remyelinating fibers were counted, three to sevenfold elevations from untreated mice. These effects were obtained even when GA-treatment was applied in a therapeutic schedule after the appearance of clinical symptoms. In conclusion, demyelination and remyelination are characteristic to relapsing-remitting PLP-induced EAE whereas in the chronic MOG-induced model axonal degeneration and somatic neuronal pathology are mainly manifested. The remyelination and neuronal preservation induced by GA are in support of neuroprotective consequences of immunomodulatory treatment in these models

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P-293
ORAL ADMINISTRATION OF HSP65-PRODUCING LACTOCOCCUS LACTIS PREVENTS EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE BY INCREASING REGULATORY T CELLS

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Introduction: Experimental autoimmune encephalomyelitis (EAE) is a rodent model of human multiple sclerosis. Heat shock proteins are evolutionary conserved proteins that maintain the cellular integrity and are possible targets for regulatory T cells.

Objective: To investigate the immunological effects of *Lactococcus lactis* that secretes Hsp65 from *Mycobacterium leprae* in EAE model.

Methods: Animals received for four days continuous feeding of either Hsp65-producing *L. lactis* (Hsp65), wild type (WT) *L. lactis* or water (control). Seven days after the last feeding, EAE was induced in C57BL/6 mice by subcutaneous injection of CFA, $MOG_{35–55}$ in the base of the tail and pertussis toxin i.p.

Results: Prophylactic treatment of mice with Hsp65, but neither with WT nor water, prevented the induction of EAE regarding the clinical scores. Such effect was followed by decreased frequencies of early activated $CD4^+CD69^+$ T cells in mesenteric lymph nodes, and late activated T lymphocytes $CD4^+CD44^+$ in lymph nodes mesenteric and inguinal, as well as in the spleen. Moreover, frequencies of Treg cells were higher in Hsp65 than in WT or water-treated mice.

Conclusion: Hsp65 *L. lactis* was effective in preventing EAE in mice through increase of Treg cells. Nevertheless, the mechanisms involved in such regulation need to be elucidated.

Disclosure of interest: None declared.

P-294
INFLAMMATORY CYTOKINES IN AUTOIMMUNE TYPE 1 DIABETES

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Inflammatory cytokines are implicated in the pathogenesis of different autoimmune diseases like Type 1 diabetes, rheumatoid arthritis and inflammatory bowel disease in humans as well as in animal models. IL-21, and IL-15 are pro-inflammatory cytokines and they exert their biological effects at various stages of the immune responses. We have shown previously that IL-21 can synergize with IL-15 or IL-7 to reduce the threshold of TCR signaling, while simultaneously increasing the production of effector cytokines such as TNFα and IFNγ. IL-21 maps to one of the shared autoimmune disease susceptibility loci in humans and mice. In non obese diabetic (NOD) mouse that develops spontaneous autoimmune type 1 diabetes (T1D), IL-21 is a candidate gene in the Idd3 locus. C57BL/6 derived Idd3 allele protects NOD mouse from T1D. As IL-2 also maps to the same locus, the relative contribution of IL-2 and IL-21 to the disease process is not well understood. It has been shown previously that NOD mouse lacking the receptor for IL-21 does not develop autoimmune diabetes. Here we report that the absence of IL-21 protects the 8.3 TCR transgenic NOD mice from diabetes. Similarly we observed that NOD mice that lack the expression of IL-15 show reduced incidence of type 1 diabetes. Our results suggest that IL-21 and IL-15 may be implicated at different stages of disease development in the NOD mouse model.

Disclosure of interest: None declared.

P-295
SCREENING EARLY PREGNANT WOMEN FOR AUTOIMMUNE THYROID DISEASE IN ATHENA HOSPITAL TIMISOARA (ROMANIA). CONTROVERSIAL OR BENEFICIAL?

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Objective: To identify a relationship between existing TPOAb among pregnant woman and its relation with their outcome of pregnancy, morbidity of autoimmune thyroiditis, to justify screening, when would be the best time for doing it.
Materials and methods: The study included 120 pregnant, romanian women, ages 18–40. We used one-time screening TPOAb, one-time screening TSH and FT4. We performed also a cost-effectiveness analysis of the screening. When probabilities were not available, we were estimated using expert clinical judgment.
Results: At initial presentation all the women were euthyroid. 10.83% had positive TPOAb level. The complications were found to be more significantly raised among positive TPOAb cases. It seems that autoimmune disease can increase the risk of reproductive failure. The administration of L-T4 prevent the risk for developing hypothyroidism during gestation and reduces pregnancy loss and preterm delivery.
Conclusions: The prevalence rate for thyroid autoimmunity in young women seems high enough to justify the screening. Screening only high-risk women, however, would fail to diagnose woman with thyroid disease. Screening pregnant women with TFTs in the first trimester of pregnancy is cost-saving compared with no screening, while screening using TPO-Ab is an economically favorable screening strategy.
Disclosure of interest: None declared.

P-296
AN ORALLY BIOAVAILABLE SMALL MOLECULE RVX-297 SIGNIFICANTLY DECREASES CLINICAL SIGNS IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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We have discovered a representative of a novel chemical scaffold (RVX-297) that prevents and treats established autoimmune disease. In vitro, RVX-297 inhibited LPS-induced IL-6 expression in human U937 cells (IC50 of 0.9 μM) and T-cell receptor activation-induced IL-17 expression in human PBMCs (IC50 of 2.3 μM). In vivo, RVX-297 treated B6 mice (75 mg/kg, PO) had 93% lower serum concentrations of IL-6 compared to the vehicle-treated controls, 4 h after 5 μg of LPS injection. RVX-297 was further evaluated in murine experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) that is known to be dependent on IL-6 and IL-17, to determine its potential to modulate autoimmune disease in vivo. EAE was induced in B6 mice via immunization with $MOG_{35–55}$ and pertussis toxin injection. Oral administration of RVX-297 from the time of immunization prevented EAE development dose-dependently from 75 to 150 mg/kg b.i.d. Therapeutic administration of RVX-297 from the time of disease onset greatly reduced clinical signs of EAE dose dependently from 75 to 125 mg/kg b.i.d dose. The efficacy of RVX-297 treatment when administered to mice either prophylactically or therapeutically was comparable or superior to the $S1P_1$ agonist, FTY720. Further analysis of spleen and lymph node culture supernatants from mice immunized with $MOG_{35–55}$ and treated with RVX-297 found greatly reduced ex vivo antigen-induced production of IL-6, IFN-γ, TNF and IL-17A protein (70, 99, 80 and 80% respectively) compared to the vehicle-treated controls. Gene expression analysis of ex vivo cultures demonstrated up to 90% inhibition of IL-17, IFN-γ, IL-1b, IL-21 and IL-4 mRNA. These cumulative results suggest that RVX-297 inhibits Th1 and Th17 responses which are believed to play a critical role in the development and pathology of autoimmune diseases. Therefore, compounds from this scaffold represent potential novel therapeutics for the treatment of autoimmune diseases including MS.
Disclosure of interest: R. Jahagirdar Employee of: Resverlogix Corp, S. Marusic: None Declared, S. Attwell Employee of: Resverlogix Corp, K. McLure Employee of: Resverlogix Corp, P. Young Employee of: Resverlogix Corp, H. Hansen Employee of: Resverlogix Corp, R. Yu Employee of: Resverlogix Corp, G. Wagner Employee of: Resverlogix Corp

P-297
NON-RESOLVING INFLAMMATION AND SELF-ORGANIZED CRITICALITY THEORY OF AUTOIMMUNITY AND SLE

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Immune system stands on 'trade-off' between infection-induced and defense-induced tissue injuries. To avoid 'too much battle against invasive organisms', immune system tries to suppress but not to eradicate invading antigens. Thus, our immune system is constantly exposed to small but continuous stimulation with antigen. We test here the integrity of immune system, i.e., how immune system responds to the overload of antigen, by stimulating maximally the immune system repeatedly with the same antigen.
Methods: BALB/c, DO11.10 TCR Tg, β_2m-deficient and *rag1/gfp* knock-in mice were i.p. immunized with SEB, OVA or KLH. Cells were transferred to naïve BALB/c or β_2m-deficient mice via i.p. or i.v. cDNA was amplified by PCR and subjected to run-off reactions with labeled Jβ primers, Jβ1.1, Jβ1.3 and Jβ2.4. To test antigen cross-presentation, $CD11c^+$ DCs were incubated with OVA followed by co-cultured with DO11.10 $CD8^+$ T, and expression of CD69 on DO11.10 $CD8^+$ T was examined, using chloroquine as inhibitor.
Results: Repeated immunization with antigen causes systemic autoimmunity in mice otherwise not prone to spontaneous autoimmune diseases. Overstimulation of $CD4^+$ T cells led to the development of autoantibody-inducing $CD4^+$ T (*ai*$CD4^+$ T) cell which had undergone TCRα but not TCRβ revision and was capable of inducing autoantibodies. *ai*$CD4^+$ T cell was induced by de novo TCR revision but not by mere cross-reaction to antigen, and subsequently overstimulated $CD8^+$ T cells, driving them to become antigen-specific CTL. These CTLs could be further matured by antigen cross-presentation, after

which they caused autoimmune tissue injury identical to SLE. The \CD4$^+$ T cell was specifically detected in SLE.
Conclusions: Autoimmunity is the inevitable consequence of over-stimulating host's immune 'system' by repeated immunization with antigen, to the levels that surpass system's self-organized criticality. SLE is induced by normal, but not autoimmune, response to antigen.
Disclosure of interest: None declared.

P-298
IS CD177 (NB1) SUFFICIENT FOR THE MEMBRANE EXPRESSION OF PR3 IN MYELOID CELLS?

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Proteinase 3 (PR3) is a granular serine-proteinase, which is also present at the plasma membrane in a stable neutrophil subset. ANCA binding to PR3 at the surface of primed neutrophils results in their activation. In neutrophils, PR3 has been shown to be associated with a number of protein partners but the function of these associations remain to be defined. CD177 is a GPI-anchored glycoprotein, which is expressed on the same neutrophil subset than PR3. It has been proposed that CD177 is essential for PR3 membrane expression in the basal state. The rat basophilic cell line (RBL) has been used to study the molecular mechanisms leading to PR3 membrane expression but unfortunately RBL-PR3 do not display PR3 membrane expression. We hypothesized that expression of CD177 would "reconstitute" the molecular "scaffold" of PR3 partners. We generated stable RBL transfectant expressing either hCD177 or hPR3 or both. Membrane expressions of CD177 or PR3 were studied by flow cytometry under basal conditions or following degranulation or apoptosis. Stable hCD177-transfected cells show a membrane expression under the basal state in WT-RBL as well as RBL-PR3. However, in RBL-PR3 CD177 failed to trigger PR3 to membrane under the basal state. After degranulation PR3 membrane expression was similar in RBL PR3 and RBL-PR3 CD177. We found the same result in apoptotic cells; membrane expression of PR3 was similar in RBL-PR3 and RBL-PR3 CD177 in flow cytometry analysis. These cells have similar intracellular PR3 storage. In keeping with data from other groups, we also found that CD177 is a receptor for extracellular PR3, as demonstrated by the binding of exogenous PR3 to RBL-CD177 in the basal state contrary to RBL WT. CD177 appears to be a partner of PR3, which could play a role as a receptor for extracellular PR3, but it seems to be insufficient for PR3 membrane targeting in the basal state in a cell model of neutrophils.
Disclosure of interest: None declared.

P-299
INVOLVEMENT OF TUMOR NECROSIS FACTOR-ALPHA AND INTERLEUKINE-17A IN BOWEL DISEASE PATHOGENESIS BY NITRIC OXIDE PATHWAY: POTENTIAL THERAPEUTIC ACTION OF INTERLEUKINE-10 AND RETINOIC ACID

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Background: Inflammatory bowel diseases (IBD) are chronic inflammatory diseases of the gastrointestinal tract, which clinically present as one of two disorders, Crohn's disease (CD) and ulcerative colitis (UC). In a previous study, we have shown correlation between NO production and intestinal tissue damages in patients reached by Crohn disease and ulcerative colitis. In this study, we investigate in vitro and in situ the modulation of nitric oxide production by TNF-α and/or IL-17A in the presence of IL-10 or retinoic acid in cultures of PBMC, macrophages and colonic mucosa from patients (CD and UC). We also evaluated IL-17 expression in inflamed colonic mucosa treated by retinoic acid.
Patients and methods: Twenty Patients with UC, 30 patients with CD and 10 healthy controls were enrolled in this study. Freshly isolated peripheral blood mononuclear cells (PBMCs) and macrophages were resuspended in RPMI 1640 and DMEM culture medium, respectively. Multiple colonic biopsies were taken from patients who underwent colonoscopy. Colonic biopsies were immediately placed in DMEM culture medium. The cultures were stimulated by human recombinant TNF-α, IL-17A in presence of IL-10 or retinoic acid. Cultures supernatants were harvested after 24 h of incubation; NO measurement was performed by modified Griess. IL-17 expression was evaluated by standard immunohistochemical procedure.
Results: Our results show that TNF-α and IL-17A up regulated NO production by PBMC, macrophages and colonic mucosa. In contrast, exogenous IL-10 and retinoic acid down regulated NO production in vitro and in situ for the two groups of patients. Retinoic acid down-regulates IL-17 expression in colonic mucosa.
Conclusion: These results suggest that proinflammatory cytokines (TNF-α, IL-17A) play a pivotal role in IBD pathogenesis disease through nitric oxide pathway. Collectively our study suggests that IL-10 and retinoic acid seem to be a useful tool for development of therapeutic strategies in IBD.
Disclosure of interest: None declared.

P-300
IDENTIFICATION OF TARGET ANTIGENS OF ANTI-ENDOTHELIAL-CELL AND ANTI-VASCULAR-SMOOTH-MUSCLE-CELL ANTIBODIES IN PATIENTS WITH GIANT CELL ARTERITIS : A PROTEOMIC APPROACH

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Background: In giant cell arteritis (GCA), immunological studies suggest the existence of a triggering antigen of unknown nature activating T-cell in the arterial wall (1). In experiments performed in animal models, a proliferation of T-cells from the xenotransplant suggests a possible recognition of a locally expressed antigen (2).

Objectives: We thus decided to use a proteomic approach in order to identify the target antigens of auto-antibodies directed against endothelial cells (EC) and vascular smooth muscle cells (VSMC).
Patients and methods: Sera from 15 GCA patients were tested in pools of 3 sera and compared to a sera pool from 12 healthy controls (HC). Serum IgG reactivity was analyzed by use of a 2-D electrophoresis and immunoblotting technique with antigens from normal human umbilical vein endothelial cells (HUVEC) and mammary-artery-VSMC. Targets antigens were identified by mass spectrometry (MALDI-TOF-TOF).
Results: Serum IgG recognized 162 ± 3 and 100 ± 17 protein spots from HUVEC and VSMC, respectively, whereas serum IgG antibodies from HC recognized 79 and 94 protein spots, respectively. Thirty spots from HUVEC were recognized by at least 2/3 pools and not by HC. Nineteen spots from VSMC were recognized by at least 3/5 pools and not by HC. Among identified proteins we found lamin A/C, voltage dependent anion selective channel protein 2, annexin V and other protein involved in cell energy metabolism and key cellular pathways.
Conclusion: IgG anti-EC antibodies and anti-VSMC antibodies are present in the serum of patients with GCA. These antibodies recognize cellular targets playing key roles in cell biology and maintenance of homeostasis. The function of these antibodies and their possible role in pathology have to be further explored.
Disclosure of interest: None declared.

P-301
IDENTIFICATION OF TARGET ANTIGENS OF ANTI-SMOOTH MUSCLE CELL ANTIBODIES IN PATIENTS WITH ANCA-ASSOCIATED SYSTEMIC VASCULITIS: A PROTEOMIC APPROACH

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Purpose: In an experimental model, immunization of splenocytes or CD4 T cell with vascular smooth muscle cell (VSMC) is responsible for a pulmonary vasculitis after adoptive transfer (1, 2).
Objectives: We thus decided to identify the target antigens of anti-VSMC in patients with ANCA-associated vasculitis.
Patients and methods: Sera from 33 ANCA-associated vasculitis patients, 15 with Wegener's granulomatosis (WG), 9 with microscopic polyangiitis (MPA), 9 with Churg Strauss syndrome (CSS), tested in pooled sera for groups of 3, were compared to a sera pool from 12 healthy controls (HCs). Serum IgG reactivity was analyzed by use of a 2-D electrophoresis and immunoblotting technique with immortalized mammary artery-VSMC antigens. Target antigens were identified by mass spectrometry.
Results: Serum IgG antibodies in the pools of patients with WG, MPA, CSS recognized 121 ± 70, 57 ± 29 and 66 ± 14 protein spots, respectively, whereas healthy controls recognized 94 protein spots. Thirty one, 18 and 22 protein spots were specifically recognized by at least 3/5, 2/3 and 2/3 pools of WG, MPA and CSS, respectively. Among identified proteins are lamin A/C, vimentin, annexin and alpha-enolase. Target antigens are involved in cytoskeleton, cell energy metabolism, and other key cellular pathways.
Conclusion: Anti-VSMC IgG antibodies are detected in patients with ANCA-associated vasculitis. They recognize cellular targets playing key roles in cell biology and maintenance of homeostasis. The functions of these antibodies and their possible role in pathology need further investigation.
Disclosure of interest: None declared.

P-302
IDENTIFICATION OF TARGET ANTIGENS OF ENDOTHELIAL CELLS ANTIBODIES IN SYSTEMIC SCLEROSIS AND IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

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Purpose: To identify target antigens of anti-endothelial cells antibodies (AECA) in systemic sclerosis (SSc) and in idiopathic pulmonary arterial hypertension (iPAH).
Material and methods: we have used a two-dimensional electrophoresis and immunoblotting technique with protein extracts of human umbilical vein endothelial cells (HUVEC). We have tested sera from 18 SSc patients with (n = 9) or without PAH (n = 9), 9 sera from iPAH patients (3 sporadic, 3 familial and 3 dexfenfluramin-associated PAH) and 12 sera from healthy blood donors as negative controls. These sera were tested at a dilution of 1/100 by pools of 3 for patients and in a pool of 12 for healthy controls. Targeted spots were identified by mass spectrometry.
Results: Serum IgG from SSc patients with or without PAH recognized 42 ± 4, 37 ± 7 protein spots, respectively. IgG from patients with sporadic, familial or dexfenfluramin-associated PAH recognized 46, 30 and 30 protein spots, respectively, whereas 53 protein spots were recognized by IgG from healthy donors. 64 protein spots were recognized specifically by IgG from SSc patients including 17 spots which were recognised specifically by PAH patients. Only six protein spots were recognized by IgG from more than 75% of patients' pools of sera and not by the IgG from healthy donors. Antigens targeted by IgG AECA from SSc and/or PAH patients have been identified by mass spectrometry (MALDI-toff) including triosephosphate isomerase, peroxiredoxin, cathepsin D, prohibitin and phosphoglycerate mutase 1.
Conclusions: We identified target antigens of AECA in SSc and in idiopathic PAH patients. Additional experiments are needed to characterize their potential pathogenetic role.
Disclosure of interest: None declared.

P-303
IDENTIFICATION OF NEW AUTOANTIBODY SPECIFICITIES DIRECTED AT PROTEINS INVOLVED IN THE TRANSFORMING GROWTH FACTOR BETA PATHWAY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Introduction: Approximately 10% of SSc patients have no routinely detectable antinuclear antibodies (ANA), usually detected by indirect immunofluorescence on HEp-2 cells. For 20 to 40% of those with detectable ANA, there is no identified specificity. In this work, we aimed to identify new target autoantigens in SSc patients.
Methods: Using a proteomic approach combining 2-D electrophoresis and immunoblotting with HEp-2 cell total and nuclear protein extracts, we systematically analysed autoantibodies in the sera of 45 SSc patients tested in 15 pools from groups of 3 patients with the same phenotype. A sera pool from 12 healthy individuals was used as a control. Proteins of interest were identified by mass spectrometry and analysed using Pathway Studio software.
Results: We identified 974 and 832 protein spots in HEp-2 cell total and nuclear protein extracts, respectively. Interestingly, alpha-enolase was recognised by IgG from all pools of patients in both extracts. Fourteen and 4 proteins were recognised by IgG from at least 75% of the 15 pools in total and nuclear protein extracts, respectively, whereas 15 protein spots were specifically recognised by IgG from at least 4 of the 10 pools from patients with non-identified ANA. The IgG intensity for a number of antigens was higher in sera from patients than in those from healthy controls; these antigens included triosephosphate isomerase, superoxide dismutase mitochondrial precursor, heterogeneous nuclear ribonucleoprotein L and lamin A/C. In addition, peroxiredoxin-2, cofilin-1 and calreticulin were specifically recognised by sera from phenotypic subsets of patients with non-identified ANA. Interestingly, several identified target antigens are involved in the transforming growth factor-beta pathway.
Conclusions: We identified several new target antigens shared among SSc patients or specific to a given phenotype. These autoantibodies could represent new diagnostic and/or prognostic markers for SSc.
Disclosure of interest: None declared.

P-304
AUTOIMMUNITY AND PULMONARY ARTERIAL HYPERTENSION: THE ROLE OF LEPTIN

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Pulmonary arterial hypertension (PAH) is defined as an increased mean pulmonary arterial pressure with normal pulmonary artery wedge pressure. It is widely accepted that immune and inflammatory mechanisms could play a significant role in PAH genesis or progression, but the pathophysiology is still unclear. Recent evidence has demonstrated a detrimental involvement of leptin in promoting the pathogenesis of various autoimmune diseases by controlling the regulatory T cells (Treg) onset. Despite this knowledge, the role of leptin in PAH is unknown. Here, we considered whether leptin promotes the immunopathogenesis of PAH by regulating Treg. To test this hypothesis, we withdrew blood samples from patients with PAH with normal body mass index (BMI) (idiopathic (I), heritable (H) and connective tissue disease-related (CTD)) and healthy control subjects. To measure serum leptin levels, we performed an ELISA test on serum samples. To detect circulating Treg and those expressing leptin receptor (ObR), we isolated the peripheral blood mononuclear cells, stained the latter with surface fluorescently tagged antibodies and selected Treg expressing ObR as $CD4^+CD25^+CD127^{low}ObR^+$ cells by flow cytometry (FACS). Leptin was higher in PAH patients compared to controls. Furthermore, leptin was higher in IPAH and CTD-PAH patients compared to HPAH patients, whereas it did not differ between IPAH and CTD-PAH patients. FACS analysis revealed that Treg number was comparable in PAH patients and controls. However, the percentage of Treg ObR^+ was higher in PAH patients compared to controls and interestingly, it was even higher in IPAH and CTD-PAH compared to HPAH patients. IPAH and CTD-PAH patients were comparable. No correlation was found with BMI. Our findings show for the first time that leptin and its receptor are increased in PAH patients. Interestingly, IPAH and CTD-PAH are comparable. Therefore, leptin and its receptor could play an important role in the immunopathogenesis of PAH.
Disclosure of interest: None declared.

P-305
USEFULNESS OF ANTI-CITRULLINATED PROTEIN ANTIBODIES DETERMINATION IN SYNOVIAL FLUID OF PATIENTS WITH RHEUMATOID ARTHRITIS AND NON-RHEUMATOID ARTHRITIS

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Objective: Anti-citrullinated protein antibodies (ACPA) seem to be produced locally at the site of joints inflammation in the first stage of rheumatoid arthritis (RA). A strong correlation between serum ACPA (S-ACPA) and ACPA in the synovial fluid (SF-ACPA) is now suggested. A case–control study was conducted to evaluate the usefulness of ACPA determination in SF of patients with RA.
Methods: A total of 52 patients with a knee-joint effusion (25 RA, 18 peripheral spondyloarthropathies (SPA) and 9 osteoarthritis (OA)) were included in our study. SF samples were obtained by performing therapeutic arthrosynthesis. IgG S-ACPA and SF-ACPA levels were determined by the enzyme-linked immunosorbent assay (ELISA). We have also determinate IgG levels in serum and SF by nephelometry.
Results: Higher levels of IgG ACPA in SF ($p = 0.045$) and serum ($p = 0.045$) were found in patients with RA respect to SPA and OA patients. There was a significant and positive correlation between ACPA in serum and SF ($rho = 0.516$; $p = 0.007$) not only in the RA group but also in patients with SPA. Serum ACPA discriminated RA from non-RA at cut off value of 2.7 U/ml (sensitivity 69%; specificity 78%; area under the curve (AUC) 0.72) whereas SF-ACPA discriminated RA from non-RA at a higher cut off value of 4.95 U/ml (sensitivity 73%; specificity 61%; AUC 0.71).
Conclusion: Our study suggests that the determination of IgG SF-ACPA didn't give complement information to serum ACPA in patients with RA.
Disclosure of interest: None declared.

P-306
STUDY OF ANTI-PERIPLAKIN AUTO-ANTIBODY ISOTYPES DURING THE COURSE OF IDIOPATHIC PULMONARY FIBROSIS

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Some studies have suggested the role of autoimmunity against alveolar epithelium in the pathophysiology of the idiopathic pulmonary fibrosis (IPF). We recently evidenced the presence of a new circulating autoAb directed against periplakin (PPL) in both serum and BAL fluid of 16/40 (40%) IPF patients. The detection of IgG anti-PPL Abs was associated with a more severe disease (Am J Resp Crit Care Med, 2010). As, during auto-immune cutaneous bullous diseases, the various isotypes of autoAbs are associated with different clinical phenotypes, we wanted to evaluate the anti-PPL isotypes and their potential evolution during the course of the disease. Circulating IgA, IgG and IgG4 autoAbs directed against PPL were searched by immunoblot using human placental amnion extract as an epithelial antigen source in the 40 patients with IPF. Among the 16 patients with anti-PPL IgG, 6 of them had IgG4 autoAbs and 9 of them had also IgA. Moreover, we could follow the serum of 2 patients with IPF during the course of their disease. A semi quantitative evaluation could be performed. Mrs D was treated by steroids and immunosuppressive drugs for 4 years but finally died with a severe exacerbation; she had an anti-IgA profile throughout the disease course as anti-IgG4 were absent. Mr G. was followed for 2 years and became totally stable at the respiratory level under treatment; he had high levels of IgG4 and IgA anti-PPL at the beginning of the treatment, which almost completely disappeared at the last evaluation. After the evidence of this new antigen target during IPF, we now suggest that the isotypes could be also of importance. In particular, our preliminary data suggest the importance of follow up of the IgG and the IgA isotype during the course of the disease to distinguish the more aggressive forms of the disease. Further evaluations are under study to determine an eventual isotypic switch according to the response to treatment.

Disclosure of interest: None declared.

P-307
TOLERANCE INDUCTION BY CO-IMMUNIZATION WITH DNAHSP65 AND RECOMBINANT HSP65 DID NOT AVOID FULL EXPERIMENTAL ENCEPHALOMYELITIS EVOLUTION

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Experimental autoimmune encephalomyelitis (EAE) is a demyelinating inflammatory disorder of the central nervous system, which involves autoimmune response to myelin antigens. It has been demonstrated that simultaneous co-immunization with a DNA vaccine and its cognate coded protein antigen can induce antigen-specific regulatory T cells (Treg) that are able to control the destructive inflammatory reaction. Heat shock proteins (hsps), in special hsp65, are possible targets for Treg cells due to their enhanced expression in inflamed tissues and the evidence that hsp induce immunoregulatory T cell responses. In this investigation we evaluated the prophylactic potential of DNAhsp65 plus recombinant hsp65 co-immunization strategy on the development of EAE. Female C57BL/6 mice were immunized with 2 i.m. doses of DNAhsp65 + rhsp65. Animals were then submitted to EAE induction with a s.c. emulsion containing myelin oligodendrocyte glycoprotein (MOG) in Complete Freund's Adjuvant with BCG and 2 i.p. doses of *Bordetella pertussis* toxin, 0 and 48 h after MOG immunization. Co-immunization triggered a downmodulatory effect characterized by decreased IFN-γ and augmented IL-10 levels in cultures stimulated with rhsp65 compared to cultures from animals immunized only with DNAhsp65. However, this vaccinal strategy did not affect neither clinical score nor immune response to MOG. Mice previously co-immunized with DNAhsp65 + rhsp65 lost less weight than the other groups submitted to EAE induction, but clinical scores were very similar among all animals with EAE. Anti-MOG IgG1 and IgG2a serum levels were similar between all groups with EAE. Cytokine production (IFN-γ, TNF-α, IL-17, IL-10 and IL-5) from spleen cell cultures stimulated with MOG also did not reveal any immunomodulatory effect of this co-immunization strategy. We concluded that co-immunization with DNAhsp65 plus rhsp65 triggered a downmodulatory response but this was not able to avoid EAE development. Supported by FAPESP.

Disclosure of interest: None declared.

P-308
CIRCULATING ANTIBODIES INTERFERE WITH DIFFERENTIATION, AMPLIFICATION AND FUNCTION OF HUMAN TH17 CELLS: A THERAPEUTIC VIEW

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Th17 cells play a critical role in the pathogenesis of several autoimmune and inflammatory diseases. Circulating antibodies that mainly include IgG play an important role in immune homeostasis. A therapeutic preparation of polyclonal IgG known as intravenous immunoglobulin (IVIg) is increasingly used in the treatment of diverse autoimmune and inflammatory diseases, including those diseases where Th17 cells are also implicated. Therefore, we examined whether IVIg interferes with the development and function of human Th17 cells. To address this, Th17 cells were differentiated from naïve human CD4+ T cells in the presence of TGFβ and IL-21. Th17 cells were amplified by stimulating memory CD4+ T cells in the presence of IL-1β and IL-6. The effect of IVIg was examined on differentiation

and amplification of Th17 cells, expression of Th17 lineage-specific transcription factor RORC, secretion of Th17 effector cytokines and phosphorylation of STAT3, a transcription factor that plays an important role in Th17 development and function. We demonstrate that IVIg inhibits the differentiation and amplification of human Th17 cells, as well as the production of their effector cytokines IL-17A, IL-17F, IL-21 and CCL20. The inhibitory effects of IVIg on Th17 cells are F(ab′)2-dependent and involve interference with the expression of RORC and activation of STAT3. Also, IVIg significantly enhanced Foxp3+ regulatory T cells (Treg) among the memory CD4+ T cells. Thus, circulating antibodies can reciprocally regulate human Th17 cell and Treg to maintain immune homeostasis. These results reveal a novel mechanism of action of IVIg in achieving therapeutic effect in autoimmune and inflammatory diseases.
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P-309
THE PROLIFERATION OF PRIMARY HUMAN T CELLS INDUCES THE REDISTRIBUTION FATTY ACIDS IN GLYCEROPHOSPHOLIPID SPECIES

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T cell activation is an important event in autoimmune diseases such as rheumatoid arthritis. Transformed malignant cells are known to greatly alter their metabolism of fatty acids as a mechanism that allows rapid cell proliferation. In this study, fatty acid metabolism was evaluated in rapidly proliferating T cell receptor-stimulated primary human T-cells. The distribution of fatty acids in these cells changed significantly following cell stimulation and resembled that measured in the human Jurkat T cell line. The cellular content of saturated and monounsaturated fatty acids was significantly increased in proliferating cells, suggestive of an increased expression of fatty acid synthase and steroyl-CoA desaturase as is seen in cancer cells. Additionally, cellular arachidonate (20:4n-6) was redistributed in a pattern that was unlike any other fatty acid. This was characterized by an accumulation in 1-acyl-PI, 1-alkyl-PC and 1-alk-1-enyl-PE species and a loss from 1-acyl-PC species in stimulated cells. This arachidonate mass redistribution is accompanied by enhanced CoA-independent transacylase-mediated arachidonate-phospholipid remodelling. When the stimulus was removed from proliferating T cells, they stopped proliferating and the fatty acid distribution largely reverted back to that of resting cells. To begin to identify enzymes involved in this remodeling, we measured significant changes in the expression of phospholipases A_2 (IVA, IVB, IVC) and lysophospholipid acyltransferases (LPEAT2, MBOAT7) by qPCR. Overall, these results suggest that metabolic pathways regulating the cellular content and distribution of fatty acids may be potential therapeutic targets for disorders associated with T cell proliferation. (Supported by the Canadian Institutes of Health Research, the New Brunswick Health Research Foundation and the Canada Research Chairs Program).
Disclosure of interest: None declared.

P-310
DIFFERENTIAL JOINT DISEASE EXPRESSION IN PRISTANE-INDUCED ARTHRITIS—A SYSTEMATIC PROFILE OF DISEASE PROGRESSION IN DA/OLAHSD RATS

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Pristane-induced arthritis (PIA) is an MHC dependent polyarthritis with infiltrating activated CD4+ T cells, erosion of peripheral joints and greater susceptibility in females. Unlike collagen induced arthritis it has a chronic relapsing course. A dose-ranging study was carried out to determine the pristane dose that would induce relapsing arthritis without excessive adverse effects as defined by preset humane endpoints and aimed to track disease progression on all individual peripheral joints. Pristane (75, 100 or 150 μl) was administered under light isoflourane anaesthesia, i.d. to the tail base to DA/OlaHsd rats. Controls received no injection. Rats were scored with a 16 point system recording swollen or red digits, knuckles, midfoot and ankle/wrist between day 5–25 every 2 days and between day 25–100 twice every week. Paw swelling was measured with 0.02 mm graduation callipers. Adverse effects were monitored to Institutional ethical guidelines and approved by the UK Home Office. Paws were frozen or fixed with paraformaldehyde. For all pristane doses, inflammation followed an acute phase followed by a chronic relapsing phase. Front and hind paws responded with signs of disease by day 20 followed by a decline finishing by day 40. Ankle swelling persisted to day 70. Relapse occurred in front and hind digits with some wrist involvement, as opposed to ankles and midpaws which did not. Hind paw swelling only occurred in the acute phase, fully declining by day 40. In the chronic phase, front paws were often characterised by swollen knuckles and there was a high incidence of severe distortion of front digits. Pristane was best tolerated at 75 and 100 μl.
Conclusion: In PIA in DA/OlaHsd rats, chronic relapsing disease follows a course that differentiates between digits, wrists, ankles, and foot thickness. Doses of 75ul and 100ul elicited a less severe and more manageable disease.
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Disclosure of interest: None declared.

P-311
ENDOGENOUS GALECTIN-1 INHIBITS EXPERIMENTAL ARTHRITIS: A POTENTIAL SWEET THERAPEUTIC TARGET?

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Galectin-1 (Gal-1), an endogenous b-galactoside binding lectin, has emerged as a leading player in the modulation of a variety of immune

responses. Previous work has shown that the therapeutic administration of exogenous Gal-1 to mice with arthritis can in fact reduce disease severity through the induction of T cell apoptosis. In this current study we aimed to examine the functions of endogenous Gal-1, by performing the collage-induced arthritis model (CIA) in Gal-1 KO mice. Animals received an initial immunisation of chicken type II collagen (CII) in complete Freund's adjuvant (FA) followed by a booster on Day 21, which consisted of CII in incomplete FA. Animals were monitored daily for signs of arthritis from Day14 onwards. In terms of disease incidence, 92% of Gal-1 KO mice developed arthritis compared to 72% in the WT over the complete time course. The mean day of disease onset was also earlier in the Gal-1 KO (Day 25 $\pm$ 1.56) compared to WT (Day 29 $\pm$ 3.27) mice. A more severe arthritis was also apparent with the mean clinical score (7.55/12 $\pm$ 1.11) in Gal-1 KO mice compared to WT (5.38/12 $\pm$ 1.86). Histological analysis of joints taken from both phenotypes with similar clinical scores showed no differences in degree of synovitis and joint destruction. Counts of total lymph node cells revealed double the number of cells in Gal-1 KO compared to WT. Collectively these data indicate for the first time, that endogenous Gal-1 has tonic inhibitory roles in the development arthritis affecting, in part, disease severity.
Disclosure of interest: None declared.

P-312
PREVALENCE OF ANTINUCLEAR ANTIBODY IN HEALTHY POPULATION OF WESTERN IRAN

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Antinuclear antibodies (ANA) are frequently found in the sear of healthy populations and also in a wide verity of autoimmune diseases like systemic lupus erythematosus and juvenile rheumatoid arthritis. To define the prevalence of ANA in healthy population in west of Iran we studied 230 individuals, 98 blood donors and 132 hospital personnel, which included doctors, laboratory technicians, and nurses. 5 ml of blood samples were collected and sera separated by centrifugation. The titer of ANA were measured using specific ELISA kits. The ELISA kit used in this study was from genesis diagnostics which detects IgG antibodies against dsDNA, histones, SSA/Ro, SSB/La, Sm, Sm/RNP, Scl70, Jo-1, and centromeric antigens. Six of 213 healthy volunteers ware positive for IgG ANA (2.6%). Of these 6 positive cases, 2 were having high titer of ANA, and 4 samples were moderately high titer of ANA. All of the positive individual's age was above 30 that can be due to the effect of aging on autoantibody production in healthy population. Further investigations with larger number of samples can be helpful for precise determination of ANA prevalence in population of western Iran. Moreover, future study can be conducted for clarification of immunofluorescence pattern of positive samples.
Disclosure of interest: None declared.

P-313
THE ROLE OF MONOCYTES IN KIDNEY DAMAGE—CELLULAR MARKERS AND THERAPEUTIC TARGETS IN LUPUS NEPHRITIS?

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Numerous studies highlight that monocytes are key-mediators of tissue damage in nephritis, including lupus nephritis. Previous published work in our lab has shown that $CX_3CR1^{hi}Gr1^-$ monocytes in mice and $CD14^{dim}CD16^+$ monocytes in humans patrol blood vessels. These patrolling monocytes may scavenge dead cells and/or immune complexes. Furthermore, it has been shown that murine $Gr1^-$ monocytes selectively expand and accumulate inside renal capillaries during nephritis, and that they are activated by immune complexes in an FcγR-dependent way. Our lab recently published that $CD14^{dim}CD16^+$ monocytes detect nucleic acids and mediate kidney inflammation in lupus patients. Nevertheless, little is known about the events that govern monocyte recruitment and their activation in the kidney. By using intravital single- and two-photon microscopy and fluorescent-labelled high and low molecular weight dextran we have been able to identify and image in real time the glomerulus and associated structures in $CX_3CR1^{gfp/+}Rag_2^{-/-}\gamma_c^{-/-}$ mice under basal non-inflammatory conditions. Our hypothesis is that $Gr1^-$ monocytes patrol glomerular capillaries, detect immune complexes and are selectively recruited into the kidney, where they induce tissue damage. Using the novel imaging techniques developed in our lab we will be able to test our hypothesis and assess if the targeting of monocytes or a specific type of monocytes would be beneficial in kidney inflammation.
Disclosure of interest: None declared.

P-314
PREVALENCE OF ANTI- CYCLIC CITRULLINATED PEPTIDE ANTIBODY IN PATIENTS WITH RHEUMATOID ARTHRITIS IN WEST OF IRAN

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Introduction: Rheumatoid arthritis (RA) is an autoimmune disorder that affects a considerable number of populations during reproductive ages. RA is characterized by symmetric and erosive synovitis causes progressive joint destruction and disability. Anti-cyclic citrullinated peptide (CCP) antibody is a more sensitive and specific test than Rheumatoid factor for diagnosis of patients with RA. Moreover, Anti-CCP antibody titer predicts the severity of erosive arthritis and disease outcome. The objective of this study was to provide data regarding the prevalence of Anti-CCP antibodies in Iranian RA patients.
Method: A total of 112 RA patients and 206 age and sex matched healthy controls from Kermanshah province (west of Iran) were enrolled in this study. All Patients fulfilled the American College of Rheumatology diagnostic criteria for RA and healthy controls did not suffer from any known rheumatic diseases. 5 ml of blood samples were collected and sera separated by centrifugation. Anti-CCP IgG antibody was measured using specific ELISA kits (Euroimmune). The data were analyzed using SPSS version 11.5 statistical software package.
Results and discussion: Six of the controls (2.91%) and Sixty-eight out of 112 patients (60.71%) were positive for Anti-CCP antibodies. Sensitivity and specificity were 60.71 and 97.09%, respectively in this study. The results of this study showed that Anti-CCP antibody is prevalent in RA patients in west of Iran at 60.71%. Moreover, its prevalence seems to be higher in healthy controls in comparison to

other countries populations. Further investigations with higher sample numbers may be helpful in determination of precise prevalence, sensitivity and specificity of Anti-CCP in both healthy control and RA patients in west of Iran.
Disclosure of interest: None declared.

P-315
GOOD CLINICAL RESPONSE TO MTX CORRELATES WITH BOTH TH17 NORMALIZATION AND TH1 IMPROVEMENT IN PERIPHERAL BLOOD FROM RHEUMATOID ARTHRITIS PATIENTS

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Th1 systemic deficiency exerts pro-inflammatory effects in rheumatoid arthritis (RA) pathogenesis. A role of IL-17 in Th1 defects in RA patients has been recently suggested. We investigated pro-inflammatory and anti-inflammatory T helper phenotypes including Th17 and Th1 in order to clinical response to methotrexate (MTX) therapy.
Methods: Eighteen patients with active RA and 15 healthy controls were enrolled in the study. The frequencies of Th17 and Th1 cells in peripheral blood (PB) from individuals studied were determined by flow cytometry. IFN-gamma as well as IL-17 intracellular contents were measured by mean fluorescence intensity (MFI) estimation. Disease activity score (DAS28) and C-reactive protein (CRP) levels were also determined before and after at least 4 months of therapy with MTX in a stable dose 15 mg/week.
Results: Before therapy, we showed that clinical and biochemical signs of disease activity were present in all patients. Th17 population and intracellular contents of IL-17 were markedly heightened in RA patients compared to controls. In addition, imbalance in Th17/Th1 ratio with predominance of pro-inflammatory Th17 cells was found in RA. After MTX treatment, we observed a significant decrease in DAS28 and CRP levels in 17 out of 18 patients. Clinical improvement was accompanied by normalization in Th17 population as measured by both Th17 cell frequencies and IL-17 intracellular levels. In contrast, a significant increase in spontaneous IFN-gamma production in CD4+ T cells was found in RA patients treated with MTX.
Conclusions: We show that expansion of PB Th17 cells parallels the inflammation in active RA. Normalization of Th17 population after successful treatment with MTX contributes to Th1 systemic improvement, what is in agreement with IFN-gamma anti-inflammatory effects in RA. The balance between these Th cell subsets would attenuate the inflammation levels and could thus be related to the remission period of RA.
Disclosure of interest: None declared.

P-316
IN SEARCH FOR NEW, INNOVATIVE TOOLS HELPING TO DISTINGUISH EARLY RHEUMATOID ARTHRITIS (RA) FROM NON-RA PATIENTS

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Early identification of patients with undifferentiated arthritis (UA) destined to develop RA among all UA cases, enabling appropriate treatment at early stages of the disease, still remains a challenge. We attempted to find out if the analysis of lymphocyte proliferation dynamics could be a good diagnostic approach distinguishing prospective RA from non-RA patients prior to final diagnosis. Forty early UA patients were included based on routine clinical criteria and measurement of standard diagnostic markers: anti-CCP (anti-cyclic citrullinated peptide), RF (rheumatoid factor), ANA-Hep2 (antinuclear antibodies), ESR. The inclusion criteria were considered to define the population of patients with UA, for whom the early classification criteria is the main target especially in order to define patients with early rheumatoid arthritis. Peripheral blood mononuclear cells were isolated from blood samples taken at UA diagnosis, stained with CFSE, mitogen-stimulated and analyzed by FACS. $CD4^+CD28^+$ cell cycle and G0–G1 time, % of dividing cells and number of divisions per cell were calculated. Final assignment of UA patients into RA and non-RA rheumatic diseases' groups based on EULAR criteria, was done during 2-year follow-up and confronted with laboratory and clinical findings at initial UA diagnosis in search of parameters with predictive capacities. We found strong, significant differences in all analyzed proliferation features between RA and non-RA groups. By thorough regression analysis we found that proliferation parameters were more relevant than disease activity and serological markers for distinction of RA from non-RA, with both high specificity and sensitivity. Our results highlights a new innovative diagnostics tool for distinguish early RA among early UA, at the beginning of diagnosis procedure, early in the disease course, what allow to gain EULAR/ACR recommendations.
Disclosure of interest: None declared.

P-317
TREGITOPE EXPANDS REGULATORY T CELLS AND AMELIORATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Purpose: Multiple sclerosis (MS) is a T-cell mediated, autoimmune disease targeting central nervous system myelin. Auto-reactive T cells reacting to myelin antigens presented by antigen presenting cells drive the immune response. Expansion of $CD4^+FoxP3^+$ T cells (Tregs) is being considered as a means to restore tolerance.
Methods: Using myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), the animal model of MS, we investigated the effects of the recently discovered regulatory T cell epitopes (Tregitope). Tregitopes are promiscuous MHC Class II T-cell epitopes naturally located in the Fc and framework regions of Fab from IgG. Treated mice received 6 consecutive injections of human Tregitope 167 + 289 (200 μg/mouse) i.p. in PBS between days 0–10 post-immunization. Control mice received 6 × 200 μg doses of either ovalbumin (Ova, negative control) or 6 × 16 mg intravenous immunoglobulin (positive control).
Results: Using MOG-TCR transgenic XFoxP3-GFP mice, we found that, in agreement with the tolerogenic effects of IVIG,

Tregitope 167 + 289 administration expanded Tregsin vitro and in vivo and ameliorated EAE compared to Ova-treated controls. Tregitope decreased the frequency of IFNg-producing T cells and induced IL-9+ T cells in the periphery. We also found upregulation of Jagged2 on CD11b+CD11c− macrophages. Jagged 2 is a Notch ligand that has been shown to induce murine Treg cell expansion. To understand the significance of the enhanced Jagged2 expression, we treated EAE mice with anti-Jagged2 monoclonal signaling antibody. We found that anti-Jagged2 treatment expanded Tregs and suppressed IFNg-positive T cells suggesting that Tregitopes effects are at least partially mediated by Jagged2.
Conclusion: Our data provide support for the tolerogenic effects of Tregitope in autoimmunity. Further investigations into the mechanisms of Tregitope functions will open new therapeutic opportunities for autoimmune diseases and transplantation.
Disclosure of interest: None declared.

Cancer and inflammation

P-318
NEUROTENSIN (NT) STIMULATES VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) SECRETION FROM HMC-1 HUMAN MAST CELLS

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Mast cells play a key role in allergy and inflammation. When stimulated they secrete a host of mediators including histamine, arachidonic acid metabolites, cytokines, proteases, and growth factors such as the angiogenic factor, VEGF. Studies show mast cells accumulate in and around many human tumors including colorectal, breast, lung, and gastric. Angiogenesis is required for tumor growth and there is a positive relationship between the number of mast cells around tumors, microvasculature density, tumor growth and decreased patient survival. These results suggest that mast cells, via the release of angiogenic factors like VEGF, support tumor-required angiogenesis. The peptide NT is secreted by a number of human cancer cells and is a potent stimulus of mast cell secretion in vivo and in vitro. Rat and human mast cells express NT-type 1 G-protein-coupled receptors (NTS-1) and NT's stimulation of mast cells, in vitro and in vivo, is blocked by pretreatment with the NTS-1 antagonist, SR48692. The accumulation of mast cells around tumors, the secretion of VEGF by mast cells and the secretion of NT by human tumors, prompted us to ask if NT would stimulate VEGF secretion from human mast cells. HMC-1 mast cells withdrawn from serum were incubated at 37 C for up to 24 h with various concentrations of NT or with media alone. The media was then assayed for VEGF via ELISA. NT stimulated a significant, concentration-dependent increase in VEGF secretion that was blocked by pretreatment of the cells with SR48692 before stimulation by NT. VEGF secretion was also significantly reduced by pretreatment of the cells with inhibitors of protein kinase C (bisindolmelamide), P-I-3 kinase (wortmannin), MAPK (U0125), and p38 MAPK (SB202190). Our results show that NT stimulates VEGF secretion from HMC-1 mast cells by an NTS-1, PKC, PI-3-kinase, p38 MAPK pathway and suggest that tumor-derived NT may promote angiogenesis by stimulating VEGF secretion from tumor-associated mast cells.
Disclosure of interest: None declared.

P-319
HIGH MRNA EXPRESSIONS OF CCR7 IMPROVE POST OPERATIVE PROGNOSIS OF PULMONARY ADENOCARCINOMA CORRELATION WITH CRKL-C-ABL MRNA EXPRESSION

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Chemokines and chemokine receptors not only act as a potent chemoattractant for inflammatory cells, but also have the powerful ability in cancer metastasis and tumorigenesis. CrkL and c-ABL interaction is reported to transform into many malignant cells. We examined and evaluated the effect of CCR7 and CrkL, c-ABL on pulmonary adenocarcinoma specimens and the relationship of their mRNA expression in pulmonary adenocarcinoma. 120 patients with pulmonary adenocarcinoma were included in this retrospective analysis. The expression of chemokine receptors CCR7, ligands CCL21, CCL19, CrkL and c-ABL mRNA expression in pulmonary adenocarcinoma were examined and evaluated the relation to prognosis. High mRNA expression of CCR7 in lung cancer patients indicated significantly good prognosis than the groups of low expressions. The expression of CrkL and c-ABL mRNA had relationships with CCR7 mRNA expression in pulmonary adenocarcinoma. The expression of CCR7, CrkL and c-ABL were confirmed with immunohistochemical analysis of pulmonary adenocarcinoma specimens. In conclusion, we propose CCR7 as clinical good prognostic factors and the strong relation between chemokine receptor CCR7 and CrkL, C-ABL kinase mRNA expression in pulmonary adenocarcinoma.
Disclosure of interest: None declared.

P-320
ANTI-INFLAMMATORY EFFECTS OF SOLANUM NIGRUM LINN EXTRACTS IN MURINE MACROPHAGE RAW 264.7 CELLS

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Solanum nigrum Linn (SNL), also known as black nightshade, is commonly used in traditional Chinese medicine for curing inflammation-related diseases and various kinds of cancers. However, the mechanism of anti-inflammatory action of SNL is still unclear, thus in this context, the objective of our study is to reveal the mechanism of anti-inflammatory activity of SNL, which would provide the basis of its medical usage. The main aim of this study is trying to evaluate the anti-oxidant and anti-inflammatory effects about *Solanum nigrum* Linn extracts (SNE). Here, we report that SNE significantly inhibited the productions of NO and ROS on the lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. In addition, SNE suppressed the protein expressions of iNOS and COX-2 and the mRNA expression of iNOS in LPS-activated RAW 264.7 cells. The molecular anti-inflammatory mechanism of SNE might mediated by suppressing the phosphorylation of MAPKs such as ERK-1/2, JNK-1 and p38-α MAPK, and the translocation of NF-κB p65 subunit into nucleus in RAW 264.7 cells. These results indicated that SNE inhibited LPS-induced inflammatory response by blocking NF-κB and MAPKs

signaling pathways in macrophages, and demonstrated that SNE possessed anti-inflammatory properties in vitro.
Disclosure of interest: None declared.

P-321
ENZYMATIC ACTIVITY AND IMMUNE RESPONSE TO PANCREATIC CANCER

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Background: Pancreatic cancer remains the most aggressive malignancy of all human cancers. Patients have an extremely poor prognosis, with 5-year survival less than 5% and a median survival of 6 months. These dismal statistics are due to a combination of low rate of resectability at presentation and inherently aggressive tumor behavior. Pancreatic cancer is characterized by infiltrating tubular units embedded in desmoplastic stroma which is rich in immune cells, endothelial cells and fibroblasts. The question arises whether cancer cell and infiltrating macrophage enzymes may be responsible for cancer cells detachment and metastasis formation.
Aim: To evaluate enzymatic activity in pancreatic cancer tissue.
Materials: Tumor tissue samples were obtained from 25 patients who underwent macroscopically curative resection. Patients had not received any preoperative radio- or chemotherapy. Tissue specimens were analyzed with immunohistochemistry using antibodies against CD3, CD68, elastase. Gelatin zymography and zymography in situ was used for evaluation of MMP2 and MMP9 activity.
Results: Immunostaining showed CD68 elastine positive macrophages infiltrates around cancer nests and also in surrounding tissue. We also detected dense infiltrates of T lymphocyte in tumor stroma. Zymography revealed that both active metalloproteinases were present in pancreatic cancer tumor tissue.
Conclusion: The obtained results indicate that immune cell enzymatic activity have impact on tumor progression. Tissue metalloproteinases 2 and 9 may influence tumor expansion.
Disclosure of interest: None declared.

P-322
PLATELET-ACTIVATING FACTOR ENHANCES TUMOR METASTASIS VIA PROTEIN KINASE CK2-DEPENDENT NF-KB ACTIVATION

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Inflammation has been increasingly recognized as an important component of tumorigenesis. We have demonstrated previously that platelet-activating factor (PAF), a potent inflammatory mediator, has the ability to enhance tumor growth and metastasis via the activation of the transcription factor, NF-κB. The protein kinase. CK2 is a ubiquitous serine/threonine kinase. CK2 has been shown to induce NF-κB activation via direct degradation of IκBα. In this study, we investigated the role of CK2 in PAF-mediated NF-κB activation as well as enhanced tumor metastasis. PAF increased CK2 activity in vivo. CK2 inhibitor prevented PAF-induced NF-κB activation and IκBα, β degradation in vivo and in vitro. CK2 inhibitors, DMAT and TBCA, inhibited PAF-induced not only mRNA expression of proinflammatory cytokines (TNFα and VEGF), antiapoptic factors (Bcl-2, Bcl-xL) but also experimental pulmonary tumor metastasis. CK2 inhibitors also blocked PAF-mediated decreases in the etoposide-induced caspase-3 activity as well as PARP cleavage. Inhibitory activities of NF-κB inhibitor, NAC to the PAF-induced endpoints were comparable to those of CK2 inhibitors. These data suggests that PAF enhances tumor metastasis via CK2-dependent NF-κB activation.
Disclosure of interest: None declared.

P-323
INFLAMMATION IS A MANIFESTATION OF CO2-INDUCED INCREASE OF THE POSTCAPILLARY VENULES' PRESSURE

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We measured the pressure–temperature relationship of CO2 dissolved in saline for different concentrations, plotting the first against the second. We found that in each diagram there are several inflex points and that the first derivatives of all of them have one maximum always near the 37°C, independently of CO_2 content. Thus, we discovered a physical constant, explaining the human body temperature. On that base we created the following theory: (1) The capillary blood flow is promoted using the Stirling-Malone engine cycle; (2) The working medium is the containing dissolved CO_2 blood plasma, expanding by pressure fall, temperature elevation or by factors reducing the CO_2 solubility in it or producing additional CO_2, like acids; (c) The capillary glycocalyx chains (banded forward by the RBC passage) and the parachute formed RBC act as ratchet and pawl, determining their one-way motion—a capillary pump; (3) The plasma expansion is on the account of the nanobubbles at the liquid/solid site of the glycocalyx chains, being maximal at 37°C. All inflammatory agents have great solubility in blood (histamine) or react with its buffers or both, as formic acid, displacing the contained CO2 or producing new amounts of it. This raises the pressure in venules by capillary pumping and direct venules' CO2-induced pressure. We use this theory to treat inflammation and cancer, using, as well the discovered by us: (1) Extremely strong penetrative feature of formic acid molecules via biological membranes; (2) Its strong blood coagulating feature; (3) Its strong bactericidal and virucidal feature. The said, combined with the greater permeability of the inflamed tissue vessels and the specific permeability of the cancer ones, kills instantly selectively only the diseased tissue by disrupting the postcapillary venules, due to their greater diameter, when supplying to the nearest accessible place, even only 10% formic acid solution. Microbial killing conserves dead tissue until removed.
Disclosure of interest: V. Panchev Other: patent, A. Pancheva Other: patent, M. Pancheva Other: patent.

P-324
JACALIN HAS A PROTUMORIGENIC ROLE IN A MOUSE MODEL OF CHEMICALLY-INDUCED COLON CARCINOGENESIS

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Tumors are composed, besides the tumoral cells, of a population of immune cells that infiltrate to the developing tumor site and establish the tumor inflammatory microenvironment. Reciprocal interactions between neoplastic cells and their surrounding microenvironment determine the tumor progression. Inflammation can foster or inhibit cancer growth, depending on the composition of infiltrating cell populations and the stage of tumor development. Colorectal cancer represents a paradigm for these connections. Increased expression of the TF antigen is a common feature in malignant epithelia. Jacalin, a lectin from *A. integrifolia*, recognizes TF antigens and has antiproliferative effects on human colon cancer cells. In this study, we examined the effects of jacalin on the initial stages of mouse colon carcinogenesis induced by Methyl-N′-Nitro-N-Nitroso-Guanidine (MNNG). Balb/c mice were given 4 intrarectal deposits of 0.2 ml solution of MNNG (5 mg/ml) twice a week for 2 weeks. Starting 2 weeks before carcinogen administration, animals were treated orally (0.1 ml/mouse) with jacalin (0.005 and 0.05 mg/ml) twice a week for 10 weeks. At sacrifice, the colons were removed, fixed and processed for immunohistochemical analysis. When compared to animals submitted to carcinogenic action, mice additionally treated with jacalin showed results similar to those of control animals. Upon lectin treatment, we observed a decrease in cell proliferation in the colonic crypts, in the rate of stromal cell apoptosis, in the stromal expression of COX-2 and in the number of macrophages, CD4 and CD8 T lymphocytes associated with preneoplastic lesions. Intriguingly, the number of preneoplastic lesions increased when the lectin was administered to mice submitted to the carcinogenic action. These observations indicate that jacalin exerts protumorigenic effects on the colon and might reflect the manner in which it affects elements of the inflammatory tumor microenvironment. Financial Support: FAPESP. Disclosure of interest: None declared.

P-325
SPECIFIC TARGETING OF ADHERENT AND CHEMORESISTANT FEMALE LEUKEMIC PROGENITORS THROUGH GLYCOGEN SYNTHASE KINASE 3B INHIBITION BY A FLAVONOID WITH ANTI-INFLAMMATORY PROPERTIES

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Therapeutic resistance of acute myeloid leukemia stem cells, enriched in the $CD34^+38^-123^+$ progenitor population, is supported by extrinsic factors such as the bone marrow niche. Here, we report that when adherent onto fibronectin or osteoblast niche components, $CD34^+38^-123^+$ progenitors survive through an integrin-dependent activation of GSK3β by serine 9-dephosphorylation. Strikingly, GSK3β-mediated survival was restricted to female leukemic progenitors. The scaffolding protein RACK1, activated downstream of the $\alpha_5\beta_1$ integrin and the proinflammatory TNF receptor, was specifically upregulated in female but not male leukemic progenitors, and controlled GSK3β activation through the phosphatase PP2A. In a mirrored fashion, male but not female normal adherent progenitors $(CD34^+38^-)'$ survival was partially dependent upon this pathway. Thus, the GSK3β-dependent survival pathway might be gender-specific in normal immature population and flip-flopped upon leukemogenesis. In search for compounds able to reduce cell adhesion-mediated drug resistance of leukemic progenitors, we previously found that the anti-inflammatory flavonoid rutin, but not the structurally-related compound quercetin, induces apoptosis specifically in adherent leukemic cells. Importantly, we show that rutin inhibited survival of adherent female leukemic progenitors ($CD34^+38^-123^+$) but spared normal female progenitors ($CD34^+38^-$). The pro-apoptotic effects of rutin were correlated with a decrease of active GSK3β and inhibitors of GSK3β reproduced rutin-induced cytotoxicity. GSK3β inhibition by rutin restored sensitivity to etoposide, and impaired the clonogenic capacities of adherent female leukemic progenitors. Together, our results strengthen GSK3β as a promising target for leukemic stem cell therapy and reveal gender of patients as a new parameter in anti-leukemia therapy.
Disclosure of interest: None declared.

P-326
NEMO-BINDING DOMAIN PEPTIDE INDUCES APOPTOSIS OF HUMAN MELANOMA CELLS THROUGH INHIBITION OF CONSTITUTIVE NF-KB ACTIVATION

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Melanoma is the most aggressive form of skin cancer and its incidence has increased in the last decade. One important target identified in melanoma tumor progression is the Nuclear Factor-κB (NF-κB) pathway. In vitro studies have shown that IKK is constitutively active in human melanoma cells as compared to normal melanocytes, leading to NF-κB activation which in turn regulates the expression of anti-apoptotic proteins. These events are implicated as major molecular mechanisms for melanocytes transformation [1]. It has been found that a short cell-permeable peptide spanning the IKKβ NEMO binding domain (NBD), disrupted the association of NEMO with IKKs in vitro and blocked TNFα-induced NF-κB activation in vivo [2]. In the present study we investigated the effect of the NBD peptide on survival of several human melanoma cell lines (A375, WM115, SK-Mel-5) as well as on the IKK/NF-κB signalling pathway. We report that NBD peptide inhibits the proliferation of all human melanoma cell lines used. Inhibition of cell growth was associated with direct inhibition of (1) constitutive IKK activity; (2)

NF-κB DNA-binding activation; (3) induction of apoptosis in all cell lines tested. Using the A375 melanoma cell line, we show that inhibition of IKK/NF-κB signalling pathway by NBD peptide leads to down-regulation of the expression of several NF-κB-dependent anti-apoptotic gene products and to the activation of caspase-3. Our studies suggest that a selective inhibition of IKK/NF-κB activation can be an effective strategy to be developed in the fight against cancer.

1. Ueda Y, Richmond A. Pigment Cell Res. 2006;19: 112–124.
2. May M, Gao X, Cao Z, Rothe M, Goeddel DV. Science. 2000;289:1550–1554

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P-327
13-HODE DERIVED FROM LIPOXYGENASE PATHWAY IS INVOLVED IN INTESTINAL EPITHELIAL CELL GROWTH

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Arachidonic acid (AA) derived metabolites produced by lipoxygenase (LOX) pathways are synthesized by intestinal epithelial cell (Caco-2), and are involved in cell growth [1]. 15-LOX also converts the abundant linoleic acid to 13-hydroxyeicosatetraenoic acid (13-HODE), a metabolite produced by Caco-2 cells cultured in 10% fetal bovine serum (8.22 ± 1.73 nM). The biological effects of 13-HODE on cell growth/cancer development are controversial. Thus, Hsi et al. [2] described a mitogenic effect in combination with EGF in human colorectal cells whereas Nixon et al. [3] reported an anti-tumorigenic effect by 13-HODE. Considering these findings we aimed to study the role of 13-HODE on Caco-2 cell growth. Our results show that exogenous addition of 13-HODE (10–1,000 nM) induced a concentration dependent cell proliferation and DNA synthesis in Caco-2 cultures. 13-HODE has two enantiomeric forms. Interestingly, 13 (R)-HODE presents higher mitogenic effects than 13 (S)-HODE, and these effects were blocked by ketoprofen, a cyclooxygenase inhibitor, suggesting that prostaglandins are involved in the effects of 13-HODE on Caco-2 cell growth.

1. Cabral M, Martín-Venegas R, Moreno JJ. Proc Nutr Soc. 2010;69:E309
2. Hsi LC, Wilson L, Nixon J, Eling TE. J Biol Chem. 2001; 272:19269–19276
3. Nixon JB, Kim KS, Lamb PW, Bottone FG, Eling TE. Prostaglandins Leuk Essential Fatty Acids. 2003;70:7–15

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P-328
IDENTIFICATION OF GENETIC SUSCEPTIBILITY FACTORS IN SUBGROUPS OF BLADDER CANCER CHARACTERIZED BY DIFFERENTIAL EXPRESSION OF CYCLOOXYGENASE-2 (COX-2)

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Background: Accumulating evidence has linked chronic inflammation to carcinogenesis in a variety of tissues including the urothelium. COX-2 mediates the inflammatory state via the production of prostaglandins and exhibits elevated expression in urothelial cell carcinomas (UCC) of the bladder. We aimed at determining the association between SNPs in inflammatory pathway genes and bladder cancer risk according to COX2 expression levels in UCC.
Methods: COX-2 expression was determined in 608 bladder cancer cases from the Spanish Bladder Cancer/EPICURO study using immunohistochemical analysis in tissue microarrays. Information on recurrent cystitis was obtained for 334 low-COX2 and 274 high-COX2 expressing cases and 988 matched controls. 911 SNPs in 214 inflammatory genes were genotyped in the same population. Polytomous logistic regression models were applied to estimate the odds ratios (OR) and 95% confidence intervals (CI) of bladder cancer risk.
Results: A SNP in *TLR9* (1486T > C, rs187084) was associated with a significantly reduced risk of bladder cancer (OR = 0.63, 95% CI 0.52–0.78, P = 4.7e–06) in low-COX2 expressors. An interaction analysis considering 53 SNPs in 10 genes of the TLR pathway yielded an OR = 13.01 (95% CI 1.62–104.62, P = 0.016) in those individuals harboring variant alleles in *TLR9*-rs187084 and *MAPK14*-rs2237094. Interestingly, an interaction between *TLR9*-rs187084 and a history of recurrent cystitis was also found among high COX2 expressors (OR = 2.33, 95% CI 1.03–5.32, P = 0.043).
Conclusion: Bladder cancer subphenotypes defined by COX-2 expression present differential genetic susceptibility, highlighting the complex etiology of UCC.
Disclosure of interest: None declared.

P-329
MAST CELLS AND CD47 RECEPTOR INDUCE GROWTH AND PRO-INFLAMMATORY CYTOKINES SECRETION BY HUMAN ASTROCYTOMA CELL LINES

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Recent data have expanded the concept that inflammation is a critical component of tumour progression. Now it is clear that the tumour microenvironment is one of the main participants implied in the neoplastic process. A marked infiltration of mast cells has been observed in the stroma surrounding many tumors, but the precise effects of these cells are unknown. Our objective was to study 1-the effect of mast cells on astrocytomas growth cell lines by targeting histamine and inflammatory mediators 2- the influence of CD47 receptor activation by its agonist 4N1 on cytokines secretions. Rat peritoneal mast cells were co-cultured with human astrocytomas CCF-STTG1 and U87 cells. Proliferation was assessed by the colorimetric MTS test and [^{3}H]thymidine incorporation under conditions

allowing or not cell-to-cell contact between the different cell types. Cytokines secretion was measured using membrane array assays. Cytokines and CD47 receptor expression was assessed by RT-PCR. Mast cells increase the growth of U87 and CCF-STTG1 cell lines by about 50 and 40%; this effect required cell-to-cell contact between the 2 cell types. Several pro-inflammatory cytokines such as IL6, IL7, IL15, IFNγ and MCP-1 were highly secreted from CCF-STTG1 and U87 cells lines in the presence of mast cells. Histamine increases the proliferation of both U87 and CCF-STTG1 human astrocytomas cell lines. Moreover, IL6 expression was dose-dependently increased by histamine in these cells. The CD47 receptor is expressed by mast cells, U87 and CCF-STTG1 cell lines. His activation by the agonist 4N1 induces the secretion of histamine by mast cell and increases the level of pro-inflammatory cytokines RNAm expression by human astrocytomas. Our data indicate that mast cells and CD47 receptor activation induce the growth of human astrocytomas cells lines and increase the level of pro-inflammatory cytokines by these cells, thereby likely contributing to tumour aggressiveness by enhancing the proliferation.
Disclosure of interest: None declared.

P-330
IMMUNE MODULATION BY HUMAN PAPILLOMAVIRUS ASSOCIATED TUMORS: FROM THE INFLAMMATORY INFILTRATE TO SECONDARY LYMPHOID ORGANS

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Human Papillomavirus, HPV, is the main etiological cause of cervical cancer. Asymptomatic women infected with HPV generate CD4 Th1 cells against tumor antigens, while women with tumors show a specific CD4 regulatory response. Mechanisms that generate regulatory responses in some women are not known. Our laboratory has been using the TC-1 mouse tumor model to study mechanisms of immune suppression by tumors. We have seen that about 10% of the total TC-1 tumor population is composed by a M2-like macrophage population that expresses, among other cytokines, IL-10 and TGFb, both potential inducers of regulatory T cells. Aiming to understand the relationship between tumors and lymphoid organs, we transplanted purified naïve T cells into RAG1$^{-/-}$ mice and observed threefold more regulatory T cells being formed when recipients had TC-1 tumors. On the other hand, transplant of E6/E7 peptides loaded tumor macrophages or CD11b+ cells from spleen of tumor bearing mice into naïve recipients did not induce significant increase in regulatory T cell phenotype, instead, we observed that in vitro antigen stimulated T cells expressed Th1 cytokines, but not IL-2 and did not proliferate. Cytokine expression profile showed that tumor macrophages express a mix of pro and anti-inflammatory cytokines, while tumor cells express cytokines like GM-CSF. Indeed, mice with tumors have approximately 3 fold more myeloid cells in the spleen than naïve mice. In the spleen, we observed downregulation of CXCL10 and CXCL9, upregulation of CCL2, IL-1Ra, TREM-1 in mice with tumors in comparison to naïve mice. Cytokine concentration alteration in the spleen of tumor bearing mice may contribute to deficiency in T cell activation, increased numbers of myeloid cells, poor myeloid cell activation. Our data indicate that conditioning of the host's lymphoid organs is important for generation of regulatory responses towards tumor antigens.
Disclosure of interest: None declared.

P-331
THE DIFFERENT EFFECT OF THROMBOXANE (TX)A2 ON THE ANGIOGENESIS IN THE WOUND HEALING AND CANCER ANIMAL MODELS

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Objective: Thromboxane A_2 (TXA_2)is known to be stimulator concerning vasoconstriction and angiogenesis via thromboxane prostanoid receptor (TP) activation. In this study we evaluated the role of TXA_2 in different physiological states using wound healing and cancer models.
Materials and methods: Mouse TXA_2 synthase (mTXAS) gene was retrovirally introduced into C57 BL/6 J (C57) cells, then designated as C57-mTXAS. Only retrovirus vector introduced C57 cells were designated as C57-EV and used for control. Mouse bladder carcinoma cells (MBT) were subcutaneously injected to C3H mice for bladder carcinoma model. One x 10^6 cells of C57-mTXAS or C57-EV were directly inoculated to the tumor as the size of 0.5 $\times$ 0.5 cm. Tumors were extracted after 14 days of cell inoculation for thrombus rate measurement and vascular endothelial growth factor (VEGF) in sera was also measured. The urethane sponges were implanted to the back of C57 mice for wound healing model. C57-mTXAS or C57-EV inoculated to the sponges after 4 days of the implantation and sponges were extracted then used for VEGF measurement and microvascular density (MVD). The TXAS synthase inhibitor (OKY-046) or TP antagonist (S-145) were also used.
Results and discussion: C57-mTXAS cell therapy successfully suppressed for the bladder tumor growth rate followed by induction of thrombonecrosis in vessel inside of tumors but not outside. Production of VEGF in sera was suppressed for 1–2 weeks after therapeutic cells inoculation. Secretion of VEGF in C57-mTXAS was significantly increased after arachidonic acid administration. Inoculation of C57-mTXAS into implanted sponges, significantly induced VEGF and MVD after 6 and 12 days, respectively. The induction of VEGF and MVD were abolished with administration with OKY-046 and S-145. These result suggested that TXA_2 may play different physiological role and its signal pathway such as stromal derived factor-1 (SDF-1) would be clarified in the future.
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P-332
INFLIXIMAB ATTENUATES THE INFLAMMATORY COMPONENT OF IRINOTECAN-INDUCED INTESTINAL MUCOSITIS WITHOUT AFFECTING DIARRHEA

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Intestinal mucositis (IM) is a limiting side effect of anticancer therapy with Irinotecan (IRI). Previous studies reported that TNFα seems to be a key mediator in many inflammatory responses, such as IM, rheumatoid arthritis and colitis. We have shown that non-selective inhibitors of cytokines attenuate IRI-induced IM. Besides, there is a lack of information about the effect of selective cytokine target therapy on anticancer drug toxicity. Then, we aimed to evaluate the role of a selective TNFα inhibitor, Infliximab, on IRI-induced IM. C57BL/6 mice ($n = 6$) were divided into groups: saline (5 mL/kg, i.p.); IRI (75 mg/kg/4 days, i.p); Infliximab (5 mg/kg, i.v) + IRI. Diarrhea was assessed daily. Animals were killed on day 5 and the duodenum was collected for myeloperoxidase (MPO, U/mg tissue), IL-1β dosage (pg/mL), western blot of the inducible nitric oxide synthase, in vitro duodenal contractility and white blood cell count (cells/mm^3). Data were analyzed with ANOVA/Student Newman Keul or Kruskal–Wallis/Dunn's test. $P < 0.05$ was accepted. Ethics Committee 99/10. IRI induced a significant ($p < 0.05$) diarrhea (1[0–2]), increased MPO activity (16.5 ± 1.7), IL-1β dosage (431.5 ± 158), iNOS expression (1.2 ± 0.3), intestinal contractility (144 ± 25.6), and leukopenia (7267 ± 1180) compared with saline (0[0-0], 6.3 ± 1.2, 1.8 ± 1.8, 0.03 ± 0.02, 55.7 ± 11.8, 11100 ± 1412, respectively). These effects were abrogated ($p < 0.05$) by Infliximab (MPO: 8.7 ± 1.9, IL-1β: 72.6 ± 23.9, and iNOS expression: 0.14 ± 0.1) compared with IRI. However, gut dysfunction (diarrhea: 1[0–2], intestinal contractility: 154 ± 47.3) were not affected by infliximab ($p > 0.05$). In addition, Infliximab potentiated ($p < 0.05$) IRI-induced leukopenia (4067 ± 518). Thus, we showed the prominent anti-inflammatory effect of the target therapy anti-TNFα on IRI-induced IM. However, it did not counteract intestinal dysfunction and even potentiated the leukopenia, which might limit its use together with cancer chemotherapy. Support: CNPq/FUNCAP/CAPES
Disclosure of interest: None declared.

P-333
DISSECTING THE ROLE OF NEUTROPHILS IN TUMOUR GROWTH AND METASTASIS WITH THE AID OF OPTICAL CELL TRACKING AND 2-PHOTON MICROSCOPY

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The immune system is critically involved in multiple stages of tumour growth and spread and the outcomes of this involvement can be both beneficial and harmful. Although neutrophils are abundant in many human and animal tumours, their role in cancer pathogenesis remains controversial. This is likely due to the fact that neutrophils can play a dual role in primary tumours: they can mediate tumour rejection but also promote angiogenesis and tissue remodelling which favour tumour growth. Furthermore, emerging evidence suggests that neutrophils may also have the ability to promote metastasis. Hence there is great potential for novel neutrophil-based therapies in the treatment of cancer if the contrasting roles of neutrophils in tumour growth are properly understood. We have designed a system which utilizes 2-photon imaging together with a photoswitchable transgenic mouse Kaede to track neutrophils in real time inside tumours deposits and also to follow their fate as they egress from the tumours. Using this method we show that neutrophils form dynamic swarms inside primary tumour deposits. This swarming behavior may lead to tissue remodeling, as was previously observed in infections, and promote tumour growth and metastasis. These results provide the first information about the dynamics of neutrophil behaviour inside solid tumours and their fate as they egress primary tumour deposits.
Disclosure of interest: None declared.

P-334
IL10 PROMOTOR POLYMORPHISM IN INFLAMMATORY BREAST CANCER

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Inflammatory breast cancer is characterized by clinical symptoms including inflammation is the hallmark. This type of cancer remains poorly understood from a biological point of view: both the process of carcinogenesis than inflammation. In Tunisia, the incidence of inflammatory breast cancer is 5.7% and non-inflammatory breast cancer is 25–27%. Interleukin-10 has immunosuppressive properties, anti-inflammatory and antiangiogenic. To test whether IL-10 promoter region polymorphisms are associated with susceptibility to inflammatory breast cancer, we examined the contribution of interleukin-10 (IL-10) gene polymorphisms in Tunisian patients with localized or metastatic breast cancer and patients with Inflammatory breast cancer IBC. In this study we looked SNP at position −627 (C > A) at the promoter regulatory region of IL-10 that has the ability to modulate the expression of IL10. The methodological approach to answer our question is the case–control study. We analyzed by RFLP, SNP-627 (C > A) in peripheral blood samples collected from two groups of patients 22 with Inflammatory cancer (IBC) and 37 with non-inflammatory breast cancer (non-IBC) belonging to different histological stages. Genotypic and allelic frequencies were compared between groups of patients and 55 healthy controls; and analyzed according to the presentation of the disease. In Tunisian population, comparison of allele frequencies showed a contribution of the A allele in the IL-10 promoter as a risk allele for the inflammatory form of breast cancer, the C allele in the IL-10 promoter was found protective against this type of cancer ($p = 0.015$). The expression level of IL-10 differs between individuals and this polymorphism in relation to the promoter region of the gene where several variations in the level of expression of the gene have been described. The low expression alleles may be risk factors for inflammatory breast cancer.
Disclosure of interest: None declared.

P-335
ANTITUMORIGENIC EFFECTS OF THE ATYPICAL CANNABINOID O-1602 IN A COLITIS-ASSOCIATED COLON CANCER MODEL

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Cannabinoids have anti-inflammatory and anti-tumorigenic properties, but their therapeutic use is hampered because of their psychotropic side effects. Atypical cannabinoids act via novel cannabinoid receptors and are devoid of psychoactivity. O-1602 is such an atypical cannabinoid and its anti-inflammatory potential has been recently demonstrated in an experimental form of colitis. We were interested whether this compound may effect tumor growth in a mouse model of colitis-induced colon cancer. Male CD1 mice received 10 mg/kg of the carcinogen azoxymethane followed by a 7-day exposure to 2% dextran sulfate sodium supplemented in their drinking water. Mice were kept for another 10 weeks during which tumors developed. Multiple tumors were found predominantly in the distal part of the colon. Treatment with O-1602 (3 mg/kg i.p. every second day over 3 weeks) significantly diminished tumor incidence by more than 30% (11 ± 1.5 after vehicle vs. 7 ± 1.2 after O-1602 treatment) and tumor area by more than 50% (67.3 ± 13.7 mm^2 after vehicle vs. 30.3 ± 16 mm^2 after O-1602 treatment; means $\pm$ sem; $n = 12$; *t* test). O-1602 is therefore able to reduce tumor burden in an inflammation-driven colon cancer model. Since atypical cannabinoids are not centrally active, they may become an attractive drug for the treatment of colonic and possibly other cancers. The study is supported by the Austrian Science Fund (grant P 22771-B18 to RS) and the F. Lanyar Foundation.
Disclosure of interest: None declared.

P-336
INFLAMMATORY MEDIATORS IN MURINE SQUAMOUS CELL CARCINOMA

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Squamous cell carcinoma (SCC) is one of the most common human cancers worldwide. The development of this tumor is accompanied by an immune response, which causes a massive infiltration ofi nflammatory cells into the tumor environment, leading to local and systemic production of cytokines, chemokines and other mediators. Studies in humans and animal models indicate that increased inflammatory enzymes, such as elastase, and an imbalance in the cytokines are related to the development of cancer. We used a multistage model of squamous cell carcinoma (SCC) to examine the involvement of elastase (ELA), myeloperoxidase (MPO), nitric oxide (NO), and cytokines (IL-10, IL-13, IL-17, TGF-β and TNF-α) during tumor development. We detected significantly higher ELA, but not MPO, activity in SCC lesions than control epithelial tissues. We also found increased NO, IL-17, TNF-α and TGF-β levels in SCC lesions compared with controls. Similar levels of IL-10 and IL-13 were detected in tumor microenvironment compared with control tissue. These results show an imbalance of inflammatory mediators' production in SCC development and might indicate an escape mechanism contributing to the persistence of SCC.
Disclosure of interest: None declared.

P-337
DUAL EFFECT OF SERUM AMYLOID A PROTEIN ON GLIOMAS

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The acute phase protein serum amyloid A (SAA) has been proposed as a marker of tumor progression and there are evidence sustaining its participation in processes that favor carcinogenesis and metastasis, in spite of this, the possibility of direct activities of SAA on tumor cells remains to be addressed. Here, we have investigated the effect of SAA on two human glioma cell lines, A172 and T98G. SAA stimulated the proliferation of both lines, but had different effects on migration and invasiveness behavior depending on cell line. SAA increases migration and invasion of T98G, whereas it decreased these in A172. Both lines expressed all the three isoforms of SAA and the production the protein was detected intracellularly. SAA affected the production of compounds present in the tumor microenvironment that can orchestrate tumor progression, such as cytokines, nitric oxide (NO) and matrix metalloproteinases (MMPs). The effect of SAA on IL-8 production was remarkable for A172; whereas these cells did not produce basal amounts of this cytokine, they were extremely responsive to SAA. On the other hand, T98G produced large amounts of IL-8 that was unaffected by SAA addition. SAA induced the production of (NO), increased the expression of (MMPs)-2 and -9, and down regulated the expression of the MMPs regulator gene RECK. These data based on in vitro assays are suggestive of the contribution of SAA in cancer progression depending on the cell type and concentration of SAA. In the case of gliomas, the most common brain cancer in adults, our data support previous findings indicating a role for the inflammatory process in tumor susceptibility and therapy.
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Disclosure of interest: None declared.

P-338
ELASTASE AND CYTOKINES IN ACTINIC CHEILITIS AND ORAL SQUAMOUS CELL CARCINOMA

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Inflammatory cells and mediators are components of the tumor microenvironment and inflammation has been proposed to represent a mechanism to cancer development. Actinic cheilitis (AC) is an oral pre-malignant lesion characterized by discrete chronic infiltrate and cellular abnormalities that might develop into oral squamous cell carcinoma (OSCC). The characterization of inflammatory mediators in the blood serum of pre-cancerous and tumor lesions has not been fully investigated. Besides, little is known about the role of elastase (ELA) and myeloperoxidase (MPO) activities, nitric oxide (NO) and IL-6, IL-10, IL-13, IL-17, TGF-β and GM-CSF in oral tumors or pre-malignant lesions. In the present study, we investigated the expression of these molecules in the blood of patients with actinic cheilitis and oral squamous cell carcinoma. MPO, NO, ELA, IL-6, IL-10, IL-13, IL-17, TGF-β and GM-CSF levels were increased in OSCC, while in the AC only ELA was elevated. Therefore, oral tumors strongly induced the production of inflammatory mediators related to neutrophils and macrophages functions. Further studies are necessary to establish the significance of these cells in human oral cancer. The understanding of the differences between a pre-malignant lesion and multiple mechanisms of OSCC-induced immune suppression may provide a range of novel targets for cancer prevention and therapies.
Disclosure of interest: None declared.

P-339
PROCOAGULANT AND PROTHROMBOTIC ACTIVATION OF HUMAN PLATELETS BY DOXORUBICIN

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The prothrombotic risk associated with chemotherapy including doxorubicin (DOX) has been frequently reported, yet the exact mechanism is not fully understood. Here, we report that DOX can induce procoagulant activity in platelets, an important contributor to thrombus formation. In human platelets, DOX increased phosphatidylserine (PS) exposure and PS-bearing microparticle generation. Consistently, DOX-treated platelets and generated microparticles induced thrombin generation, a typical feature of procoagulant activity. DOX-induced PS exposure was determined to be from intracellular Ca^{2+} increase and ATP depletion which resulted in the activation of scramblase and inhibition of flippase. Along with this, apoptosis was induced by DOX as determined by the dissipation of mitochondrial membrane potential ($\Delta\Psi$), cytochrome c release, Bax translocation and caspase-3 activation. Ca^{2+} chelator (EGTA), caspase inhibitor (QVD) and antioxidants (Vitamin C and Trolox) can attenuate DOX-induced PS exposure and procoagulant activity significantly, suggesting that ROS generation, Ca^{2+} and apoptosis were associated in DOX-enhanced procoagulant activity. Rat in vivo thrombosis models confirmed the prothrombotic effects of DOX through the mediation of platelet procoagulant activity, where DOX administration resulted in increased venous thrombus formation along with increased PS exposure and $\Delta\Psi$ dissipation, reflecting that DOX-enhanced procoagulant activity of platelets may lead to increased thrombus formation, indeed.
Disclosure of interest: None declared.

P-340
MODULATION OF MACROPHAGE TUMORICIDAL ACTIVITIES BY JACALIN

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Within the context of tumors, macrophages have been increasingly recognized as central regulators. These cells are able to dramatically affect the course of the disease and depending on their functional orientation, can present both pro- and anti-tumor activities. The aim of this study was to analyze the modulation of macrophage tumoricidal activity by the lectin jacalin. We show that in vitro, jacalin (2.5–40 μg/ml) induced the production of both pro- and anti-inflammatory mediators by human macrophages. Lower concentrations of the lectin (up to 5 μg/ml), when compared to the higher (from 10 to 40 μg/ml), induced the secretion of higher levels of the anti-inflammatory cytokines IL-10 and TGF-β. Similar amounts of the pro-inflammatory cytokines TNF-α and IL-6 were secreted by cells stimulated with jacalin at all of the concentrations tested. For IL-12, high concentrations of the lectin determined the maximal responses. As assessed by MTT assays, when supernatants from macrophages stimulated with higher, but not with lower concentrations of the lectin were added to cultures of human colon adenocarcinoma cells (HT-29), up to 25% reduction of cell viability was observed. These results indicate that jacalin, through its ability to exert a pro-inflammatory activity, can direct macrophages to an anti-tumor phenotype.
Financial support:FAPESP.
Disclosure of interest: None declared.

P-341
ANTI-INFLAMMATORY EFFECT OF LATEX PROTEINS (LP) ISOLATED FROM CALOTROPIS PROCERA IN 5-FLUOROURACIL-INDUCED ORAL MUCOSITIS IN HAMSTERS

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Mucositis induced by antineoplastic drugs is an important, dose-limiting, and costly side effect of cancer therapy. Generally, 100% of patients submitted to cancer chemotherapy develop oral mucositis (OM) and 30% of the patients with OM of grade 3 and 4 have to suspend the treatment. *Calotropis procera* (CP) is the laticiferous plant belonging to the Asclepiadaceae family. It is widely distributed in Asia, Africa and South America, and abundant in the Northeast of

Brazil. Relevant properties have been detected in protein fraction (LP) isolated from latex of CP. The anti-inflammatory activities of the proteic fraction of CP was studied in OM model, in male *Hamsters*. This work was approved by the local Ethical Committee (protocol 036/10). OM was induced, in hamsters, by 2 intraperitoneal (i.p) administrations of 5-FU on the 1st and 2nd days of the experiment (60 and 40 mg/kg, respectively). LP was i.p injected 24 h before and 24 h after mechanical trauma of the cheek pouches. Ten days after 1st 5-FU injection, tissues from the cheek pouches were harvested. The anti-inflammatory activity was evaluated by myeloperoxidase (MPO) activity, macroscopical, Histopathological and immunohistochemical analysis of cheek pouche tissue. LP significantly inhibited macroscopical and histopathological parameters when compared to non treated group with maximum effect reaching 75% (macroscopic scores; 5 mg/kg; $p < 0.001$) and 66% (histopathological analysis; 5 mg/kg; $p < 0.001$). The MPO activity was also significantly inhibited in 91% at the same dose ($p < 0.001$) and also inhibited immunostaining for TNF, iNOS and COX-2. LP from CP showed anti-inflammatory effect inhibiting the development of OM in hamsters, with only 2 injections. These preliminary data show an important inhibitory effect that should be explored in another pre-clinical assay in order to obtain sufficient results to elaborate a possible clinical trial. Financial support: CNPq and CAPES.
Disclosure of interest: None declared.

P-342
CANCER CHEMOPREVENTIVE POTENTIAL, POLYPHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF PROSOPIS CINERARIA

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Prosopis cineraria (L) Druce, commonly called as Khejri, widely distributed and used in Rajasthan, India, as folk medicine and food. Cancer chemopreventive potential of *Prosopis cineraria* leaf extract was investigated using two stages skin carcinogenesis model system. Animals were divided into five groups (control, throughout, pre, peri and post). A significant reduction in tumour incidence, tumour yield, and tumour burden, weight and size of tumour were observed in all treatment groups as compared to control. Average latent period was also increased significantly in all treatment groups. Significant increased in GSH, SOD, CAT and decrease in LPO was also observed in all treatment groups as compared to control. Total phenolic, flavonoid and flavonol contents were found significantly higher in leaf followed by flower, pod and stem bark. The leaf extract showed significant free radical scavenging activity followed by flower, pod and stem bark as evidenced by low IC_{50} for DPPH and high percentage inhibition of DPPH and ABTS. The results from the present study suggest significant chemopreventive potential, higher polyphenolic contents and antioxidant activity of *Prosopis cineraria*.
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P-343
EXPRESSION OF CHÉMOKINES CCL2, CCL5 AND THEIR RECEPTORS CCR2, CCR5 IN INFLAMMATORY BREAST CANCER

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Breast cancer is the major reason of female death, it could take different forms, the most fatal type is inflammatory that can grow and spread rapidly. In Tunisia, the incidence of inflammatory breast cancer (IBC) is 5.7% and non-inflammatory breast cancer (non-IBC) is 25–27%. Chemokines regulate many biological processes such as proliferation, apoptosis, angiogenesis, leukocyte migration; they are also heavily involved in inflammation and in the development of metastases. Our aim in this study was to evaluated and compared CCL2 and CCL5 and their receptors major CCR2 and CCR5 genes expressions in Tunisian patients, with localized or metastatic breast cancer and patients with IBC. We analyzed by RT-PCR the expression of two chemokines CCL2 and CCL5 genes and their receptors in biopsy samples collected from two groups of patients 19 with IBC and 34 with non-IBC. Results obtained allowed us to assess the correlation of expression of these chemokines to the histological classification and their prognostic implications especially in IBC. Our results show that an almost universal expression of CCL5 and CCR5 mRNA with 100% of IBC tumors express at least one of these markers, and 95% of these tumors express both markers at once, whereas this expression is less frequent in non-IBC. A statistically significant difference between the two groups of breast cancer for the expression of chemokine CCL5 (p value $= 0.037$) and its receptor CCR5 (p value $= 0.049$). These results suggest that expression of CCL5 and its receptor CCR5 might be a factor associated with IBC.
Disclosure of interest: None declared.

P-344
LATEX FROM CALOTROPIS PROCERA ATTENUATES DIARRHEA AND INFLAMMATORY EVENTS ON IRINOTECAN-INDUCED INTESTINAL MUCOSITIS

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Intestinal mucositis (IM) is a commonly side effect of irinotecan (CPT-11) based cancer chemotherapy. There is an incidence of IM

associated-diarrhea in up to 25% of patients. However, there is not a pattern clinical management of this side effect. *Calotropis procera* (CP), a plant found in Africa, Asia and South America, and abundant in the Northeast of Brazil shows anti-inflammatory activities in animal models. Thus, we aimed to evaluate the anti-inflammatory effects of a protein fraction from CP in CPT-11-induced IM. Swiss mice ($n = 10, 23 \pm 2$ g) were treated for 4 days with saline (Sal, 5 mL/kg, i.p.) or CPT-11 (75 mg/kg, i.p.). In other experimental groups, CP (1, 5 and 50 mg/kg/day, i.v.) was administered for 6 days, 30 min before the CPT-11. On the 7th day, we evaluated the total leukocyte count ($\times 10^3$/mL) and diarrhea (by scores). After sacrifice, the duodenum was collected for measurement of myeloperoxidase activity (MPO), morphometric analysis (villi/crypt), IL-1b level (pg/mL), and in vitro contractility (% contraction in relation to KCl 60 mM). ANOVA/Bonferroni or Kruskal–Wallis/Dunn was used as statistical tests. $P < 0.05$ was accepted. Ethics Committee 99/10. CP attenuates diarrhea scores and MPO activity at 5 mg/kg (diarrhea: 1[0–2]; MPO: 4.05 ± 1.07) and 50 mg/kg doses (diarrhea: 1[0–3]; MPO: 5.57 ± 1.50) when compared with CPT-11 (diarrhea: 3[2–3]; MPO: 24.45 ± 3.0, respectively). In addition, CP decreased over contractility (5 mg/kg: 165.1 ± 57.7), IL-1b level (5 mg/kg: 143.5 ± 41.5 and 50 mg/kg: 182 ± 65.7) and villi/crypt ratio (5 mg/kg: 2.79 ± 0.17) increased in comparison with only CPT-11 treated mice (contractility: 906.1 ± 225.4, IL-1b: 806.1 ± 247.6 and villi/crypt: 1.63 ± 0.17). However, CP did not change leukopenia induced by CPT-11 at doses tested. These findings show anti-inflammatory and anti-diarrhea effects of latex from *Calotropis procera* in CPT-11-induced IM. New approaches are being undertaken to elucidate the possible mechanism of action involved. Support: CNPq/CAPES.
Disclosure of interest: None declared.

P-345
AGE-SPECIFIC IMMUNOTHERAPY CAN PROMOTE PROTECTIVE ANTI-TUMOR IMMUNITY IN AGED MICE

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Cancers promote suppressive mechanisms hampering protective anti-tumor immunity. Reversing tumor-associated immune dysfunction is an attractive cancer immunotherapy strategy. Although most patients with cancer are elderly, most preclinical studies are performed in young animals.

We studied two immunotherapies for B16 melanoma in young (<6 months) versus aged (>20 months) C57BL/6 and FoxP3-IRES-RFP mice: depleting regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs), two immune regulators known to impede anti-tumor immunity. Treg numbers were comparable at baseline and following tumor challenge in young and aged mice. Denileukin diftitox (DT) reduced Treg numbers equivalently in young versus aged animals. However, Treg depletion reduced tumor growth and improved tumor-specific immunity only in young mice. Thus, either aged anti-tumor immunity is ineffective even with reduced Tregs, or other suppressive mechanisms exist. Aged mice had higher MDSC numbers both at baseline and upon tumor challenge. Depleting MDSCs with anti-Gr-1 antibody improved tumor-specific immunity and reduced tumor growth significantly better in aged versus young mice. Strikingly, only in aged mice, Treg depletion led to an unexpected and undesired increase in MDSC, suggesting that Treg depletion could fail in older patients from increased MDSCs. Tregs depletion also resulted in myeloid cells increase in naïve young and aged mice. Combining Treg depletion with MDSC depletion increased clinical response in aged tumor-bearing mice, consistent with the hypothesis that Tregs negatively regulate MDSC in aged but not young tumor-bearing mice. These data demonstrate that anti-tumor immunity can be effective in aged individuals provided that underlying immune dysfunction is correctly identified and reversed. Treg depletion efficacy could be enhanced in aged individuals by simultaneous MDSC depletion. Cancer immunotherapies must be tailored to account for age-dependent immune differences.
Disclosure of interest: None declared.

P-346
SPACE–TIME ORGANIZATION OF LYMPHOCYTES IN TERTIARY LYMPHOID STRUCTURES IN HUMAN LUNG CANCER PATIENTS

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The secondary lymphoid organs (SLO) are classically described as the place of initiation of adaptive immune responses. Aside from the classical SLO, there is a rapidly growing body of evidence that under inflammatory condition, the infiltrating immune cells are able to organize into structures called "tertiary lymphoid structures" presenting a high degree of similarity with the SLO. Accumulating evidence indicates that adaptive immunity can be initiated independently of SLOs. Here, in human lung cancer, we have characterized the immune cell infiltrate as well as their in situ organization. We have described that immune cells can be organized into tertiary lymphoid structures in some tumors. We called these structures "Tumor-induced Bronchus-Associated Lymphoid Tissues" (Ti-BALT) as they were not observed in non-tumoral lung. More importantly, we demonstrated that a high density of these structures was associated with long-term survival in lung cancer patients. Thus, we wanted to explore the structural and cellular organization of these structures compared to canonical secondary lymphoid organ, as well as to determine which chemokines and receptors are involved in T- and B-cell recruitment into Ti-BALT. By flow cytometry and confocal microscopy, we showed that Ti-BALT are predominantly composed of CD4+ TIL with naive and central-memory phenotypes. The ratio CD4/CD8 T cells is unchanged in Ti-BALT whereas the ratio CD45ro/CD45ra T cells is much more in favor of memory T cells in Ti-BALT compared to lymph nodes. We also demonstrated that TIL in or out Ti-BALT have a distinct chemokine receptor profile. This data is in accordance with the expression of chemokines on micro-dissected Ti-BALT versus non-Ti-BALT areas. A better knowledge about the immunologic function of tertiary lymphoid structures in a tumor context is an important prerequisite for the development of immunological intervention strategies involving creation or suppression of these structures
Disclosure of interest: None declared.

P-347
AQUEOUS EXTRACT OF SHALLOT (ALLIUM ASCALONICUM) CAN ABOLISH IN VITRO ANGIOGENESIS THROUGH INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND MATRIX METALLOPROTEINASES 2 AND 9 (MMP-2, 9)

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Today, inhibition of angiogenesis is an important approach for cancer treatment and prevention. Epidemiological and animal studies have indicated that consumption of Allium plants like shallot is associated with a reduced risk of cancer development. Here, we investigated the effects of aqueous extract of shallot on critical steps and mediators of in vitro angiogenesis. Human umbilical vein endothelial cells were treated with aqueous extract of shallot at 100–1,500 μg/ml for anti-proliferation, anti-migration and anti-tubulogenesis. In addition the effect of the extract on VEGF secretion, matrix metalloproteinases (MMP-2, 9) expression was assayed using Elisa, gelatin zymography and RT-PCR, respectively. Treatment with aqueous extract of shallot at 500 μg/ml and higher concentrations resulted in significant decrease of endothelial cells proliferation, migration and tubulogenesis. Moreover this extract showed a dose dependent inhibitory effect on VEGF secretion and MMP-2, 9 expression. Altogether, these results indicated that shallot is a potent anti-angiogenic herb of genus Allium. Aqueous extract of shallot exerts its inhibitory effect through reducing production of essentials mediators of angiogenesis including VEGF and MMPs.
Disclosure of interest: None declared.

P-348
AMIFOSTINE ATTENUATES INFLAMMATORY EVENTS AND XEROSTOMIA ON 5-FLUOROURACIL-INDUCED ORAL MUCOSITIS THROUGH THE INHIBITION OF TNF-ALPHA, IL-1BETA AND NOS-2

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Oral mucositis (OM) is a common side effect of anticancer agents. Amifostine (AMF) is an antioxidant that has being studied for the prevention of cancer chemotherapy toxicity. This study evaluated the effect of AMF on inflammation and xerostomia observed in 5-Fluorouracil (5-FU)-induced OM and its modulating effect on cytokine and inducible nitric oxide synthase (iNOS). *Golden siriam* hamsters were divided into groups: I: Saline (10 mL/kg, i.p.); II: 5-FU (60 and 40 mg/kg, i.p., days 1 and 2, respectively) + Mechanical trauma (MT) of cheek pouch on day 4; III: MT; IV: AMF (12.5, 25 or 50 mg/kg, s.c) daily/10 days + 5FU. Animals were weighed daily. The salivary rate was recorded (days 4 and 10). They were killed on the 10th day to observe the macroscopic damage to the mucosa and samples were collected for histopathology, immunohistochemistry, myeloperoxidase activity (MPO), TNF-α and IL-1β tissue concentration. Data were analyzed with ANOVA/Student Newman Keul or Kruskal–Wallis/Dunn's test. $P < 0.05$ was accepted. Ethics Committee (01/11). 5-FU was able to induce a significant ($p < 0.05$) increase in tissue TNFα (1471 ± 452.7) and IL-1β (6870 ± 2417) compared to saline (0.0 ± 0.0, 787.5 ± 356.1, respectively). AMF (50 mg/kg) significantly reduced ($p < 0.05$) the levels of TNFα (428.6 ± 246.3) and IL-1β (395.9 ± 86.06) when compared to 5-FU group. In addition, AMF (50 mg/kg) significantly reduced the MPO activity (1.79 ± 0.32), macroscopic (2.5[1–5]), and microscopic (2[0–3]) injury, immunoexpression for TNF-α(0[0–1]), IL-1β(0[0–2]) and iNOS (1[0–3]), in comparison to 5-FU (5.96 ± 1.81; 5[3–5]; 3[2–3]; 2[1–3]; 2 [1–3]; 2[2–3], respectively). AMF, at all doses used, reversed the salivary rate reduction due to the 5-FU injection, but did not alter weight loss ($p > 0.05$). In conclusion, AMF attenuates the inflammatory events observed in the OM and xerostomia induced by 5-FU. This inhibitory effect seems to occur via modulation of TNF-α, IL-1$\beta$and iNOS. Support: CNPq/CAPES/FUNCAP.
Disclosure of interest: None declared.

P-349
IFN-G REDUCES THE METASTATIC PHENOTYPE OF HYPOXIC LUNG TUMORS AND POTENTIATES CHEMOTHERAPY IN VITRO AND IN VIVO

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Integrins expressed by tumor cells are strongly implicated in various cell functions including those promoting metastasis and have also been linked to chemoresistance. Produced by activated T cells, natural killer cells, and dendritic cells, IFN-g has a pleiotropic role in the immune response. In addition, IFN-g is a key player in Inflammation as it activates macrophage and neutrophil that then secret Reactive oxygen species and other cytokines. Inflammation is often associated with hypoxia which is link to metastatic, aggressive and resistant cancers. Our studies demonstrated that hypoxia increases the expression of integrins at the cell surface of lung cancer cells. In this study, we describe a new anti-cancer role for interferon gamma (IFN-g); we show that IFN-g markedly inhibits the expression of aVb5, aVb3, and b1 integrins on lung cancer cells both in normoxia and in hypoxia, inhibits metastases and invasion, and chemosensitise lung cancer cells in vitro and in vivo. Our studies revealed the anti-invasion and anti-metastatic activity of IFN-g on lung cancer cells as treated cancer cells lose their ability to adhere, proliferate, migrate, invade, form colonies, and are significantly less efficient at infiltrating the lungs. We also demonstrate that IFN-g significantly increases the efficacy of several drugs on resistant cancer cells both in vitro (normoxia and in hypoxia) and in vivo. We propose to combine IFN-g with currently used low efficient chemotherapeutic agents to potentiate their antitumor efficiency for the treatment of chemoresistant tumors. This would allow the use of lower doses of chemotherapy, and consequently prevents their associated side effects. Such IFN-g combined therapies would efficiently eradicate primary and metastatic resistant lung cancers.
Disclosure of interest: None declared.

Pain and inflammation

P-350
COMPARISON OF MANUAL THERAPY WITH TAPING IN THE TREATMENT OF OSTEOARTHRITIS OF THE KNEE: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Objectives: Conservative treatment should be tried prior to surgical treatment in knee osteoarthritis. This study was designed to evaluate the short-term effects of manual therapy on pain relief and functional improvement in knee osteoarthritis in comparison with taping.
Methods: The study included 36 patients who were diagnosed as having knee osteoarthritis according to the ACR (American College of Rheumatology) criteria, and had complaints lasting for more than 1 year. All the patients had stage 2 or 3 osteoarthritis radiographically according to the Kellgren-Lawrence criteria. Eighteen patients (mean age 50 ± 0.6 years; range 40 to 60 years) received manual therapy. Manual therapy applied on knee for 30 min daily for a total of 10 sessions in 2 weeks. Eighteen patients (mean age 50 ± 0.9 years; range 40 to 60 years) received a total of 6 sessions of taping for a total of 2 weeks. Pain with numerous rating system and functional performance using the WOMAC questionnaires was assessed before and immediately after each treatment. The patients were evaluated before and after treatment in terms of pain by using of numerical pain scale (NRS) and functionality using the WOMAC (Western Ontario and McMaster Universities) osteoarthritis index.
Results: The scores of all instruments showed significant improvements following treatment in both groups ($p < 0.001$). Post treatment changes in relation to baseline scores differ significantly between the two groups. ($p < 0.05$).manual therapy was better than taping in treatment of osteoarthritis of knee.
Conclusion: Both manual therapy and taping had significant effect on osteoarthritis of knee but manual therapy is better than taping on improvement of pain and disability of this patients. Manual therapy is a noninvasive, complication-free, and cost-effective alternative modality for the conservative treatment of knee osteoarthritis.
Keywords: Knee pain, knee osteoarthritis, manual therapy, taping.
Disclosure of interest: None declared.

P-351
ANTINOCICEPTION EFFECTS OF LOCAL ANESTHETIC AGENTS, CAPSAICINE AND LINGUAL DENERVATION IN ANGIONEUROTIC TONGUE EDEMA MODEL

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Introduction: The inflammatory response induced by *Dieffenbachia picta*, san ornamental plant, is a result of tissue mechanical lesion caused by calcium oxalate needles. The toxicity arrises from its ability to cause painful edema of oral mucous membranes after chewing on the stem. Angioneurotic tongue edema caused by *D. picta* sap in live mice was standartized (DIP et al. 2004, 2011).
Methods: The tongue edema measures were done with a digital tachymeter, 2 h after topical application (0.1 mL) of *D. picta* sap. The mice were treated 15 min after edema induction, by injection or topical application of lidocaine 2–10%, bupivacaine 0.5%, ropivacaine 1% (50 µl/tongue). Neonatal mice received daily subcutaneously administration of capsaicin (1% 50 mg/kg) for 5 weeks; bilateral sections (5 mm) of lingual nerve were done in rats, 21 days before tongue edema induction. *D. picta*-induced mechanical hyperalgesia was assessed in mice by measuring hind paw withdrawal thresholds to an increasing pressure stimulus using an analgesymeter with a wedge-shaped probe. Paw withdrawal thresholds were measured before and up to 30 min after an intraplantar injection of 0.01–0.1 mL of the plant sap associated with anesthetic solutions.
Results: All local anesthetic solutions showed anti-edematogenic effects. Partial reduction of tongue edema was observed after pre treatment with capsaicine and after lingual denervation. Sap-induction hipernociception was dose response and completely abolished by ropivacaine. *Conclusions* The key to reduce *D. picta* inflammatory reaction is inhibit antidromic vasodilation and axon reflex flare, reducing mastocyte degranulation and release of tachykinins from nerve endings. Ropivacaine maybe showed better results because it seems to function not only as a local anesthetic, blocking neurotransmission in the damaged tissue, but also leading to a local vasoconstriction, as well as an unspecific antagonist response on nociceptors involved in peripheral pain.
Disclosure of interest: None declared.

P-352
A RAPID AND RELIABLE MODEL OF ORAL OEDEMA TO SCREEN FOR NEW ANTI-INFLAMMATORY DRUGS

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We have developed a model to screen for anti-inflammatory effects in the oral cavity. A range of doses (25–1,000 mcg in 0.1 ml) of carrageenan (CG) were injected into the upper lips of anesthetized animals (mixture of ketamine/xylazine, 15/90 mg/kg, subcutaneous), or intraplantarly (ipl) in Holtzman rats (150–180 g, male) at time zero. Control rats (C) received the same volume locally (0.1 ml), either in the lip or the hind paw of physiological saline at time zero. Increased thickness of the lip or hind paw was measured with a digital caliper (Mytutoyo, Japan) for up to 6 h following injections. CG induced a significant and dose-dependent increase in lip thickness in comparison with controls ($Delta_{CG}$ at 500 mcg/site = 4.12 ± 0.05 mm; $Delta_C = 0.023 \pm 0.005$ mm) with a maximal effect occurring at 1 h after injection, in contrast to 3 h ($Delta_{CG}$ at 500 mcg/site = 1.68 ± 0.04 mm; $Delta_C = 0.08 \pm 0.004$ mm) in rat paws. At these times, non-selective (2 mg/kg indomethacin; 20 mg/kg ibuprofen), selective (12 or 30 mg/kg celecoxib) non-steroidal anti-inflammatory drugs (NSAIDs) or dexamethasone (1 mg/kg), given s.c. 30 min before CG, reduced oral and paw oedema to a similar extent. Although hind paw oedema is highly predictive of the

clinical efficacy of NSAIDs (Mukherjee et al., 1996), our results would suggest that oral oedema in rats could be as useful a model and would take less time in screening for new anti-inflammatory drugs.
Mukherjee, et al. Predictability of the clinical potency of NSAIDs from the preclinical pharmacodynamics in rats. Inflamm Res. 1996;45:531–40.
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Disclosure of interest: None declared.

P-353
OXADIAZOLONE DERIVATIVES: NOVEL CANDIDATES, WITH DOUBLE POTENTIALITY, FOR ANTI-INFLAMMATORY JOINT THERAPY

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Osteoarticular diseases (OD) such as arthritis and osteoarthritis are characterized by inflammation leading to articular cartilage destruction. Nonsteroidal anti-inflammatory drugs (NSAIDs) targeting cyclooxygenases (COXs), improve rheumatic symptoms by reducing the production of prostaglandin E_2 (PGE_2), the main eicosanoid contributing to pain and inflammation in OD. However these drugs are associated with various side effects (gastrointestinal and cardiovascular toxicities) caused mainly by an impaired balance between COX-1 and COX-2. Thus, the strategy of novel anti-inflammatory drugs development could be to inhibit production of COX substrates without modifying the COX-1 to COX-2 ratio. Group IIA secreted phospholipase A2 (GIIAPLA2) is involved in the synthesis of eicosanoids upstream COXs. This enzyme is known to be involved in the pathogenesis of many inflammatory diseases including OD. We have developed novel GIIAPLA2 specific inhibitors such as the C8 oxadiazolone derivative. C8 inhibits IL-1b-stimulated PGE2 secretion in rabbit articular chondrocytes without modifying the COX-1 to COX-2 ratio. Interestingly, oxadiazolone derivatives have structural homology with PPAR ligands like rosiglitazone. Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors. They have an anti-inflammatory activity in several inflammatory diseases, including OD. We hypothesize that oxadiazolone derivatives could activate PPAR. In order to test our hypothesis, we transfected rabbit articular chondrocytes with a PPAR-responsive construct (PPRE-luc) with/without overexpression of PPARs or PPAR dominant negative mutants. We show that C8 activates the PPRE-dependent transcription more powerfully than rosiglitazone. C8 effect on PPRE involves at least in part PPARγ activation, but not PPARα nor PPARβ/δ. In conclusion C8 appears as candidate for new anti-inflammatory drugs with double potentiality (GIIAPLA2 inhibition and PPAR activation).
Disclosure of interest: None declared.

P-354
INTRAGANGLIONAR RESINIFERATOXIN PREVENTS TRIGEMINAL INFLAMMATORY AND NEUROPATHIC HYPERALGESIA

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Trigeminal ganglion C-fiber neurons bearing TRPV1 channels are selectively destroyed by resiniferatoxin (RTX), a potent capsaicin analogue (Karai et al., 2004). The current study, approved by UFPR's Animal Use Ethics Committee, assesses the effects of RTX injection into the trigeminal ganglion in inflammatory and neuropathic models of thermal hyperalgesia.
Methods: RTX (200 ng) or vehicle (2 µl) were microinjected into the right trigeminal ganglion of anesthetized male Wistar rats (200 g). After injection, rats were submitted to surgery for placement of two loose silk 4.0 ligatures around the right infraorbital nerve. Ipsilateral thermal stimulation of snout was conducted daily by application of either heat ($\sim$50°C, from source placed 1 cm from vibrissal pad) or cold (1-s tetrafluoroethane spray) stimuli. Decreased latency for head withdrawal or vigorous snout flicking, or increased facial grooming behavior duration indicated heat or cold hyperalgesia. One week after RTX, other rats received a 50 µl injection of carrageenan (50 µg), formalin (2.5%) or vehicle into the upper lip and carrageenan-induced heat hyperalgesia or formalin-evoked nociception were recorded.
Results and conclusion: The eye wipes test confirmed the success of trigeminal ganglion C-fiber deletion in RTX-treated rats (4.1 ± 1.2 wipes) versus vehicle-treated rats (22.8 ± 2.6 wipes). Nerve injury surgery induced heat and cold hyperalgesia in saline-treated rats, starting on Day 2 and lasting until Day 9, but these responses on Days 3, 6 and 9 were abolished in RTX-treated rats. Carrageenan induced heat hyperalgesia in vehicle-treated, but not RTX-treated, rats. Nociceptive responses to orofacial formalin were unchanged by RTX treatment. Thus, trigeminal ganglion neurons expressing TRPV1 are crucial for the development of orofacial inflammatory and neuropathic thermal hyperalgesia.
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Disclosure of interest: None declared.

P-355
DESIGN, SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NEWLY SYNTHESIZED THIAZOLO[3,2-B][1,2,4]TRIAZOLE DERIVATIVES OF IBUPROFEN

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Thiazoles are important class of heterocyclic compounds exhibiting a wide range of biological properties, such as anti-inflammatory, analgesic, antimicrobial, antitumor, anticancer and found in many potent biologically active molecules such as fentiazac, meloxicam (anti-inflammatory agent), nizatidine (antiulcer agent), sulfathiazole (antimicrobial agent), ritonavir (antiretroviral drug) and bleomycine and

tiazofurin (antineoplastic agents). Literature survey reveals that 1,2,4-triazoles represent an important pharmacophore, and play a vital role as medicinal agents showing diverse biological activities such as anti-inflammatory, analgesic, antibacterial, antifungal, antitubercular and anticancer. Furthermore, condensed heterocyclic compounds having a thiazolo[3,2-b][1,2,4]triazolering system have also been reported to possess anti-inflammatory and antibacterial activities. Arylalkanoic acids are an important class of NSAIDs, but their use has been associated with gastro-intestinal toxicity. It has been reported in literature that modification of carboxyl function of representative NSAIDs results in retained anti-inflammatory activity and reduced ulcerogenic potential. To reduce their toxic effects a number of derivatives have been prepared and in some of these carboxylic group has been replaced by 5-membered heterocyclic moieties such as thiazole and 1,2,4-triazole. In the present study we have synthesized some newer analogues of 2-(4-isobutyl-phenyl)-propionic acid (ibuprofen) having thiazolo-triazole moiety. The tested compounds showed potent anti-inflammatory activity as compared to standard drug ibuprofen at the same oral dose. The compounds showing significant anti-inflammatory activity also showed maximum reduction in severity index along with minimum lipid peroxidation, with no hepatocyte necrosis or degeneration. Thus these compounds represent a fruitful matrix for development of new class of non acidic anti-inflammatory agents.
Disclosure of interest: None declared.

P-356
ANTI-INFLAMMATORY ACTIVITY OF RIPARIN I (O-METHYL N-BENZOYL TYRAMINE) ON PAW EDEMA MODELS IN MICE

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Introduction/aim: Riparin I (ripI) is an alcamid compound that was isolated from unripe fruit of *Aniba riparia*. We decided to evaluate the anti-inflammatory effect of ripI in models of inflammation based on a prior anti-inflammatory effect shown in the second phase of the formalin test (Araujo et al., Naunyn-Schmied Arch Pharmacol, 2009).
Methods: Male mice (20–25 g) were pre-treated with vehicle (tween 80 3%, i.p.), ripI-25 mg/kg, i.p., ripI-50 mg/kg and indometecin 10 mg/kg, i.p. or cyproheptadine 10 mg/kg, i.p. One hour after treatment, the paw edema was induced by intraplantar injection of 50 μL of carrageenan 1%, dextran 1.5%, histamine 4 μg/μL or serotonin 4 μg/μL. The edema volume was measured in microliter s(μL). Data were analyzed by two-way ANOVA followed by Bonferroni as the post hoc test. This work was approved by the local ethics committee (protocol number 15/09).
Results: RipI decreased carrageenan-induced paw edema at 4 h (ripI-25: 35.71 ± 9.72; ripI-50: 44.29 ± 12.12) as compared to control (95.71 ± 10.66). RipI inhibited dextran-induced paw edema at 3 h (ripI-25: 60.0 ± 7.99; ripI-50: 60.5 ± 5.84) and 4 h (ripI-25: 43.5 ± 7.56; ripI-50: 45.0 ± 6.7) comparing to controls (3 h: 90.5 ± 2.73; 4 h: 72.0 ± 4.95). Also, ripI decreased the edema induced by histamine in all measure times, 15 min (ripI-25: 35.5 ± 4.56; ripI-50: 40.0 ± 9.63), 30 min (ripI-25: 26.0 ± 3.23; ripI-50: 21.00 ± 3.39), 60 min (ripI-25: 17.50 ± 5.97; ripI-50: 26.00 ± 5.36) and 90 min (only ripI-25: 11.50 ± 3.16) as compared to controls (15 min: 64.0 ± 4.46; 30 min: 56.0 ± 4.87; 60 min: 47.50 ± 6.29; 90 min: 43.00 ± 6.37) but did not alter serotonin-induced paw edema.
Conclusion: RipI is more effective on dextran-induced edema whose main mediators are serotonin and histamine. RipI decreased the histamine-induced paw edema but not serotonin-induced paw edema showing that its anti-inflammatory effect is mediated, in part, by inhibiting the actions of histamine.
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P-357
ROLE OF TACKYKININ NK1 RECEPTORS ON INFLAMMATORY OROFACIAL PAIN

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There is accumulating evidence that substance P (SP) released from trigeminal ganglia neurons participates of orofacial inflammatory pain. Here, we have investigated the ability of SP to induce orofacial nociception and thermal hyperalgesia and the role of NK1 receptors on two models of orofacial inflammatory pain. Male Wistar rats (200–250 g) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization # 424). Rats, pre-treated or not with captopril (5 mg/kg, i.p.), received SP (1–100 μg/50 μl, into the upper lip) or vehicle and were placed in observation cages for evaluation of the facial grooming behavior up to 15 min. Thirty minutes later, rats were submitted to ipsilateral application of either heat (~50°C heat source placed 1 cm from vibrissal pad) or cold (1-s tetrafluoroethane spray) stimuli to the snout. Heat and cold hyperalgesia were estimated as decreases in the latency to display head withdrawal or vigorous snout flicking, or increases in duration of bilateral facial grooming behavior, respectively. Additional groups of rats received the selective NK1 receptor antagonist SR140333 (10 μg/50 μl or 1–3 mg/kg) 30 min before the injection of carrageenan (50 μg/50 μl) or SP (1 μg/50 μl) or 1 h before the injection of formalin (2.5%, upper lip) and the heat hyperalgesia or formalin-induced nociception was assessed. SP (at 1 μg/50 μl) induced heat hyperalgesia, but failed to evoke significant facial grooming or cold hyperalgesia, even in rats pre-treated with captopril. Systemic treatment with SR140333 prevented SP and carrageenan-induced heat hyperalgesia and reduced by about 50% the first and the second phase of nociception induced by formalin. Our results suggest that NK1 receptors may represent a potential target for the control of orofacial inflammatory pain. Financial support: Sanofi-Aventis, Fundacao Araucaria. T.F.C. and M.F.T.J. have scholarships from CAPES and UFPR-TN, respectively.
Disclosure of interest: None declared.

P-358
COMPARISON OF SUBLINGUAL KETOROLAC VERSUS SUBLINGUAL PIROXICAM DURING POST-OPERATIVE PAIN MANAGEMENT AFTER LOWER THIRD MOLAR EXTRACTION

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Lower third molar extraction provides a clinical model for studying analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs). In this double-blinded, randomized, crossover investigation, 47 patients received ketorolac sublingually and piroxicam sublingually during two separate appointments after lower third molar extraction of symmetrically positioned lower third molars. A surgeon evaluated objective parameters and patients documented subjective parameters comparing postoperative results for a total of 7 days after surgery. Patients treated with either sublingual ketorolac (40 mg) or piroxicam (20 mg) reported low pain scores during postoperative period. Similarly, the subset of patients who received ketorolac sublingually exhibited similar average amount of analgesic rescue medication (paracetamol) when compared to the subset of patients who received piroxicam sublingually ($p > 0.05$). Additionally, values for mouth openings measured just prior to surgery and immediately following suture removal 7 days later were similar among patients ($p > 0.05$), and the type of NSAID used in this study showed no significant differences between swellings on the second or seventh postoperative days ($p > 0.05$). In summary, pain, trismus and swelling after lower third molar extraction, independent of surgical difficulty, could be successfully controlled by ketorolac administered sublingually (40 mg) or piroxicam administered sublingually (20 mg) and no significant differences were observed between the type of NSAID used in this study.
Disclosure of interest: None declared.

P-359
A LINK BETWEEN PRIMARY HYPERALGESIA AND BLOOD FLOW IN ARTHRITIS

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We have previously shown that i.art. injected CFA can cause secondary mechanical and thermal hyperalgesia in the mouse paw via distinct TRPV1- and TRPA1-dependent mechanisms (Keeble et al., 2005; Fernandes et al., 2010). We are now trying to establish a link between primary mechanical hyperalgesia and blood flow in animals with arthritis. CD1 mice (12 weeks old) received a unilateral intra-articular injection of CFA (10 µl/joint; ipsilateral joint) in the knee. The contralateral joint received 10 µl of saline. Primary hyperalgesia was assessed by using a pressure application measurement device once a week, over 4 weeks. Animals developed primary mechanical hyperalgesia for 3 weeks after arthritis induction. We also assessed blood flow in the knee joint in these animals by using a Moor laser Doppler flowmeter. Two weeks after arthritis induction, baseline knee blood flow was recorded. At this time point, primary hyperalgesia was more accentuated in the ipsilateral joint. We found that blood flow was increased in both ipsilateral and contralateral knee joints (1.3- and 1.7-fold increase, respectively). Interestingly, blood flow recordings were 25% higher in the contralateral when compared to the ipsilateral joint. We therefore, suggest a link between primary hyperalgesia and blood flow regulation. Further investigations are necessary to fully understand the link between blood flow and pain in arthritis. Work supported by the Arthritis Research UK and British Heart Foundation.

Keeble, et al. Arthr Rheum. 2005;52:3248–56.
Fernandes, et al. Arthr Rheum. 2010. doi:10.1002/art.30150.

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P-360
A POTENTIAL LINK BETWEEN THE TRPA1 AND TRPV1 RECEPTORS IN VIVO

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Recent evidence suggests a potential interaction between the TRPA1 and TRPV1 receptors as the TRPA1 receptor is known to be co-expressed in 50% of all TRPV1-positive sensory neurons [1]. Both receptors are activated or sensitised in inflammation and involved in cutaneous neurogenic vasoactive responses [2]. This study investigated (i) if in vivo deletion of TRPA1 affects TRPV1 (capsaicin)-mediated responses and (ii) the role of TRPV1 in TRPA1 (mustard-oil)-induced cutaneous blood flow in pharmacogenetic studies. Peripheral blood flow was measured, as a marker of in vivo activation in the ears of anaesthetised mice using laser Doppler techniques for 30 min following topical application of agents. Ear swelling was also determined as a measure of oedema formation. Topical application of capsaicin (10 mg/ml) induced a significant ($p < 0.05$) and similar increase in blood flow and swelling in the ipsilateral ears of wild-type and TRPA1 knockout mice. Furthermore, mustard-oil (1%) induced vasodilatation was greater in TRPV1 knockout and in CD1 mice pre-treated with TRPV1 antagonist SB366791 (5 mg/kg, i.p., 2 h) as compared to wild-type and CD1 mice pre-treated with vehicle (2% DMSO, i.p., 2 h), respectively. No significant change in oedema formation was observed. This study provided evidence that capsaicin-induced vasodilatation and swelling do not involve TRPA1 receptor. Our previous findings showed that mustard-oil induced vasodilatation was dependent on TRPA1 and vasodilator neuropeptides [2, 3]. The present study shows that TRPV1 may also be involved in regulating TRPA1-mediated neurogenic responses in mouse vasculature. Our results highlight that genetic deletion or pharmacological blockade of TRPV1 may potentiate TRPA1-mediated responses in the mouse skin at least. This study was supported by a BBSRC-led IMB capacity building award & the BHF.

1. Andersson, et al. J Neurosci. 2008;28:2485–89.
2. Grant, et al. Eur J Pharmacol. 2005;507:273–80.
3. Pozsgai, et al. Cardiovasc Res. 2010;87:760–68.

Disclosure of interest: None declared.

P-361
INHIBITION OF INFLAMMATORY PAIN BY A NATURAL PRODUCT EXTRACTED FROM THE RED ALGAE LAURENCIA GLANDULIFERA: AN OPIOID-MEDIATED MECHANISM

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Diterpenoids extracted from the red algae *Laurencia* sp. were isolated and tested for their anti-inflammatory and analgesic properties in models of visceral pain. The structure and the relative stereochemistry

of this compound were established by means of spectral and data analyses.
Methods: The analgesic effects of the diterpenoid extract were evaluated on two in vivo models of abdominal pain induced by the intraperitoneal injection of acetic acid (1%) or visceral pain induced by the intracolonic administration of mustard oil (0.001%). In vitro, calcium mobilization recordings in primary cultures of mouse dorsal root ganglia sensory neurons were performed.
Results: Mouse treatment with the diterpenoid extract (intraperitoneal, 1 mg/kg per mouse) inhibited nociception induced by both acetic acid and mustard oil. Further, the pretreatment of sensory neuron with this compound (300 μM) inhibited significantly free intracellular calcium mobilization induced by PAR_2 agonist (200 μM) or histamine (100 μM) treatment. The effects of the *Laurencia* sp. extract treatment on histamine-induced calcium flux was blocked by naloxone, an antagonist of opioid receptors. The use of specific antagonist of opoids receptors delta, Kappa and mu (naltrindole, nor-binaltorphimine dihydrochloride and CTOP) confirmed the implication of mu and kappa opioid receptors.
Conclusions: These results showed strong analgesic effects for a diterpenoid extracted from red algae, demonstrating opioid receptor activation properties for this natural product. This diterpenoid extract could be considered as a new natural product that could be use in the management of pain.
Disclosure of interest: None declared.

P-362
UMBELLULONE, THE OFFENSIVE PRINCIPLE OF THE HEADACHE TREE (UMBELLULARIA CALIFORNICA), STIMULATES THE TRIGEMINOVASCULAR SYSTEM VIA TRPA1 ACTIVATION

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The California bay laurel (*Umbellularia californica*), has been known by native Americans as the headache tree', because the scent of its leaves produces violent headache crises. However, the mechanism by which *U. californica* causes headache is unknown. We recently described a case of cluster headache-like attacks triggered by the inhalation of the scent of *U. californica*. The major volatile constituent of the leaves of *U. californica* is umbellulone, a monoterpene ketone. The irritant properties of umbellulone are recapitulated by its ability to affect respiration, heartbeat, and blood circulation in laboratory animals, eventually causing death. Despite the β,β-dialkyl substitution, a structural feature known to suppresses Michael reactivity, umbellulone reacted quickly with thiols, suggesting that it could act as a trigeminovascular stimulator via TRPA1 activation. We found that umbellulone selectively stimulates TRPA1 expressing HEK293 cells and rat trigeminal ganglion neurons, but not untransfected cells or neurons after TRPA1 blockade. Umbellulone also released CGRP from trigeminal nerve terminals. In wild type mice, but not in TRPA1 deficient mice, umbellulone excited cultured trigeminal neurons. Finally, the increase in rat meningeal blood flow induced by umbellulone applied to the rat nasal mucosa was selectively inhibited by TRPA1 and CGRP receptor antagonists. The TRPA1-dependent ability of umbellulone to activate the trigeminovascular system, thereby causing nociceptive responses and CGRP release, represents a plausible mechanism for *U. californica*-induced headache.
Disclosure of interest: None declared.

P-363
PSORIATIC ARTHRITIS: TEMPOROMANDIBULAR JOINT INVOLVEMENT AS THE FIRST ARTICULAR PHENOMENON

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Aim: The aim is to underline the importance of the paediatric dentist and orthodontist in the contribution to the early diagnosis of Psoriatic Arthritis (PA), avoiding and preventing the orofacial and systemic complications.
Materials and methods: Psoriatic Arthritis is a chronic systemic disease that is difficult to detect. The diagnosis is made mainly on clinical grounds based on the findings of psoriasis and inflammatory arthritis of the joints. Many reports have described the damaging effects of PA on the temporomandibular joints (TMJs), but no study has clearly reported the TMJ as the first articulation to be involved in PA. This work reports a case of PA that was diagnosed several years after a TMJ onset because no other signs apart from psoriasis were present.
Results: The missed early diagnosis resulted in severe TMJ damage. The TMJ can be the first joint involved in PA. It is often unilateral, with a sudden onset. Symptoms include pain and tenderness of the joint area and the muscles of mastication, morning stiffness, tiredness in the jaws, joint crepitation, occasional painful swelling of the TMJ capsule and painful mandibular movements associated with a progressive decrease in the interincisal opening. In severe cases, ankylosis of the TMJ may occur.
Discussion: the diagnosis of PA of the TMJ is difficult and is made mainly on the basis of the systemic presentation of the disease. In general, the diagnosis is based on a triad of psoriasis, radiographic evidence of erosive polyarthritis, and a negative serologic test for RF.
Conclusions: For a correct, early diagnosis of PA, collaboration between the dentist and rheumatologist it is very important. The dentist should recommend in addition to exercise and local pain treatment, an occlusal splint to help keep the TMJs working properly, improve function, relieve pain, reduce swelling, and prevent further severe TMJ damage.
Disclosure of interest: None declared.

P-364
EFFECT OF LIGHT EMISSION DIODE (LED) THERAPY ON PAW EDEMA AND HYPERALGESIA INDUCED BY BOTHROPS MOOJENI VENOM

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Envenoming caused by snake from *Bothrops* sp. induces local effects in the victims, which are represented by intense edema formation, pain and necrosis. The most effective treatment for *Bothrops* snakebites is antivenom (AV) therapy. However, this procedure does not reverse the local reaction caused by the venom. Other alternatives are being investigated with the purpose of diminishing the local effect induced by bothropic snakebites that include the LED therapy. The aim of this study was to investigate the effectiveness of LED therapy to revert the edema formation and hyperalgesia caused by *Bothrops moojeni* venom (BmV). Also, the effect on edema formation and hyperalgesia induced by BmV when the LED treatment is combined with the use of AV was also evaluated. Male swiss mice were used. To measure the edema formation and hyperalgesia, 1 μg/paw (50 μL) of BmV was injected into the right paw of mice. The edematogenic effect was evaluated by pletismograph at 15, 30 min, 1, 3, 6 and 24 h after BmV injection or saline. The LED therapy on hypernociceptive effect induced by BmV was evaluated using a Von Frey test applied before, 1, 3, 6 and 24 h after venom injection. The animals had been treated with red LED (λ635 nm, density of energy of 4 J/cm^2, irradiation time of 41 s and area of 1.2 cm^2) and infrared LED (λ945 nm, density of energy of 4 J/cm^2, irradiation time of 38 s and area of 1.2 cm^2) applied: 30 min and 3 h after the venom injection. The results showed that paw edema and hyperalgesia caused by BmV was significantly reduced by red and infrared LED. Treatment with AV did not modify the edema formation or hypernociception caused by BmV. The combined therapy with LED and AV did not modify the LED effect. In conclusion, both LED therapy significantly reduced edema formation and hyperalgesia caused by BmV, suggesting that LED therapy should be considered as a potentially therapeutic approach for the local effects of *Bothrops* species.
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Disclosure of interest: None declared.

P-365
OXALIPLATIN ELICITS MECHANICAL AND COLD ALLODYNIA IN RODENTS VIA TRPA1 RECEPTOR STIMULATION

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Platinum-based anticancer drugs cause neurotoxicity. In particular, oxaliplatin produces early-developing, painful and cold-exacerbated paresthesias. However, the mechanism underlying these bothersome and dose-limiting adverse effects is unknown. We hypothesized that the transient receptor potential ankyrin 1 (TRPA1), a cation channel activated by oxidative stress and cold temperature, contributes to mechanical and cold hypersensitivity caused by oxaliplatin and cisplatin. Oxaliplatin and cisplatin evoked glutathione-sensitive relaxation, mediated by TRPA1 stimulation and the release of calcitonin gene-related peptide from sensory nerve terminals in isolated guinea pig pulmonary arteries. No calcium response was observed in cultured mouse dorsal root ganglion neurons or in naïve Chinese hamster ovary (CHO) cells exposed to oxaliplatin or cisplatin. However, oxaliplatin, and, with lower potency, cisplatin, evoked a glutathione-sensitive calcium response in CHO cells expressing mouse TRPA1. One single administration of oxaliplatin produced mechanical and cold hyperalgesia in rats, an effect selectively abated by the TRPA1 antagonist HC-030031. Oxaliplatin administration caused mechanical and cold allodynia in mice. Both responses were absent in TRPA1-deficient mice. Administration of cisplatin evoked mechanical allodynia, an effect that was reduced in TRPA1-deficient mice. TRPA1 is therefore required for oxaliplatin-evoked mechanical and cold hypersensitivity, and contributes to cisplatin-evoked mechanical allodynia. Channel activation is most likely caused by glutathione-sensitive molecules, including reactive oxygen species and their byproducts, which are generated following tissue exposure to platinum-based drugs from cells surrounding nociceptive nerve terminals.
Disclosure of interest: None declared.

P-366
THE EFFECT OF THE TRPV1 ANTAGONIST, SB366791, ON THERMAL NOCICEPTIVE THRESHOLDS AND CAPSAICIN-INDUCED VASCULAR RESPONSES IN MICE

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The Transient Receptor Potential Vanilloid 1 (TRPV1) receptor is expressed predominantly on sensory nerve fibres and is activated by a variety of noxious stimuli including heat >43°C, protons and a variety of inflammatory mediators. Therein, TRPV1 plays an integral role in the pain response and, thus, has been highlighted as a therapeutic target for the treatment of pain. However, TRPV1 antagonists cause hyperthermia in humans and animals which is associated with increased thermogenesis and decreased cutaneous blood flow (Romanovsky et al., 2009). The present study has investigated the effect of the TRPV1 antagonists SB366791 (Gunthorpe et al., 2004) on thermal nociception, body temperature and capsaicin-induced vascular responses in mice. Female, CD1 mice (Charles River, UK) were used for all experiments in accordance with the Scientific Procedures Act 1986. Mice were injected i.p. with SB366791 (5 mg/kg) or vehicle (10 ml/kg). Thermal nociceptive responses in both paws were measured 4 h after injection of SB366791 or vehicle using the Hargreaves test. Body temperature was measured using radiotelemetry via subcutaneously implanted transmitters (DSI). For measurement of vascular responses, capsaicin or vehicle was applied to the ears and exposed knee joint synovial membranes of urethane-anaesthetised mice and blood flow was measured by laser Doppler imagery (Moor instruments). SB366791 increased thermal nociceptive thresholds ($P < 0.05$) without increasing core body temperature. Concomitantly, SB366791 had no significant effect on capsaicin-induced vascular responses. Thus, to conclude, SB366791 can increase thermal nociceptive thresholds in mice without increasing core temperature. This may be due, at least in part, to the lack of inhibition of vascular responses by SB366791.
Gunthorpe, et al. Neuropharmacology. 2004;46:133–49.
Romanovsky, et al. Pharmacol Rev. 2009;61:228–61.
Disclosure of interest: None declared.

P-367
ANTI-INFLAMMATORY ACTIVITY OF RIPARIN II (N-2-HYDROXYBENZOYL TYRAMINE) IN RATS

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Introduction/aim: Riparin II (ripII) is a alkamide compound isolated from *Aniba riparia*, collected from the Amazonas. We decide evaluate the anti-inflammatory effect of riparin II in animals models.
Methods: Male rats (180–240 g) were divided into groups: controls (tween 80 2%), ripII 25 mg/kg, ripII 50 mg/kg and indometacin 10 mg/kg, cyproheptadine 10 mg/kg or dexametasone 5 mg/kg. All drugs were injected orally. The anti-inflammatory activity of ripII were studied in animals models of inflammation: carrageenan- and dextran-induced edemas (Winter et al., 1962), carrageenan-induced peritonitis (Souza and Ferreira, 1985) and MPO and Protein measurement (Bradley et al., 1982) and (Lowry et al., 1951). Data were analyzed using one-way ANOVA and Newman–Keuls test. This work was approved by the local ethics committee (protocol number 40/10).
Results: The previous administration of ripII reduced the paw edema (2 h: ripII25: 2.258 ± 0.284; ripII50: 2.161 ± 0.1769; Ctrl: 3.54 ± 0.2706; 3 h: ripII25: 2.683 ± 0.2308; ripII50: 2.31 ± 0.2151; Ctrl: 4.18 ± 0.2459; 4 h: ripII25: 2.615 ± 0.2683; ripII50: 2.549 ± 0.255; Ctrl: 4.271 ± 0.309). The pre-treatment with ripII 25 and 50 mg/kg had a reduction in edema formation by dextran (2 h: ripII25: 2.212 ± 0.1957; ripII50: 2.047 ± 0.3502; Ctrl: 3.371 ± 0.2737; 4 h: ripII25: 2.108 ± 0.2062; ripII50: 1.71 ± 0.3759; Ctrl: 3.356 ± 0.3457). The treatment with ripII 25 and 50 mg/kg had a decreased in migration of leucocytes to peritoneal cavities and the amount of total protein: ripII25: $8.750 \times 10^6 \pm 1.027 \times 10^6$; ripII50: $6.88 \times 10^6 \pm 854{,}342$; Ctrl: $1.145 \times 10^7 \pm 1.042 \times 10^6$; ripII25: 59.2 ± 11.4; ripII50: 53.31 ± 12.12; Ctrl: 91.28 ± 5.534, respectively. RipII decreased the MPO activity (Ctrl: 240.5 ± 26.86; ripII25: 173.8 ± 13.59; ripII50: 190.0 ± 6.063).Conclusion: RipII in both doses was able to reduce the edemas induced by carrageenan and dextran. RipII reduced the influx of leukocyte and myeloperoxidase activity as well as the protein extravasations in response to carrageenan.
Financial support: CNPq/CAPES.
Disclosure of interest: None declared.

P-368
NEUROPROTECTIVE EFFECT OF AMIFOSTINE UPON OXALIPLATIN-INDUCED PERIPHERAL SENSORY NEUROPATHY IN MICE

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Oxaliplatin (OXP), a platinum-based agent, plays a wide range of antitumor activities, limited due to a prominent peripheral sensory neuropathy (PSN). Amifostine (AMF) is an antioxidant agent used in management of some anticancer side effects. This study aimed to evaluate the protective effect of amifostine on OXP-induced PSN. The study was approved by the Ethics Committee (protocol 27/08). Swiss male mice (25–35 g) were divided into 3 groups, I: Saline (0.1 mL/kg, i.v.); II: OXP (1 mg/kg, i.v.) twice a week for 4.5 weeks; III: AMF (1, 5, 25, 50 or 100 mg/kg, s.c.) injected 30 min before OXP. Mechanical hyperalgesia, thermal allodynia and Rota-rod tests were performed weekly. Histopathology for dorsal horn of spinal cord and immunohistochemical assay for Fos, CASP3, IL-1β, nitrotyrosine, iNOS, nNOS and NMDA was performed. OXP significantly decreased mechanic and thermal nociceptive threshold from 14th day on ($p < 0.01$) when compared to control group. AMF treatment inhibited these effects in all doses tested ($p < 0.001$), and the best dose was 25 mg/kg, which increased mechanic (94.33% of AUC) and thermal (100.85% of AUC) nociceptive threshold compared to OXP group. Locomotor impairment was not evidenced through Rota-rod test. Furthermore, we observed edema and neurons atrophy in dorsal horn of OXP group, not showed in control and improved in AMF group. OXP group had overexpression of Fos, CASP3, nNOS, iNOS, and nitrotyrosine, but a reduced NMDA expression when compared to control group. AMF group had hypoexpression of Fos, nitrotyrosine and increased NMDA expression, but not altered CASP3, nNOS, iNOS expression when compared to OXP group. These results suggest that AMF protects tissue and sensory changes on OXP-induced NSP, probably through an antioxidant effect, since it inhibited c-Fos, nitrotyrosine expression, and through an antiapoptotic signaling mediated by NMDA receptor. Support: CNPq/CAPES/FUNCAP.
Disclosure of interest: None declared.

P-369
ANTI-INFLAMMATORY AND ANTINOCICEPTIVE PROPERTIES OF NEW SULPHONYLHYDRAZONE DERIVATIVES RATIONALLY DESIGNED AS PPAR AGONISTS

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Peroxisome Proliferator Activated Receptors (PPARs) have been arisen as promising targets to treat chronic inflammatory diseases acting by mechanisms pro-resolution. Fasted swiss and BALBc mice (20–30 g) were used in in vivo tests of inflammation and pain ($n = 7$–10 animals/group; $^*p < 0.05$ ANOVA one-way). Compounds were orally administered at a dose of 100 μmol/kg. All the studies have been approved by the UFRJ ethic committee. In vitro studies for TNF-α were carried out in LPS-stimulated murine macrophages (100 ng/ml) (Gallily, JPET:283, 1997). Among seven derivatives evaluated in the writhing test (Coolier, Br J Pharmacol Chem:32, 1968) we emphasize the antinociceptive effect displayed by LASSBio-1474 and LASSBio-1470 which inhibited the writhing behavior in 50 and 30%*, respectively. The PPAR-α agonist fenofibrate inhibited by 30%*. In order to better characterize the antinociceptive and anti-inflammatory profile of the compounds, we performed the formalin-induced nociception and carrageenan-induced peritonitis tests (Tjolsen, Pain:51, 1992; Vinegar, Proc SEBM:143, 1973). LASSBio-1523 inhibited the neurogenic phase by 30% without interfering with inflammatory phase. LASSBio-331, LASSBio-1471 and LASSBio-1474 significantly inhibited the inflammatory phase in 72, 42 and 53%*, respectively. These compounds also inhibited the

leukocyte migration in peritonitis such as Rosiglitazone, a PPARγ agonist. Compounds were evaluated in TNF-α production given the ability of PPAR agonists modulate inflammatory cytokines. LASSBio-1473 and LASSBio-1474 (100 μM) inhibited the TNF-α in 83%*, being more effective than thalidomide (300 μM; 67% of inhibition), an anti-TNF-α drug. None of the compounds showed cytotoxicity. This study identified derivatives with anti-inflammatory and antinociceptive activities that can be useful in treating inflammatory conditions and chronic pain associated with diseases such as diabetes.
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Disclosure of interest: None declared.

P-370
PROSTANOIDS AND LIPOXYGENASE DERIVED MEDIATORS ARE INVOLVED IN NOCICEPTIVE EFFECTS OF SMALL PEPTIDES ISOLATED FROM SOCIAL WASP

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Hymenoptera venoms are complex mixtures of biochemically and pharmacologically active components such as biogenic amines, peptides and proteins. Some of these peptides are involved with the activation of different basophiles types, chemotaxis of polymorphonucleated leukocytes, smooth muscle contraction and neurotoxicity. Stinging accidents produce acute pain, local tissue damage and occasional deaths in large vertebrates, including man. The goal of this work is to evaluate the hemolytic, mast cell degranulation, chemotaxis, antinociceptive/nociceptive, and inflammatory/antiinflammatory effects of small peptides such as Protonectin, Protonectin (1-5)-OH and Protonectin (1-6), isolated from the social wasp *Agelaia pallipes pallipes* venom. Also, some of the mechanisms involved in nociceptive and inflammation phenomena were evaluated for these peptides. Hyperalgesia and edema were determined, after application of peptides, by electronic von Frey apparatus and a paquimeter. Results showed that Protonectin, Protonectin (1-5)-OH, and Protonectin (1-6) induced hemolytic, mast cell degranulation and chemotactic activity. Also, these peptides produced a hyperalgesic and edematogenic responses. Both phenomena were detected 2 h after peptides injection. In order to evaluate the role of prostanoids and the involvement of lipidic mediators in hyperalgesia induced by the peptides, indomethacin and zileuton were used. Results showed that indomethacin blocked protonectin-induced hiperalgesia and induced a decrease of the edematogenic response. Zileuton inhibited protonectin (1-5)-induced hyperalgesia and decreased oedema. On the other hand, zileuton and indomethacin did not interfere with these phenomena using Protonectin (1-6) peptide. These results indicate that Protonectin, Protonectin (1-5)-OH and Protonectin (1-6) peptides play fundamental roles in the wasp venoms contributing to inflammation and pain induced by insect venoms with different mechanisms.
Disclosure of interest: None declared.

P-371
DOWNREGULATION OF OXIDO-INFLAMMATORY CASCADE IN ALCOHOLIC NEUROPATHIC PAIN BY EPIGALLOCATECHIN-3-GALLATE

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Introduction: Alcoholic neuropathy is one of the devastating complications of long term alcohol consumption which involves decreased nociceptive threshold characterized by spontaneous burning pain, hyperalgesia and allodynia. Alcoholic neuropathy has been associated with behavioral alterations, increase in oxidative-nitrosative stress and pro-inflammatory cytokines.
Objectives: The present study was designed to explore the protective effect of epigallocatechin-3-gallate against alcoholic neuropathy in rats.
Methods: Adult male Wistar rats (150–200 g) were administered alcohol (10 g/kg, 35%, v/v) for 10 weeks and pain was measured by using Tail immersion test, Randall-Sellito test and von-Frey hair test at 6th, 8th and 10th week. After 10 weeks, rats were sacrificed under deep anesthesia and sciatic nerves were isolated for various biochemical and molecular estimations.
Results: Chronic alcohol (35%) treated rats developed neuropathy after 6 weeks, which was evident from decreased tail flick latency (thermal hyperalgesia), paw withdrawal threshold in Randall-Sellito test (mechanical hyperalgesia) and von-Frey hair test (mechanical allodynia) along with enhanced in oxidative-nitrosative stress and inflammatory mediators (TNFα, IL-1β and TGF-β1 levels). Co-administration of epigallocatechin-3-gallate significantly and dose-dependently prevented behavioral, biochemical and molecular changes associated with alcoholic neuropathy.
Conclusions: The current findings suggest the neuroprotective potential of epigallocatechin-3-gallate in attenuating the behavioral and biochemical alterations associated with alcoholic neuropathy through modulation of oxido-inflammatory cascade.
Disclosure of interest: None declared.

P-372
OPPOSITE ROLES OF HISTAMINE AND SEROTONIN IN FORMALIN-INDUCED RAT ARTICULAR NOCICEPTION

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Antihistamines have been shown to produce analgesia in some situations, but also hypernociception in experimental models, which may explain why they are not always effective analgesics in clinic situations. Herein, we investigated the effect of antihistamine on the articular incapacitation, edema, and plasma leakage (PL) induced by formalin in the rat knee joint. Low and high doses of the brain permeant H1 antihistamine promethazine increased and decreased the incapacitation, respectively. However, both non-permeant H1 antihistamines loratadine and cetirizine only increased this formalin effect. None of the treatments changed the edema and PL. Loratadine given locally with formalin increased incapacitation, but had no effect when administered in the contralateral knee. Systemic loratadine was also without effect when formalin was co-injected with sodium chromoglycate. Histamine (0.2 and 2 nmol/knee), and the selective H1 agonist 2-pyridylethylamine (0.05; 0.5 and 5 nmol/knee) decreased incapacitation with no change in the edema or PL. Cetirizine prevented the antinociceptive effect of the H1 agonist. Serotonin (1, 10 and 100 nmol/knee) administered alone did not cause incapacitation, but increase the edema and the highest dose changed the PL. When co-injected with formalin, serotonin only enhanced

incapacitation and the highest dose also increased the edema. NAN 190 (0.1 and 0.6 pmol/knee), cyproheptadine (0.16 and 0.5 pmol/knee), and ondansetron (0.26 pmol/knee) decreased the incapacitation without changing the edema or PL. These results suggest that in the rat knee joint the histamine H1 receptor seems to play an antinociceptive role, while serotonine is important for the formalin-induced effect.
Disclosure of interest: None declared.

P-373
ESTROGEN-RELATED MODULATION OF MENINGEAL MAST CELLS: A ROLE IN THE PATHOPHYSIOLOGY OF MENSTRUAL MIGRAINE

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Menstrual-related migraine headaches occur in about half of all women migraineurs. The mechanisms that play a role in mediating the increased propensity of women to develop migraine headaches during this hormonal phase is unknown, but the abrupt decline (withdrawal) in estrogen levels during the premenstrual phase has been suggested to play a role. Mast cells (MCs) play an important role in inflammation and are found throughout the intracranial meninges where they are thought to play a key role in migraine headache triggering. We postulated that changes in meningeal MC density could serve as one factor that modulates the propensity to develop migraine headache such as during menstrual migraines. We therefore examined whether changes in estrogen levels modulate the density of meningeal MCs and whether this effect increases the propensity of meningeal sensory neurons to become activated by common migraine triggers. In cycling female rats, meningeal MCs exhibited fluctuations in their density with increases noted during the stages of reduced estrogen levels of estrous and diestrus 1. An increase in meningeal MC density was also noted in ovariectomized (OVX) females subjected to an experimental estrogen repletion and withdrawal paradigm. In electrophysiological experiments, systemic administration of the migraine triggers nitroglycerin or CGRP resulted in enhanced activation of meningeal sensory neurons during diestrous 1 compared to the high estrogen stage of proestrous. Migraine triggers-evoked neuronal responses were also enhanced in OVX animals subjected to exogenous estrogen withdrawal paradigm when compared to untreated OVX rats. The MC stabilizing agent sodium cromolyn or depletion of meningeal MCs ameliorated the estrogen-related enhanced responses of meningeal sensory neurons. We propose that estrogen-related recruitment of MCs to the meninges play an important role in the pathophysiology of menstrual migraines.
Disclosure of interest: None declared.

P-374
EFFECTS OF CXCR2 AND TRPV1 SELECTIVE ANTAGONISTS IN THE MODEL OF HEMORRHAGIC CYSTITIS INDUCED BY CYCLOPHOSPHAMIDE IN RATS

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Hemorrhagic cystitis (HC) is a common side effect observed in patients under chemotherapy with cyclophosphamide (CYP). The urotoxic side effects of CYP are attributed to the metabolic compound acrolein and can be partially prevented by the uroprotector agent 2-mercaptoethene sulfate (Mesna). This study analyzed the anti-inflammatory and the antinociceptive effects of the selective CXCR2 and TRPV1 receptor antagonists—SB225002 and SB366791—in the rat model of CYP-induced HC. Male Wistar rats were used (200–220 g) and HC was induced by a single injection of CYP (200 mg/kg, ip). Breathing rate, closing of the eyes, and specific posture were scored at different time points after cystitis induction as nociception indexes. The mechanical hypernociception was measured with Von Frey filaments in the bladder area and in the rat paw. As inflammatory parameters, hemorrhage presence, edema formation, and bladder weight were determined macroscopically at 24 h after CYP administration. The neutrophil migration and inflammatory cytokines profile was assessed 4 h after cystitis induction by means of myeloperoxidase (MPO activity) and IL-1β and TNFα, respectively. As expected, Mesna treatment was able to reduce in a significant manner all the inflammatory and the nociceptive parameters induced by CYP. Of note, the pre-administration of SB225002 (1 mg/kg ip) and SB366791 (500 μg/kg ip) significantly attenuated the hemorrhage, the edema formation and the neutrophil recruitment, as well the increase in MPO activity and the elevation of IL-1β. Interestingly, the treatment with either SB225002 or SB366791 markedly reduced the bladder wet weight and the nociceptive responses. The present results indicate that CXCR2 and TRPV1 antagonists might represent important alternatives to prevent nociception following chemotherapy with CYP.
Disclosure of interest: None declared.

P-375
EFFECT OF INTRA-ARTICULAR MEN16132 AND DEXAMETHASONE ON MIA-ENDUCED OSTEOARTHRITIS PAIN IN RATS

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Background: Osteoarthritis (OA) is a degenerative joint disease causing pain and disability. Inflammatory mediators are responsible for synovitis and pain transmission, and contribute to the pathology development.
Aim: This study aimed to evaluate the effect of intra-articular administration of the kinin B2 receptor antagonist MEN16132 and dexamethasone on pain response in the experimental model of OA induced by monosodium iodoacetate (MIA) in rats.
Material and methods: MIA (1 mg) and 7 days later MEN16132 (1–3–10 μg), dexamethasone (10–30–100–300 μg) or their combination were injected in the right knee in a volume of 25 μl. Pain was measured with the incapacitance tester for the following 21 days.
Results: MIA was able to produce pain and to increase the levels of bradykinin and prostaglandin metabolite in synovial fluid. MEN16132 or dexamethasone caused a dose-dependent and long lasting analgesia with a maximum effect of about 60% inhibition of pain at the doses of 3–10 and 100–300 μg/25 μl, respectively. In this model MEN16132 was about 30-fold more potent than dexamethasone. The combination

of submaximal doses of MEN16132 (1 μg/25 μl) and dexamethasone (10–30 μg/25 μl), each producing a mean 20–25% inhibition of pain, caused analgesia of about 40–45%, ascribable to the sum of the respective antinociceptive effects.
Conclusion: Intra-articular combination of low doses of MEN16132 and dexamethasone induces an additive increase of analgesia that might allow to reduce the doses of corticosteroids for the treatment of pain and inflammation associated to diseases such as osteoarthritis.
Disclosure of interest: None declared.

Neuroinflammation: central and peripheral nervous systems

P-376
MMP-9 LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS: EMERGING EVIDENCE FOR NEUROINFLAMMATION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting the motor neurons of the spinal cord and brain. Degeneration of spinal motor neurons leads to muscular atrophy and weakness without accompanied sensory changes, whereas degeneration of motor neurons in the cerebral cortex leads to hyper-reflexia. Because of its rapid and progressive clinical course, ALS patients generally die within 2–5 years of onset. Matrix metallo proteinases (MMPs) are a relatively large family of more than 20 Zn^{2+}- containing, Ca^{2+}-requiring endopeptidases, characterized by their ability to digest components of the extracellular matrix. Excessive expression of MMPs may result in tissue damage. Therefore, precise control of the regulation of expression and activity of MMPs is important physiologically and pathologically. ALS patients were selected using the World Federation of Neurology (WFN) clinical criteria. Elisa kit for the estimation of MMP-9 was obtained from R&D Systems, Minneapolis, MN, USA and was measured as per the manufacturer's specifications. Standard curve was prepared as per the instructions and the MMP-9 levels were expressed as ng/ml. Results show that the neuroinflammatory marker, MMP-9 levels in the serum of ALS patients were significantly ($P < 0.01$) increased with respect to control subjects and also showed significant correlation ($R^2 = 0.9$) with the duration of the disease. Neuroinflammation can both be a cause, and a consequence of chronic oxidative stress which is produced by glutamate excitotoxicity. Cytokine-stimulated microglia generate copious amounts of reactive oxygen and reactive nitrogen species, creating a stress upon ambient neurons. Conversely, oxidants can stimulate pro-inflammatory gene transcription in glia, leading to various inflammatory reactions including production of MMP-9.
Disclosure of interest: None declared.

P-377
NAAG REDUCES NEUTROPHIL INFILTRATION AND EDEMA RESPONSE IN CARRAGEENAN-INDUCED ACUTE INFLAMMATION

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Introduction: *N*-Acetylaspartylglutamate (NAAG) is widely distributed peptide transmitter in the mammalian nervous system, activates mGlu3 receptors at presynaptic sites which results to inhibit glutamate release. Evidence suggests that glutamate receptors are involved in inflammatory response carried through the COX-2 up regulation. In present study we aimed to investigate the effect of NAAG (10 mg kg^{-1}) on carrageenan-induced acute inflammation.
Methods: Acute inflammation was induced by subplantar injection of carrageenan 1% (w/v), in the right hind paw of the rats. The animals received an intraperitoneal injection of NAAG 20 min before induction of inflammation. The severity of inflammation was assessed 4 h later using maximum paw edema volume, Area Under Curve(AUC), microscopic changes of inflamed paw, measurement of oxidative stress markers; myeloperoxidase(MPO), lipid peroxidation(MDA level), SOD and GPx activity in paw tissue.
Results and conclusion: NAAG reduced maximum inflammatory response and AUC by 52.56 and 58% ($P < 0.001$), respectively, compare to control. Microscopic studies indicated significant reduction in neutrophil infiltration. MPO activity in NAAG treated group was inhibited by 80.19% ($P < 0.01$) and MDA level was decreased by 88.13% ($P < 0.01$) compare to controls, but there were no significant reduction in SOD and GPx activity. The results, for the first time, suggest that NAAG may be involved in inhibiting peripheral inflammation and it is possible that exerts this effect by modulating systemic glutamate levels.
Keywords: *N*-acetylaspartylglutamate, inflammation, myeloperoxidase, carrageenan.
Disclosure of interest: None declared.

P-378
THE EFFECT OF NEUROPEPTIDES AND BICYCLIC MONOTERPENE DIOLS ON NITRIC OXIDE

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Nitric oxide (NO) is an important signaling messenger with a broad range of functions in both the central and peripheral nervous systems. In the skin, NO is involved in vasodilation, melanogenesis, and immune/inflammatory processes. NO is synthesized from L-arginine by 3 isoforms of NO synthase (NOS)—neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Keratinocytes constitutively express nNOS and can be induced to express iNOS by inflammatory cytokines. Preliminary studies using human immortalized keratinocytes (hTERT) demonstrates that iNOS is induced in a time- and dose-dependent manner by UVB and cytokines such as CGRP, substance P, and VIP. To extend these findings, we selected primary cortical neurons as a primary culture paradigm with cellular lineage distinct from keratinocytes. Expression of all NOS isoforms following neuropeptide treatment (24 vs. 48 h) was assessed by Western blot. Primary cortical neurons were treated in vitro with the nociceptive neurotransmitter substance P or bicyclic monoterpene diols (BMTd) reported to increase NO levels in neural crest-derived cells and compared to neurons that received brain-derived neurotrophic factor (BDNF, 100 ng/μl), a growth

factor known to increase nNOS. BMTd is known to increase nitric oxide levels in both melanoma and neuronal cells. Compared to BDNF control, 0.1–3 mM BMTd and 1–50 nM substance P selectively upregulated iNOS at the protein level while have less of an effect on nNOS or eNOS. Our results provide additional support for the involvement of iNOS upregulation in the development and testing of agents of interest, which may specifically modulate NO-related pathways.
Disclosure of interest: None declared.

P-379
NEUROTOXICITY CAUSED BY NEUROINFLAMMATION COULD BE MEDIATED BY GLIAL CELL-SECRETED PHOSPHOLIPASE A2 AND IS INHIBITED BY NOVEL COBALT-CONTAINING COMPOUNDS

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Activation of microglia and astrocytes contributes to neuroinflammation observed in a number of neurodegenerative disorders including Alzheimer's and Parkinson's diseases. Such chronically activated glial cells secrete an array of cytokines and inflammatory mediators, some of which may be directly toxic to neurons and could therefore exacerbate neurodegeneration. Identifying the neurotoxins released by stimulated glial cells and searching for novel compounds that could be used to inhibit the neurotoxic activity of glia were the goals of this study. We used immortalized monocytic and astrocytoma cell lines to model microglia and astrocytes respectively. In addition, we used primary human astrocytes. Lipopolysaccharide and interferon-gamma, two potent proinflammatory mediators, were used to induce glial cell activation, which was accompanied by secretion of substances that were toxic to neuronal cells. We demonstrated that incubation of supernatants with phospholipase A2 group IIA-specific immunosorbents diminished their toxicity. We also prepared a series of four-coordinate cobalt compounds bearing beta-ketoaminato ligands which varied in the number of –CF3 ligand substituents. We identified a cobalt complex that inhibited the neurotoxic activity of glial cells at low micromolar concentrations without affecting their viability. The anti-neurotoxic activity of this cobalt complex was reduced by specific inhibitors of mitogen-activated protein kinases (MAPK). The corresponding ligand that did not contain cobalt was not effective. Our data indicate that phospholipase A2 group IIA could be one of the toxins responsible for glial cell-induced neurotoxicity. We also identified a novel cobalt-containing compound that inhibited the neurotoxic activity of glial cells in a MAPK-dependent manner. Cobalt-containing metallocomplexes may have potential as anti-inflammatory agents beneficial in neurodegenerative diseases. Supported by NSERC and Jack Brown and family AD foundation.
Disclosure of interest: None declared.

P-380
RELATIONSHIP BETWEEN THE PATTERNS OF CYTOKINES SECRETED BY ACTIVATED MICROGLIA CELLS IN VITRO AND THE GENERATION OF PATHOGENIC TH17 CELLS

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Introduction: Currently microglial cells have received great attention within the immune responses, due to the fact that its activation by inflammatory cytokines can promote the infiltration and destruction of the central nervous system (CNS) specially in the case of multiple sclerosis (MS). Besides, the it has been shown that they are also capable of expressing suppressive molecules such as indoleamine-2,3-dioxygenase (IDO), able to suppress the proliferation of T cells. However, little is known about its true role in the pathogenesis of MS.
Aims: This study aims to better understand the relationship between the secretion of cytokines by activated microglial cells and their connection with the pathogenic Th17 cells.
Methods: C57Bl6 animals had their spinal cords and brains removed and their mononuclear cells extracted. Later, cells were stimulated or not with LPS or rm-IFN-g, for 24 h. IDO functional activity was also performed after co-cuture with MOG 35-55 specific 2D2 CD4 in the presence or absence of 1-methyl tryptophan, an IDO inhibitor.
Results: Our results showed that, except for the TLR-4 and TLR-2, all the other molecules showed a significant increase in its expression after stimulation with LPS, which was not observed after IFN-g, except for TGF-beta and socs3. TLR-4 was decreased after IFN-g.
Conclusions: Our data suggest that activation of microglial cells through the TLR-4 is able to induce the synthesis of a series of cytokines both pro-(IL-6, IL-23) as anti-inflammatory (IL-10, IL-27, TGF-b and IDO). The results obtained here shows the ability of inflammatory microglial cells to produce inflammatory cytokines which are important for th17 cells maintenance. On the other hand, it has also a regulatory role, due to its capacity to inhibit T CD4 specific response. In summary, our data show the importance of microglial cells during EAE, both as promoter or regulator of CNS inflammation.
Disclosure of interest: None declared.

P-381
CYCLOOXYGENASE-1 EXPRESSION IN ACTIVATED MICROGLIA/MACROPHAGES DURING NEUROINFLAMMATION

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Neuroinflammation occurs as a defensive response to pathogenic insult in brain and plays a crucial role in a variety of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases

(PD). Cyclooxygenase (COX), prostanoid-synthesizing enzyme, is well known to be involved in the regulation of inflammatory process. Two distinct COX isoforms, COX-1 and COX-2, are characterized as a constitutively expressed housekeeping enzyme and an inflammatory inducible enzyme, respectively. Recently, changes of COX-1 and COX-2 in the brain of patients with ischemic, traumatic brain injury and of several neurodegenerative diseases such as AD, PD and amyotrophic lateral sclerosis (ALS), have been reported, indicating that not only COX-2 but also COX-1 mediated neuroinflammation is a critical component in neuronal degeneration. In the present study, to determine the involvement of COX-1 in neuroinflammatory process, we performed positron emission tomography (PET) study with ^{11}C labeled ketoprofen-methyl ester (KTP-Me), its acid form rather specific to COX-1, during neuroinflammatory process of rats which were intracranially injected lipopolysaccharide (LPS) or quinolinic acid (QUIN). In addition, the cell type of COXs expression during neuroinflammation was determined by immunohistochemical studies. In the PET studies, both LPS and QUIN increased ^{11}C-KTP-Me accumulation in the inflamed hemisphere. It peaked at day 1 after the injury at which microglia/macrophages was activated with COX-1 but not with COX-2 expression. In addition, the increased accumulation of ^{11}C-KTP-Me was decreased rapidly from day 3 after the injury. This time-course of ^{11}C-KTP-Me was closely corresponded to that of changes in number of COX-1 expressing activated microglia/macrophages in the inflamed area. These results strongly indicate that COX-1 but not COX-2 expression in microglia/macrophages is critically involved in the neuroinflammatory process in the brain.
Disclosure of interest: None declared.

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22(*R*)-HYDROXYCHOLESTEROL INDUCES MAPK PHOSPHATASE-1 EXPRESSION BY HUMAN ANTIGEN R TRANSLOCATION IN CULTURED BRAIN ASTROCYTES

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Oxysterols are oxidized cholesterol metabolites generated enzymatically or non-enzymatically during bodily metabolic processes. They function as ligands for liver X receptor (LXR), thus perform important roles during lipid metabolism and transport. Recently, anti-inflammatory actions of them attracted the interests of many researchers, but the precise mechanism of actions are largely unknown. In the present study, we unraveled that 22(*R*)-hydroxycholestrol (HC), a family member of oxysterol, increased MAPK phosphatase (MKP)-1 induction in brain astrocytes. Transcript levels of MKP-1 showed peak levels in 3 h, but protein levels were sustained up to 12 h and more prominent, suggesting that MKP-1 expressions are regulated at post-transcriptional levels. Moreover, experiments using actinomycin D revealed that 22(*R*)-HC increased MKP-1 mRNA stability in a LXR receptor-independent manner. mRNA stability is regulated by RNA-binding proteins (RBPs), of which HuR is a representative RBP that increase mRNA stability. As expected, 22(*R*)-HC increased cytoplasmic translocation of HuR in immunohistochemistry and nuclear/cytoplasmic fractionation experiments. Since nucleus/cytoplasm shuttling of HuR is regulated by its phosphorylation on Ser residues in the hinge region, we first tested the involvement of PKC, using its pharmacologic inhibitors. Immunoprecipitation assays using antibodies against phospho-serine and HuR revealed that 22(*R*)-HC increased cytoplasmic translocation of phosphor-HuR, whereas nuclear phospho-HuR was decreased. Much remains to be clarified to reveal in-depth mechanisms underlying HuR translocation, our data show that 22(*R*)-HC-induced MKP-1 expressions are regulated at the post-transcriptional levels via HuR translocation. Considering the importance of MKP-1 during inflammatory responses, the post-transcriptional regulation of MKP-1 by 22(*R*)-HC suggest a novel target during post-transcription, which might be efficient because rapid modulation is possible.
Disclosure of interest: None declared.

P-383
INTERACTIONS BETWEEN DORSAL ROOT GANGLION NEURONS AND GLIAL CELLS IN VITRO

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Isolated dorsal root ganglion (DRG) cells are a valuable model for studying the neuronal sensitizing effects of inflammatory agents such as prostaglandin E2. Under in vivo conditions, the soma of each DRG neuron is closely enveloped by a sheath of satellite glial cells. Recent studies suggest that inhibiting glial cell communication may attenuate pain responses to nerve injury. When DRG cell cultures are prepared from adult rats, the non-neuronal (glial) cells make up the majority of cells. Purified cultures of these glial cells appeared to proliferate more slowly than those cultured with neurons, therefore the aim of this study was to determine if neurons regulated proliferation and activation of glial cells. Three groups of DRG cells were prepared: mixed DRG cell cultures (neurons were $31 \pm 3\%$ total cells); neuron-enriched cell cultures (neurons were $73 \pm 5\%$ total cells); and glial cell cultures (glial cells were $97 \pm 1\%$ total cells). After 6 days in culture, there was a significant increase in the number of glial cells (as %Day 1) only in the mixed DRG cell and neuron-enriched cell cultures ($P < 0.05$). Protein expression and viability (MTT assay) data was also significantly greater in the mixed DRG cell group compared with the glial cell preparation ($P < 0.05$). The intensity of GFAP-immunoreactivity increased from Day 1 to Day 7, with no obvious difference between the three cell groups. In conclusion, our results suggest that DRG glial cells proliferate faster in the presence of DRG neurons when assayed in vitro, which may reflect the increased glial cell proliferation reported in intact DRG following nerve damage. Taken together, these results suggest that either intact DRG neurons release factor/s to prevent glial cell proliferation, or damaged DRG neurons release factor/s to stimulate glial cell proliferation. This study was supported by a direct grant from The Chinese University of Hong Kong (CUHK2041584).
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P-384
ANTI-INFLAMMATORY ACTION OF PALMITOYLETHANOLAMIDE IN AN EXPERIMENTAL MICE MODEL OF SPINAL CORD TRAUMA

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Spinal cord injury (SCI) has a significant impact on quality of life, expectancy, and economic burden, with considerable costs associated with primary care and loss of income. The complex pathophysiology of SCI may explain the difficulty in finding a suitable therapy for limiting neuronal injury and promoting regeneration. Although innovative medical care, advances in pharmacotherapy have been limited. The aim of the present study was to carefully investigate molecular pathways and subtypes of glial cells involved in the protective effect of PEA on inflammatory reaction associated with an experimental model of SCI. The compression model induced by applying an aneurysm clip to the spinal cord in mice is closer to the human situation, since it replicates the persistence of cord compression. Spinal cord trauma was induced in mice by the application of vascular clips to the dura via a four-level T5-T8 laminectomy. Repeated PEA administration (10 mg/kg i.p., 6 and 12 h after SCI) significantly reduced the degree of the severity of spinal cord trauma through the reduction of mast cell infiltration and activation. Moreover, PEA treatment significantly reduced the activation of microglia and astrocytes expressing cannabinoid CB_2 receptor after SCI. Importantly, the protective effect of PEA involved changes in the expression of neurotrophic factors, and in spinal cord dopaminergic function. Our results enhance our understanding about mechanisms related to the anti-inflammatory property of the PEA suggesting that this N-acylethanolamine may represent a crucial therapeutic intervention both diminishing the immune/inflammatory response and promoting the initiation of neurotrophic substance after SCI.
Disclosure of interest: None declared.

P-385
GILZ INHIBITS INFLAMMATION CONSEQUENT UPON SPINAL CORD INJURY (SCI)

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Inflammation is involved in a large array of pathological conditions as a homeostatic response aimed to tissue repair and to defend organ functional integrity. Many signals and stimuli, including traumatic events, can be responsible of inflammatory process activation. An uncontrolled and excessive post-traumatic inflammatory reaction may play an important role in the secondary injury processes, which develop after spinal cord injury (SCI). Glucocorticoids (GC), among the most used drugs for SCI patients treatment, exert their anti-inflammatory action through the inhibition of lymphocyte activation/proliferation, production of proinflammatory cytokines and down-regulation of specific adhesion molecules resulting in modification of lymphocyte migration and redistribution. Using GILZ transgenic mice, in which GILZ is selectively over-expressed in the T-cell lineage, we show that GILZ antagonizes the development of SCI in mice further suggesting that GILZ is an anti-inflammatory molecule that mediates the effect of GC and represents a potential pharmacological target for immune responses modulation in inflammatory disorders such as SCI. In particular, leukocyte infiltration, NF-κB activation, pro-inflammatory cytokine production and tissue damage were significantly reduced in GILZ-TG mice. These results indicate GILZ acts as an anti-inflammatory molecule in experimental SCI. Results could suggest for new therapeutic approaches alternative to treatment with GC.
Disclosure of interest: None declared.

P-387
PARTICIPATION OF SCAVENGER RECEPTOR CLASS A AS A MODULATOR OF THE INFLAMMATORY RESPONSE OF ASTROCYTES

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Introduction: Scavenger receptor A (SR-A) is involved in recognition of endogenous molecules and pathogens, and inflammatory activation. It was considered to be expressed almost exclusively in myeloid cells. However, we described for the first time the presence of SR-A in astrocytes, which provide key functions for the modulation of the neuroinflammatory response.
Results: Astrocytes derived from SR-A$^{-/-}$ or SR-A$^{+/+}$ mice were stimulated with LPS or Polyinositic acid (Poly I, a SR-family-A ligand). In contrast to microglial cells, which showed little dependence on SR-A mediated activation, we demonstrated the participation of SR-A on the activation of NF-κB pathways, and the modulation of nitric oxide (NO) and IL-1β production by astrocytes. MAPKs (JNK, ERK1/2) signaling pathways were evaluated. The absence of SR-A had no effect on the activation of ERK1/2 or JNK induced by inflammatory stimulation. In contrast, NF-κB transcriptional factor was unable to translocate into the nucleus of SR-A$^{-/-}$ astrocytes, as it normally does in SR-A$^{+/+}$ astrocytes exposed to LPS. At the functional level, astrocytes from SR-A$^{-/-}$ mice exposed to proinflammatory ligands produced 50% less NO than SR-A$^{+/+}$ astrocytes, whereas NO production by microglia was similar for both genotypes. On the other hand, LPS or Poly I stimulated SR-A$^{-/-}$ astrocytes increased by 4-fold their production of IL-1β compared with SR-A$^{+/+}$ counterparts.
Discussion: Our results show that stimulation with proinflammatory molecules of astrocytes from SR-A$^{-/-}$ animals activated MAPK but not NF-κB signaling, resulting in higher levels of IL-1β but lower NO production compared with SR-A$^{+/+}$ astrocytes. Altogether, we propose that SR-A is involved in the modulation of the inflammatory response of astrocytes.
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Disclosure of interest: None declared.

P-388
MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF NEW INVERTEBRATE FACTORS INVOLVED IN THE SPECIFIC MICROGLIAL CELL RECRUITMENT

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In vertebrates microglial cells are a major glial cell element of the central nervous system (CNS). Reports in animal models underlined their involvement in various steps of neuron repair. The ability of nerve cells to survive damage is different across phyla. Higher vertebrates have a limited ability to regenerate efficiently their CNS. In contrast, some invertebrates as leeches can completely repair its CNS

after injury. From a specific leech nervous system EST library, we characterized two specific factors. First, *Hm*C1q (for *H. medicinalis*), homologous to vertebrate C1q, was detected in neurons and glial cells. In chemotaxis assays, leech microglial cells were shown to respond to human C1q. The effect was reduced when microglia was preincubated with signalling pathway inhibitors (pertussis toxin or wortmannin) or a specific anti-human gC1qR antibody suggesting the involvement of gC1qR in C1q-mediated migration. By using a recombinant HmC1q, the chemotactic effect was shown to be dependent on a specific C1q binding cell surface protein. Second, *Hm*IL-16, a homologous of interleukin-16 (IL-16) human active form was detected in leech nervous system and was involved in microglia recruitment. By using either an anti-human IL-16 antibody or anti-*Hm*IL-16 antibody, the cell migration was significantly reduced. In human, mature IL-16 might bind to CD4 (as receptor) to stimulate migration of immune cells. Interestingly *Hm*IL-16 was shown to promote human CD4 + T cell migration. Indeed an anti-human IL-16 antibody, an IL-16 antagonist peptide or the soluble CD4 highly reduced the chemotactic effect. Thus *Hm*IL-16 effect might be the result of binding to a protein homologous to human CD4. This study emphasizes the role of leech factors in the microglial cell recruitment known to be essential for neuron repair and, in invertebrates, highlights the presence of potential "cytokines" that are highly evolutionarily and functionally conserved in CNS.
Disclosure of interest: None declared.

P-389
MODULATION OF NEUROINFLAMMATORY ACTIVATION OF GLIAL CELLS BY TGFB1 IS MEDIATED BY THE INDUCTION OF MKP-1 EXPRESSION

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Rationale: Chronic neuroinflammation has been suggested as a driving force for Alzheimer's disease (AD). AD is a neurodegenerative disease characterized by amyloid-β (Aβ) deposition, neurofibrillary tangles, neuronal loss and glial cell (microglia and astrocytes) activation. Aβ causes the phosphorylation of MAPKs and activation of NFκB pathway inducing the expression of inflammatory cytokines and reactive species like nitric oxide (NO) that have been shown to accelerate neurodegeneration and contribute to progression of pathology. Negative regulation of MAPK signaling is exerted by MAPK phosphatases (MKPs), being MKP-1 the archetype of the family. TGFβ1 is an inflammation modulator whose level is increased in AD. We have previously shown that TGFβ1 has a neuroprotective role against Aβ toxicity, and recently it has been demonstrated that its signaling pathway is impaired in AD. These findings suggest that TGFβ1 has a key role in the pathogenesis of AD, although the molecular mechanism underlying its neuroprotective effect has not been completely elucidated. Here we studied the potential role of MKP-1 in the modulatory effect of TGFβ1 on cytokine- and Aβ-stimulated glial cells. Results: Using rat primary glial cultures, we found that TGFβ1 prevented the NO production in a time- and dosis-dependent manner, being most effective after 48 h of pretreatment. This was correlated with an increase in MKP-1 expression, prevention of MAPKs activation and attenuation of NFκB p65 translocation to nucleus. Suppression of MKP-1 expression by siRNA altered the modulation of inflammatory response by TGFβ1.
Conclusions: We show for the first time that TGFβ1 induce the expression of MKP-1 in glial cells, inhibiting MAPK signaling and preventing cytokine- and Aβ-mediated neuroinflammation. These findings reveal mechanisms of the protective actions of TGFβ1 and point out TGFβ1 signaling and MKP-1 as new targets for the treatment of AD.
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P-391
ACTIVATION OF NF-KB INDUCED BY REACTIVE GLIA OR TNFALPHA IN ALPHA SYNUCLEIN OVEREXPRESSED SH-SY5Y CELLS

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α-Synuclein (α-syn) is a small soluble protein expressed primarily at presynaptic terminals in the central nervous system and its dysfunction is related with several neurodegenerative diseases, including Parkinson's disease (PD). The microglial neuroinflammatory responses affect the onset and progression of PD. Nuclear-kB factor (NF-kB) is a transcription factor involved in cellular defense response, apoptosis and regulation of inflammatory genes expression. The aim of the present study is to evaluate the influence of reactive glia cells in the α-syn expression and NF-kB activation in SH-SY5Y cells infected with lentiviral (LV) vectors expressing wild-type (WT) or A30P α-syn. Glial cells were prepared from 1 day Wistar pups. Cortices were isolate and cells were dispersed with 0.25% trypsin. Cells were placed onto flasks and maintained in DMEM supplemented with 10% Fetal Bovine Serum. We challenged SH-SY5Y cells transduced with LV system with conditioned medium (CM) from reactive glia previously treated with LPS (1 mg/ml). The expression of α-syn was evaluated by Western blot assay. Cell nuclear extract was used to run EMSA to measure NF-kB binding activity. Exposures of SH-SY5Y cells transduced with LV vectors expressing wild-type or mutant (A30P) α-syn protein to CM from treated glia cells produced a pronounced increase in α-syn expression when compared to control treated cells. In addition, CM induced an increase in NF-kB activation in nuclear extracts from SH-SY5Y cells infected with LV vectors expressing WT protein when compared to control group. Our results suggest a putative interaction between α-syn expression and NF-kB signaling in response to an inflammatory stimulus. Therefore, α-syn can be converted to a toxic compound in the presence of a chronic inflammatory condition which might be linked to NF-kB signaling cascade.
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Disclosure of interest: None declared.

P-392
INFLAMMATORY PARAMETERS AND NEUROTROPHIC FACTORS IN BIPOLAR DISORDER: SEARCHING TRAIT AND STATE BIOMARKERS

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Introduction: There is a growing body of evidence indicating the involvement of immune and/or inflammatory dysfunction and impaired neuroplasticity in the physiopathology of bipolar disorder (BD), a condition that affects millions worldwide.
Objective: The aim of this study was to evaluate circulating levels of a series of cytokines, chemokines and neurotrophic factors in BD patients in comparison with healthy controls. Secondary analysis was performed to evaluate predictors of BD diagnosis and state (euthymia or mania).
Methods: Fifty-three BD patients (19 in euthymia and 34 in mania) and 30 healthy controls were comprehensively assessed with a structured psychiatric interview (Mini-International Neuropsychiatric Interview) and psychopathological scales (Young Mania Rating Scale and Hamilton Depression Rating Scale). Plasma levels of BDNF, GDNF, NGF, TNF-alpha, sTNFR1, sTNFR2, CCL2, CCL3, CCL11, CCL24, CCXL8 and CCXL10 were determined by ELISA.
Results: There were no significant differences in the proportion of gender, age and years of schooling between controls and BD patients. In univariate analysis, BD patients presented increased levels of BDNF, GDNF, sTNFR1, CCL24 and CXCL10, but decreased levels of NGF, CCL3 and CXCL8 in comparison with controls. In multivariate analysis, elevated plasma levels of sTNFR1 ($p < 0.001$) and BDNF ($p < 0.001$), and reduced plasma levels of CCL3 ($p = 0.003$) were associated with BD diagnosis. The logistic regression model presented good adjustment in Hosmer–Lemeshow test and a predictor power of 85.7%. No variable was associated with mania state in the multivariate analysis.
Conclusion: The present study corroborates the view of involvement of inflammatory and neurotrophic mediators in BD physiopathology. Inflammatory mediators might be regarded as promising biomarkers of BD trait.
Disclosure of interest: None declared.

P-393
TNF ALPHA AND NO INFLUENCES ON PILOCARPINE-INDUCED SEIZURES

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Introduction: The inflammatory process is involved in neurological disorders such as Parkinson's and Alzheimer's disease. The control and knowledge of these changes can help treat these and other diseases. The aim of this study was to investigate the neuroinflammatory dependence on pilocarpine-induced seizure model in mice.
Methods: Mice (28–32 g) were pretreated with thalidomide (25, 100 and 200 mg/kg, ip), an inhibitor of the release of TNFα, Infliximab (2, 5 and 10 mg/kg, sc), an antibody anti-TNFα, Aminoguanidine (25 and 100 mg/kg, ip), an inhibitor of inducible nitric oxide synthase, or ODQ (2.5, 10 and 20 mg/kg, ip), an inhibitor of guanylate cyclase, and 30 min after was induced the seizure with pilocarpine (400 mg/kg, sc). In the behavioral assessment was observed following parameters: Latency for a first seizure and latency to death. For statistical analysis, we used analysis of variance (ANOVA) and Student–Newman–Keuls as post hoc. This work was approved by the local ethics committee (protocol number 41/10).
Results and discussion: Our results show the attenuation of the behavioral parameters (increased latency to first seizure and decreased of mortality) at higher doses of the groups pretreated with thalidomide and infliximab. In groups pretreated with the higher dose of Aminoguanidine and in a dose of 10 mg/kg of ODQ showed the increased latency and survival of animals death.
Conclusion: We demonstrate the neuroprotective activity of thalidomide and infliximab, which may be related to the decreased amount of TNF, while, aminoguanidine and ODQ to increase the survival rate can be related to an aggressor effect of nitric oxide via cGMP.
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Disclosure of interest: None declared.

Cardiovascular diseases

P-394
EXACERBATION OF THROMBOTIC EVENTS BY DIESEL EXHAUST PARTICLE IN MURINE MODEL OF HYPERTENSION

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The adverse cardiovascular events associated with acute exposure to particulate air pollution are exacerbated among individuals with hypertension. This study was undertaken to determine the cardiovascular effect of diesel exhaust (DEP; 15 μg/mouse) on TO mice made hypertensive by implanting osmotic minipump infusing angiotensin II or vehicle (control). On day 13, the animals were intratracheally instilled with DEP (15 μg/mouse) or saline. 24 h later, pulmonary exposure to DEP insignificantly decreased systolic blood pressure (SBP) in normotensive (NT) mice. However, it caused a significant decrease of SBP in hypertensive (HT) mice (−16 %, $p < 0.005$). The PaO_2 was decreased, and $PaCO_2$ was increased in DEP-treated HT mice compared to NT mice treated with DEP ($P < 0.05$). Number of circulating platelets was significantly increased in DEP-treated HT versus saline-treated HT and DEP-treated NT mice. Moreover, DEP exposure of NT mice showed a prothrombotic effect in pial arterioles compared to saline-treated NT mice ($P < 0.05$). Interestingly, in DEP-treated HT mice the prothrombotic events were significantly aggravated compared to saline-treated NT and DEP-treated HT mice. The direct addition of DEP (0.1–1 μg/ml) to untreated mouse blood significantly induced in vitro platelet aggregation in a dose-dependent fashion, and these effects were potentiated in blood of HT mice. DEP caused the activation of intravascular coagulation which was confirmed by a shortening of activated partial thromboplastin time (aPTT) and the prothrombin time (PT) following in vitro exposure to DEP (0.25–1 μg/ml). The effect of DEP on aPTT was potentiated in the plasma of HTmice. It can be concluded that the thrombotic events caused by DEP are exacerbated in HT mice. Our findings, therefore, provide a plausible explanation for the cardiovascular morbidity and mortality accompanying urban air pollution.
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P-395
IS PENTRAXIN 3 A MORE SENSITIVE MARKER OF INFLAMMATORY RESPONSE TO PERCUTANEOUS CORONARY INTERVENTION THAN HIGH-SENSITIVITY C-REACTIVE PROTEIN

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Background: C-reactive protein (CRP) and pentraxin 3 (PTX3) are members of a highly conserved pentraxin superfamily. CRP is synthesized in the liver and may represent a systemic response to local inflammation, whereas PTX3 is synthesized locally at the inflammatory sites by endothelial and smooth muscle cells or by monocytes/macrophages and may represent a rapid marker for local inflammation at sites of vessel injury. We compared plasma high-sensitivity CRP (hsCRP) and PTX3 concentrations after bare-metal stent (BMS) and drug-eluting stent (DES) implantation. 33 patients with stable coronary artery disease who underwent percutaneous coronary intervention (PCI) were divided into two groups: 1–16 patients (BMS group); and 2–17 patients (DES group). All patients were scheduled for an elective, 6-month clinical follow-up. Major adverse cardiovascular events (MACEs) (death, re-hospitalization for acute coronary syndrome, target vessel revascularization) were assessed.
Results: Baseline demographics and clinical characteristics were similar in both groups. Patients after BMS implantation had a higher median PTX3 concentration 1.19 ng/mL (0.72–1.33) compared to patients after DES implantation 0.85 ng/mL (0.55–1.02, $p = 0.04$). Median hsCRP concentrations were similar in both groups: 0.9 mg/L (0.78–1.1) versus 0.89 mg/L (0.7–1.0), respectively ($p = 0.7$). Six out of 33 patients had MACEs during follow-up. The cut-off value to predict MACEs for PTX-3 was >1.291 ng/mL (AUC 0.907, sensitivity 83%, specificity 96%, $p < 0.0001$) and for hsCRP was >0.9 mg/L (AUC 0.92, sensitivity 83%, specificity 81%, $p < 0.0001$).
Conclusions: DES showed significantly lower plasma PTX3 levels after PCI compared with BMS. hsCRP levels were comparable between the two groups. Thus, PTX3 may be a more sensitive marker of local inflammatory response due to vessel injury than hsCRP. Both PTX3 and hsCRP had a high predictive value for MACE during follow-up.
Disclosure of interest: None declared.

P-397
SPARC PROTECTS AGAINST ADVERSE INFLAMMATION IN VIRAL MYOCARDITIS

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Acute viral myocarditis is a serious condition, which can cause sudden cardiac death in previously healthy adults. Despite intense research to unravel the mechanisms underlying the severe inflammation by cardiotropic viruses, treatment for acute myocarditis is primarily supportive, and specific therapies targeting inflammation are needed. Our laboratory has previously demonstrated a role for extracellular matrix proteins such as Osteonectin (SPARC) and Thrombospondin-2 in the wound healing process after myocardial infarction and in pressure overload. Here we investigate the role of matricellular protein SPARC in the coxsackie B3 (CVB3) murine model of viral myocarditis. Exposure to the CVB3 virus resulted in a biphasic response of SPARC cardiac transcript levels, with a twofold decrease after 24 h and a fourfold increase at 7 days. This was also observed in protein levels at 5 and 11 days post infection, respectively. Immunohistochemical analysis showed low SPARC expression under normal conditions with an abundant presence after 11 days of viral presence. Immunofluorescence studies confirmed SPARC expression on CD-3 positive lymphocytes and V-CAM positive cells, but not on a-smooth muscle actin-positive capillaries, CD45-positive leukocytes or F4/80-positive macrophages. SPARC WT and null mice were exposed to the CVB3 virus, and an increased mortality was observed in the SPARC null mice, associated with increased cardiac inflammation. To further demonstrate the role of SPARC in myocarditis, adenoviral over-expression of SPARC improved the mortality in both SPARC WT and null mice. These data demonstrate the protective role of SPARC in viral myocarditis and suggesting that SPARC may be a novel target for the treatment of viral myocarditis.
Disclosure of interest: None declared.

P-398
LYMPHOCYTE SUBPOPULATIONS IN LYMPH NODES AND PERIPHERAL BLOOD. A COMPARISON BETWEEN PATIENTS WITH STABLE ANGINA AND ACUTE CORONARY SYNDROME

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Atherosclerosis is characterized by a chronic immune response. An increased Th1 activity has been associated with plaque instability and risk of acute cardiac events. Lymphocyte analyses in blood are widely used to evaluate the immune status in patients with coronary artery disease. We investigated whether thoracic lymph nodes (LN) from patients with stable angina (SA) and acute coronary syndrome (ACS) revealed changes in the immune response that were not detectable in peripheral blood (PB). PB and thoracic LN were collected in 13 patients with SA and 14 patients with ACS during coronary by-pass surgery. PB and LN lymphocytes were assessed by flow cytometry using antibodies to CD3, CD4, CD8, CD19, CD16/56, CD25, Foxp3, CD69, HLA-DR, IL-18R and CCR4. LN showed a cell composition that was different from PB with a higher proportion of B cells, lower proportions of CD8+ T cells and NK cells and a twofold higher CD4/CD8 ratio. CD4+CD69+ cells were markedly enriched in LN [47(39–55)%] versus [1.1(0.9–1.7)%] while Th1 (CD4+IL−18R+) cells were more frequent in PB [5.9(3.7–7.3)%] versus [2.4(1.6–4.8)%]. Foxp3+ regulatory T cells were somewhat increased in LN [4.5(2.7–6.0)%] compared with PB [2.0(1.3–2.7)%]. Tregs were inversely related to Th1 cells in PB ($p < 0.05$), but not in LN. There were no significant differences in either PB or LN lymphocyte distributions between ACS and SA patients except for NK cells that were

reduced in ACS PB [13(10–19) vs. 20(12–23)%]. To conclude, there were several diversities in lymphocyte composition between PB and LN. However, the few changes observed in PB of ACS patients compared with SA patients were not mirrored in thoracic LN indicating that LN analyses in patients with advanced coronary artery disease do not add any information to PB analyses.
Disclosure of interest: None declared.

P-400
CHARACTERIZATION OF ENDOTHELIAL DYSFUNCTION IN LIGATURE-INDUCED PERIODONTITIS IN RATS

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Introduction: Epidemiological studies have shown that periodontitis, an inflammatory disease from an infection of the oral cavity, is a risk factor for cardiovascular diseases (CVDs). Endothelial dysfunction is the initial step in the development of CVD. The aim of the present study was evaluated endothelial function in an experimental model of periodontitis.
Methods: Procedures were approved by ethic committee. A ligature was placed around rat mandibular and maxilar molars to induce periodontitis. A simulated procedure was performed in the Sham (Sh) group. Seven, 14 or 28 days after procedure, effects of intravenous injection of acetylcholine (Ach), sodium nitroprusside (SNP) or phenylephrine (Phe) on blood pressure (BP) were evaluated. Acetylcholine and phenylephrine response were also assessed in constantly perfused mesenteric bed. The blood was obtained for analyze serum lipid profile.
Results and discussion: There was no difference in BP reduction to SNP in either group and in none of the evaluated times. Fourteen days after ligation, Ach response in BP was significantly reduced in ligature-induced periodontitis when compared tom Sh group (Ach 30 nmol/kg: Sh 50.6 ± 4.1; Ligature 31.5 ± 3.3 mmHg, $n = 8$; $p < 0.05$; expressed as change in MAP). There was no difference in Ach response in other times (7 and 28 days). In mesenteric beds removed 28 days after ligature procedure, the response to Phe was significantly increased while the response to Ach was decreased. Seven and 14 days after ligature no changes in response to Ach or Phe were observed. The ligature resulted in plasma LDL increase 14 days after procedure. Plasma cholesterol, HDL, and triglycerides were not changed in any time. Periodontitis-induced endothelial dysfunction and increase in LDL levels may be involved in the early development of CVD.
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Disclosure of interest: None declared.

P-401
THE FREE RADICAL SCAVENGER CAN EFFECT ON THE INFLAMMATORY BIOMARKERS IN ACUTE ISCHEMIC STROKE FOLLOWING THROMBOLYSIS WITH RT-PA

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Background: It has been reported that the better outcome after rt-PA therapy could relate to the reduction of inflammatory response. The rt-PA administration was reported to decrease the activation of astrocytes after ischemic insult. In this study, we explored whether the inflammatory response is altered by rt-PA therapy and whether the free radical scavenger administration can reduce the inflammatory response providing better outcome or not.
Methods: Acute ischemic stroke patients were consecutively enrolled in this study ($n = 57$, 72 ± 12.2 yo). Thrombolysis with rt-PA intravenous administration was performed on 11 patients. IL-6, oxidative LDL (OxLDL) and the radical productivity were measured as inflammatory biomarkers at 3 time points: before medication, 4 and 24 h following medication. All patients were classified into cardiogenic embolism ($n = 39$), atherothrombotic infarction ($n = 18$) and lacunar infarction ($n = 10$) by clinical examinations and records. Neurological alteration was assessed by comparing NIH Stroke Scale (NIHSS) on admission and at 1 month later.
Results: IL-6, OxLDL and the radical productivity were significantly increased at 4 and 24 h following medication in rt-PA administration compared with conventional therapy ($p < 0.02$). Whereas, patients with both rt-PA and free radical scavenger administration showed 32% reduction in IL-6 and 18% reduction in OxLDL at 24 h following medication as compared to patients with only rt-PA. Moreover, rt-PA and free radical scavenger treated patients exhibited better outcome compared with only rt-PA treated patients (NIHSS reduction: 9.4 and 1.7, respectively), although NIHSS was not different on admission (15.3 and 19.8, respectively).
Conclusion: The free radical scavenger could decrease the inflammatory biomarkers which were amplified by rt-PA administration. Reduction of inflammation may provide better outcome.
Disclosure of interest: None declared.

P-402
THROMBIN INDUCES OSTEOPROTEGERIN PRODUCTION VIA PHOSPHATIDYLINOSITOL 3-KINASE PATHWAY IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS

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Background and objectives: Thrombin is a procoagulant and proinflammatory molecule and is also a potential mediator of bone resorption and vascular healing. Osteoprotegerin (OPG) is a key molecule that binds to the receptor activator of the nuclear factor-kB (NF-kB) ligand (RANKL), and it inhibits osteoclast differentiation. In addition, OPG protects against vascular calcification. This study aimed to evaluate the biological effects of thrombin on OPG production in human dermal microvascular endothelial cells (HuDMECs).

Materials and methods: Cells were treated with various concentrations (0, 0.1, 2.4, and 4.8 U/mL) of thrombin for 24 h. Thrombin-induced OPG production in HuDMECs was determined using an enzyme-linked immunosorbent assay (ELISA). RANKL expression in HuDMECs was estimated using a reverse-transcriptase polymerase chain reaction (RT-PCR). The influence of thrombin on OPG production in HuDMECs and the thrombin signaling pathway were investigated using inhibitors.
Results: Thrombin induced OPG production in the HuDMECs in a dose-dependent manner. RANKL mRNA expression was also upregulated by thrombin. The phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002, and the Src kinase inhibitor, PP1, exerted an inhibitory effect on thrombin-induced OPG expression. In addition, thrombin-induced OPG production was inhibited by the protease-activated receptor (PAR)-1 antagonist.
Conclusion: Thrombin induces OPG expression in HuDMECs, possibly through PAR-1. Thrombin-induced OPG production is regulated by the PI3K and Src pathways. This finding suggests that thrombin-activated HuDMECs may contribute to bone metabolism and vascular healing, partly through the OPG/RANKL system. This research was supported by a Grant-in-Aid for Scientific Research, Japan (22592097).
Disclosure of interest: None declared.

P-403
ROLE OF MAST CELLS ON THE CINC-2 PRODUCTION, NEUTROPHILS MIGRATION AND BONE RESORPTION IN SHR ANIMALS SUBMITTED TO PERIODONTAL DISEASE

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The aim of this study was to evaluate the levels of CINC-2 and neutrophil migration (NM) in the gingival tissue (GT) from spontaneously hypertensive rats (SHR) and Wistar (W) (180–200 g) (Protocol 52/06) submitted to periodontal disease (PD) and the role of mast cells (MAST) on this process. Thus, a group of animals was depleted of MAST by treatment with compound 48/80 (48/80), i.p. After 5 days, the animals were performed ligation of the first molars counterparts with silk thread to induce PD. The induction was also performed in animals of the groups that did not receive treatment with the 48/80 and after 7 and 14 days, GT were collected for the determination of CINC-2 production by ELISA, the MN was evaluated by the level of myeloperoxidase (MPO), and the mandibles were dissected and radiographed to evaluate the level of the bone resorption (BR) using Digora software. We observed that the levels of CINC-2 tends to increase in W and SHR animals in the presence of PD. CINC-2 is increased in the absence of MAST in SHR with 7 days after induction of PD and tends to increase in animals W without MAST 7 days after induction of PD. In animals with 14 days of induction, the SHR with PD showed a significant increase over its control. NM observed into the GT in SHR with PD is higher when compared with W animals with PD, both 7 and 14 days after disease induction. This migration was decreased in MAST-depleted SHR animals but not in animals W. The induction of PD, since we observed higher BR in all animals with PD. The presence of MAST increases BR only in SHR 14 days after induction of PD. In conclusion, we observed that the presence of MAST enhances the inflammatory process in SHR and BR with PD but not in animals W. In contrast, the presence of MAST decreases the production of CINC-2 suggesting that this chemokine is not related to bone resorption and neither MN in this model (SHR animals).
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Disclosure of interest: None declared.

P-404
S100A12 SUPPRESSES PROINFLAMMATORY BUT NOT PROTHROMBOTIC FUNCTIONS OF SERUM AMYLOID A ON MONOCYTES/ MACROPHAGES

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Serum amyloid A (SAA) is an acute-phase reactant. Levels are elevated in coronary artery disease (CAD) patients and deposits are localised in macrophages in atheroma. SAA is an important mediator of inflammation and coagulation. It increases monocyte/macrophage cytokine production and tissue factor (TF). Similarly, the calcium-binding protein, S100A12, is expressed in foam cells and macrophages in vessels with atherosclerotic lesions, and circulating levels are elevated in CAD patients. S100A12 has pro-inflammatory properties, but our recent studies indicate its pleiotropic activities. Immunoaffinity experiments using human serum showed that SAA bound S100A12, suggesting functional interaction. Western blotting of sera from CAD patients confirmed low (10 kDa) and high mass SAA (200 kDa) and S100A12 (200 kDa), suggesting complex formation with components of HDL. We assessed whether S100A12 influenced the pro-inflammatory and pro-thrombotic functions of SAA in vitro. S100A12 did not directly induce cytokines in human PBMC, monocytes/macrophages but significantly suppressed SAA-induced cytokine production. IL-8 staining suggested that the secretory pathway was unaffected by S100A12. Conversely, S100A12 did not suppress LPS-induced cytokines or alter SAA-induced TF pro-coagulant activity, indicating selective effects. S100A12 and SAA share similar putative receptors, but competition studies indicated that this was unlikely. SAA activates the NFκB and MAPK pathways. S100A12 suppressed SAA-induced phosphorylation of ERK1/2; no effects on NFκB, p38 and JNK MAPK pathways were observed. S100A12 and SAA mediate inflammatory processes. Since S100A12 downregulated SAA-induced cytokine production, and bound SAA in plasma, it may protect circulating monocytes from activation when SAA and S100A12 levels are elevated during inflammatory episodes. However, S100A12 is unlikely to moderate the reported SAA-induced prothrombotic tendencies of circulating monocytes in patients with CAD.
Disclosure of interest: None declared.

P-405
PREVALENCE AND ASSOCIATED FACTORS FOR ASYMPTOMATIC PULMONARY ARTERIAL HYPERTENSION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Pulmonary arterial hypertension (PAH) is one of the rare but devastating complications in connective tissue diseases. PAH in systemic lupus erythematosus (SLE) is underrecognized in the clinical field. Early identification of PAH is important and can alter the natural course.
Objectives: To estimate point prevalence of asymptomatic PAH and determine the associated factors for PAH in a cohort of SLE patients
Methods: A prospective cross-sectional study of 101 patients with SLE were recruited in a single tertiary centre. Transthoracic echocardiography was performed to estimate the pulmonary arterial pressures. PAH was defined as resting systolic pulmonary artery pressure (sPAP) $\geq$ 40 mmHg, in the absence of left heart disease. The patients were also evaluated with respect to their clinical and serologic features, and disease treatment.
Results: PAH was identified in eight patients (7.9%) who had no history of interstitial lung disease. Fourteen patients (13.8%) had sPAP of 30–40 mmHg. Higher SLEDAI score (13.14 $\pm$ 3.4 vs. 5.8 $\pm$ 0.5, p = 0.001) and lower positivity of anti-Ro/SSA antibody (12.5 vs. 66.3%, p = 0.03) was observed in patients with PAH than patients without PAH. Of note, serum uric acid (UA) was significantly higher in patients with PAH than in those without PAH (7.8 $\pm$ 2.2 vs. 5.4 $\pm$ 1.9 mg/dl, p = 0.002). However, no significant differences in disease duration, Raynaud's phenomenon, antiphospholipid antibodies were found between these groups. In multivariate analysis, higher serum UA level was independently associated with the presence of PAH. Interestingly, serum UA level correlated significantly with plasma B-type natriuretic peptide level (r = 0.453, p < 0.001).
Conclusions: Clinically silent PAH is not uncommon in SLE patients. Serum UA level may be useful as a surrogate marker for screening of PAH.
Disclosure of interest: None declared.

P-406
ZERO FLOW GLOBAL ISCHEMIA-INDUCED INJURIES IN RAT HEART ARE ATTENUATED BY NATURAL HONEY

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Objectives: In the present study, effects of preischemic administration of natural honey on cardiac arrhythmias and myocardial infarction size during zero flow global ischemia were investigated in isolated rat heart.
Materials and methods: The isolated hearts were subjected to 30 min zero flow global ischemia followed by 120 min reperfusion then perfused by a modified drug free Krebs-Henseleit solution throughout the experiment (control) or the solution containing 0.25, 0.5, 1 and 2% of natural honey for 15 min before induction of global ischemia (treated groups), respectively. Cardiac arrhythmias were determined based on the Lambeth conventions and the infarct size was measured by computerized planimetry.
Results: Myocardial infarction size was 55.8 $\pm$ 7.8% in the control group, while preischemic perfusion of honey (0.25, 0.5, 1 and 2%) reduced it to 39.3 $\pm$ 11, 30.6 $\pm$ 5.5 ($P < 0.01$), 17.9 $\pm$ 5.6 ($P < 0.001$) and 8.7 $\pm$ 1.1% ($P < 0.001$), respectively. A direct linear correlation between honey concentrations and infarction size reduction was observed ($R^2 = 0.9948$). In addition, total number of ventricular ectopic beats were significantly decreased by all used concentrations of honey ($P < 0.05$) during reperfusion time. Honey (0.25, 0.5 and 1%) also lowered incidence of irreversible ventricular fibrillation ($P < 0.05$). Moreover, number and duration of ventricular tachycardia were reduced in all honey treated groups.
Conclusion: Preischemic administration of natural honey before zero flow global ischemia can protect isolated rat heart against ischemia/reperfusion injuries as reduction of infarction size and arrhythmias. Maybe, antioxidant and free radical scavenging activities of honey, reduction of necrotized tissue and providing energy sources may involve in these cardioprotective effects of honey.
Keywords: Natural honey, global ischemia, preischemic administration, arrhythmia, isolated rat heart.
Disclosure of interest: None declared.

P-407
CHANGES IN SERUM GROWTH FACTORS CONCENTRATIONS AFTER STEM CELLS TRANSPLANTATION IN A RAT MODEL OF MYOCARDIAL INFARCTION

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There are some data on beneficial effects of mesenchymal stem cells (MSCs) for the myocardial infarction. One mechanism is ability of MSCs to secrete cytokines. It is important assess changes of growth factors in serum after therapy.
Purpose: Assess TGF β, VEGF-C and GM-CSF concentrations in the serum of rats after postinfarct intramyocardial injection of mesenchymal stem cells.
Materials and methods: The experiment were conducted on 32 adult male Wistar rats. Myocardial infarction was simulated by the cryodestruction of the left ventricular anterior wall. Mesenchymal stem cells were derived from rat bone marrow and injected into myocardium 21 days after cryoinjury. In the control group, normal saline was infused. The blood levels of TGF β, VEGF-C and GM-CSF were measured at 3, 7 and 14 days.
Results: The mean serum TGF β in group with MSCs was 40.53 $\pm$ 7.9 ng/ml on day 3, and this significant ($p < 0.05$) increase a compared with control group (15.99 $\pm$ 6.9 ng/ml). The maximum TGF β concentration observed on day 7 after transplantation (62.35 $\pm$ 33.02 ng/ml). On day 14 concentration of TGF β made up 20.8 $\pm$ 10.02 ng/ml. The maximum VEGF-C concentration in the group with MSCs was noted on day 3 after transplantation 132.2 $\pm$ 18.94 pg/ml. By 7 days VEGF-C levels reduced to 72.88 $\pm$ 29.48 pg/ml. There was an increase the content of VEGF-C by day 14. No statistically significant differences between control and groups with MSCs were observed. GM-CSF concentration was 14.05 $\pm$ 6.08 pg/ml and there were no significant changes on 7 and 14 days. No statistically significant differences between control and treated groups were observed.
Conclusions: Introduction of mesenchymal cells into the infarcted myocardium stimulated increase TGF β in the peripheral blood of rats. MSCs do not demonstrate a significant effect on the level of VEGF-C and GM-CSF in the peripheral blood.
Disclosure of interest: None declared.

P-408
RELATIONSHIP BETWEEN INFLAMMATION AND VITAMIN D IN ALGERIAN PATIENTS WITH HYPERTENSION

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Introduction: Hypertension is a multifactorial trait resulting from the combined influence of environmental and genetic determinants. It remains a major risk factor for cardiovascular diseases and is therefore one of the most important causes of premature morbidity and mortality in developed and developing countries. Very recent studies have focused on the relationship between inflammation and arterial stiffness in essential hypertension. Furthermore, according to the latest research, Vitamin D deficiency may increase the risk of developing hypertension. Because of lingering uncertainty of these associations, we examined in this study, the link between serum concentration of vitamin D and inflammation in blood samples of Algerian patients with hypertension. The Inflammation status was evaluated with serums levels of ferritin and c-reactive protein.
Patients and methods: The study was conducted on 53 Algerian patients suffering from hypertension: 34 women and 19 men. The middle age was: 49 $\pm$ 7.5 years. For each patient, we measured serum levels of: CRPus and Ferritin by immunoturbidimetric assay on integra 400. Vitamin D by electrochemiluminescence technology on elecsys 2010. Cholesterol, LDL cholesterol, HDL cholesterol and triglycerides by enzymatic colorimetric method on integra 400.
Results: In our cohort of hypertensive patients, we noted a significant elevation of CRP levels on the one hand and low levels of vitamin D on the other. The correlation coefficient between CRP and vitamin D was ($r = 0.39$, $p < 0.0005$). A positive correlation was found between CRP and LDL cholesterol ($r = 0.13$, $p < 0.001$) but not with other lipid parameters. And no correlation was observed between ferritin and vitamin D in both sexes.
Conclusion: In conclusion, our work reveal the existence of a close association between low serum levels of vitamin D and inflammation assessed by CRP levels and not by ferritin levels in hypertensive patients in Algeria.
Disclosure of interest: None declared.

P-409
P16INK4A-DEFICIENCY PROMOTES MACROPHAGES FROM CLASSICAL TO ALTERNATIVE ACTIVATION

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The *CDKN2A* locus, which contains the tumor suppressor gene $p16^{INK4a}$, is associated with an increased risk of age-related inflammatory diseases, such as cardiovascular disease and type 2 diabetes, in which macrophages play a crucial role. Monocytes can polarize towards classically (CAMΦ) or alternatively (AAMΦ) activated macrophages. However, the molecular mechanisms underlying the acquisition of these phenotypes are not well defined. Here, we show that $p16^{INK4a}$-deficiency ($p16^{-/-}$) skews macrophages towards an alternative phenotype. Transcriptome analysis revealed that $p16^{-/-}$ bone marrow-derived macrophages (BMDM) exhibit a phenotype resembling alternatively activated macrophages. In line with this observation, $p16^{-/-}$ BMDM displayed a decreased response to classically polarizing IFNg and LPS and an increased sensitivity to alternatively polarizing IL-4. Furthermore, mice transplanted with $p16^{-/-}$ bone marrow displayed higher hepatic AAMΦ marker expression levels upon *Schistosoma mansoni* infection, an in vivo model of AAMΦ phenotype-skewing. Surprisingly, $p16^{-/-}$ BMDM did not display increased IL-4-induced STAT6 signaling, but rather a decrease in STAT1 and IKKa,b phosphorylation, which correlated with higher levels of inhibitory acetylation of STAT1 and IKKa,b and with decreased phosphorylation of JAK2. These findings identify $p16^{INK4a}$ as a modulator of macrophage activation and polarization via the JAK-STAT pathway with possible roles in inflammatory diseases.
Disclosure of interest: None declared.

P-411
EVIDENCE THAT THE CGRP RECEPTOR FAMILY GENE EXPRESSION IS ALTERED IN ATHEROSCLEROSIS

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Background: The CGRP family of peptides, signal via unique receptors that comprise of a G-protein-linked calcitonin-like receptor (CLR) with a single transmembrane protein receptor associated modifying protein (RAMP) that forms 3 distinct types, RAMP1, RAMP2 and RAMP3. Of these, CLR linked with RAMP1 leads to a high affinity CGRP receptor, CLR with RAMP2 or RAMP3 leads to ADM receptors (McLatchie et al., 1999). We and others have good evidence that ADM mediates vascular protection through the CLR-RAMP2 receptor (Tam et al., 2006; Liang et al., 2009).
Material and methods: RAMP1, 2 & 3, CLR, ADM and CGRP gene expressions were monitored in human carotid endarterectomy specimens in stable and unstable lesions (Collot-Teixeira et al., 2009). Total RNA was extracted from unstable ($n = 15$) and stable ($n = 12$) atherosclerotic lesions, retrotranscribed and gene expression was assayed using quantitative real time PCR. Gene expression data normalisation was carried out against the geometric mean of three reference genes (18S, GAPDH and PPIA) and analysed using the DDCt method. Statistical analyses were performed using unpaired t-test with Welch's correction.
Results: Results show a significant decrease of RAMP1 gene expression and increase in RAMP2, CLR and ADM gene expressions in "unstable" atherosclerotic lesions compared to "stable" parts of the lesions. RAMP3 and CGRP did not show any change in expression.
Conclusion: CGRP is a potent vasodilator with vascular protective properties and structurally related to the vascular protective peptide ADM, with which it shares a unique family of receptors. This study suggests that the CGRP receptor pathway may be downregulated as the atherosclerotic plaque develops, but conversely the ADM pathway may be upregulated. The switch in the signalling activities of this peptide family as the plaque develops is of potential interest to understanding possibly lack of adverse effects for CGRP antagonists, currently under development for migraine.
Disclosure of interest: None declared.

P-412
ANTI-INFLAMMATORY AND CLINICAL EFFICACY OF NONSELECTIVE CYCLOOXYGENASE INHIBITOR IN PATIENTS WITH ACUTE CORONARY SYNDROMES PRESENTED WITHOUT ST-SEGMENT ELEVATION, NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN ACUTE CORONARY SYNDROME (PLEATRIAL)

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Introduction: NSAIDs are the most frequently prescribed drugs in the world. There are a lot of controversial information published during recent years about NSAID cardiosafety. It is still unclear do NSAIDs develop cardioprotective or negative effects in acute coronary patients. Aim of the study was to investigate safety and efficacy of lornoxicam, nonselective COX-inhibitor, in patients with acute coronary syndrome (NSTEACS) and to evaluate the influence of lornoxicam on the inflammatory markers.
Methods: Overall 85 patients with NSTEACS were randomized to the prospective single-blind trial to receive lornoxicam (8/12 mg/day) plus aspirin (100 mg) or aspirin alone for 15 days. Primary end points were: cardiovascular death, non-fatal myocardial infarct, nonfatal stroke, unstable angina.
Results: According to the 6 months follow-up there were a significant reduction of primary end points in patients receiving lornoxicam compared with placebo (8.5 vs. 33.3%, OR = 0.18, P = 0.006). There was also significantly lower unstable angina event rate in lornoxicam group (4.2 vs. 18.1%, OR = 0.20, P = 0.04). CRP levels in the lornoxicam group (n = 47) were significantly lower at 15 days compared to placebo and base line levels [from 19.3 to 7.62 mg/l in lornoxicam group (60.5%) vs. from 41.1 to 26.1 mg/l in placebo group (36.5%), $p < 0.01$]. There were also nonsignificant lower IL-6 levels at 15 days in the lornoxicam group [from 15.3 to 5.0 pg/ml in lornoxicam group (67.4%) vs. 18.4 to 9.02 pg/ml in placebo group (50%), $p < 0.15$]. The anti-inflammatory IL-10 levels were significantly higher at 15 days in lornoxicam group compared with placebo ($p = 0.04$). No adverse complications associated with the lornoxicam treatment were observed.
Conclusion: Lornoxicam plus aspirin was associated with significant reductions in adverse outcomes in acute coronary syndrome patients without ST-segment elevation. Additional larger trials are required to confirm the findings of this study.
Disclosure of interest: None declared.

P-413
THE EFFECTS OF NKT CELLS ON ATHEROGENESIS IN PORPHYROMONAS GINGIVALIS INFECTION MODEL

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Objectives: Atherosclerosis is an inflammatory disease which is accelerated by chronic infection such as periodontitis. NKT cells have been shown to accelerate atherosclerosis in mouse model and human. NKT cells can recognize lipid antigens derived from bacterial pathogens presented by CD1d, and have a potent ability to produce IFN-γ, a potentially pro-atherogenic cytokine. The aim of the present study is to elucidate the role of NKT cells in infection-related atherogenic response in mouse model.
Materials and methods: CD1d−/− mice with C57BL/6 background and C57BL/6 mice which were administered α-galactosylceramide (αGalCer) were orally infected with *Porphyromonas gingivalis*, a representative pathogen of periodontitis, 10 times at 3-day intervals. After completion of infection alveolar bone loss and atherogenesis-related gene expression in liver and artery were examined.
Results: CD1d−/− mice demonstrated less alveolar bone resorption whereas αGalCer -administered mice showed increased resorption compared with C57BL/6 mice. Serum IL-6 level was increased in both CD1d−/− and αGalCer-administered mice. In liver, gene expressions of IFN-γ, IL-4 and TNF-α were down-regulated in CD1d−/− mice, whereas up-regulated in αGalCer-administered mice. In artery, CCL2 and early growth response 1 gene expression was down-regulated and natriuretic peptide precursor B was up-regulated in CD1d−/− mice compared to wild type mice, however, opposite tendency was seen in αGalCer-administered mice.
Conclusion: In accelerating process of atherogenesis by chronic periodontal infection, NKT cells play a role as effectors which promote inflammatory response in liver and artery.
Disclosure of interest: None declared.

P-414
TARGETING OF C-KIT+ HEMATOPOIETIC PROGENITOR CELLS PREVENTS HYPOXIC PULMONARY HYPERTENSION

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Hematopoietic c-kit+ progenitor cells may contribute to pulmonary vascular remodeling and pulmonary hypertension. Stromal derived factor-1 (SDF-1/CXCL12) and its receptors CXCR4 and CXCR7 have been shown to be critical for homing and mobilization of hematopoietic c-kit+ progenitor cells in the perivascular niche. We administered AMD3100, a CXCR4 antagonist, and CCX771, a CXCR7 antagonist, to chronic hypoxia exposed mice in order to study the role of c-kit+ progenitor cells in pulmonary hypertension. CXCL12, CXCR4 and CXCR7 protein expression, hemodynamic parameters, right ventricular mass, extent of vascular remodeling and perivascular progenitor cell accumulation were studied. Chronic hypoxia-exposed mice showed increased total lung tissue expression of CXCR4, CXCR7 and CXCL12 after development of pulmonary hypertension. This was associated with significantly increased right ventricular systolic pressure, and evidence of right ventricular hypertrophy, vascular remodeling and perivascular c-kit+/sca-1+ progenitor cell accumulation. CCX771 administration did not abrogate these effects. In contrast, administration of AMD3100, whether alone or combined with CCX771, prevented vascular remodeling, pulmonary hypertension and perivascular accumulation of c-kit+/sca-1+ progenitor cells, with a synergistic effect of these agents. This study offers important pathophysiological insights into the role of

hematopoietic c-kit+ progenitors in hypoxia-induced vascular remodeling and may have therapeutic implications for pulmonary hypertension.
Disclosure of interest: None declared.

P-415
EFAVIRENZ INDUCES HUMAN LEUKOCYTE ACCUMULATION THROUGH MAC-1 AND GP150.95 ACTIVATION

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Background: Highly active antiretroviral therapy (HAART) has been linked to the development of cardiovascular diseases such as myocardial infarction and atherosclerosis. Efavirenz (EFV) is one of the most widely used antiretroviral agents and, thus, cannot be ruled out as a causal agent of these side effects. The present study was designed to assess the role of clinically relevant doses of EFV on human leukocyte-endothelial cell interactions in vitro, which are a hallmark of the early stages of these pathological states.
Methods: The interaction between human leukocytes-peripheral blood polymorphonuclear (PMN) and mononuclear (PBMC) cells-and human umbilical vein endothelial cells (HUVEC) was analysed using the parallel plate flow chamber system. Leukocyte β_2 integrin and endothelial expression of adhesion molecules was evaluated by flow cytometry. A one-way ANOVA followed by Newman–Keuls post hoc test were performed and statistical significance was set at $^*p < 0.01$ (vs. vehicle).
Results: Clinical concentrations of EFV induced a significant dose-dependent increase in PMN rolling (veh: 57.5 ± 4.8, 15 μM: 129.0 ± 10.3*) and adhesion (veh: 10.8 ± 1.4, 15 μM: 25.4 ± 1.6*), and in PBMC rolling (veh: 56.3 ± 5.8, 15 μM: 99.5 ± 4.4*) and adhesion (veh: 10.4 ± 1.8, 15 μM: 24.3 ± 1.8*). When adhesion molecules were analysed, EFV was found to increase Mac-1 and gp150.95 expression in both neutrophils and monocytes, despite producing no effect on the expression of E-selectin, ICAM-1 or VCAM-1. Blocking antibodies against Mac-1, gp150.95 or their ligand ICAM-1 prevented the rolling and adhesion of PMN and PBMC induced by EFV.
Conclusions: EFV induces significant human leukocyte–endothelial cell interactions at clinically relevant doses and promotes the activation of Mac-1 and gp150.95. This leukocyte activation may be responsible for the leukocyte recruitment that leads to the vascular damage associated with atherosclerosis and myocardial infarction observed in HIV patients treated with HAART.
Disclosure of interest: None declared.

P-416
IFN-GAMMA PLAYS A PROTECTIVE ROLE IN PRESSURE OVERLOAD-INDUCED CARDIAC HYPERTROPHY

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The ubiquitin-proteasome system (UPS) mediates the main proteolytic pathway of targeted protein degradation in the cell. UPS dysfunction has been observed in animal models of pressure-overload cardiac hypertrophy and failure. UPS dysfunction has also been implicated in failing human hearts. Both clinical observation and some animal experiments have suggested that proteasome functional insufficiency may play an important role in the development of congestive heart failure. It has been well elucidated that IFN-γ results in replacement of the constitutive catalytic subunits β1, β2, and β5 with the immunosubunits β1i, β2i, and β5i, respectively. The different proteasome complexes, such as the regular proteasome and immunoproteasome, exhibit different catalytic efficiencies. These catalytic alternations in the immunoproteasome produce well-defined effects on the antigen processing for major histocompatibility complex class I. However, a precise function of immunoproteasome in a pressure overload-induced heart remodeling has not been identified. To address this, we examined mouse model of pressure overload cardiac hypertrophy induced by transverse aortic banding (TAB) using Balb/c mice (WT) and IFN-γ deficient mice. Three weeks after TAB, substantial hypertrophic changes of the hearts were observed in WT mice with mild fibrotic changes of the left ventricle. On the other hand, IFN-γ deficient mice exhibited more significant hypertrophic change with exaggerated fibrosis in the hearts, compared with WT mice. After TAB, both mRNA and protein levels of immunosubunit β1i (LMP2) significantly increased in left ventricles of WT mice but not in those of IFN-γ deficient mice. Furthermore, more significant polyubiquitinated peptides accumulated in the hearts of IFN-γ deficient mice, compared with WT mice. These observations suggest that IFN-γ plays a protective role in pressure overload-induced cardiac hypertrophy though the induction of immunoproteasome activation.
Disclosure of interest: None declared.

P-417
MAJOR DIFFERENCES AMONG PROTEOMES OF MACRO-VASCULAR AND MICRO-VASCULAR ENDOTHELIAL CELLS: 2D-DIGE APPROACH

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Rationale: Progress on the isolation and culture of various endothelial cells (EC) has allowed comparison of their biochemical and physiologic properties. However, very few studies compared the proteomes of EC from different sources.
Objective: To compare proteomes of macro-vascular and micro-vascular EC.
Methods: Proteomes of human umbilical vein EC (HUVEC) and 2 sources of micro-vascular EC, human pulmonary (HMVEC-P) and dermal micro-vascular EC (HMVEC-D) from healthy caucasian donors (4 in each group) were compared using two-dimension differential in gel electrophoresis (2D-DIGE) at pH ranges of 3-11 and 4-7 and mass spectrometry.
Results: Among the protein spots detected in pH 4–7 and in pH 3–11 gels, we identified 17 and 64 proteins which were differentially

expressed between HUVEC and HMVEC-P and between HUVEC and HMVEC-D with a ratio ≥ 2 and a T-test ≤ 0.01. Between HMVEC-P and HMVEC-D, 6 and 3 different proteins were differentially expressed in pH 4–7 and in pH 3–11 gels respectively with a ratio ≥ 2 and a T-test ≤ 0.01. Among identified proteins are cytoskeleton proteins and enzymes implicated in glycolysis. Ingenuity software analysis interestingly showed that numerous proteins overexpressed in HMVEC-D compared to HUVEC are implicated in the retinoic acid pathway.
Conclusion: Major differences were observed between proteomes of macro-vascular and micro-vascular EC. Some of the differentially expressed proteins might be of great importance in the immune recognition of EC antigens by IgG from patients with systemic sclerosis.
Disclosure of interest: None declared.

P-418
SERUM AMYLOID A STIMULATES THE RELEASE OF ENDOTHELIAL MICROPARTICLES, WHICH REFLECT EARLY PROCOAGULANT AND LATE PROINFLAMMATORY RESPONSES OF HUMAN CORONARY ENDOTHELIAL CELLS

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Background: Elevated serum amyloid A (SAA), a systemic inflammatory marker, strongly associates with increased cardiovascular (CV) risk, endothelial dysfunction, future CV events and worse coronary artery disease (CAD) prognosis. Increased plasma endothelial microparticles (EMPs) are biomarkers of endothelial dysfunction in CV diseases, associate with high-risk angiographic lesions and predict future CV events in CAD.
Aim: To investigate the role of SAA in EMP generation in cultured primary human coronary artery endothelial cells (HCAEC) and determine whether EMPs could reflect SAA-induced procoagulant and/or proinflammatory responses in HCAEC.
Methods: HCAEC were stimulated with human recombinant SAA (1,000 nM; 4, 8, 16, 24 h). EMPs were isolated from culture supernatants by differential centrifugation. Flow cytometry was used to measure EMP number, Annexin V binding, CD31, CD62E and tissue factor (TF) surface exposure. Gene expression (TF, CD62E, IL-6, IL-8) was measured by Real-Time PCR; TF activity by Actichrome TF Activity Assay, IL-6 and IL-8 levels by ELISA.
Results: SAA-stimulated HCAEC released significantly increased numbers of Annexin V+ and CD31+ EMPs. Time-dependent rise in SAA-induced Annexin V+ EMPs highly correlated with IL-6 and IL-8 levels ($p < 0.01$). CD62E antigen was expressed only on SAA-induced EMPs and reflected time-dependent changes in CD62E mRNA levels, indicating cell activation. TF mRNA expression, TF surface exposure and activity in HCAEC peaked at 4 h following SAA stimulation and were accompanied by TF surface exposure and activity on EMPs. Later during SAA stimulation TF expression and activity progressively decreased in SAA-induced HCAEC and EMPs, suggesting tight TF regulation.
Conclusion: SAA induces the release of EMPs from primary HCAEC. SAA-induced EMPs reflected early procoagulant and late proinflammatory HCAEC responses and may serve as surrogate biomarkers and/or mediators of SAA-induced changes of endothelial function in CV diseases.
Disclosure of interest: None declared.

P-419
HEME MODULATES SMC PROLIFERATION VIA NADPH OXIDASE ACTIVATION: COUNTER-REGULATORY ROLE FOR HO-1 SYSTEM

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Cardiovascular diseases represent the major cause of mortality in western countries. Among these conditions, atherosclerosis is the most prominent one. A hallmark of atherosclerosis is the atheromatous plaque formation, characterized by different disturbances, like vascular smooth muscle cell (VSMC) accumulation. Under the effect of different pathological stimuli, VSMC are able to migrate and proliferate, contributing to the fibrous cap formation. Notably, these events occur mainly in sinuous vessels, and are associated to turbulent blood flow, what may lead to hemolysis and consequent free heme accumulation. Our group has characterized heme as a proinflammatory molecule that mediates its effect via NADPH oxidase complex activation and reactive oxygen species (ROS) production. In this work we aim to elucidate the putative role of free heme in VSMC physiology and the molecular mechanisms underlying the affected processes. We observed that free heme induces VSMC migration and proliferation in a ROS-dependent manner. Heme-induced ROS production relies on NADPH oxidase activation, once diphenyleneiodonium (DPI), a NADPH oxidase inhibitor completely abolished this effect, and also inhibited heme chemotactic and proliferate effect. Additionally, heme activates proliferation-relationed and redox-sensitive signaling pathway, such as MAP kinases and NFkB. It was also observed a critical crosstalk between NADPH oxidase and heme oxygenase (HO-1) system. Heme induces HO-1 expression, and the pretreatment of VSMC with HO inhibitors potentiated heme proliferative effect. In summary we show that free heme induces VSMC proliferation via NADPH oxidase activation, which is elegantly counter-regulated by HO-1 activity, and the depiction of the signaling events underlying this process may lead to the development of novel and more effective therapeutic interventions in cardiovascular diseases (Financial Support: FAPERJ, CAPES, CNPq).
Disclosure of interest: None declared.

P-420
MANAGEMENT OF INFLAMMATORY STATE IN HYPERCHOLESTEROLEMIC PATIENTS WITH ATORVASTATIN TREATMENT

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Statins which inhibit the enzyme HMG CoA reductase are used in the treatment of hypercholesterolemia. These HMG-CoA reductase inhibitors have antiinflammatory properties, but their effect on neopterin which are produced by interferon-g stimulated macrophages largely unknown. Also chitotriosidase and myeloperoxidase (MPO) are produced during the inflammatory process in pro-atherogenic state within the vasculature. In this regard we aimed to search effects of atorvastatin terapies on inflammation which induce cardiovascular disease risk in hypercholesterolemic patients.
Material and methods: The study included 30 patients who were diagnosed as hypercholesterolemic (0 month) (>200 mg/dl). All patients were treated by atorvastatin (10 mg/day) for 3 months (3 months). Control group was composed of healthy individuals ($n = 30$). Bloods samples were taken before and 3 months after treatment. Neopterin levels were measured by HPLC method, MPO activity was measured by spectrophotometric method and chitotriosidase activity were measured by fluorometric method in both groups.
Results: Chitotriosidase activity was higher in hypercholesterolemic patients compared to control. (37.8 ± 12.3 vs. 21.5 ± 8.4 nmol/L, $p < 0.001$). Chitotriosidase activity was decreased after 3 months in atorvastatin treatment in hypercholesterolemic patients (32.3 ± 10.6 vs. 37.8 ± 12.3 nmol/L, $p < 0.05$). Both MPO and neopterin levels decreased after 3 months atorvastatin therapy in hypercholesterolemic patients ($p < 0.01$ U/L for both).
Conclusions: Atorvastatin therapy is effective therapeutic strategy in decreasing inflammation which increases the incidence of cardio vascular diseases in hypercholesterolemic patients beyond decreasing serum cholesterol levels.
Disclosure of interest: None declared.

P-421
ANTIINFLAMMATORY CONSEQUENCES OF ATORVASTATIN AND GLITAZONE THERAPIES ON HYPERCHOLESTEROLEMIC RATS

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Introduction: Hypercholesterolemia usually occurs prior to the onset of atherosclerosis and renders tissue more susceptible to inflammation. In this process some markers of activated macrophages like chitotriosidase and myeloperoxidase are released into circulation. In this regard we aimed to assess the antiinflammatory effects of atorvastatin and glitazone using an in vivo hypercholesterolemic rat model.
Material and methods: The control group (group I) fed basal diet. All other animals fed with hypercholesterolemic diet which contains 2% (w/w) cholesterol and 1% cholic acid in basal diet for 8 weeks. The hypercholesterolemic group (group II) was continued taking hypercholesterolemic diet while the group III, IV and V, VI, VII, VIII, IX received 1 mg rosiglitazone, 4 mg rosiglitazone, 10 mg atorvastatin, 1 mg rosiglitazone plus 10 mg atorvastatin, 4 mg rosiglitazone plus 10 mg atorvastatin, 3 mg pioglitazone and 3 mg pioglitazone plus 10 mg atorvastatin, respectively, by oral gavage in addition to hypercholesterolemic diet for 3 weeks. The plasma concentrations of Myeloperoxidase were measured by spectrophotometric method. Chitotriosidase enzyme activity was measured by fluorometric method.
Results: Serum Myeloperoxidase levels of control group, hypercholesterolemic group and group III, IV, V, VI, VII, VIII, IX were 80 ± 40^a, 440 ± 180, 290 ± 70, 200 ± 40^a, 290 ± 60, 190 ± 50^a, 170 ± 40^a, 102 ± 20^a, 104 ± 30^a mU/L, respectively. Also serum chitotriosidase levels of these groups were 5.97 ± 1.48^a, 16.25 ± 2.5, 9.67 ± 1.68^a, 7.20 ± 1.55^a, 8.57 ± 1.20^a, 8.74 ± 1.26^a, 8.24 ± 2.63^a, 9.41 ± 1.33^a, 8.61 ± 1.58^a nmol/mL, respectively.[a]Significant different from hypercholesterolemic group $p < 0.05$.
Conclusions: Glitazones decreased inflammatory process but combined atorvastatin and glitazone therapy was more effective on inflammation. Also pioglitazon is better than rosiglitazone for decreasing inflammation. Glitazones may be strategical target for decreasing inflammation in hypercholesterolemia.
Disclosure of interest: G. Akalin Ciftci Other: Unıversity Grant, I. Erdogan: none declared, A. Musmul: none declared, O. Alatas: none declared.

P-422
ACCUMULATION OF CD56+CD8+T CELLS IN PATIENTS WITH CORONARY ARTERY DISEASE: HIGH PRODUCTION OF IFN-GAMMA BUT DIFFERENTIAL EXPRESSION OF ANNEXIN 1

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Atherosclerosis is considered a Th1-dominated inflammatory disease, but there is a growing interest for the role of $CD8^+$T cells. The cytotoxicity of $CD8^+$T cells has been reported to be influenced by CD56. We hypothesized that the distribution and cytokine-producing capacity of $CD56^+CD8^+$T cells was altered in patients with coronary artery disease compared with healthy individuals. We also examined if the expression of Annexin 1 in $CD56^+CD8^+$T cells was related to a Type 1 response. Blood was collected from 30 patients with acute coronary syndrome (ACS), 34 patients with stable angina (SA) and 36 healthy controls. Blood sampling was repeated in patients at 3 and 12 months. T cells were analyzed by flow cytometry using antibodies to CD3, CD8 and CD56. Whole blood from 9 ACS, 14 SA and 10 controls was stimulated ex vivo and intracellular staining for IFN-γ, TNF, IL-10, IL-13 or IL-17, as well as double-staining for IFN-γ and Annexin 1 was determined by flow cytometry. Data are given as mean (SD). Total numbers of $CD56^+CD8^+$T cells were similar in ACS and SA but higher compared with controls, 184 (160) versus 109 (83) cells/µl, $p = 0.003$. The proportions of $CD56^+CD8^+$T cells were expanded in patients, 30 (19) versus 23 (11)%, $p = 0.05$, not changing over time. Upon mitogenic stimulation, the majority of $CD56^+CD8^+$T cells produced IFN-γ and TNF, but no IL-10, IL-13 or IL-17, with no difference between ACS, SA and controls. After stimulation, an increased expression of Annexin1 was seen in IFN-γ^+ cells, $p < 0.001$. However in ACS, the Annexin 1 MFI expression was markedly lower compared with SA and controls, 45735 (8381), 65581 (9369), and 70806 (20372), $p = 0.001$. In both ACS and SA patients, accumulations of peripheral $CD56^+CD8^+$T cells seem to be a constitutive phenomenon independent of clinical manifestation. The cells are high producers of IFN-γ and TNF which may indicate an increased Type 1 activity in the patients. The differential expression of Annexin 1 highlights its potential role in ACS.
Disclosure of interest: None declared.

P-423
ENDOTHELIN-1 RELEASE FROM HUMAN EMBRYONIC STEM CELL DERIVED-ENDOTHELIAL CELLS (HESC-EC): COMPARISONS WITH HUMAN ENDOTHELIAL CELLS

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Human embryonic stem cell-derived endothelial cells (hESC-EC) are being investigated as research tools and cell therapy for vascular inflammation. It is known that hESC or hESC-EC do not respond to LPS [1]. Thus, the full extent of how these hESC-EC function is not known. Endothelial cells release the vasoconstrictor hormone endothelin-1 (ET-1), elevated levels of which are associated with vascular inflammation. In the current study we have compared the ability of unstimulated human endothelial cells from umbilical vein (HUVEC), human aorta (HAEC) and the microvasculature of the lung (HMVEC) with hESC-EC to release ET-1. ET-1 is produced by endothelial cells under basal culture conditions, but can be increased further by agonists including thrombin. hESC-EC were derived as described previously [1]. All cells were cultured for 24 h in endothelial cell specific medium (Lonza-EGM2). ET-1 release in conditioned media was measured by ELISA. For hESC-EC and HUVEC, experiments were run in parallel and cell number was assessed by nuclear staining with DAPI and automated cell counting. HAEC and HMVEC were run in separate experiments but under similar conditions. HUVEC (215.2 $\pm$ 70.0 pg/ml), HAEC (248.7 $\pm$ 14.2 pg/ml) and HMVEC (339.8 $\pm$ 19.5 pg/ml) all released detectable levels of ET-1 (data are mean $\pm$ SEM). However, hESC-EC failed to release detectable levels of ET-1 in this time period (<42 pg/ml). There was no significant difference ($p > 0.05$; Student's *t* test) in cell number between HUVEC (151.5 $\pm$ 30.7 cells/field) and hESC-EC (256.0 $\pm$ 82.5 cells/field). Compared with other types of human endothelial cells, hESC-EC released relatively low levels of ET-1. As ET-1 can contribute to cardiovascular disease this could represent a benefit of hESC-EC over native endothelium in a damaged organ. However, the findings remain preliminary and a detailed examination of the ET-1 pathway in these cells remains the subject of investigation. [1] PLoS One. 2010;5:e10501.
Disclosure of interest: None declared.

P-425
LYMPHOTOXIN-ALPHA GENE THR26ASN POLYMORPHISM AND ACUTE MYOCARDIAL INFARCTION

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Vascular inflammation plays an important role in the development of myocardial infarction. Lymphotoxin-alpha (LTA), a member of the tumor necrosis factor family, is a cytokine produced by lymphocytes that mediates a wide variety of inflammatory, immunostimulatory, and antiviral responses. Acute myocardial infarction (AMI) is the clinical manifestation of the chronic development of coronary artery atheroma, with the final process of plaque rupture and coronary thrombosis. The aim of this study sought to assess the association of LTA Thr26Asn polymorphism with AMI. One hundred and sixty eight AMI patients were compared to 169 healthy controls. The Frequencies of the LTA Thr/Thr, Thr/Asn, and Asn Asn genotypes among AMI patients were 45.8, 46.4, and 7.8%, respectively, and in controls were 43.1, 45, and 10.9%, respectively. There was no significant difference in the distribution of LTA Thr26Asn genotypes or in the allelic frequencies between AMI patients and controls ($P = 0.098$). The frequency of Asn/Asn genotype not increased with the number of stenoses in coronary vessels in AMI patients. In conclusions, the LTA gene is not associated with susceptibility to AMI and not influences the severity.
Disclosure of interest: None declared.

P-426
ACTIVATION OF MONOCYTES AND CYTOKINE PRODUCTION IN PATIENTS WITH PERIPHERAL ATHEROSCLEROSIS OBLITERANS

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Introduction and objectives: Arterial peripheral disease is a condition caused by the blocking of blood flow as a result of cholesterol deposit in the arteries of the arms, legs and aorta. Studies have shown that macrophages in the atherosclerotic plaque are highly activated, and this process makes these cells important antigen presenting cells, with the development of a specific immune response, having LDLox as a possible inducing antigen. As functional changes of cells which participate in the atherogenesis process may occur in the peripheral blood, the objective of the present study was to evaluate plasma levels of anti-inflammatory and inflammatory cytokines such as, interleukin-6 (IL-6), IFN-γ, IL-10 and TGF-β in patients with peripheral arteriosclerosis obliterans, as well as the level of monocyte activation in the peripheral blood through the ability of these cells to release hydrogen peroxide (H_2O_2) and to develop fungicidal activity (FA) against *Candida albicans* (*C. albicans*).
Methods: IL-6, IFN-γ, IL-10 and TGF-β from plasma of patients were detected by ELISA. Monocyte cultures activated with TNF-α and IFN-γ were evaluated by FA against *C. albicans* using plating and CFU recovery and by H_2O_2 production.
Results: Plasma levels of all cytokines were significantly higher in patients compared to those detected in control subjects. Monocytes from control group did not release substantial levels of H_2O_2, however, these levels were significantly increased after activation with IFN-$\gamma$and TNF-α. We found that patient's monocytes, before and after the activation process, showed lower responses than control subjects. Similar results were found when FA was evaluated. The results of the patients were always significantly smaller than those detected for controls.
Conclusions: The results revealed an unresponsiveness state of patient's monocytes in vitro probably due to the high activation process occurring in vivo as evidenced by high plasma levels of cytokines.
Disclosure of interest: None declared.

P-427
ANTI-THROMBOTIC EFFECT OF BLACK SOYBEAN THROUGH INHIBITION OF PLATELET ACTIVATION

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Many clinical trials have demonstrated the beneficial effects of soybean (*Glycine max*) on general cardiovascular health. Among a variety of soybeans, black soybean displays superior biological activities to yellow or green soybeans such as in antioxidant capacity, anti-inflammatory activity and anticancer activity. However, few studies have been directed on the effect of black soybean on cardiovascular function. In this study, we aimed to investigate the effect of black soybean extract (BB) on platelet activation, a critical contributor to thrombotic diseases. In the freshly isolated human platelets, 20% ethanol extract of black soybean has shown potent and selective inhibitory activities on collagen-induced platelet aggregation while yellow bean extract had no activities. Serotonin secretion and P-selective expression, important factors for platelet-tissue interaction were also decreased along with reduced thromboxane A_2 formation by BB treatment. These in vitro results were further confirmed in ex vivo platelet aggregation measurement and in vivo venous thrombosis model where oral administration of BB reduced collagen-induced platelet aggregation and $FeCl_3$-induced thrombus formation. Notably, clotting time and bleeding time was not affected by BB administration, indicating that black soybean can be a novel dietary supplement for the prevention of thrombotic diseases and improvement of blood circulation.
Disclosure of interest: None declared.

P-428
MOLECULAR PATTERN AND HLA-DR IN B- THALASSEMIA PATIENTS COMPLICATED BY MYOCARDITIS IN UPPER EGYPT

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Background: IVS-I-6 (T-C), IVS-I-110 (G-A), IVS-II-1 (G-A) and IVS-II-745 (C-G) B globin gene mutations are common in the Mediterranean population and were reported to be common in Egyptians. In dilated cardiomyopathy, immune related disorders show preferential associations with HLA genes. It was known that the mutations occurred with β- thalassemia major have a strong relation to severity and complication accompanying the disease. We aimed to study the frequency of the previous mentioned mutations and HLA-DR in Upper Egyptian β- thalassemia patients.
Patients and methods: The study included 68 Egyptian patients with β- thalassemia major classified into two groups; the first group was 35 patients suffering from left sided heart failure, while the second group formed from 33 patients without heart failure. PCR amplification using five oligonucleotides primers to the concerned mutations. Dot–blot analysis was carried out with allele-specific probes and detection was done by using streptavidin-peroxidase and Chemiluminescent liquid detection. HLA class II-DR was investigated for all patients and controls using sequence specific oligonucleotide (SSO).
Results: The following frequencies of the investigated mutations were noticed IVS-I-110 (G–A): 41%, IVS-II-1 (G–A): 18%, IVS-I-6 (T–C): 14%, and lastly IVS-II-745: 12%. DR4 and DR7 were significantly increased in patients with heart failure with a P value of <0.02, Odds Ratio (OR) = 3.3 and $p < 0.01$, OR = 2.1, respectively. A statistical significant difference was found between patients who are positive for IVS-I-1 and patients with heart failure ($p < 0.02$).
Conclusions: The recorded frequencies of tested mutations are very close to the previous studies which were performed in Egyptian β-thalassemia patients. Immunogenetic pathogenesis mediated mechanism attributed to myocarditis must be considered in addition to other risky factors as iron overload.
Disclosure of interest: None declared.

P-429
ESTABLISHMENT OF A CORONARY ARTERITIS MURINE MODEL BY ORAL ADMINISTRATION OF PURE INNATE IMMUNE LIGANDS

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Rationale: There is a line of evidence that activation of innate immune Toll-like receptors (TLRs) contributes to the development and progression of cardiovascular diseases. With respect to nucleotide-binding domain, leucine-rich repeat-containing (NLRs) protein family, an exact role in the cardiovascular system remains to be clarified.
Objective: We investigated the effects of stimulants for NLRs on human artery endothelial cells in vitro and murine arteries in vivo.
Methods and Results: Human coronary artery endothelial cells (HCAEC) were challenged in vitro with microbial components that stimulate NLRs or TLRs. We found the stimulatory effects of NLR and TLR ligands on the adhesion molecule expression and cytokine secretion by HCAEC. Based on these in vitro results, we examined the in vivo effects of these ligands in mice. Among NLR and TLR ligands, FK565, one of the nucleotide-binding oligomerization domain (Nod)1 ligands, induced strong site-specific inflammation in the aortic root. Furthermore, coronary arteritis/valvulitis developed after direct oral administration or ad lib drinking of FK565. The degree of respective vascular inflammation was associated with persistent high expression of proinflammatory chemokine/cytokine and matrix metallopeptidase genes in each tissue in vivo by microarray analysis.
Conclusions: This is the first coronary arteritis animal model induced by oral administration of a pure synthetic Nod1 ligand. The present study has first demonstrated an unexpected role of Nod1 in the development of site-specific vascular inflammation, especially coronary arteritis and valvulitis.
Disclosure of interest: None declared.

P-430
DEVELOPMENT OF A PRIMARY HUMAN MACROPHAGE-DERIVED FOAM MODEL TO STUDY INFLAMMATORY AND PRO-RESOLVING MEDIATORS ISSUED FROM ARACHIDONIC, EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACIDS

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Introduction: Atherosclerosis involves the cellular infiltration in the arterial intima of several cell types, including monocytes. Then, monocytes differentiate into macrophages and become foam cells after oxidized Low Density Lipoprotein (oxLDL) uptake. The aim of this work was to develop a model of primary human macrophage-derived foam cells and to study their inflammatory profile. We have focused our attention on the capacity of macrophage-derived foam cells to metabolize polyunsaturated free fatty acids into inflammatory and pro-resolving molecules.
Methods: For this purpose, monocytes from human donor were differentiated into macrophages, which were then incubated in the presence of oxLDL. The uptake of oxLDL was associated with an increase of total cholesterol and a higher level of cholesterol ester into the cells. We have then incubated those cells in presence of arachidonic, eicosapentaenoic and docosahexaenoic acids to evaluate the capacity of macrophage-derived foam cells to synthetize lipid mediators after their stimulation by a phorbol myristate and a calcium ionophore. Supernatant were then collected and mediators analyzed using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) methodology to quantitatively evaluate bioactive lipids production.
Results: We showed that in presence of oxLDL, macrophage-derived foam cells produce more inflammatory (TXB2, PGE2) and oxidative stress mediators (5-oxo-ETE) than cells that have not received ox-LDL. On the other hand, those cells still have their capacity to produce pro-resolving mediators such as the precursors of resolvins, protectins and maresin.
Conclusions: Taking together these results demonstrate that the model of human macrophage-derived foam cells developed here is a valuable tool to screen the effects of polyunsaturated fatty acid on the resolution of inflammation in an atherosclerotic plaque.
Disclosure of interest: None declared.

Respiratory diseases

P-431
ANTI-INFLAMMATORY POTENTIAL OF A NEW PTEROCARPANQUINONE IN A PULMONARY INFLAMMATION MODEL

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The search of new anti-inflammatory agents is target of many researches. Natural products and their analogues modified synthetically are molecules of great interest. The aim of our investigation is to study the anti-inflammatory effect of the natural product extracted from *Tabebuia*, lapachol, and its synthetic derivative, a new pterocarpanquinone. LQB 118, in the inhaled lipopolysaccharide (LPS)-induced lung inflammation model, using C57J/BL6 mice. Our results demonstrated that intraperitonial treatment with 1 mg/kg of lapachol or LQB 118 reduced neutrophil influx to lungs in mice submitted to inhaled LPS (0.5 mg/mL). Additionally, LQB 118 reduced the concentration of the inflammatory mediators TNF-a and KC in the bronchoalveolar lavage fluid (BALF). These effects of LQB 118 are comparable to those of dexamethasone or aspirin, two well-established anti-inflammatory drugs. The reduction of mediators concentration by LQB 118 is partially due to reduction of NF-kB activation in the cell lungs. In vitro, LQB 118 also inhibited TNF-a production in LPS-stimulated mononuclear cells of human peripheral blood (PBMC). The highest concentration tested (100 mM) was able to inhibit almost 100% of TNF-a liberation. These data confirm the anti-inflammatory action of lapachol observed in a paw edema model (Almeida de et al., 1990), and reveal the lapachol derivative, LQB 118, as a potent modulator of inflammation, through the reduction of inflammatory mediators.
Disclosure of interest: None declared.

P-433
EPITHELIAL-DENDRITIC CELL CROSSTALK: MODULATION BY PARTICULATE MATTER

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The airway epithelium acts as a barrier protecting the lung from inhaled substances notably airborne pollutants. In some respiratory inflammatory disease such as asthma, these substances can either induce and/or exacerbate the disease. Situated beside airways epithelial cells and at the junction of innate and adaptative immunity, dendritic cells (DCs) have an important role in setting up how immune responses are initiated and perpetuated. The aim of our study is to better understand how bronchial epithelial cells (BECs) could interact with DCs particularly when epithelium is submitted to environmental risk factor such as particulate matter (PM). For this purpose, we used a coculture system composed of BECs differentiated in air–liquid interface and monocyte-derived immature dendritic cells (iDC), placed on basolateral side of BECs. Particulate matter are then added on apical surface of BECs. The expression of different cytokines/chemokines (IL-8, CCL-20, TSLP for example), which are notably involved in T cell activation and DCs chemoattraction, was studied in BECs by RT-PCR or ELISA. We have shown that coculture of BECs with iDC could regulate release of some molecules involved in inflammation process or DCs chemoattraction. However, addition of PM on this system seems to have little effect on these expressions. Devising of BECs/DCs coculture system could allow imitating first step of airways epithelium responses to inhaled substances and notably better understanding the modulation of antigenic presentation and immune system activation.
Disclosure of interest: None declared.

P-434
CRUCIAL ROLE OF IL-1 AND IL-23 IN EARLY IL-17A EXPRESSION LEADING TO PULMONARY INFLAMMATION AND FIBROSIS

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Idiopathic pulmonary fibrosis is a devastating as yet untreatable disease. Using the murine model of bleomycin-induced acute lung injury resulting in pulmonary fibrosis we reported before that neutrophil airway influx, remodeling and lung fibrosis depend on the activation of the NLRP3 inflammasome leading to mature IL-1b production. Since interleukin-17 (IL-17) is a major pro-inflammatory cytokine involved in neutrophil recruitment and chronic lung pathologies, we asked whether IL-17 plays a role in lung injury-induced inflammation and fibrosis. We find that IL-17A pulmonary expression is upregulated after bleomycin or rmIL-1b administration in the airways. Lung inflammation, remodeling and fibrosis are diminished in IL-17RA deficient mice or after IL-17A antibody neutralization. Early IL-17A producing cells in response to lung injury are mostly gdT cells and in a lesser extent CD4abT cells. Finally local bleomycin or IL-1b administration enhanced pulmonary inflammation, remodeling and fibrosis which are IL-23p19 dependent. In conclusion, we highlighted the existence of an IL-1b-IL-23-IL-17A axis. IL-17A and IL-17RA signaling play a pivotal role in bleomycin-induced acute lung inflammation and fibrosis, IL-1b and IL-23p19 expression being critically involved in IL-17A dependent lung pathology.
Disclosure of interest: None declared.

P-435
EFFECTS OF NCX 226, A COMPOUND TARGETING ENDOTHELIN RECEPTORS AND NO PATHWAY, ON MONOCROTALINE-INDUCED PULMONARY HYPERTENSION AND BLEOMYCIN-INDUCED LUNG FIBROSIS MODELS

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Background: NCX 226 is a novel nitric oxide (NO)-donating bosentan. NO is known to exert anti-inflammatory and anti-fibrotic actions and improve microcirculation. We tested the effects of NCX 226 in monocrotaline-induced pulmonary hypertension (PAH) in the rat and bleomycin-induced lung fibrosis in the mouse, in comparison with bosentan.
Methods: *PAH*: monocrotaline s.c. was administered to Sprague–Dawley rats then treated orally with test compounds (NCX 226 and bosentan in equimolar doses to 100 and 300 mg/kg) for 21 days. Endpoints: right ventricular pressure (RVP) and cardiac mass ratio. *Lung fibrosis*: Bleomycin was instilled intra-tracheally in C57BL/6 mice then treated orally for 14 days with NCX 226 or bosentan (equimolar at 10 and 30 mg/kg). *Endpoints*: airways resistance, histological (collagen, microvascular density, arterial dilation) and biochemical analyses.
Results: *PAH*: NCX 226 and bosentan reduced RVP but only NCX 226 had significant reductions in weight ratio of right ventricle/left ventricle + sectum. Both compounds did not affect systolic blood pressure. *Lung fibrosis*: Both test drugs prevented bleomycin-induced airway stiffness, being NCX 226 better. High dose NCX 226 was significantly more effective than equimolar bosentan in reducing lung tissue levels of myeloperoxidase (10.9, 4.8*$^{\#}$ and 6.9* mU/mg prot), TGF-β (357.5, 134*$^{\#}$ and 200.6* pg/mg prot) and thiobarbituric-acid reactive substances (10, 4.4*$^{\#}$ and 6.6* nmol/mg prot); values for vehicle, 37 mg/kg NCX 226 and 30 mg/kg bosentan, respectively ($p < 0.01$ vs. vehicle*or equimolar bosentan$^{\#}$). Both compounds decreased lung sclerosis and inflammation-induced vasculogenesis.
Conclusion: NCX 226 and bosentan were active in the two models. NCX 226 showed improved efficacy over bosentan on RVP and cardiac mass ratio in PAH and in reducing key inflammatory parameters (e.g., leukocyte infiltration, TGF-β and oxidative stress) in lung fibrosis, confirming a potential beneficial role for NO in these pathologies.
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P-436
THE LUNG INFLAMMATION INDUCED BY INTESTINAL ISCHEMIA/REPERFUSION MODIFIES OVER THE DAYS OF REPERFUSION

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Introduction: Experimental and clinical studies have reported that intestinal ischemia/reperfusion (I/R) induces acute lung inflammation (ALI), which is characterized by the release of many inflammatory mediators, neutrophil infiltration and increased vascular permeability. It is known that inflammatory mediators, generated at the site of intestinal I/R, are transported by lymphatic system and, reaching the lungs, contributes to ALI. Nevertheless, data on late effects of intestinal I/R in ALI are still fragmented.
Material and methods: Male Wistar rats were subjected to 45 min of ischemia of the superior mesenteric artery and then to 2, 24, 72 or 120 h of reperfusion. After these times of reperfusion, the activity of myeloperoxidase (MPO) and the extravasation of Evans blue dye (EB) in lungs were determined. Some inflammatory mediators in lymph and in lung explants were quantified.
Results: The MPO activity and the EB extravasation in lung after 2 h of reperfusion increased, but have not changed from basal after 24 and 72 h. However, after 120 h of reperfusion they increased again. Lymph collected from intestinal I/R animals contains significant amounts of IL-1b, IL-6, VEGF and LTB_4 compared to basal. Lung explants from reperfused animals revealed that while the levels of IL-1b increased during the hours of reperfusion, the levels of IL-10 decreased. The amount of IL-6 is higher after 2 h of reperfusion than in the others periods studied, whereas the levels of VEGF increased only after 120 h of reperfusion.

Conclusion: Our data indicate that the time of reperfusion mediates lung inflammation. Lymph and blood-borne inflammatory mediators participate in the onset/maintenance of pulmonary inflammation. It is possible that the regulation of endogenous control of inflammation is altered and may contribute to pulmonary inflammation observed after 120 h of reperfusion.
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Disclosure of interest: None declared.

P-437
NEUTROPHIL ELASTASE REGULATES CFTR FUNCTION IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR). CFTR protein is a membrane chloride channel notably expressed on mucosal epithelia as lung. Defective membrane CFTR is instrumental in mediating lung inflammation. Inflammatory cells secrete serine-proteases as neutrophil elastase (NE), which have well described deleterious effects on the integrity of lung tissue. However, the effect of proteases on CFTR has not yet been addressed. The aim of the present study was to characterize the effect of NE on CFTR both at structural and functional levels. Our results show that NE is able to induce the degradation of CFTR protein in lung epithelial cells leading to a loss of CFTR function. We also demonstrate that NE-induced CFTR degradation involves intracellular calpaïn proteases since a specific calpaïn inhibitor abolishes CFTR degradation. Moreover, we show that NE is able to up-regulate calpaïn activity by inhibiting calpastatin, the endogenous inhibitor of calpaïns. For the first time we demonstrate here that NE, a serine-protease, induces the degradation of CFTR protein via a calpaïn-dependent pathway in airway epithelial cells. NE-induced CFTR degradation also leads to a loss of CFTR function. These results are of crucial importance since the absence of CFTR plays a pivotal role in the induction of lung inflammation.
Disclosure of interest: None declared.

P-438
CLINICAL CHARACTERISTICS OF COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN WITH CONNECTIVE TISSUE DISORDERS

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Aim: To study peculiarities of clinical course of community-acquired pneumonia (CAP) in children with connective tissue disorders (CTD).
Methods: 64 children aged 1–18 with CAP were studied during 1 year. Patients were divided for analysis into two age groups: children up to 3 years old (group A, n = 16), and aged 4–18 (group B, n = 48). All patients had manifestations of CTD. CAP was clinically and radiographically diagnosed with detection of serum antibodies (IgG and IgM) against intracellular pathogens measured by enzyme-linked immunosorbent assay (ELISA). The presents of CTD made necessary assessment of status of cardiovascular system in all patients.
Results: All patients had CAP caused by atypical pathogens with indistinct clinical manifestations and symptoms. Recurrent course of CAP was in 54 (84.4%) patients. CAP caused by *Chlamydophila* pneumonia (Cp) was more frequent in group A—in 15 (93.7% of the group) patients. CAP caused by *Mycoplasma pneumonia* (Mp) was more frequent in group B—in 44 patients (91.6% of the group), in 6.8%—together with Cp, in 27.3%—with *Cytomegalovirus*. Asthma was diagnosed in 29 (45.3%) patients mainly of group B, with recurrent CAP in 10 (34.5% of patients with asthma); 62.1% of these children had pulmonary hypertension (PH). 44.4% of patients with PH had evidence of pulmonary fibrosis (PF), which led to pneumatocele (PC) in 27.7%, polyserositis (PS) in 11.1% and spontaneous pneumothorax (SP) in 3.4% of patients.
Conclusions:
1. Close relationship between CTD and CAP is revealed.
2. Cp CAP was more frequent in children up to age of 3 years, Mp CAP—in children aged 4–18.
3. More than one-third of patients with asthma had recurrent CAP.
4. Asthma in children with CAP and CTD was more severe with development of PH, PF, PS, SP.

Disclosure of interest: None declared.

P-439
ACTIVATION OF CALPAINS TRIGGERS LUNG INFLAMMATION INDUCED BY HYPERINFLATION

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Proinflammatory cytokine generation and neutrophil infiltration into the lung during mechanical ventilation are thought to play important roles in the development of ventilator-induced lung injury, although the exact cellular and molecular mechanisms which trigger lung inflammation are still not clear. Calpains, a family of Ca^{2+}-dependent intracellular cysteine proteases, have been shown to be involved in the inflammatory response and multiple organ failure. The aim of the present study was to determine the role of calpain in the pathogenesis of lung inflammation due to mechanical ventilation. Male C57BL/6J mice were subjected to high (28 ml/kg) tidal volume ventilation for 2 h in the absence and presence of pharmacological calpain inhibitor. Additionally, depletion of calpain expression in pulmonary microvascular endothelial cells in vivo using siRNA was performed at 48 h before ventilation. Mechanical ventilation caused a rapid (within minutes), persistent calpain activation and lung inflammation as evidenced by neutrophil recruitment, production of the cytokines TNF-α and IL-6, pulmonary vascular hyperpermeability, and lung edema formation. Pharmaceutical calpain inhibition as well as depletion of calpain-1 or calpain-2 significantly attenuated these inflammatory responses caused by lung hyperinflation. Inhibition of calpain activation also reduced eNOS-mediated NO production and subsequent ICAM-1 phosphorylation induced by mechanical stretch. This study demonstrates that calpain activation during mechanical ventilation may trigger lung inflammation via eNOS/NO-mediated ICAM-1 phosphorylation and neutrophil recruitment. Thus, inhibition of calpain activation may be a novel therapeutic strategy for the

prevention and treatment of ventilator-induced lung injury. (Supported by NIH NHLBI grant 5R01HL104092 and AHA Grant 0730331N.)
Disclosure of interest: None declared.

P-440
INCREASED IL-33 EXPRESSION IN CYSTIC FIBROSIS LUNG DISEASE

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Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by a mutation of the CF transmembrane conductance regulator (CFTR). Defective CFTR in the airway epithelium results in CF lung disease featuring mucus hypersecretion, neutrophilic inflammation, and impaired lung function, which can be fatal. Early life-*Pseudomonas aeruginosa* (Psa) infection of the lungs occurs in 70% of individuals with CFTR mutation. Understanding the mechanisms by which CFTR mutation and Psa infections lead to airway inflammation and tissue injury is key in the process of designing future therapies. Interleukin (IL)-33 is an IL-1-like cytokine promoting neutrophil recruitment and survival during sepsis. Other studies suggested that biologically active IL-33 would only be released upon cell necrosis. The potential role of IL-33 in CF disease has not yet been explored. Therefore, we sought determining whether IL-33 expression is elevated in CF lungs and if Psa infections contribute to its release. Our results showed increased IL-33 expression in airway epithelial cells (AECs) of CF lung tissue compared to healthy biopsies. Upon exposure to Psa diffusible material, AECs expressing the most common mutation of CFTR, CFTRΔF508, secrete more IL-33 than cells with wild-type CFTR gene. Upon infection with Psa, CFTR-deficient mice also displayed increased up-regulation of IL-33 expression compared with wild-type littermates. Our results demonstrate for the first time that IL-33 is elevated in the airways of CF patients and propose that it is a Psa infection-triggered inflammatory time-bomb as IL-33 is released following tissue injury during exacerbations. Our data suggest that targeting IL-33 may provide a novel avenue to limit exaggerated airway's neutrophilic inflammation and morbidity in CF lung disease.
Disclosure of interest: None declared.

P-441
INHIBITION OF MITOGEN-ACTIVATED PROTEIN (MAP) KINASES IN ACUTE AND CHRONIC LUNG INJURY IN MICE

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The aim of this study is to investigate the effects of 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98059), which is a specific inhibitor of the activation of mitogen-activated protein kinase kinase (MAPK3/MAPK1), on the development of acute lung inflammation caused by carrageenan injection or pulmonary fibrosis induced by instillation of bleomycin (BLEO). Mice were injected with carrageenan into the pleural cavity or were subjected to intratracheal instillation of BLEO (1 mg/kg), a glycopeptide produced by the bacterium *Streptomyces verticillus*. Mice subjected to injury developed significant inflammatory response characterized by marked neutrophil infiltration and tissue edema. An increase in immunoreactivity to nitrotyrosine, iNOS, TNF-alpha and IL-1β were also observed in the lungs from injured mice. In contrast, administration of PD98059 (10 mg/kg, 10% DMSO, i.p.) 1 h after carrageenan injection or BLM instillation significantly reduced: (1) cytokines production, (2) IκB-α degradation and NF-κB nuclear expression (3) iNOS expression (4) nitrotyrosine and PARP localization (5) the degree of apoptosis, as evaluated by Bax and Bcl-2 balance, FAS ligand expression and TUNEL staining. Taken together, all our results clearly show that inhibitors of the MAPK3/MAPK1 signaling pathways, such as PD98059, may be useful in the treatment of lung injury and inflammation .
Disclosure of interest: None declared.

P-442
GLPG0259, AN ORAL SMALL MOLECULE INHIBITOR OF MAPKAPK5, INHIBITS TOBACCO SMOKE-INDUCED LUNG INFLAMMATION IN A MOUSE MODEL OF COPD

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Smoking and air pollutants are recognized as key pathogenic factors for chronic obstructive pulmonary disease (COPD), leading to gradual loss of lung function. GLPG0259, a potent inhibitor of MAPKAPK5 currently in Phase II trials for rheumatoid arthritis, was characterized in various in vivo and in vitro models of inflammation and showed strong capacity to inhibit cytokine-induced MMP expression as well as innate immune response. In view of similar processes in COPD pathogenesis, we investigated the efficacy of GLPG0259 in models of COPD. GLPG0259 was profiled in normal human bronchial epithelial cells (NHBECs) with the addition of poly(dI-dC). IL-6 and CCL5 RNA levels were determined by qPCR. The in vivo efficacy was characterized in the mouse LPS model (serum TNFα levels) and in a 4-day tobacco smoke mouse model of COPD (inflammatory cell counts and cytokine levels in bronchoalveloar lavage fluid (BALF), inflammatory gene expression in lung tissue). In NHBECs, GLPG0259 concentration-dependently inhibited both IL-6 and CCL5. In the LPS model, GLPG0259 strongly reduced the TNFα release (ED_{50} 3 mg/kg). In the COPD model, GLPG0259 dose-dependently (0.1–10 mg/kg b.i.d.) inhibited the cell influx into BALF. At 1 mg/kg, GLPG0259 reduced the total cell number (40%), neutrophils (51%), lymphocytes (61%), macrophages (29%) and epithelial cells (50%), comparable to the p38 inhibitor BIRB796. Disease and compound effects were also assessed by cytokine measurements as well as gene expression profiling. GLPG0259 is active on pathways involved in the COPD pathogenesis both in vitro and in vivo, indicating that this compound might have therapeutic benefit in COPD.
Disclosure of interest: None declared.

P-443
IL-32 IN CHRONIC OBSTRUCTIVE PULMONARY DISEASES

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Background: Interleukin-32 (IL-32) is a newly described proinflammatory cytokine which is expected to have an important role in autoimmune disorders. Recently, it was shown that chronic obstructive pulmonary diseases have a component of autoimmunity, though the role of IL-32 in those diseases is not known. The aim of this study was to investigate IL-32 concentration in different tissue compartments from patients with COPD, asthma and compare with healthy subjects.
Methods: Outpatients aging from 40 to 80 years with stable COPD ($n = 51$), asthma ($n = 31$), and healthy subjects ($n = 9$) were studied. IL-32 concentration in serum, induced sputum and BAL was analyzed by enzyme-linked immunosorbent assay.
Results: IL-32 concentration was significantly increased in all studied tissue compartments from patients with COPD in comparison to asthmatics and healthy subjects (serum: 26.77 ± 2.56 vs. 6.09 ± 1.16 and 4.63 ± 1.03 pg/ml; induced sputum: 19.66 ± 1.69 vs. 5.82 ± 1.15 and 3.59 ± 0.66 pg/ml; BAL: 22.46 ± 2.48 vs. 6.25 ± 1.08 and 4.21 ± 1.13 pg/ml, respectively), and significantly correlated with smoking history (pack years). There was no statistically significant difference of IL-32 concentration between asthmatics and healthy subjects.
Conclusion: The fact that IL-32 concentration is increased in patients with COPD and related to the smoking history let us suggest that IL-32 may play an important role in the pathogenesis of COPD.
Disclosure of interest: None declared.

P-444
OCID 2987—A NOVEL, SELECTIVE, POTENT, ORALLY ACTIVE PDE4 INHIBITOR IN MURINE MODELS OF ASTHMA AND COPD

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Rationale: PDE4 inhibitors increase cAMP levels, and as a consequence have shown anti-inflammatory effect in various animal models of inflammation. PDE4 inhibitors are in various stages of development with roflumilast in particular being approved in Europe for treatment of COPD. OCID 2987 a novel PDE4 inhibitor was evaluated in murine models of asthma and COPD.
Methods: In LPS induced acute lung injury model Sprague-Dawley rats were exposed to LPS (100 mg/ml) for 40 min. For evaluation of neutrophil influx BALF (broncho-alveolar lavage fluid) was collected at 6 h and for TNF-α estimation at 1.5 h. In COPD model C57 mice were exposed to the tobacco smoke of 10 cigarettes/day for 20 days. 24 h after last cigarette smoke exposure BALF was collected and differential cell counts conducted. In guinea pig model of asthma, animals were sensitized and challenged with aerosolized ovalbumin. BALF was collected 48 h after last ovalbumin challenge for total cell count and differential cell count.
Results: In LPS induced acute lung injury model OCID 2987 produced inhibition of neutrophil influx in BALF with ED_{50} value of 0.81 mg/kg, p.o. and inhibition of TNF-α in BALF with ED_{50} value of 1.9 mg/kg, p.o. OCID 2987 at 1 mg/kg, p.o. produced 66.96% inhibition of neutrophil influx in BALF of cigarette smoke exposed mice. 1 mg/kg dose of OCID 2987 resulted in 70.24% inhibition of eosinophils in BALF at 48 h ovalbumin post challenge in guinea pigs.
Conclusion: OCID 2987 is a potent, selective PDE4 inhibitor with demonstrated efficacy in animal models of asthma and COPD. OCID 2987 has completed the Phase I regulatory requirement and is expected to start FIH dosing by April 2011.
Disclosure of interest: None declared.

P-445
NEONATAL MALNUTRITION AND PRODUCTION OF IFN-G, IL-12 AND IL-10 BY ALVEOLAR MACROPHAGES: STUDY OF IN VITRO CELLULAR INFECTION BY METHICILLIN-RESISTANT/METHICILLIN-SENSITIVE STAPHYLOCOCCUS AUREUS

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Objective: To compare levels of IFN-γ, IL-12, and IL-10 produced by alveolar macrophages (AM) among groups of well-nourished (N) and malnourished (MN) rats, followed by in vitro cellular infection by methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA).
Methodology: Male Wistar rats ($n = 12$) were breastfed by rats whose diet contained 17 and 8% of protein during lactation (N and MN group). After weaning, both groups were administered a normoproteic diet. Following the macrophage isolation, four systems were formed: negative control composed by macrophages (C−), positive control added with lipopolysaccharide (C+) and two systems, MSSA and MRSA, stimulated by the strains. Cytokine release was evaluated after 24 h of incubation.
Results: Malnutrition led to the decrease of body mass as well as reduction in the production of IFN-γ in the systems C+ ($p = 0.001$) and MRSA ($p = 0.001$). However, it was not observed significant difference among systems C− ($p = 0.707$) and MSSA ($p = 0.065$). IL-12 levels analysis demonstrated that malnutrition caused a decrease of such cytokine in all systems: C+ ($p = 0.009$), C− ($p = 0.006$), MSSA ($p = <0.001$) and MRSA ($p = <0.001$). In addition, no significance was observed on the production of IL-10, C+ ($p = 0.729$), C− ($p = 0.341$), MSSA ($p = 0.680$) and MRSA ($p = 0.908$). Among MSSA and MRSA strains, statistically significant differences were only found in the production of IFN-γ (MSSA vs. MRSA (N) $p = <0.001$, MSSA vs. MRSA (MN) $p = <0.001$) and IL-10 (MSSA vs. MRSA (N) $p = 0.012$, MSSA vs. MRSA (MN) $p = 0.037$) ($p < 0.05$, Student t test).
Conclusion: The model of neonatal malnutrition revealed disorders in body mass and compromised the production of IFN-γ and IL-12. Results suggested that an intense production of IFN-γ and IL-10 cytokines may be stimulated by MRSA strain in the alveolar macrophages.
Disclosure of interest: None declared.

P-446
AQX-1125, A FIRST-IN-CLASS CLINICAL-STAGE SHIP1 ACTIVATOR SMALL MOLECULE FOR THE TREATMENT OF PULMONARY DISEASES: SUPPRESSION OF CHEMOTAXIS IN VITRO AND EFFICACY IN RODENT MODELS OF ASTHMA AND COPD IN VIVO

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Pharmacological modulation of the phosphoinositide 3-kinase (PI3K)/Akt pathway is emerging as a means to control inflammatory disorders. The SH2-containing inositol-5′-phosphatase 1 (SHIP1) metabolizes PI[3,4,5]P3 to PI[3,4]P2. SHIP1-deficient mice exhibit a marked degree of inflammatory cell recruitment into the lung, leading to pulmonary inflammation. Pharmacological, allosteric activation of SHIP1 exerts anti-inflammatory effects (Ong et al., Blood, 2008). Here we overview the biological effects of AQX-1125, a small molecule SHIP1 activator, a current clinical development candidate. AQX-1125 induced a concentration-dependent increase in the catalytic activity of human recombinant SHIP1 enzyme. AQX-1125 suppressed Akt phosphorylation in SHIP1-proficient MOLT-4 cells, but not in SHIP1-deficient Jurkat cells. AQX-1125 inhibited degranulation of wild-type bone marrow-derived mast cells, but not of SHIP1-deficient cells. As phosphoinositide signaling plays a key role in cytokinesis, the effect of AQX-1125 was tested on leukocyte chemotaxis. AQX-1125 was found to be a nanomolar, a pan-selective inhibitor of leukocyte chemotaxis. AQX-1125 exhibited high oral bioavailability (>80%) and long terminal half-life (>5 h) in rodents and dogs, and yielded high concentrations in the lung. AQX-1125 exerted anti-inflammatory effects and reduced inflammatory cell infiltration into the BAL in a rat ovalbumin-mediated airway inflammation model, in a rat pulmonary inflammation model induced by LPS, and in a mouse model of cigarette smoke-induced airway inflammation. AQX-1125 markedly reduced the production of multiple chemokines/cytokines in the lung. Thus, SHIP1 activation may have utility for the treatment of respiratory disorders such as chronic obstructive pulmonary disease and asthma. The first-in-class SHIP1 activator AQX-1125 demonstrates ideal drug-like properties and has recently entered Phase I clinical testing.
Disclosure of interest: C. Szabo Consultant/Speaker's bureau/Advisory activities with: Aquinox; employee of: Aquinox; stock ownership or royalties of: Aquinox. L. MacKenzie employee of: Aquinox, stock ownership or royalties of: Aquinox, G. Stenton employee of: Aquinox, stock ownership or royalties of: Aquinox, P. Tam employee of: Aquinox, stock ownership or royalties of: Aquinox, J. Cross Grant/research support from: Aquinox, C. Harwig employee of: Aquinox, stock ownership or royalties of: Aquinox, J. Raymond: none declared, J. Toews employee of: Aquinox, D. Chernoff Consultant/Speaker's bureau/advisory activities with: Aquinox, stock ownership or royalties of: Aquinox, T. MacRury employee of: Aquinox, stock ownership or royalties of: Aquinox

P-447
USE OF NEW COLLAGEN DEPOSITION AS NOVEL READ-OUT IN BLEOMYCIN-INDUCED LUNG FIBROSIS

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The bleomycin-induced pulmonary fibrosis model in the mouse is the most common model to investigate potential new therapies for idiopathic pulmonary fibrosis (IPF). The main read-outs used to quantify the severity of fibrosis are based on the amount of collagen deposition (either measured using histological sections or by biochemical assay). However, this becomes a problem when the model is run using a therapeutic treatment protocol in which treatment is only started after fibrosis has been allowed to establish. Since the production of collagen (the main component of fibrous tissue) already starts during in the initial inflammatory phase, the window for measuring effects of new compounds is much smaller in the therapeutic protocol versus preventive treatment. In this study, we investigated the kinetics of collagen deposition during bleomycin-induced lung fibrosis in C57Bl/6 mice using deuterated water (D_2O) to label newly formed collagen synthesis. Furthermore, we used whole gene array analysis in combination with the ToxProfiler software to differentiate between the different pathways induced during fibrosis. Array analysis showed that inflammatory processes were maximal during the first week of disease, while extracellular matrix related pathways were maximal during the second and third week after induction, after which they slowly returned to base line levels. The new collagen assay showed that during the first week already substantial collagen formation took place, despite strong upregulation of inflammatory processes. The production of collagen was further upregulated during the second and third week, after which collagen synthesis returned to pre-induction levels. This high level of collagen synthesis during the first week explains the difficulties in obtaining sufficient window during therapeutic treatment.
Disclosure of interest: None declared.

P-448
COMMD1 AND CSN5: TWO ANTI-INFLAMMATORY PROTEINS IN THE CONTEXT OF CYSTIC FIBROSIS?

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *CFTR* gene, which encodes an epithelial anion channel. Morbidity is mainly due to the lung disease, characterized by a chronic neutrophilic inflammation. Deregulation of inflammatory pathways is observed in the airways of CF patients, as evidenced by an

increased AP-1 activity and NF-κB response. Consequently, pro-inflammatory cytokines such as IL-8 are increased. CSN5 and COMMD1 are two proteins involved in the trafficking of CFTR but also in inflammatory processes. We observed a larger proportion of COMMD1 and CSN5 in the nucleus of CF cells. We studied the influence of COMMD1 or CSN5 overexpression/extinction on NF-κB pathway in CF and non-CF bronchial epithelial cells (IB3-1 and S9, respectively). To decipher the underlying mechanisms, we have first performed a set of luciferase assays and NF-κB binding assays in order to test NF-κB transcriptional activity in the presence or absence of each protein. TNFα stimulation produced a higher increase of NF-κB activity in CF versus non CF cells. Overexpression of COMMD1 and CSN5 in the same experimental conditions decreased NF-κB activity by half in both cell lines. As IL-8 promoter contains AP-1 and NF-κB responsive elements, we performed such experiments with the wild-type and mutant constructs. Our preliminary results did not show a clear-cut effect on IL-8 promoter. However, new experimental conditions such as IL-1β stimulation are currently investigated. Our results show for the first time the anti-inflammatory properties of COMMD1 and CSN5 in bronchial cells. These data could expand investigations to other chronic inflammatory diseases such as asthma or chronic obstructive pulmonary disease. This work was supported by public grants from Institut National de la Santé et de la Recherche Médicale (INSERM) and Chancellerie des Universités de Paris, and the French Association "Vaincre la Mucoviscidose".
Disclosure of interest: None declared.

P-449
ROLE OF COX-1 AND COX-2 IN THE RELEASE OF PROSTANOIDS IN MURINE LUNG AND ISOLATED LUNG FIBROBLASTS

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Cyclooxygenase (COX) is the first enzyme in the conversion of arachidonic acid to prostanoids. There are two isoforms of COX; COX-1, which is constitutively expressed with a homeostatic role in most tissues, and COX-2, which while constitutively expressed in some discreet sites is generally inducible by growth factors and during inflammation. In the current study, we have used tissues and cells from knock-out mice to investigate the relative contributions of COX-1 and COX-2 to PGE_2 production by lung tissue ex vivo and by proliferating lung fibroblasts in vitro. Lung tissues from WT (C57Bl6), COX-$1^{-/-}$ and COX-$2^{-/-}$ mice were immediately dissected (<15 min after death) and incubated (37°C) for 30 min in DMEM containing 50 μM calcium ionophore (A23187). Release of PGE_2 was determined by competitive immunoassay. In parallel studies, murine lung fibroblasts from COX-$1^{-/-}$ and COX-$2^{-/-}$ mice were explanted and cultured before being seeded in 96-well plates at sub-confluence (5,000–8,000/well) and incubated for 24–48 h in the presence of 10% FCS. Accumulated release of PGE_2 was then measured as above. Over 30 min PGE_2 was released by lung pieces from wild type (1,117 ± 55 pg/ml) and COX-$2^{-/-}$ (2,013 ± 255 pg/ml) but not from COX-$1^{-/-}$ (<61 pg/ml) mice (n = 4). In contrast, proliferating lung fibroblasts from COX-$1^{-/-}$ (4,978.9 ± 1,392 pg/ml) mice released higher levels of PGE_2 than cells from COX-$2^{-/-}$ (1,194 ± 617 ng/ml) mice (n = 4 using cells from 2–3 separate mice for each genotype). These results show that COX-1 activity underpins the stimulated release of PGE_2 in healthy mouse lung tissue. Conversely, COX-2 activity predominates in proliferating lung fibroblasts, which may be important as COX-derived PGE_2 mediates proliferation of lung fibroblasts [1]. Our results suggest a switch in COX isoform in lung cells during proliferation which could be relevant to our understanding of conditions such as idiopathic pulmonary fibrosis [1] Trends Immunol. 2004;25(1):40–6.
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P-450
OXIDATIVE STRESS MARKERS IN PULMONARY FIBROSIS INDUCED BY BLEOMYCIN ARE STRAIN DEPENDENT

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Introduction: Pulmonary fibrosis has high rates of mortality and morbidity, but there is no established therapy at present. Oxidative stress plays an important role in the pathogenesis of and pulmonary fibrosis. The aim of the present study was to determine whether there is any significant genetic influence on the development of lung fibrosis related to oxidative stress after bleomycin stimulus.
Methods and Results: C57BL/6, DBA/2 and BALB/c male mice were divided in control group (50 μL saline; i.t., n = 10 each strain) and bleomycin (Bleo) group (0.1 U/kg i.t., n = 10 each strain). After 21 days the mice were sacrificed. The lungs of 20 animals were homogenized for oxidative stress marker determinations and compared with the respective control groups by with Tukey post-test. Lung ventilation was performed in 10 animals of each group and elastance and resistance determined. Malondialdehyde equivalent levels were increased in the C57BL/6 Bleo group ($p < 0.01$), and were decreased in DBA/2 and BALB/c ($p < 0.0001$) when compared with the respective control strain group. Both activity and expression of catalase were increased in C57BL/6 Bleo group ($p < 0.01$), decreased in DBA/2 and BALB/c Bleo groups when compared with control groups ($p < 0.01$). Superoxide dismutase activity increased in C57BL/6 and BALB/c Bleo groups ($p < 0.01$). Nitrite levels were increased in C57BL/6 and BALB/c ($p < 0.01$) Bleo groups. Lung resistance was increased in C57BL/6 Bleo group ($p < 0.01$) and in DBA/2 ($p < 0.05$).
Conclusion: Our results shown that C57BL/6 mice are more susceptible to redox imbalance and oxidative damage and bleomycin response in DBA/2 and BALB/c strains we not associated with oxidative stress injury.
Disclosure of interest: None declared.

P-451
INHIBITION OF THE NUCLEAR KINASE MSK1 REDUCES INFLAMMATORY INFILTRATE AND FIBROPROLIFERATION IN A MOUSE MODEL OF OBLITERATIVE BRONCHIOLITIS

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Introduction: Obliterative bronchiolitis (OB) is the manifestation of chronic allograft rejection after lung transplantation and is characterized by obstruction of the small airways with inflammatory infiltrate and fibrosis. Our hypothesis proposes the nuclear kinase MSK1 (Mitogen- and Stress-Activated Kinase) as an actor in OB via the activation of pro-inflammatory genes.
Methods: A mouse model of heterotopic tracheal transplantation (iso- and allograft) was used. Mice were treated intraperitoneally up to 21 days (D) with compounds inhibiting MSK1: H89 (10 mg/kg/day) and fasudil (30 mg/kg/day) vs solvent (DMSO 5%). Total RNA was extracted from tracheas and MSK1 and IL-6 mRNA levels were quantified by qPCR. Tracheal sections were observed after hematoxylin–eosin staining. CD3+ lymphocyte infiltration was quantified at D7 after immunohistochemistry.
Results: MSK1 mRNA is increased in the allografts by +68 ± 8% at D7 and +85 ± 4% at D21 in comparison to D0, whereas the expression of MSK1 mRNA is unmodified in the isografts. Likewise, the expression of IL-6 mRNA in the allografts at D7 is increased by +88 ± 2% as compared to D0. Histologically, the allograft shows inflammatory infiltration at D7 and lumen fibroproliferative obstruction at D21. Upon H89 and fasudil treatment, a decreased lymphocyte infiltration is observed, of 92 ± 3% for H89 and 90 ± 5% for fasudil at D7 as compared to controls. At D21, H89 and fasudil inhibit the tracheal obstruction in allografts by 80 and 45%, respectively, compared to controls. No effect of H89 or fasudil is observed in isografts.
Conclusion: Our data show that the nuclear kinase MSK1 is overexpressed upon graft rejection in our model of OB, as well as the pro-inflammatory cytokine IL-6. Pharmacological inhibition of MSK1 causes a reduced lymphocytic infiltration and tracheal obstruction. Thus, MSK1 is proposed as a potential therapeutic target to combat OB after lung transplantation.
Disclosure of interest: None declared.

P-452
HEME OXYGENASE 1 INDUCTION REDUCES LUNG OXIDATIVE STRESS AND INFLAMMATION AFTER INTESTINAL ISCHEMIA AND REPERFUSION INJURY

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Intestinal ischemia and reperfusion (intestinal I/R) frequently occurs in human pathological conditions, and it has been considered as a significant cause of Acute Respiratory Distress Syndrome (ARDS). *It is thought that polymorphonuclear neutrophils and mediators generated in the setting of oxidative stress, such as reactive oxygen species* (*ROS*), have important roles in its pathophysiology. In this sense, we hypothesize that heme oxygenase-1 (HO-1) up regulation, an enzyme with antiinflammatory and antioxidant activities, could modulate the expression of antioxidant enzymes and neutrophils recruitment in the lung after an intestinal I/R, therefore inducing cytoprotection. Upon anesthesia male Wistar rats were subjected to 45 min occlusion of the superior mesenteric artery (SMA) followed by 2 h of reperfusion. Before ischemia, one group of rats had the lymphatic thoracic duct sectioned and another group of rats was treated 48 and 24 h before induction of SMA occlusion with an HO-1 inductor, Hemin (10 mg/kg, i.p.). Neutrophils recruitment to the tissues was indirectly measured by myeloperoxidase (MPO) activity. HO-1, superoxide dismutase (SOD) 1 and 2, catalase (CAT) mRNA were determined in the lung tissue. Intestinal I/R determined a significant increase of MPO activity in lung that was reduced in rats upon lymphatic duct sectioned. Hemin treatment of rats increased the activity of HO-1, SOD-1 and SOD-2, but did not modify the expression the CAT in lung tissue. In addition, the MPO activity in lung tissue was significantly reduced in hemin-treated group as compared with non treated rats upon intestinal I/R. Our data indicate that HO-1 decreases neutrophils recruitment and upregulates the expression of antioxidant enzymes, reducing inflammation of lung tissue after intestinal I/R. Moreover lymph factors seem not be involved with HO-1 effects.
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Disclosure of interest: None declared.

P-453
ROFLUMILAST N-OXIDE REDUCES RELEASE OF IL-8 AND VEGF FROM AIRWAY EPITHELIAL A549 CELLS EXPOSED TO CIGARETTE SMOKE EXTRACT (CSE) AND LIPOPOLYSACCHARIDE (LPS)

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Introduction: Cigarette smoke is a leading cause of chronic obstructive pulmonary disease (COPD) where bacterial infections account for acute exacerbations. IL-8 and VEGF are increased in COPD exacerbations. Roflumilast is a selective PDE4 inhibitor approved for severe COPD in EU. We investigated the effects of Roflumilast N-oxide (RNO), the active metabolite of roflumilast on the release of IL-8 and VEGF from airway epithelial A549 cells by CSE and LPS.
Methods: CSE was prepared from the smoke of Kentucky 2R1 cigarettes bubbled into FK-12 medium. A549 cells were preincubated with RNO (100 nM, 300 nM, 1 μM), prostaglandin E2 (10 nM) and exposed to CSE (0.4% > 10%) with or without LPS (0, 1 μg/ml) for 24 h. IL-8 and VEGF were determined by ELISA in culture supernatants.
Results: CSE (0.4–10%) increased VEGF release from A549 cells to a maximum of 1.7-fold versus control but did not induce IL-8 release. LPS at 0.1 μg/ml moderately increases VEGF release but not IL-8. However, adding LPS (0.1 μg/ml) to CSE (2% or 4%) resulted in a synergistic increase in IL-8 (control: 76.36 ± 14.32 pg/ml;

LPS: 157.7 ± 47.5 pg/ml, CSE: 73.83 ± 15.44 pg/ml, LPS + CSE: 301.4 ± 28.59 pg/ml) and VEGF (control 45.00 ± 3.40 pg/ml; LPS 131.7 ± 11.9 pg/ml, CSE 72.16 ± 1.45 pg/ml, LPS + CSE 339.2 ± 20.48 pg/ml) release. RNO at 100 nM, 300 nM and 1 μM reduced release of IL8 by about 85% and of VEGF by 70–80%
Conclusions: CSE increased the release of VEGF but not IL-8 from A549 cells. The additional presence of LPS synergistically enhanced the release of both mediators. The PDE4 inhibitor roflumilast N-oxide reduced CSE/LPS-induced IL-8 and VEGF release from A549 cells.
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Disclosure of interest: None declared.

Musculoskeletal diseases

P-455
COMPARATIVE STUDY OF MYCOPHENOLATE MOFETIL (MFF) VERSUS CYCLOPHOSPHAMIDE (CYC) IN TREATMENT OF LUPUS NEPHRITIS

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Background: Optimal management of lupus nephritis with immunosuppressive agents remains a challenge.
Objective: To study the efficacy, safety, and tolerability of oral MMF in comparison to CYC injection as a therapeutic tool for active lupus nephritis.
Method: 80 patients with biopsy proven lupus nephritis were recruited. 40 patients received MFF (MFF group) and other 40 received intravenous CYC as monthly pulses of 1gm/m^2 (CYC group).
Eligibility criteria: * SLE meeting four classification criteria of ACR. * Renal biopsy documenting lupus nephritis according to the classification of WHO as proliferative glomerulonephritis class III (focal), IV (diffuse) or V (membranous).
Exclusion criteria: * creatinine clearance of less than 30 ml/minute.
* Serum creatinine greater than 3 mg/dl.
* Pregnancy and lactation.
All patients were assessed, 12 and 24 weeks after commencement of treatment, by urinary protein/24 h, serum anti-ds-DNA, C3 and C4. Clinical and laboratory evaluation for drug toxicity have been done.
Results: In MFF group, there was a significant reduction of urinary protein/24 h if compared with CYC group 12 and 24 weeks after commencement of treatment. MFF group showed significant early response after 12 weeks and complete remission if compared to CYC group. Infection and gastrointestinal intolerance have been encountered in CYC group rather MFF group. Hematologic toxic effects were uncommon in both groups.
Conclusion: Induction therapy with MFF was superior to CYC in induction of complete remission and appeared to be better tolerated than CYC.
Disclosure of interest: None declared.

P-456
PROTECTIVE EFFECTS OF CORM-3 IN AN ANIMAL MODEL OF POSTMENOPAUSAL ARTHRITIS

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Introduction: The incidence of rheumatoid arthritis (RA), an autoimmune disease characterized by persistent synovial inflammation as well as articular cartilage and bone destruction, is greater in women compared with men and can be related to menopause. Estrogen deficiency has demonstrated to increase incidence and progression of RA. We have shown previously that CO-releasing molecule, CORM-3 exerts anti-inflammatory effects in vitro and in vivo. The aim of this study was to investigate the effects of CORM-3 on the inflammatory response as well as on cartilage and bone metabolism in comparison with alendronate (ALN).
Methods: Two weeks after ovariectomy (OVX), collagen-induced arthritis (CIA) was induced in DBA/1J mice. ALN (100 μg/kg po once a day) and CORM-3 (10 mg/kg ip once a day) were administered from day 22. The clinical score was studied using a scale of 0–2 in each paw. Mice were killed at day 36 or 50. Histological analyses were performed on hind paws. Trabecular microarchitecture was analyzed by μCT.
Results: CORM-3 treatment resulted in a significant reduction of the clinical score with respect to the control animals (OVX + CIA). The bone parameters of tibia of animals treated with CORM-3 were similar to naïve group at day 36. At day 50, CORM-3 reduced chondrocyte death compared with control group. ALN administration did not improve the clinical score nor the articular cartilage damage. At day 50, ALN administration showed to be an effective treatment to reduce bone resorption.
Conclusions: These data corroborate the anti-inflammatory role of CORM-3 and demonstrate protective effects against cartilage destruction. In addition, CORM-3 is able to reduce the bone loss in the early phase of the disease. ALN confirms its antiresorptive action in this animal model of arthritis + osteoporosis but it fails to protect joint cartilage damage. Therefore, these data suggest that a combination of both drugs could be an effective strategy in postmenopausal arthritis.
Disclosure of interest: None declared.

P-457
ELEVATION OF BOMBINA VARIEGATA PEPTIDE 8 IN MICE WITH COLLAGEN-INDUCED ARTHRITIS

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Background and objective: *Bombina variegata* peptide 8 (Bv8)/prokineticin-2 is a protein isolated from skin secretions of the frog. Bv8 has diverse functions, being involved in angiogenesis, gastrointestinal motility, neurogenesis, circadian rhythm regulation, hormone release, and the pain threshold. Recently, Shojaei et al. reported an interesting study on angiogenesis: tumor-derived G-CSF mobilized bone marrow Bv8-positive cells to tumor sites, and these cells were CD11b+/Gr1+, promoting tumor angiogenesis via the mediation of Bv8. The purpose of this study was to investigate Bv8 in mice with type II collagen-induced arthritis (CIA).
Methods: We induced CIA in male DBA/1J mice. The number of CD11b+/Gr-1+ cells in peripheral blood from these mice on days 28 and 35, were examined by flow cytometry. RNA was extracted from the joint on days 21, 24, 28, and 35, and examined for G-CSF, Bv8, PKR1, and PKR2 mRNA expression by real-time RT-PCR. Synovial tissue and bone marrow were immunohistochemically examined using anti-Bv8, Gr-1, PKR1 and PKR2 antibody. To determine bone marrow-derived Bv8 positive cells in synovial tissue, we induced CIA in bone marrow chimera mice and performed histological analysis.
Results: The level of G-CSF mRNA expression was elevated and the number of CD11b+/Gr-1+ cells in peripheral blood increased in the CIA group. The level of Bv8, PKR1 and PKR2 mRNA expression in the joint was elevated in the CIA group. In addition, an increase in Bv8-positive cells was observed in the synovium and bone marrow in the CIA group. Bv8-positive cells in the synovium partially derived from bone marrow and most Bv8-positive cells observed in the synovium were positive for Gr-1.
Conclusion: Bv8 was elevated in the synovium and bone marrow of CIA mice, suggesting that Bv8 plays an important role in the pathogenesis of arthritis.
Disclosure of interest: None declared.

P-458
ULTRASONOGRAPHIC EVALUATION OF ASYMPTOMATIC HAND AND WRIST JOINT IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Articular manifestation is common in systemic lupus erythematosus (SLE) and results from variable involvement of joints and tendons. Musculoskeletal ultrasound (MSUS) is a sensitive technique in detecting abnormalities of joints and tendons.
Objectives: To evaluate sonographic abnormalities in SLE patients who were currently asymptomatic in hand and wrist joints, and to correlate those with clinical parameters.
Methods: Eighty SLE patients without arthralgia were enrolled. Age and sex-matched 17 healthy controls and 11 SLE patients with arthralgia were included as controls. MSUS using a 5–13 MHz linear array probe was performed in wrist, 2nd and 3rd MCP joint, and flexor tendons of 2nd, 3rd and 4th finger of nondominant side.
Results: Of 80 SLE patients, wrist synovitis was found in 42 patients (52.5%) and MCP synovitis was noted in 41 patients (51.3%). Tendinitis and tenosynovitis were detected in 32 patients (40%) and 12 patients (15%), respectively. These abnormalities were observed more frequently in selected SLE patients with arthralgia ($n = 11$). Moreover, synovial thickness in joints and tendon thickness were greater in patients with arthralgia compared to those without ($P < 0.006$ and $P < 0.03$). However, only alteration found in healthy subjects was synovitis in MCP joint (5.9%). Among patients without arthralgia, patients with synovitis had shorter disease duration ($P = 0.022$), while patients who showed tendinitis had higher mean age of disease onset ($P = 0.002$). Importantly, tendon thickness was positively correlated with mean daily prednisolone dosage ($r = 0.23$, $P = 0.037$).
Conclusions: Asymptomatic joint and tendon involvement of hand and wrist is common in SLE patients. Longitudinal study is needed to determine if silent sonographic abnormalities will develop relevant musculoskeletal symptoms in those patients and progress to more serious pathology leading to deformity.
Disclosure of interest: None declared.

P-459
DUAL INHIBITION OF IL-1-INDUCED NF-KB ACTIVATION AND INOS ACTIVITY IN HUMAN CHONDROCYTES BY ALPHA-PINENE

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Nuclear factor-kappaB (NF-kB) plays a major role in arthritic diseases by promoting the expression of inflammatory and catabolic mediators, namely nitric oxide (NO). Our previous study showed that a fraction (named Falpha-p) of the essential oil from the leaves of *Juniperus oxycedrus*, mainly composed of a racemic mixture of alpha-pinene [2,6,6-trimethyl-bicyclo(3.1.1)hept-2-ene] decreases IL-1-induced NF-kB activation and NO production in human chondrocytes. This study aimed at further elucidating the mechanism of action of alpha-pinene. NF-kB activation was evaluated in the human chondrocytic cell line, C-28/I_2, by western blot for cytoplasmic phospho-IkB-a levels and by ELISA for DNA binding activity. To determine the ability of the test compounds to directly inhibit NO production, regardless of their effect on NF-kB-dependent iNOS expression, nitrite levels were measured by the Griess reaction in human chondrocytes treated with the test compounds after pretreatment with IL-1 for 18 h to induce iNOS expression. Falpha-p decreased phospho-IkB-a, NF-kB DNA binding and nitrite concentration to 22.1% ± 7.8, 46.4% ± 13.2 and 11.3% ± 0.4 of the levels induced by IL-1 alone, respectively. When added 18 h after IL-1, Falpha-p decreased nitrite levels to 42.1% ± 7.3. The commercial racemic and (+) enantiomer of alpha-pinene were less effective while the (−) enantiomer had no significant effects. (+) alpha-pinene probably accounts for most of the ability of Falpha-p to inhibit IL-1-induced NF-kB activation and NO production. The later seems to involve NF-kB dependent and independent mechanisms, probably through direct inhibition of iNOS activity. Supported by grants PTDC/SAU-OSM/67936/2006 and PTDC/EME-PME/103578/2008 from FCT.
Disclosure of interest: None declared.

P-460
EXPRESSION OF NOTCH FAMILY MEMBERS IN HUMAN ARTICULAR CHONDROCYTES

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Aim: The Notch family is involved in cell differentiation during embryogenesis. Osteoarthritic chondrocytes undergo morphological and biochemical changes leading to the de-differentiation process. In this study, we were interested in the involvement of the Notch pathway in human articular chondrocyte de-differentiation.
Methods: Human articular cartilage was obtained from cadavers after informed consent of the family. Chondrocytes were isolated then subjected to several cell culture passages and treated with or without the [*N*-[*N*-(3,5-difluorophenacetyl-L-alanyl)]-*S*-phenylglycine *t*-butyl ester] (DAPT): a Notch inhibitor. Chondrocyte morphology was studied using optical microscopy. Immunoblot was performed to study the expression of differentiated chondrocyte markers (Collagen II, aggrecan) and de-differentiated chondrocyte markers (collagen I, metalloproteinases and nitric oxide synthases) as well as Notch family members.
Results: Without DAPT treatment, chondrocyte de-differentiation resulted in fibroblast-like morphology. This was confirmed by immunoblot analysis, which showed an increase in collagen type I, metalloproteinases, nitric oxide synthases and a decrease in collagen type II and aggrecan expression. With DAPT treatment, de-differentiation was delayed. Immunoblot analysis showed during the first passages inhibition of collagen type II and aggrecan expression, which then was re-expressed during the last passage, suggesting chondrocyte re-differentiation.
Conclusion: In this study, we showed that inhibition of the Notch receptor not only delayed the de-differentiation process, but also leaded to chondrocyte re-differentiation, which confirms the involvement of the Notch pathway in chondrocyte de-differentiation. This suggests that modulation of this pathway could constitute a new therapeutic target for the treatment of osteoarthritis.
Disclosure of interest: None declared.

P-461
INHIBITION OF PROSTAGLANDIN D2 SIGNALING PREVENTS THE PROGRESSION OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscle disease characterized by progressive skeletal muscle atrophy and weakness. DMD is caused by mutations in the dystrophin gene, which encodes the cytoskeletal protein dystrophin. We have found that hematopoietic prostaglandin (PG) D synthase (HPGDS) was induced in grouped necrotic muscle fibers in DMD patients. Cytosolic form of phospholipase A_2 and cyclooxygenase-2, the upstream enzymes of the arachnoid acid cascade, were similarly observed in the HPGDS-positive fibers. We performed functional analysis of prostaglandin D_2 produced by HPGDS in DMD and developed a novel therapy for DMD based on the inhibition of PGD_2 signaling. HPGDS was induced in the necrotic muscle fibers of chemically induced rat and mouse models of DMD and in genetically dystrophin-deficient mdx mice and DMD dogs. The necrotic muscle in DMD models constitutively expressed HPGDS. Oral administration of HPGDS inhibitor for 5 days prevented the expansion of muscular necrosis in *mdx* mouse, as measured by X-ray computed tomography (CT) imaging. *mdx* mice were treated with HPGDS inhibitor for 30 days, and then the recovery of muscle strength, necrotic muscle volume evaluated by X-ray CT, locomotor activities and muscle histology were examined. The HPGDS inhibitor decreased the volume of necrotic muscle, improved locomotor activity and the histological analysis revealed that the inhibitor prevented the expansion of muscle damage. These data demonstrate the pathological role of PGD_2 produced by HPGDS in the expansion of muscle necrosis. These results indicate that PGD_2 produced by H-PGDS is involved in the expansion of muscle necrosis in DMD and that inhibition of PGD_2 signaling is a novel therapy for DMD.
Disclosure of interest: None declared.

P-462
ANTI-INFLAMMATORY EFFECTS OF OLEOCANTHAL IN C2C12 MYOBLASTS

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Objective: Oleocanthal has been shown to mimic the actions of a non-steroidal anti-inflammatory drug (NSAID) and acts as a natural NSAID through the inhibition of cyclooxygenase (COX). NSAIDs attenuate proliferation in C2C12 myoblasts through COX inhibition. COX-2 dependent prostaglandin synthesis is required in muscle regeneration and is implicated in myoblast proliferation. Therefore the use of anti-inflammatory COX-2 inhibitors may be detrimental to muscle growth and regeneration. The objective of the present study is to determine if a natural NSAID such as oleocanthal has adverse effects on myoblast proliferation in vitro.
Methods: Mouse C2C12 myoblasts were grown in 96 well plates. Cell viability was determined by fluorescent assay (CytoTox-Fluor, Promega). Myoblasts were treated with 0.1, 0.5, 1, 5, 10 μM oleocanthal for 24 h. DMSO (0.24%) was used as a vehicle control. Myoblast proliferation was determined by the incorporation of 25 μM 5-bromo-2′-deoxyuridine (BrdU) for 24 h in the presence of oleocanthal. BrdU levels were measured a 405 nm with a reference wavelength of 690 nm.
Results and discussion: Concentrations of oleocanthal between 0.1–5 μM were not toxic to myoblasts over 24 h. Additionally, these concentrations did not affect myoblast proliferation. NSAIDs are prescribed for the treatment of pain associated with inflammatory related disease states such as arthritis leaving patients susceptible to potential adverse effects on skeletal muscle. Long term ingestion of a naturally occurring NSAID such as oleocanthal at low concentrations may have pharmacological benefits without compromising muscle growth or regeneration. Ongoing studies are presently determining the anti-inflammatory effects of oleocanthal in attenuation of inflammatory markers and proteins associated with proteolysis including p38-MAPK, NFκB, antrogin-1 (MFAbx) and MuRF-1 in C2C12 cells and

the attenuation of *Lipopolysaccharide* (LPS) induced TNFα and IL-6 production.
Disclosure of interest: None declared

P-463
FREE INTERLEUKIN-18 AS A DISEASE ACTIVITY MARKER FOR ADULT ONSET STILL'S DISEASE

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Objective: Recent investigations have shown that the serum level of interleukin-18 (IL-18) is extremely high in adult-onset Still's disease (AOSD) patients and is closely related to disease activity. However, IL-18 binding protein (IL-18 BP), a natural antagonist of IL-18, and free IL-18 in AOSD have not been studied yet. The aim of this study was to investigate the usefulness of free IL-18 as an activity marker of AOSD.
Methods: A total of 133 Korean subjects were enrolled in this study. They are consisted of 43 AOSD patients meeting Yamaguchi's criteria, 30 unaffected controls, 30 rheumatoid arthritis (RA) patients, and 30 ankylosing spondylitis (AS) patients. The AOSD patients were divided into three groups according to disease course, and into active or inactive groups. The inactive patients were further subdivided into a remission group and a maintenance group. IL-1β, IL-6, TNF-α, IFN-γ, IL-18 and IL-18BP concentrations were measured by ELISA kit. Free IL-18 levels were calculated by applying the law of mass action.
Results: Despite high levels of IL-18BP, the level of free IL-18 was significantly higher in the AOSD patients than controls (AOSD 10757.3 $\pm$ 11430.0 pg/mL, healthy control 175.1 $\pm$ 74.2 pg/mL, AS 192.6 $\pm$ 98.8 pg/mL, RA 402.4 $\pm$ 450.0 pg/mL, $p < 0.0001$).Free IL-18 levels were significantly lower in the inactive group than the active group (active 14779.9 $\pm$ 11679.2 pg/mL, inactive 6543.1 $\pm$ 9730.6 pg/mL, $p = 0.016$), but still higher than in the controls. Free IL-18 levels in the remission group were lower than in the maintenance group (remission 660.6 $\pm$ 713.3 pg/mL, maintenance 8482.9 $\pm$ 11138.0 pg/mL, $p = 0.021$).When we followed patients showing normal range of acute phase reactants, most patients still showed higher serum levels of free IL-18, and part of them had a flare up later on.
Conclusions: Free IL-18 level represented the disease activity in the AOSD patients, and could be a useful marker in AOSD patients whether continuous immune modulating therapy is necessary.
Disclosure of interest: None declared.

P-464
PK AND PD PROFILE OF GLPG0259, A SMALL MOLECULE MAPKAPK5 INHIBITOR FOR THE TREATMENT OF RHEUMATOID ARTHRITIS, IN HEALTHY VOLUNTEERS

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GLPG0259, an inhibitor of the kinase MAPKAPK5, blocks the release of mediators of inflammation (IL-6, TNFα) and cartilage erosion (MMPs) in disease-relevant cells and in synovial cultures. In the mouse collagen-induced arthritis (CIA) model, GLPG0259 reduces paw inflammation and protects bone and cartilage. Therefore, GLPG0259 may offer a promising new approach for the treatment of rheumatoid arthritis (RA). Here we determined the safety, pharmacokinetic (PK) and pharmacodynamic (PD) profile of GLPG0259 in healthy volunteers, including exploration of interaction with methotrexate (MTX). GLPG0259 solution was administered orally in a dose range of 1.5–150 mg as single doses and in a dose range of 25–75 mg q.d. for 14 days as multiple doses. Single doses of 50 mg as an oral solution were compared to capsule formulations in fasted and fed state. Single doses of MTX were administered before and at steady-state exposures of 50 mg GLPG0259. The PD effects of GLPG0259 were assessed by monitoring a gene signature in blood samples. GLPG0259 was generally well tolerated with no adverse effects on ECG, vital signs or laboratory parameters in healthy volunteers. GLPG0259 showed a dose-proportional PK profile over the dose range tested, substantiating a once-daily oral dosing regimen. Exposures reached steady state after one week of q.d. dosing and exceeded levels seen in the mouse CIA model. The combination of MTX and GLPG0259 did not influence the PK profile of either compound, allowing for the combination therapy in patient studies. Similar PK and safety profiles were observed for the capsule formulations. Furthermore, GLPG0259 altered a specific gene signature. GLPG0259 showed good safety and a PK profile that supports once daily dosing. Based on these results, a Phase II dose-finding study was initiated, evaluating three dose levels of GLPG0259 in RA patients with an inadequate response to MTX.
Disclosure of interest: None declared.

P-465
ACTIVE THERAPEUTIC IMMUNIZATION WITH TNF-KINOID IN PATIENTS WITH RHEUMATOID ARTHRITIS WITH SECONDARY RESISTANCE TO A TNFA MONOCLONAL ANTIBODY

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Background: Blocking TNF alpha (TNFα) with monoclonal antibodies (mAbs) has been very successful in the treatment of severe rheumatoid arthritis. However, nearly 30% of the patients failed to respond and most of them escape mAb efficiency during time. Active immunization with a TNF-Kinoid induces self polyclonal anti-TNFα antibodies and effectively treats TNFα mediated arthritis in transgenic mice. This novel therapeutic strategy could be an attractive alternative to anti-TNFα mAbs.

Objectives: We evaluate the safety, immunogenicity, clinical response to TNF-K in patients with rheumatoid arthritis who develop secondary resistance to one TNFα mAb.
Material and methods: TNFα-Kinoid (TNF-K, Neovacs SA, Paris, France) is an immunotherapeutic composed of recombinant human TNFα conjugated to KLH as a carrier protein, inactivated and adjuvanted with ISA-51 emulsion.
Patients with active rheumatoid arthritis (DAS28 $\geq$ 3.2) with history of positive clinical response to one anti-TNFαmAb followed by secondary failure are being enrolled in a double-blind, placebo-controlled, phase 2, dose escalation study to evaluate three different doses of TNF-K. Immune responses are being evaluated through titration of anti-TNFα and anti-KLH antibodies with isotyping, evaluation of neutralizing capacity and of cellular responses. Clinical response is being assessed by regular evaluation of DAS28, ACR and EULAR scores and titration of C reactive protein
Results: Up to now, no related serious adverse event has been reported. Few minor and transient local and systemic reactions have been observed following immunization. Safety, immunological and clinical results will be presented.
Conclusions: Active immunization with TNFα kinoid to induce a polyclonal, self anti-TNFα antibody response is an attractive and promising new therapeutic concept in rheumatoid arthritis.
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P-466
ADIPONECTIN IS ASSOCIATED WITH JOINT INFLAMMATION AND CARTILAGE DEGRADATION IN OSTEOARTHRITIS

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Objectives: Adipocytokine adiponectin is involved in the regulation of energy metabolism and appetite, and possibly in the development of insulin resistance. Recently, it has also been shown to regulate inflammatory responses. The aim of the present study was to investigate the association and effects of adiponectin on inflammation and cartilage destruction in osteoarthritic joints.
Methods: Blood and cartilage samples were collected from 35 male patients with OA, undergoing total knee replacement surgery. Plasma concentrations of adiponectin and biomarkers of cartilage degradation, i.e. COMP (cartilage oligometric matrix protein) and MMP-3 (matrix metalloproteinase 3) were measured by immunoassay. Cartilage samples were cultured for 48 h, and adiponectin released in the culture medium was measured and correlated to nitric oxide (NO), interleukin-6 (IL-6), MMP-1 and MMP-3 production. Finally, the effects of adiponectin on NO, IL-6, and MMP production in cartilage were studied in tissue culture experiments.
Results: Plasma adiponectin concentrations correlated positively with biomarkers of cartilage degradation, i.e. with COMP (Pearson $r = 0.54$, $p < 0.001$, ln transformation) and with MMP-3 ($r = 0.34$, $p = 0.046$). In addition, OA cartilage was found to produce adiponectin, and the adiponectin amounts released from cartilage in tissue culture correlated positively with the production of NO ($r = 0.34$, $p = 0.049$), IL-6 ($r = 0.39$, $p = 0.021$, ln transformation) and MMP-3 ($r = 0.36$, $p = 0.037$, ln transformation). Further, adiponectin was found to enhance production of NO, IL-6, MMP-1 and MMP-3 in OA cartilage in vitro by a p38 kinase dependent manner.
Conclusions: These findings suggest that adiponectin is involved in the pathogenesis of joint inflammation and cartilage destruction in osteoarthritis.
Disclosure of interest: None declared.

Gastrointestinal diseases

P-467
DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PATTERN IN CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI ISOLATED FROM CHILDREN WITH ACUTE DIARRHEA

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Background: For monitoring the drug resistance among Campylobacter populations which has become a serious concern worldwide, there is a need to reliable and reproducible laboratory techniques. There are several methods; including disk diffusion, broth micro-dilution, agar dilution and E-test to apply to determine in vitro susceptibility profiles of Campylobacter to a range of antimicrobial agents.
Objectives: The aim of present study was isolation of *Campylobacter jejuni* and *Campylobacter coli* from children with diarrhea and determination of antimicrobial susceptibilities of the isolates to clinically relevant antimicrobials.
Methods: In total 220 stool samples of children with diarrhea were cultured on Preston agar and the isolated *Campylobacter* spp. were identified by further standard identification test. Susceptibility testing was carried out using Kirby–Bauer disk diffusion method and E-test.
Results: In this study, 14 Campylobacter strains were isolated (6.36%), of which 9 cases (64.3%) were identified as *C. jejuni* and 5 cases (35.7%) as *C. coli*. Using disk diffusion, all the Campylobacter isolates were fully resistant to cephalothin, oxacillin and ampicillin followed by ceftazidime with resistance rate of 71.42%. Gentamicin and ciprofloxacin were the most effective antibiotics against both isolated Campylobacter species. According to E-test results, Campylobacter isolates demonstrated the greatest resistance to cephalothin (92.85%), oxacillin (92.85%) and ampicillin (78.57%).
Conclusions: Our study reveals a high-level correlation between the E-test and agar disk diffusion method in evaluating the resistance of *Campylobacter* spp. to tested antimicrobial agents.
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P-468
INVESTIGATION OF BRADYKININ 1 RECEPTOR ROLE IN RODENT MODELS OF INTESTINAL INFLAMMATION

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The BKR1 is an inducible receptor, activated in immune system by an inflammatory stimulus or after tissue damage (i.e. inflammatory bowel diseases). Thus, we investigated in rodents the role of BKR1 on intestinal inflammatory models using the selective antagonist SSR240612 (SSR).
Methods: Acute intestinal inflammation was induced by indomethacin (20 mg/kg sc) in rats or by DNBS (6 mg/mouse rectal) in mice. A chronic model of colitis was evoked by TNBS (15 mg/rat rectal) in rats or by TNBS (1 mg/mouse rectal) in mice pre-sensitized with TNBS on dorsal skin. SSR and its vehicle were given orally by single or repeated oral treatment. Sulfasalazine was used as reference oral compound. Animals wasting syndrome was monitored daily; intestinal macroscopic damage and colon histological analysis was evaluated after colon withdrawal. ANOVA and Kruskal–Wallis tests were used for statistics. The results are expressed as mean ± SEM of 7–10 animals for group.
Results: In the indomethacin model, SSR (3, 10 and 30 mg/kg) dose dependently prevented small intestinal ulcers formation; % of inhibition vs control 11, 31 and 60 respectively, $p < 0.05$. In DNBS colitis, SSR (30 mg/kg/bid for 3 days) significantly reduced mice colon ulcers score (52%, $p < 0.05$), colon thickness and the loss of body weight. In rat TNBS colitis, SSR (30 mg/kg/bid for 4 days) significantly inhibited ulcers (38%, $p < 0.05$), colon adhesion (62%, $p < 0.05$) and wasting syndrome; diarrhoea score 1.2 ± 0.37 versus control 2.8 ± 0.13, $p < 0.05$. Histological analysis indicated a significant reduction of total microscopic damage (SSR 10.3 ± 0.4; control 13.3 ± 0.4, $p < 0.05$) and ulcers lesion in SSR treated rats. In mice chronic colitis model, SSR (30 mg/kg/bid for 3 days) induced again an overall recovery from the disease.
Conclusion: These results suggest a possible role for BKR1 receptors in the pathogenesis of intestinal inflammation and indicate a potential therapeutic application of antagonists for treating inflammatory bowel diseases.
Disclosure of interest: None declared.

P-470
LYMPHATIC PUMPING INHIBITION: THE ROLE OF KATP CHANNELS AND NITRIC OXIDE

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Intrinsic contractile activity of lymphatic vessels is critical in tissue fluid homeostasis, immune cell trafficking and nutrient uptake. Correlation between inflammatory bowel disease (IBD) and dilated and/or obstructed mesenteric lymphatics is described in clinical settings and animal models. In the guinea pig model of TNBS-ileitis, mesenteric lymphatic vessels are enlarged and their contractile function is impaired. This dysfunction can be caused by the action of inflammatory mediators. Among them, nitric oxide (NO), an inflammatory mediator upregulated in intestinal inflammation can decrease lymphatic muscle contractility through activation of ATP-sensitive potassium channels (K_{ATP}). Our objective is to understand the role of K_{ATP} in inflammation-induced lymphatic dysfunction and their possible dysregulation by NO using the guinea pig model of TNBS ileitis. Following initiation of the inflammation, animals were sacrificed at day 1 for PCR and day 3 for in vitro studies. Lymphatic tissues were microdissected out and real time PCR was performed for K_{ATP} subunits Kir6.1 and SUR2B, as well as iNOS. Pharmacological studies were performed in vitro on isolated, cannulated mesenteric lymphatic vessels. The contractile activity was assessed in the presence of 10 μM of the K_{ATP} antagonist glibenclamide, the iNOS inhibitor 1400 W, or the sGC inhibitor ODQ. Our data show that expression of Kir6.1, SUR2B and iNOS mRNAs was significantly upregulated in TNBS versus sham animals ($p < 0.05$). Administration of glibenclamide induced a large contractile response in the quiescent lymphatic vessels from TNBS animals ($p < 0.0001$). Administration of 1400 W and ODQ also improved lymphatic contractile activity in TNBS animals ($p < 0.05$). Our findings suggest that K_{ATP} and iNOS play a major role in the lymphatic contractile dysfunction seen in TNBS animals. Identifying molecular targets to reverse lymphatic dysfunction can lead to pharmacological tools to treat intestinal inflammation.
Disclosure of interest: None declared.

P-471
GIARDIA DUODENALIS ALTERS HUMAN INTESTINAL MUCOSAL BIOFILMS TO MODULATE INTESTINAL EPITHELIAL CELL HOMEOSTASIS: A ROLE IN POST-INFECTIOUS-IRRITABLE BOWEL SYNDROME?

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Acute enteric infections may initiate post-infectious irritable bowel syndrome (PI-IBS) via unknown mechanisms. *Giardia duodenalis* (*G. intestinalis, G. lamblia*) is the most common cause of parasitic diarrheal disease worldwide. Follow-up after recent outbreaks have implicated giardiasis in the development of PI-IBS in a number of patients. Whether disruptions in the resident intestinal microbiota structure and/or species distribution by enteropathogens may be implicated in the pathogenesis of PI-IBS has yet to be determined. This study examined (1) whether *Giardia* trophozoites modify human intestinal mucosal biofilms retrieved from intestinal biopsies, and (2) whether these biofilm changes may disrupt human epithelial monolayers.
Methods: Experiments in vitro used Caco-2 and HCT-8 intestinal monolayers. Representative, multi-species biofilms were cultured anaerobically (72 h) on the Calgary Biofilm Device from human intestinal mucosal biopsies collected during routine colonoscopies.
Results: Cell counts reveal *Giardia* promotes the planktonic growth phase of bacteria over that associated with the biofilm structure. Scanning electron microscopy indicates that *Giardia* inhibits the secretion of the normal extracellular matrix covering the biofilm. ELISA revealed that monolayers co-incubated for 3 h with *Giardia*-exposed biofilms (24 h) exhibited greater levels of apoptosis, compared to those incubated with biofilms not exposed to *Giardia*.
Conclusion: In this study, multi-species anaerobic biofilms, representative of the endogenous mucosal microbiota, were successfully grown from human intestinal biopsies. Our findings indicate that

Giardia modifies the structure and phenotype of these communities. Lastly, *Giardia*-exposed biofilms disrupt the homeostasis of intestinal monolayers by enhancing their apopotic decay. These results pave the way towards a better understanding of the ramifications that complex host-polymicrobial interactions have in chronic disease manifestation.
Disclosure of interest: None declared.

P-472
COLITIS MODULATES LOCAL METABOLISM OF GLUCOCORTICOIDS NOT ONLY IN COLON BUT ALSO IN SECONDARY LYMPHOID ORGANS

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Glucocorticoids exert anti-inflammatory and immunomodulatory effects that are regulated in part by activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11HSD1). This enzyme is responsible for the interconversion of inactive glucocorticoids (cortisone, 11-dehydrocorticosterone) into biologically active steroids (cortisol, corticosterone) and plays an important role in various diseases. We have shown recently that 11HSD1 is upregulated in bioptic samples taken from patients with ulcerative colitis. The aim of the present study was (1) to characterize the role of proinflammatory cytokines in upregulation of colonic 11HSD1 and (2) to identify the effect of inflammation associated with colitis on 11HSD1 in spleen and mesenteric lymph nodes (MLN). Experimental colitis was induced in rats by the intracolonic administration of 2,4,6-trinitrobenzenesulfonic acid. Our results showed that colitis upregulated in the large intestine not only 11HSD1 mRNA and 11-reductase activity but also mRNA levels of proinflammatory cytokines tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β). Similarly, treatment of colon tissue explants with TNFα significantly increased 11HSD1 mRNA expression. In contrast, IL-1β was without any effect even if both cytokines upregulated cyclooxygenase-2 (COX-2) mRNA. Inflammation also increased mRNA levels of 11HSD1, TNFα, IL-1β and COX-2 in intraepithelial (IEL) and lamina propria lymphocytes (LPL). Similar increase of 11HSD1 mRNA expression and 11-reductase activity was found also in mesenteric lymph nodes and spleen, particularly in T cells. Comparison of 11HSD1 levels showed that enzyme expression was lower in MLN than in IEL and particularly in LPL. Thus, inflammation stimulates the reactivation of glucocorticoids in lymphoid organs and in gut-associated lymphoid tissue. This reactivation system seems to exhibit a higher activation in the cells of the effector regions of mucosal immune system. *Supported by GA CR P303/10/0969.*
Disclosure of interest: None declared.

P-473
EFFECTS OF INTERFERON GAMMA ON AQUAPORIN 1 EXPRESSION IN MOUSE INTESTINAL EPITHELIUM

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Aims: The water channel protein aquaporin 1 (AQP1) is widely expressed on epithelial cells with the primary purpose of regulating transmembrane water flux. Chronic inflammatory states like inflammatory bowel disease (IBD) are characterized by high levels of interferon (IFN)γ and exhibit abnormal transepithelial fluid flux. We sought to determine the effect of IFNγ on AQP1 expression in the intestinal epithelium.
Methods: (1) C57Bl6 mice were given 4% dextran sodium sulfate (DSS) in their drinking water to induce acute colitis and were euthanized at 3 days. Colons were removed and AQP1 expression was assessed by immunofluorescence. (2). Confluent CMT93 cells (mouse cecal epithelial cell line that constitutively expresses AQP1) on Transwell membranes were pre-treated with either siRNA to knock down STAT1 (signal transducer and activator of transcription 1) or a JAK2 (Janus Kinase 2) inhibitor and then treated with IFNγ (10 ng/mL, 24 h) in serum free media. Cells were then lysed and protein levels for AQP1 and STAT1 were assessed by Western blot.
Results: (1) In C57Bl6 mice, AQP1 immunoreactivity was observed in vascular endothelial cells and on the apical membrane of colonic crypt epithelial cells. Acute colitis induced by DSS was associated with a loss of crypt epithelial, but not vascular endothelial, AQP1 immunoreactivity. (**2**). CMT93 cells demonstrated a significant suppression in AQP1 protein expression after treatment with IFNγ at 24 h. Pre-treatment with JAK2 inhibitor, but not STAT1 siRNA, prior to IFNγ administration resulted in the amelioration of the IFNγ-induced suppression of AQP1 protein expression.
Conclusions: Epithelial AQP1 expression is reduced by DSS colitis in mice and by IFNγ in CMT93 cells through a unique JAK2-dependent, STAT1-independent mechanism. Thus, inflammatory cytokines present during IBD are able to influence AQP1 levels and may contribute to the water transport deficiencies observed in these diseases.
Disclosure of interest: None declared.

P-474
SRT2104, A NOVEL AND SELECTIVE SMALL MOLECULE SIRT1 ACTIVATOR, INHIBITS DSS-INDUCED COLITIS IN A SIRT1-DEPENDENT MANNER

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The NAD-dependent deacetylase Sirtuin-1 (SIRT1) has been implicated in a number of critical physiological functions including control of inflammation. Genetic and biochemical studies show a key role of SIRT1-mediated deacetylation in the inflammatory response. Most notably, SIRT1 mediated deacetylation of p65/RelA directly reduces NF-kB transcriptional activity thus suppressing pro-inflammatory responses. SRT2104 is a novel small molecule that activates SIRT1 in biochemical and cell-based assays and has demonstrated selectivity against over 200 GPCRs, nuclear hormone receptors, enzymes and ion channels. In the present study the efficacy of SRT2104 to attenuate inflammation in DSS-induced colitis was compared to the corticosteroid prednisolone. Colitis was induced by administering 3% DSS in drinking water for 5 days following which the severity of the inflammatory response was assessed by endoscopy. Treatment was then begun with SRT2104 (1–30 mg/kg), prednisolone (3 mg/kg) or vehicle administered orally for 9 days followed by endoscopic analysis on days 7 and 13

with mice sacrificed on day 14 for histology. Prednisolone reduced colitis severity but worsened the body weight loss seen in this model. By contrast, SRT2104 produced a greater reduction in the colitis score compared to prednisolone while improving the loss in body weight. On histological examination, SRT2104 also produced a greater effect than did prednisolone. The ability of SRT2104 and prednisolone to reduce colitis was then examined in mice in which SIRT1 had been genetically knocked out in the gastrointestinal tract. Prednisolone was equally efficacious in wild-type and SIRT1 KO mice whereas the anti-inflammatory effect of SRT2104 was absent in the SIRT1 KO mice demonstrating a SIRT1-dependent efficacy for SRT2104. These data demonstrate that small molecule activators of SIRT1, like SRT2104, may have therapeutic value in inflammatory disorders such as ulcerative colitis.
Disclosure of interest: J. Ellis employee of: GSK, D. Gagne Employee of: GSK, V. Suri employee of: GSK, J. Cermak employee of: GSK, G. Lyng employee of: Biomodels, L. Guarente Grant/Research Support from: GSK, Consultant/Speaker's bureau/Advisory activities with: GSK, G. Vlasuk employee of: GSK

P-475
PROBIOTICS INHIBIT THE DEVELOPMENT OF INFLAMMATORY PROCESSES IN RECURRENT TNBS-COLITIS: INSIGHTS FROM INTESTINAL TRANSCRIPTOME ANALYSIS

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A recurrent TNBS colitis model was established in BALB/c mice, by weekly intrarectal installation of increasing dosages TNBS. Development of disease in this model is evident from transient weight loss and increased weight and thickness of the colons. Histopathological evaluation of the colons show increased numbers of T cells (CD4+ and CD8+), mast cells, macrophages and dendritic cells. In addition, multiplex analysis reveals elevated serum levels of pro-inflammatory cytokines, including IL-17, IFN-γ, IL-1β, and MIP-1α, suggesting the involvement of Th1 and Th17 cells. The development of these aspects of colitis is sensitive to suppression by intrarectally or orally administered budesonide. Prophylactic treatment by oral administration of *L. plantarum* or the VSL#3 probiotic mixture suppressed the development of recurrent TNBS colitis, evident from less macroscopic alterations, reduced mucosal infiltration by inflammatory cells and serum levels of IL-17, IFN-γ, IL-1β, MCP-1 and MIP-1α to concentrations comparable to those found in healthy mice. Transcriptome analysis of colon showed increased expression of mast cell proteases and α-defensins in mice with colitis, but significantly less in mice treated with probiotics. Our data indicate that the recurrent TNBS colitis model shows several aspects of inflammatory bowel disease, which are sensitive both to corticosteroids and even to subtle modulation by probiotics.
Disclosure of interest: None declared.

P-476
THE ANTI-INFLAMMATORY EFFECTS OF GIARDIA DUODENALIS OCCUR VIA THE SECRETION OF A CYSTEINE PROTEASE

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Giardia duodenalis is a non-invasive protozoan parasite of the small intestine and common cause of diarrheal disease. Despite a parasite load that may exceed one million trophozoites per centimetre of gut, the small intestinal mucosa of *Giardia*-infected individuals is devoid of overt inflammation, for reasons that remain obscure. In the small intestine, acute inflammatory responses include the secretion of the potent neutrophil chemoattractant interleukin-8 (CXCL8) by intestinal epithelial cells. Following exposure to enterocytes, *G. duodenalis* trophozoites express several cathepsin-like proteases of unknown function. We hypothesized that *G. duodenalis* trophozoites attenuate pro-inflammatory responses in intestinal epithelial cells by releasing cathepsin-like proteases.
Methods: Experiments were performed in vitro using Caco-2 and HCT-8 human intestinal epithelial monolayers, and ex vivo, using human small intestinal biopsy tissues. Cells were co-incubated with assemblage A or B *G. duodenalis* trophozoite isolates and subsequently stimulated with pro-inflammatory IL-1β.
Results: Co-incubation of *Giardia* trophozoites (both assemblage A and B genotypes) with cell monolayers or small intestinal mucosal biopsy tissues results in the secretion of an unknown, heat-sensitive factor that attenuates IL-1β-induced CXCL8 secretion. Recombinant CXCL8 administered to cell monolayers co-incubated with *Giardia* trophozoites was significantly reduced versus control groups. Cathepsin B/L activity was increased in cell supernatants and monolayers co-incubated with *Giardia* trophozoites. Pre-treatment with a cysteine protease inhibitor, E-64, abolished cathepsin B/L activity in these groups and reversed *Giardia*-mediated attenuation of IL-1β-induced CXCL8 secretion.
Conclusion: The interaction between *Giardia* trophozoites and IECs results in the secretion of a cysteine protease that cleaves CXCL8. This factor may represent a potent anti-inflammatory compound.
Disclosure of interest: None declared.

P-477
CARBON MONOXIDE (CO) AND HYDROGEN SULPHIDE (H2S) DONORS PREVENT ETHANOL-INDUCED GASTROPATHY IN MICE BY NITRIC OXIDE SYNTHASE (NOS) DEPENDENT PATHWAY

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It was demonstrated that NO, CO and H_2S are gastric defense mediators. However, the interactions of these three endogenous gases in the gastric protection mechanisms are not totally defined. In this study, we evaluated the participation of NOS in CO and H_2S gastroprotective effect against ethanol-induced gastric injury in mice. Swiss mice, (20–30 g), previously fasted for 24 h, were pre-treated with L-NAME (NOS inhibitor, 3 mg/kg, i.p.) or saline. After 30 min., received saline, hemin (HO-1 inducer, 3 mg/kg, i.p.), dimanganese decacarbonyl (DMDC) (CO donor, 12.5 μmol/kg) or NaHS (H_2S donor, 150 μmol/kg, i.p). One hour after, ethanol 50% (0.5 ml/25 g, by gavage) was administrated. At the end of 1 h, the mice were sacrificed and the stomachs removed. Mucosal lesion area was measured by planimetry using an Image J program. Gastric corpus fragments were removed and stored until assayed for malondialdehyde (MDA) and glutathione (GSH) concentration. Data were analyzed using One-Way ANOVA and Newman–Keuls test. All animal treatments and surgical procedures were approved by the local ethics committee (protocol No 63/07). CO inducer/donor (hemin: 35.0 ± 5.7 mm^2, MDA $= 55.3 \pm 6.9$ nmol/g; GSH $= 523.3 \pm 43.9$ mg/g; DMDC: 13.2 ± 2.7 mm^2, MDA $= 45.5 \pm 5.7$ nmol/g; GSH $= 501.8 \pm 25.2$ mg/g) or H2S donor (NaHS: 22.3 ± 7.1 mm^2, MDA $= 50.2 \pm 2.4$ nmol/g, GSH $= 621.1 \pm 58.1$ mg/g) significantly ($p < 0.05$) decreased the gastropathy induced by ethanol (103.9 ± 5.9 mm^2, MDA $= 89.1 \pm 5.6$ nmol/g, GSH $= 323.6 \pm 23.9$ mg/g). The pre-treatment with L-NAME reversed the gastroprotective effect induced by hemin (80.2 ± 6.8 mm^2, MDA $= 75.3 \pm 4.3$ nmol/g, GSH $= 387.1 \pm 23.24$ mg/g), DMDC (67.7 ± 5.5 mm^2, MDA $= 70.7 \pm 6.0$ nmol/g, GSH $= 352.6 \pm 30.1$ mg/g), and NaHS (89.0 ± 17.0 mm^2, MDA $= 78.4 \pm 4.6$ nmol/g, GSH $= 388.5 \pm 23.0$ mg/g). Our results suggest carbon monoxide (CO) and hydrogen sulphide (H_2S) play a protective role against ethanol-induced gastropathy through mechanisms that can be dependent of constitutive NO synthase activation. SUPPORT: Capes, CNPq and FUNCAP.
Disclosure of interest: None declared.

P-478
FYN KINASE-DEFICIENT MICE ARE LESS SUSCEPTIBLE TO DEXTRAN SODIUM SULFATE (DSS)-INDUCED COLITIS

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Introduction: Development of inflammatory bowel disease involves interaction of factors including dysregulation of immune inflammatory responses and intestinal barrier function. We have been studying the effects of the cytokine interferon gamma (IFNγ) on intestinal barrier permeability, and recently identified the Src kinase Fyn as a crucial mediator of IFNγ-induced intestinal permeability increases.
Hypothesis: We hypothesized that mice deficient in Fyn kinase would exhibit reduced disease in a T_H1-polarizing murine colitis evoked by DSS.
Methods: Twelve-to-fifteen week-old, Fyn-deficient and wild-type control mice were either supplied tap water or water supplemented with 2.5% (w/v) DSS for a ten day period (n = 5–6/treatment group). Throughout the study, animals were monitored for weight loss, stool appearance and rectal bleeding. Upon autopsy animals were assessed for colonic macroscopic damage, colonic length, hematocrit levels and myeloperoxidase activity. Further, colonic tissue was dissected and subjected to microscopic assessment of histopathology (0–12 scale) based upon epithelial structure, cell infiltrate, ulceration and changes to intestinal smooth muscle.
Results: Wild-type mice treated with DSS lost significantly more body weight relative to Fyn-deficient mice and had significantly greater decreases in hematocrit. Wild-type mice also had significantly shorter colon lengths following DSS treatment relative to Fyn$^{-/-}$ mice. Finally microscopic analysis indicated wild-type animals had substantially greater histopathology than Fyn$^{-/-}$ mice, typified by marked loss of epithelial architecture, cell infiltrate, edema and smooth muscle thickening.
Conclusions: Fyn-deficient mice exhibit reduced inflammatory disease following exposure to DSS. Further examination of whether this phenotype is due to improved intestinal barrier function or a modulation of the pro-inflammatory response will define the protective, anti-colitic effect of Fyn kinase deficiency.
Disclosure of interest: None declared.

P-479
AQUAPORIN 3 EXPRESSION AND LOCALIZATION IS ALTERED EARLY IN THE DEXTRAN SODIUM SULFATE MODEL OF COLONIC INFLAMMATION

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Aims: Inflammatory bowel diseases (IBD) are characterized by altered water transport leading to the development of diarrhea. However, the status of aquaporin (AQP) 3 expression, localization and its role in the barrier dysfunction that characterizes IBD remains unknown. *We hypothesized that AQP3 expression and localization were altered in IBD.*
Methods: C57Bl/6 mice were treated with 2.5% dextran sodium sulfate (DSS) in their drinking water for up to 7 days to induce colonic inflammation. The establishment of colitis was verified morphologically and biochemically. AQP3 expression was assessed using real-time RT-PCR and localization was assessed by immunofluorescence of formalin-fixed paraffin-embedded colonic tissue. Furthermore, AQP3 expression was assessed both in human biopsy samples and in serum-starved HT29 colorectal adenocarcinoma cells exposed to human recombinant tumour necrosis factor (TNF) α and/or interferon (IFN) γ.
Results: DSS-treated mice had significantly increased granulocyte infiltration at 5, 7 and 14 days, but not at 3 days following commencement of DSS. AQP3 mRNA expression in mucosal scrapings was unaltered at both 3 and 7 days following the start of treatment. However, immunofluorescence studies revealed overall downregulated expression of AQP3 in 3 day DSS-treated tissues, with diminished basolateral staining in cells lining colonic crypts. Human biopsy sample analysis revealed no significant difference in AQP3 mRNA expression between healthy control, active and quiescent ulcerative colitis biopsy samples. Time-course experiments with a single treatment of TNFα (25 ng/mL) or IFNγ (500 U/mL) resulted in significantly reduced expression of AQP3 mRNA at 6–12 h post-treatment, although total protein expression levels in these cells remained unaltered at 24–96 h post-treatment.

Conclusions: Our data suggest that changes in epithelial AQP3 expression and localization represent early events that occur in colonic inflammation.
Disclosure of interest: None declared.

P-480
EFFECT OF LIPOXYGENASE PATHWAY METABOLITES ON INTESTINAL EPITHELIAL BARRIER FUNCTION

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We recently demonstrated that cyclooxygenase metabolite prostaglandin E_2 induces the disruption of intestinal epithelial barrier function through the interaction with EP_1 and EP_4 receptors. In the present work, our objective was to study the effect on epithelial barrier function of eicosanoids produced by the lipoxygenase pathway: leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) and cytochrome P-450 monooxygenase pathway: 20-HETE and epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs). Moreover, we further investigated the receptors and the signaling pathways involved in this event. Paracellular permeability (PP) was estimated from fluorescein isothiocyanate–dextran fluxes and transepithelial electrical resistance in differentiated Caco-2 cells. Our results suggest that 20-HETE and EETs/DHETs have no effect on PP whereas LTD_4, 5-HETE, 12-(R)-HETE, 12-(S)-HETE and 15-HETE increased this parameter. Moreover, our findings indicate that cysteinyl-leukotriene receptor 1 participates in the effects induced by LTD_4 while no candidate was found for 5-HETE. For both eicosanoids, these effects were mediated through the activation of the phospholipase C/Ca^{2+}/protein kinase C pathway in addition to cAMP-independent PKA activation. Furthermore, we observed a correlation between increased PP and the redistribution of TJ proteins. In conclusion, on the basis of our results, we propose that lipoxygenase pathway metabolites could be contributed to the disruption of epithelial barrier function characteristic of inflammatory bowel disease.
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Disclosure of interest: None declared.

P-481
VANIN MOLECULES IN INFLAMMATORY BOWEL DISEASE

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Vanin molecules are pantetheinases which release cysteamine in tissues. Based on the use of Vanin-1 deficient mice, we showed that Vanin-1 is a major regulator of intestinal inflammation. A Vanin-1 deficiency protects mice from acute infectious or toxic stresses by dampening inflammation and ensuing lesions. Mechanistically, Vanin-1 behaves as a proinflammatory molecule by controlling glutathione levels and PPARgamma anti-inflammatory potential in gut mucosa. However, Vanin-1 role is probably more complex. Vanin-1 exerts a cytoprotective role on islet beta cells and consequently contributes to the progression of type 1 diabetes. We have investigated the contribution of human VNN1 to human gut inflammatory diseases. We sequenced the Vanin-1 gene in 400 IBD patients and controls and identified novel polymorphisms and mutations which might contribute to the progression of the disease by modulation of the enzymatic activity in gut epithelium. In parallel we developed novel tools to quantify cysteamine activity in tissues and show that VNN1 is highly expressed in epithelial cells of patients with ulcerative colitis. We also document the presence of a seric form of the enzyme which may serve as a marker in various disease conditions. Preliminary results indicate that seric pantetheinase might be involved in the control of systemic inflammation and blood homeostasis.
Disclosure of interest: None declared.

P-482
JAPANESE RICE WINE (SAKE)-DERIVED COMPONENTS HAVE ANTI-INFLAMMATORY EFFECT ON DEXTRAN SULFATE SODIUM (DSS)-INDUCED ACUTE COLITIS

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Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is partly caused by dysfunction of immune system and overproduction of inflammatory cytokines. Our previous studies identified an anti-inflammatory peptide; pyroglutamyl-leucine (pyroGlu-Leu) in Japanese rice wine (*sake*). The objective of the present study was to prepare pyroGlu-Leu rich fraction from food and check its therapeutic potency to IBD.
Constituents in Japanese rice wine were fractionated into 10 fractions on the basis of isoelectric point and charge by an ampholyte-free preparative isoelectric focusing (autofocusing) apparatus. Fr. 1–5 (pH 2.45–3.10), Fr. 6–7 (pH 2.87–2.93), Fr. 8 (pH 3.40), Fr. 9 (pH 8.66) and Fr. 10 (pH 12.14) were prepared. Then, Fr. 1–5 was fractionated by solid phase extraction using Sep Pak C18. Pyroglutamic acid and pyroGlu-Leu were rich in Fr. 1–5 and Fr. 6–7, and Fr. 8 and Fr. 9 also contained some amount of pyroGlu-Leu. Acute colitis was induced in mice by oral administration of 2.5% dextran sulfate sodium (DSS) in drinking water for 7 days. Mice were treated with each fraction at 30 mg peptide/kg body weight/day or various concentration of synthetic pyroGlu-Leu during entire colitis induction period. Fr. 9 treated group significantly improved the colon length ($p = 0.05$) and percent loss in weight ($p = 0.04$) compared with control group on DSS-induced acute colitis. 0.1 mg/kg of pyroGlu-Leu treated group also significantly improved percent loss in weight ($p = 0.05$). On the other hand, Fr. 1–5 treated group significantly reduced the colon length ($p = 0.02$). The group treated with Sep Pak C18-unabsorbed fraction of Fr. 1–5 showed high mortality while all in control group survived. This result indicates that Japanese rice wine-derived components have a potent of therapeutic agent on IBD and pyroGlu-Leu might be one of these components, though Japanese rice wine contains low amount of detrimental substance within acidic and hydrophilic properties.
Disclosure of interest: None declared.

P-483
NITROSYL-RUTHENIUM COMPLEX, A NEW NITRIC OXIDE (NO) DONOR, PREVENTS ETHANOL-INDUCED GASTROPATHY IN MICE

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NO is an endogenous mediator of gastric mucosal defense. New NO donors with ruthenium metal were produced. These compounds are soluble in water with higher chemical stability and lower toxicity. The aim of this study was to evaluate the effects of nitrosyl-ruthenium complex cis[Ru(bpy)2(SO3)(NO)]PF6 against ethanol-induced gastropathy. Swiss mice, (20–30 g), previously fasted for 24 h, were pretreated by gavage with saline, nitrosyl-ruthenium (1 mg/kg, 1.5 μmol/kg) or sodium nitroprussiate (10 mg/kg, 33.5 μmol/kg). After 30 min., ethanol 50% (0.5 ml/25 g, by gavage) was administrated. At the end of 1 h, the mice were sacrificed and the stomachs removed. Mucosal lesion area was measured by planimetry. Gastric corpus fragments were removed and stored until assayed for malondialdehyde (MDA) and glutathione (GSH) concentration. Data were analyzed using one-way ANOVA test. All animal procedures were approved by the local ethics committee (No. 33/10). Ethanol induced gastric damage (103.5 ± 14.4 mm^2), reduced glutathione (267.0 ± 26.2 mg/g) and increased MDA (241.8 ± 19.0 nmol/g) concentrations, when compared with saline (without gastric damage, MDA:142.2 ± 2.0 nmol/g, and GSH:430.8 ± 55.1 mg/g). Nitrosyl-ruthenium decreased in 72% (28.5 ± 11.1 mm^2) and sodium nitroprussiate in 59.5% (41.98 ± 14.0 mm^2) the gastric damage induced by ethanol. In addition, either nitrosyl-ruthenium or sodium nitroprussiate reversed the decrease in glutathione (nitrosyl-ruthenium $= 535.2 \pm 59.0$ mg/g; sodium nitroprussiate $= 471.0 \pm 44.2$) and increase in MDA (nitrosyl-ruthenium $= 184.8 \pm 8.5$ nmol/g; sodium nitroprussiate $= 50.2 \pm 2.4$) concentrations induced by ethanol. Our results suggest that the nitrosyl-ruthenium complex prevents ethanol-induced gastropathy, in part by decreased the ethanol-induced free radicals production. Furthermore, nitrosyl-ruthenium complex increase the ability of the gastric mucosa to resist injury at doses up to 22-fold lower than the sodium nitroprussiate.

Support: CNPq, CAPES, FUNCAP.

Disclosure of interest: None declared.

P-484
EFFECT OF VITAMIN A SUPPLEMENTATION ON INFLAMMATORY MARKERS IN PATIENTS WITH ULCERATIVE COLITIS

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Background: Ulcerative colitis (UC) is a chronic inflammatory disease of the rectal and colonic mucosa and seems to result from a complex series of interactions between susceptibility genes, the environment and the immune system. There is a paucity of data on the positive effect of vitamin A on intestinal mucosal immunity. We aimed to evaluate the effect of vitamin A supplementation on pro-inflammatory cytokine interleukin 12 (IL12) and acute phase protein, high sensitive-C-reactive protein(hs-CRP) and anti-inflammatory cytokines, IL4 and IL10 in ulcerative colitis.

Methods: Immunological assessment was done in 43 outpatients with ulcerative colitis participating in a before and after interventional survey in Sheikh al rais, a public clinic of Tabriz**.** All participants were at clinical remission stages. The severity of disease activity was assessed by Truelove–Witt Index. Vitamin A supplement was injected twice (50000 IU) with 2 weeks interval. Study period was considered 45 days. Serum interleukins and hs-CRP levels were measured by ELISA and turbidimetric methods, respectively.

Results: Vitamin A supplementation had significant effect on decrease of IL12 ($P = 0.003$) and clinical symptoms severity ($P = 0.001$). IL4 increased considerably($P = 0.001$). While hs-CRP and IL10 changes were not significant.

Conclusion: Vitamin A supplementation could suppress serum pro-inflammatory cytokines and be beneficial in reduction of clinical symptoms severity of disease in ulcerative colitis in remission and should be explored in a larger study.

Disclosure of interest: None declared.

P-485
DIFFERENTIAL EFFECTS OF INDOLES AND SEROTONIN ON INFLAMMATORY RESPONSE IN HT-29 COLON EPITHELIAL CELLS

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Inflammatory bowel disease (IBD) is a disease of chronic intestinal inflammation, which causes severe diarrhea, abdominal pain, bleeding and finally development of colon cancer. Serotonin is a neurotransmitter and gastrointestinal hormone, which regulates intestinal movements in the gut. In human IBD patients, serotonin level is increased in inflamed gut. In a previous study, we found that serotonin induces adhesion of monocytes to HT-29 colon epithelial cells, similar to the action of TNF-α. Since the presence of high levels of bacteria creates a biofilm on the surface of the gut mucosa in patients with IBD, we evaluated whether serotonin and its precursor indole inhibit bacterial biofilm formation in vitro. Indole and its derivative 3,3′-methylene-bisindole (MBI) reduced enterohemorrhagic *Escherichia coli* O157:H7 biofilm formation, whereas serotonin did not. In terms of adhesion of monocytes to HT-29 cells, indole and MBI significantly inhibited TNF-α as well as serotonin-induced adhesion of monocytes to HT-29 cells. In addition, indoles were more potent at inhibiting serotonin-induced than TNF-α-induced adhesion. Taken together, serotonin acts as proinflammatory factor in IBD by inducing inflammatory response without affecting bacterial biofilm formation. In contrast, indoles inhibited serotonin and TNF-α-induced monocytes adhesion to HT-29 cells as well as bacterial biofilm formation.

Disclosure of interest: None declared.

P-486
THE INHIBITORY EFFECTS OF WATER EXTRACTS, TRICHOSANTHES KIRILOWII AND POGOSTEMON CABLIN BENTHAM ON INFLAMMATORY PROCESS IN VITRO AND IN VIVO

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In inflammatory bowel disease (IBD), colon epithelial cells increased the expression of a variety of inflammatory mediators, cell adhesion molecules and chemokines. Several studies have shown that plant extracts and herbs possess anti-inflammatory activities. In the present study, we examined whether water extracts of traditional herbal medicine, *Trichosanthes kirilowii* root and *Pogostemon cablin* Bentham aerial parts prevent inflammatory agent-induced changes of colon epithelial cells. *Trichosanthes kirilowii* roots have been reported immunomodulatory and antitumor activities recently. *Pogostemon cablin* Bentham aerial parts are traditional medicine used for common cold and infectious disease. In this study, we found that *Trichosanthes kirilowii* water extract (TKW) and *Pogostemon cablin* Bentham water extract (PBW) effectively inhibited TNF-α-induced monocytes adhesion to HT-29 colon epithelial cells. In trinitrobenzene sulfonic acid-induced rat model of colitis, TKW and PBW reduced weight loss and increased myeloperoxidase activity in a dose-dependent manner. Furthermore, TKW and PBW suppressed TNBS-induced NF-κB nuclear translocation and mRNA expression of IL-8, IL-6 and MCP-1. When HT-29 cells were transfected with NF-κB-Luc plasmid, TKW and PBW significantly inhibited TNF-
α-induced NF-κB luciferase activity. Taken together, the results suggest that TKW and PBW suppress the colon inflammation via suppression of important pro-inflammatory cytokines expression in a NF-κB dependent manner.

Disclosure of interest: None declared.

P-487
GUT SEROTONIN INDUCES INFLAMMATORY RESPONSE AND ANGIOGENESIS

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Inflammatory bowel disease (IBD) is characterized by an excessive release of several proinflammatory cytokines which results consequently in an increased inflammatory response. Serotonin (5-hydroxytryptamine, 5-HT) is a major gastrointestinal paracrine hormone and enteric neurotransmitter. Studies on human IBD and experimental animal models have shown increased serotonin level in the inflamed gut. In this study, we investigated the effects of serotonin on intestinal inflammation in vitro by measuring the degree of monocyte adhesion to colonic epithelial cells. Serotonin (0.1–100 mM) induced monocyte adhesion to HT-29 colon epithelial cells similar to TNF-α. No additive effect was seen in case of co-treatment of the cells with serotonin and TNF-α. Serotonin-induced inflammatory response was subsided well by the treatment with different polyphenols compared to TNF-a-induced inflammation. In vivo, intra-colonic administration of serotonin in rats through rectum increased myeloperoxidase activity and expression of IL-8, IL-6 and VCAM-1. Since several lines of evidence have implicated that IL-8 induces abnormal angiogenesis in the inflamed site, we evaluated whether serotonin induces angiogenesis using in vivo chick chorioallantoic membrane (CAM) assay. In the CAM assay, serotonin (0.1 and 1.0 μg) dose-dependently induced the formation of new blood vessels. In conclusion, our results indicate that serotonin acts as a proinflammatory factor in the colon.

Keywords: Serotonin, monocyte adhesion, IBD, CAM, TNF-α.

Disclosure of interest: None declared.

P-488
EFFECT OF VITAMIN A SUPPLEMENTATION ON INFLAMMATORY CELLS IN PATIENTS WITH ULCERATIVE COLITIS

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Background: Ulcerative colitis (UC) is a disease of unknown etiology caused by an inappropriate mucosal immune response to antigens from the intestinal flora. Its etiology has not yet been resolved, but immunological and genetic factors are known to play a very important role. Active UC is often associated with elevated peripheral blood granulocytes and imbalanced inflammatory cells (CD4, CD8 Th cells). As these cells are major sources of inflammatory cytokines, selective changes of these cells in patients with UC should alleviate inflammation. According to the anti inflammatory role's of vitamin A, we decided to evaluate the effect of vitamin A supplementation on circulatory white blood cell (WBC) subtypes and inflammatory cells in this study.

Methods: Forty-three patients with ulcerative colitis were included in a before and after, interventional survey in Sheikh al rais, a public health clinic of Tabriz. All participants were at clinical remission stages. The severity of disease activity was assessed by colitis activity index (CAI).Vitamin A supplement was injected twice (50,000 IU) with 2 weeks interval. Study period was considered 45 days. Total WBC count was performed by using an automatic blood cell counter. The serum levels of hs-CRP were evaluated by immunoturbidimetry. CD4 and CD8 T cells were analysed by flow cytometry.

Results: In UC, neutrophil lymphocyte ratio (NLR) and hs-CRP levels positively correlated with CAI ($P < 0.05$). There was a negative correlation between CD4 T cell and CAI ($P = 0.01$).We found no significant effect of vitamin A supplementation on CD4 and CD8 T cell subsets, neutrophil and monocyte count after supplementation. NLR and hs-CRP levels decreased not considerably, while platelet count decreased significantly ($P = 0.004$).

Conclusion: Vitamin A supplementation does not seem to have a strong effect on inflammatory cells.

Disclosure of interest: None declared.

P-489
CONVERSION OF TOLEROGENIC RESIDENT CELLS OF THE HUMAN INTESTINAL MUCOSA INTO COLITOGENIC CELLS UPON IL-10 BLOCKADE : POSSIBLE ROLE IN THE PATHOPHYSIOLOGY OF CROHN'S DISEASE

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Crohn's disease is an inflammatory bowel disease (IBD) with a Th1 inflammation of unknown etiopathogenesis. Accumulating evidence points to breakdown of the anti-inflammatory cytokines IL-10 and/or TGFβ production/signaling in subpopulations of Crohn's disease. However, as to whether these defects can be the driving force of the inflammatory process remains to be defined. To address the issue of the consequences of depleting a normal human intestinal mucosa from IL-10 or TGFβ, we have recently setup an ex vivo model system of explant cultures of human normal colonic mucosa, microdissected from surgical resections, in combination with immunoneutralization of IL-10 or TGFβ. This model maintains cellular interactions between mucosa resident cells and is amenable to a multiparametric methodological approach (QPCR, ELISA, in situ immunofluorescence, flow cytometry after collagenase digestion). We previously demonstrated that immunoneutralization of IL-10, endogenously produced by resident mucosa cells including epithelial cells, leads to a Th1 response, mainly IFNγ, triggered by LPS from the microflora, and responsible for epithelial barrier damage. In this work, aimed at elucidating how resident mucosa cells are converted into "colitogenic" pro-inflammatory cells, we demonstrate that the IFNγ response elicited by IL-10 breakdown in explant cultures is preceded by active caspase-1 production and early secretion of mature IL-18 by epithelial cells and lamina propria macrophages. Furthermore, the IFNγ response is blocked by a caspase-1 inhibitor and by IL-18-binding protein. These results show that the Th1 response upon IL-10 breakdown depends on IL-18/caspase-1, part of the inflammasome pathway. In addition, we also show that immunoneutralization of another anti-inflammatory cytokine, TGFβ, also elicits a Th1 (IFNγ) response, but via a distinct, non redundant pathway. These distinct signalling pathways we identified can therefore represent targets in some forms of IBD.
Disclosure of interest: None declared.

P-490
SACCHAROMYCES BOULARDII AMELIORATES THE INFLAMMATION AND GASTRIC DYSMOTILITY PRESENTS IN INTESTINAL MUCOSITIS INDUCED BY 5-FLUOROURACIL IN MICE

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Saccharomyces boulardii (SB) is used widely in the treatment of gastro-intestinal disorders associated with diarrhea. The interactions of this thermophilic non-pathogenic yeast in intestinal mucositis induced by 5-FU in mice is not totally defined. Swiss male mice (30–35 g) were treated with 5-FU (450 mg/kg, ip) or 5-FU + SB (800 mg/kg, daily for 3 days), other group received saline. After animals were sacrificed and sample of jejunum (J) were collected for assessment of histopathological, MPO activity, GSH and nitrite concentration for spectrophotometry, cytokines concentrations (IL-1b, CXCL1) by ELISA. To determination of gastric emptying (retention fraction %), phenol red (PR, 0.75 mg/mL, 300 μL) by gavage was administrated and mice were sacrificed 20 min later. The stomach and intestinal were processed measurement of PR concentrations by spectrophotometry. Statistical analysis (tests ANOVA and Bonferroni, values significance with $p < 0.05$). (CEPA: Protocol 34/10). 5-FU induced significant histopathological alterations that were reverted by the treatment with SB (C = 0 (0–1), 5-FU = 2,5 (1–3), 5-FU + SB = 1 (0–2). 5-FU induced an increase on the MPO activity (no. neutrophil/mg of tissue) (C = 1.73 ± 0.37, 5FU = 7.37 ± 1.77), nitrite concentration (C = 37.00 ± 2.39, 5-FU = 59.04 ± 11.41), cytokines concentration (IL-1b: C = 589.33 ± 36.76, 5-FU = 1135.00 ± 90.70; CXCL1: C = 33.43 ± 23.42, 5-FU = 341.33 ± 146.43) and a decrease on the GSH concentration (C = 477,60 ± 25,25, 5-FU = 270,90 ± 38,50) that were reverted by SB treatment (MPO: SB + 5-FU = 4,15 ± 0,73), (Nitrite: SB + 5-FU = 37.90 ± 5.78), (cytokines: IL-1b/5-FU + SB = 726.00 ± 98.43, CXCL1/5-FU + SB = 66.30 ± 50.50) and (GSH: SB + 5-FU = 514.00 ± 38.64), respectively. Besides, the treatment with SB improved delay in gastric emptying (C = 25.21 ± 2.55%; 5-FU = 54.91 ± 3.43%; 5-FU + SB = 31.38 ± 2.80%). Our results suggest that the treatment with SB reverted the inflammatory and functional events on the intestinal mucositis induced by 5-FU in mice.
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P-491
A ROLE FOR INFLAMMATORY MEDIATORS IN LYMPHATIC DILATION DURING TNBS-INDUCED ILEITIS

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The lymphatic system is involved in many disease pathologies, including inflammatory bowel diseases (IBD). Lymphatic vessel abnormalities reported in IBD include vessel dilation, hypocontractility and flow obstruction. These may lead to inefficient removal and transport of excess interstitial fluid, antigens, and immune cells. Inflammatory mediators, particularly prostaglandins can potently modulate lymphatic vessel contractile function. Our hypothesis is that key mediators released during the inflammatory process can signal through the lymphatic vessels to modulate lymphatic function and phenotype. Inflammation was assessed in the rat ileum 24 h after surgical instillation of the hapten TNBS using a macroscopic damage scoring system and a myeloperoxidase activity assay. Mesenteric lymphatic vessels were assessed for diameter and collected for quantitative real time PCR analysis of the following mRNAs: Cyclooxygenase 1 and 2 (COX-1, COX-2), TNFα receptor 1 (TNFR1), interleukin 1-β receptor (IL1R1), interleukin 6 receptor (IL6R), interferon gamma receptor (IFNR1), prostacyclin synthase (PTGIS), prostacyclin receptor (IP), prostaglandin E2 synthase 1/2 (PGES 1/2), EP2, and EP4 receptors. Ileum from TNBS-treated rats shows an increased granulocyte infiltration, and higher macroscopic

damage scores compared to sham counterparts. Lymphatic vessels from TNBS-treated animals exhibited an increase in diameter, suggesting lymphatic contractile dysfunction. Quantitative real-time PCR shows a significant upregulation of COX-2, TNFR1, IL1R1, PTGIS and IP mRNAs in lymphatic vessels from TNBS-treated animals. Our findings suggest an increased responsiveness of the lymphatic vessels to IL-1β and TNFα, as well as an increase in lymphatic prostacyclin production and signalling. These changes could lead to the dilated lymphatic phenotype we observed in this study. Lymphatic dilation could have potential implications in modulating the course of intestinal inflammation.
Disclosure of interest: None declared.

Kidney and genitourinary tract diseases

P-492
DETECTION OF MYCOBACTERIUM TUBERCULOSIS FROM PATIENTS SUSPECTED TO GENITOURINARY TUBERCULOSIS BY CULTURE AND PCR TECHNIQUE

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Background: While renal tuberculosis (TB) is uncommon in developed countries, as many as 15–20% of TB patients in developing countries are found with *Mycobacterium tuberculosis* (MTB) in the urine. The diagnostic criterion for genitourinary tuberculosis (GUTB) is the isolation of MTB from urine. Due to the difficulties associated with laboratory diagnosis of GUTB, there has been considerable interest in applying PCR methods for the detection of the disease.
Objectives: investigation of the diagnostic value of PCR in GUTB compared with acid fast staining and culture method.
Methods: In total 200 urine samples from suspected cases of GUTB were collected. Urine pellets were used for smear preparation, culture and DNA extraction by ether–chloroform method. Nested PCR was performed according to standard protocol using primers based on IS6110 gene fragment. The results obtained by PCR were compared with those obtained by standard acid-fast bacilli stain and culture method.
Results: The positivity rate of urine samples in this study was 5.0% by using culture and PCR methods and 2.5% for acid fast staining. Four out of total samples showed positive results in all three methods (2%). The sensitivity of PCR in this study was estimated as high as culture equal to 100%, while the sensitivity for direct smear staining was 41.6%.
Conclusions: The obtained rate of GUTB in our study was 5.0%. Since the detection rate of culture and nested PCR was identical, we could suggest PCR as a rapid alternative to culture especially for confirmed cases of GUTB.
Disclosure of interest: A. D. Khosravi Grant/Research Support from: Research affairs, AJUMS, M. Hashemzadeh: none declared, A. Ghorbani: none declared, S.
Seghatoleslami: none declared.

P-494
SPINNING DOWN URINE SAMPLES COULD DIMINISH TGF-BETA 1 SIGNAL

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TGF-β1 is the important mediator of immune and anti-inflammatory responses; it is involved in normal renal function and in the development of different nephropathies. Present research was dedicated to the validation of urine TGF-β1 measurement by Quantikine Human TGF-β1 ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA). Human recombinant TGF-β1 was spiked into normal urine at different concentrations, and frozen in aliquots. Collection of 100 frozen urine samples from healthy donors were used to investigate distribution of urine TGF-β1 in normal population. Creatinine concentration in urine was determined at ARUP Laboratory. After the thawing, considerable amount of precipitate was found in some samples. Such samples were centrifuged, and supernatants or un-spun specimens were run with TGF-β1 assay in duplicates. TGF-β1 activation procedure (which precedes ELISA steps) clarified all samples no matter how much precipitation they contained. It was shown that supernatants contained only fraction of the whole sample TGF-β1 activity (77–29%, even less for the normal low concentration samples). Validation involving un-centrifuged urine samples demonstrated following TGF-β1 assay characteristics:
- linearity: from 23 to 1286 pg/mL, recovery from 95–116%;
- precision: <17%;
- analytical specificity—Limit-Of-Blank: 3 pg/mL;
- reference interval: from 0 to 39 pg/mg creatinine
- freeze–thaw stability: after first thawing, urine samples could loose from 5 to 40% of TGF-β1 activity on each following freeze–thaw circle.

Conclusion: Quantikine Human TGF-β1 ELISA assay provides accurate quantitation of human TGF-β1 in urine; urine sample preparation should not include centrifugation step; repeating freeze-thawing should be avoided.
Disclosure of interest: None declared.

P-495
PROTEASE-ACTIVATED RECEPTOR 1 (PAR1) MEDIATES ANTI-INFLAMMATORY EFFECTS IN A MOUSE MODEL OF PROSTATITIS

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The prostate is an accessory reproductive gland that is found in all male mammals. This gland is prone to infection, inflammation, and cancer, making it one of the most diseased tissues in the body. Specifically, the role of protease-activated receptor 1 (PAR1) has not been addressed in the context of prostatitis. Therefore, we have developed a murine model of prostatitis to investigate the inflammatory effects of PAR1. We hypothesized that administration of 50% ethanol (EtOH) and dinitrobenzenesulfonic acid (DNBS) into the

mouse prostate would incite an inflammatory response, and that treatment with the PAR1 activating peptide TFLLR-NH_2 would modulate this reaction. EtOH + DNBS were administered alone or in conjunction with TFLLR-NH_2 via catheter and mice were sacrificed 48 h following treatment. Prostates treated with EtOH + DNBS alone showed a 4-fold increase in weight and myeloperoxidase (MPO) specific activity and a respective 3-fold and 16-fold increase in CXCL-1 and IL-10 production over controls ($p < 0.05$). Furthermore, histological analyses of these prostates revealed edema, extensive leukocyte infiltration, and destruction of the prostatic acini. Compared with EtOH + DNBS treatment alone, prostates given EtOH + DNBS in conjunction with TFLLR-NH_2 showed a 4-fold decrease in weight and MPO specific activity, a 3-fold decrease in CXCL-1, and a 3-fold increase in IL-10 production ($p < 0.05$). Histological analyses of these prostates revealed minor edema, minimal leukocyte recruitment, and minor tissue damage. In conclusion, acute inflammation of the prostate can be induced in mice using a combination of EtOH and DNBS. Furthermore, activation of PAR1 modulates this inflammatory reaction by increasing IL-10 production, decreasing MPO and CXCL-1 production, and reducing tissue damage. These findings support an anti-inflammatory role for PAR1 in the mouse prostate.
Disclosure of interest: None declared.

P-496
PHARMACOLOGICAL MODULATION OF URINARY BLADDER INFLAMMATION IN A RAT MODEL OF CYCLOPHOSPHAMIDE-INDUCED ACUTE BLADDER PAIN

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Cyclophosphamide (CYP)-induced bladder inflammation is widely used as an in vivo model for bladder pain syndrome. We previously showed that CYP-induced bladder pain was reversed by ibuprofen and aspirin, 2 non steroidal anti-inflammatory drugs (NSAIDs), and morphine, an opioid agonist. We studied the modulation of different inflammatory mediators by these pharmacological treatments in CYP-induced bladder inflammation. Female rats were dosed with aspirin, ibuprofen, morphine or vehicle prior a single injection of CYP. Urines were then collected over 4 h and rats sacrificed. Bladders were assessed for weight, wall thickness and macroscopic damage. Inflammatory mediators were quantified in bladders and urines using enzyme immunoassays or Multiplex technology. After CYP injection, an increase in bladder weight and wall thickness was observed associated with strong edema and erythema. Bladder levels for interleukin-1β (IL-1β), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion molecule (VCAM) were significantly increased as well as urinary levels for prostaglandin E_2 (PGE_2), lipoxin A_4 (LXA_4) and 15-epi lipoxin. After NSAID treatment, macroscopic parameters were not affected except for a decreased wall thickness. Urinary PGE_2 levels were strongly reduced by both treatments, while IL-1β and IL-6 tissue levels were decreased by ibuprofen treatment only. In contrast, morphine-treated rats presented a strong increase in wall thickness and macroscopic scores, associated with increased bladder levels for IL-6 and MCP-1, and increased urinary 15-epi lipoxin. Other mediators like LXA_4 were not significantly affected by these drugs. In summary, antinociceptive effects of NSAIDs are associated with a decrease in key inflammatory mediators such as IL-1β, IL-6 and PGE_2, known to promote the sensitization of bladder nociceptors. In contrast, reversal of bladder pain by morphine may not be linked to a direct modulation of bladder inflammation.
Disclosure of interest: None declared.

P-497
ROLE OF TOLL LIKE RECEPTORS 2 AND 4, AND MYD88 MOLECULE IN AN EXPERIMENTAL MODEL OF TUBULOINTERSTITIAL NEPHRITIS

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Introduction: Tubulointerstitial nephritis (TIN) is a primary injury to renal tissue, resulting in decreased renal function, extensive tubular dilation, inflammation and fibrosis. Excessive adenine intake leads to TIN, as this purine is converted by xanthine dehydrogenase (XD) in DHA, an insoluble compound that precipitates in tubules. Toll-like receptor (TLR) 2, 4 and adaptor molecule Myd88 have pivotal role in activating the immune response and inflammation. The aim of this study is to evaluate the role of TLR 2/4 and Myd88 in an experimental model of TIN. Besides, we also evaluate the capacity of allopurinol, a XD inhibitor, in reducing inflammation.
Methods: C57/Bl6 (WT) mice and TLR2, TLR4 and Myd88 KO mice were fed with adenine enriched food and were sacrificed after 10 days. Control mice were fed with standard food. Another group received adenine chow and allopurinol diluted in drinking water. At sacrifice time, blood and renal tissue were collected for analysis. Serum creatinine was measured and histopathologic changes were evaluated. Also, RT-PCR was performed for IL-18, TNF, IL-1b, and IL-6. Furthermore, uric acid, ox-LDL, TGFb and pro-inflammatory cytokines were measured on renal tissue and immunoblotting was done for IKK.
Results: WT animals presented higher levels of serum creatinine and enhanced cellular infiltration and collagen deposition. They also showed higher gene and protein expression of inflammatory cytokines. In contrast, KO mice showed renoprotection, with serum creatinine levels, histology evaluation, and inflammatory molecules expression comparable to control. This protection was also observed on allopurinol group, which also presented decreased levels of uric acid, ox-LDL, TGFb and IKK expression.
Conclusions: TLR2/4 and Myd88 seem to participate on the development of TIN. Inhibition of XD also seems to be a promising therapeutic target to combat the developing inflammatory process.
Financial support: FAPESP/INCT Complex Fluids/CNPq.
Disclosure of interest: None declared.

P-498
TLR-2 AND TLR-4 EXHIBIT DISTINCT ROLE IN CISPLATIN-INDUCED ACUTE KIDNEY INJURY

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Acute kidney injury (AKI) presents close association with immune system including the participation of Toll-like receptors (TLRs). TLRs may interact with potential ligands released by cell death leading the production of several inflammatory cytokines, which can amplify the tissue damage. Thus, this study was designed to evaluate the influence of TLR-2 and TLR-4 receptors on prevention of cisplatin-induced AKI. So, we used wild type (WT), TLR-$2^{-/-}$ and TLR-$4^{-/-}$ mice to evaluate the morphological and functional renal parameters, besides cytokines profile and gene expression analysis. Firstly, it was observed that both KO animals showed a significant reduction of TNF-α (3.6- and 2.2-fold decreased), IL-6 (1.9- and 4.9-fold decreased) and IL-1β mRNAs (13.6- and 16.3-fold decreased) transcripts. Additionally, the production of pro-inflammatory cytokines (IL-1α, IL-1β, IL-3, IL-6, TNF-α, IFN-g, CCL-5, KC, MIP-1α and G-CSF) was considerably diminished in TLR-$2^{-/-}$ and TLR-$4^{-/-}$ mice. Although, only TLR-$4^{-/-}$ mice have a trend toward expressed anti-inflammatory molecules as Th1- and Th17-related cytokines (IL-4, IL-10, IL-12 and IL-17). Likewise, the absence of TLR-4 but not TLR-2 receptor protects mice from severe weight loss, acute tubular necrosis (ATN) and high levels of serum creatinine (SC) at 96 hs of injury ($\geq$4 to <1 mg/dL, respectively). In order to evaluate the mechanisms behind of TLRs activation, we also investigated the cytoprotective effect of Heme oxygenase-1 (HO-1). Surprisingly, the treatment with COPPIX (5 mg/kg), an intracellular activator of HO-1, decreased ATN and SC levels in WT and TLR-$4^{-/-}$ mice, but not in TLR-$2^{-/-}$ animals. All together, these results suggest that TLR-2 and -4 responded to injury signals of kidney milieu, intensifying the renal failure. However, TLR-4 have a fundamental role as pro-inflammatory agent and their cross-talking with HO-1 and TNF-α can help in better understanding of process that govern the AKI.
Support: INCT, CNPq and FAPESP.
Disclosure of interest: None declared.

P-499
CYTOMEGALOVIRUS INFECTION IN KIDNEY TRANSPLANT PATIENTS WITH AND WITHOUT GRAFT DYSFUNCTION

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Introduction: Cytomegalovirus (CMV) is an important cause of morbidity and mortality in transplant patients. High frequency and severity of CMV disease are associated with the use of anti-lymphocyte. Therefore, some have suggested the prophylactic use of ganciclovir in these patients, but a strategy is the use of early treatment based on antigenemia as a marker of infection.
Objective: To evaluate the severity of CMV infection in kidney transplant patients with graft dysfunction.
Methods: The antigenemia technique was used to follow-up. Blood samples to perform the technique of antigenemia. Blood samples for detection of antigenemia were obtained when there was clinical suspicion of cytomegalovirus infection. We evaluated 133 patients divided into 3 groups: (1) 50 patients who made use of anti-lymphocyte to treatment of rejection, (2) 12 patients who did not use anti-lymphocyte, (3) 60 patients who use anti-lymphocyte in the time of transplant (induction).
Results: Of 50 patients who had acute allograft rejection, nine patients had combined treatment of more than one anti-lymphocyte. In group 1 had more syndrome and invasive disease and lower incidence of asymptomatic. The viral load of these patients, showed that patients with invasive disease had high viral load in compared with others groups. When analysed viral load inside groups, the group with used in induction of anti-lymphocyte had increased of viral load. Patients that used anti-lymphocyte had more disease (syndrome and invasive disease). All patients with invasive disease had involvement primarily in the gastrointestinal system.
Conclusion: Patients who used anti-lymphocyte had more CMV disease (syndrome and invasive disease) with involvement of the gastrointestinal system and showed higher viral load in relation asymptomatic patients.
Financial support: FAPESP.
Disclosure of interest: None declared.

P-500
THE ROLE OF NKT CELLS IN ADENINE-INDUCED TUBULOINTERSTITIAL NEPHRITIS

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An excessive oral intake of adenine in mice leads to the accumulation of 2,8-dihydroxyadenine (DHA) in renal tubules and that causes progressive renal dysfunction accompanied by interstitial fibrosis. Physiopathology of renal diseases involves several elements in the immune response. Since NKT cells are a unique T lymphocyte sublineage that has been implicated in either up- or downregulating immune responses, the aim of our work was to study the role of NKT cells in an adenine-induced tubulointerstitial nephritis.
Materials and methods: Male C57BL/6 wild-type and Jα18 and CD1d KO mice were used in this experiment. They were allowed food and water ad libitum. A 0.25% adenine-containing diet was used in all adenine groups during 10 days. Control group received standard diet. Mice were sacrificed at day 10, after the start of adenine diet, for collecting blood and kidney. Serum urea and creatinine were measured and kidney samples were used to isolate RNA. RT-PCR assay and Western Blotting was performed as described in standard protocols.
Results: Serum urea and creatinine values were significant higher in adenine-fed WT mice compared with the control group. Although urea and creatinine were higher in adenine-fed WT than in KO groups, there was no significant difference between then. There was a significant increase in gene expression of IL-6, IL-1β, TNF-α, TLR-2, Myd88 and XDH (xanthine dehydrogenase) in adenine-fed WT mice compared with the control group. IKK protein expression was increased in adenine-fed WT mice than in control group. Our findings suggest that NKT cells may play a role on tubulointerstitial nephritis. DHA accumulation seems to be involved in the inflammation process in kidney injury since there was an increase in genes and protein expression that implicate it.
Disclosure of interest: None declared.

P-501
IL-1 RECEPTOR ANTAGONIST (IL-1RA) PROTECTS THE INFLAMMATORY PARAMETERS AND HEMORRHAGE UPON IFOSFAMIDE-INDUCED HEMORRHAGIC CYSTITIS IN MICE

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Ifosfamide (IFO) is an antineoplastic agent. Hemorrhagic cystitis (HC) is a prominent side-effect of IFO treatment. Despite the HC prevention with Mesna, a complete protection is not always achieved. The participation of cytokines on HC provided potential targets for treating this disease. This study investigated the protective effect of IL-1Ra upon inflammatory and hemorrhage response in a mice model of IFO-induced HC. Swiss male mice (25–30 g) were given saline (0.5 mL, ip) or IL-1Ra (25, 50, 100 or 200 mg/kg ip) 1 h before saline or IFO (400 mg/kg) injection. Visceral nociception was performed, 11 h after IFO injection. The animals were killed 12 h after IFO injection and bladder wet weight (BWW), vascular permeability (VP), macroscopic and microscopic parameters, myeloperoxidase assay (MPO), in vitro bladder contractility to Carbachol, and cystometrography were performed. The study was approved by Ethics Committee (Protocol 09/06). IFO increased nociceptive threshold (6.76 ± 1.10 g) when compared to saline group (1.01 ± 0.73), and IL-1Ra inhibited (3.21 ± 1.05) such effect. Furthermore, IFO increased BWW (39.11 ± 2.43), VP (28.81 ± 8.47) edema (2[1-3]), hemorrhage (3[1-3]), microscopic (2[2-2]) scores, MPO (2.84 ± 0.30); and decreased in vitro bladder contractility (165.90 ± 23.16), micturition interval (0.94 ± 0.10), and absence in amplitude of contraction when compared to saline group (12.32 ± 0.73; 2.00 ± 0.47; 0[0-0]; 0[0-0]; 0[0-0]; 0.115 ± 0.115; 285.62 ± 23.16, 30.69 ± 3.04 respectively). These effects were prevented ($p < 0,05$) with IL-1Ra (100 mg/kg) treatment (21.48 ± 1.60; 7.66 ± 0.38; 0[0-2]; 0[0-2]; 1[1-2]; 1.54 ± 0.45; and 30.77 ± 47.87, 13.78 ± 1.47, respectively). This study shows for the first time the efficacy of IL-1Ra in controlling the hemorrhage and inflammatory parameters of HC (including abdominal hypernociception and bladder dysfunction), providing perspective to clinical management of HC.

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Disclosure of interest: None declared.

P-502
GENETIC OR PHARMACOLOGICAL INHIBITION OF TNF-ALPHA REDUCES THE RENAL INJURY, DYSFUNCTION AND INFLAMMATION CAUSED BY ISCHEMIA–REPERFUSION

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The role of the cytokine tumour necrosis factor-α in the pathophysiology of renal ischemia/reperfusion (I/R) injury is unclear. Here we investigate the effects of on the degree of renal injury induced by I/R, dysfunction and inflammation in (1) tumour necrosis factor-R1 knock out (TNF-αR1KO) mice and in (2) wild-type mice administered with infliximab, a chimeric antibody which neutralise the biological activity of TNF. All mice were subjected to bilateral renal artery occlusion (45 min) and reperfusion (6 h). In addition, wild-type mice were pretreated (30 min before ischemia) with a TNF-α soluble receptor construct, infliximab, to mimic the effects that would be seen in TNF-αR1KO mice. At the end of experiments, indicators and markers of renal dysfunction, injury and inflammation were measured. Kidneys were used for histological evaluation of renal injury, for the localization of the intercellular adhesion molecules and selectins, as well as apoptosis (TUNEL, Bax, Bcl-2 and Fas-L expression) by immunohistochemistry and western blot analysis. TNF-αR1KO mice and wild-type mice administered with infliximab demonstrated significantly reduced plasma urea and creatinine levels in comparison with I/R-mice, indicating reduction of renal dysfunction. Neutrophil infiltration was also significantly reduced in TNF-αR1KO mice and wild-type mice administered with infliximab subjected to renal I/R. TNF-αR1KO mice demonstrated reduced histological tubular injury and markedly reduced immunohistochemical evidence of ICAM-1, P-selectin, and apoptosis when subjected to renal I/R. Our results clearly demonstrate that TNF-α play an important role in the renal ischemia and reperfusion injury and put forward the hypothesis that modulation of TNF-α expression may represent a novel and possible therapeutic option to attenuate the ischemia–reperfusion injury process that leads to acute kidney injury, a frequent complication with renal transplantation, also associated with graft function.

Disclosure of interest: None declared.

P-503
USING TRANSCRIPTIONAL PROFILING TO INVESTIGATE PROXIMAL TUBULAR DYSFUNCTION IN KIDNEY TRANSPLANT PATIENTS WITH HIGHER RISK FOR DEVELOPMENT OF CHRONIC ALLOGRAFT DYSFUNCTION

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Retinol binding protein (RBP) is a small tubular protein, freely filtered by the glomeruli barrier and totally reabsorbed by the proximal tubular cells. High levels of uRBP indicate proximal tubular dysfunction (PTD) and closely correlate with long term renal and heart transplant outcomes. However, at this stage of PTD, histology or renal function are not good screening tests. Therefore, we were interested in identify the gene profile of those patients, with PTD assessed by uRBP, in order to discover new biomarkers. Renal transplanted patients with good (serum creatinine <1.5 mg/dL) and stable (for at least 3 months) were screened for PTD. Sixteen patients with high levels (>1.000 mg/L) and 11 patients with normal uRBP levels were consecutive selected a graft biopsied of performed after uRBP measurement. Agilent (GE) platform of 44,000 genes were used. Each slide quality was assessed and those considered good and discovered were selected for plotting the spots. We

calculated gene fold difference between groups and those above and below 2 were selected. 135 genes were differentially expressed; 123 down regulated and 12 up regulated in patients with higher uRBP levels. Among those genes, we observed difference in cell adhesion molecules, cell communication, cytosolic calcium ion concentration and ribosome pathways. PTD is associated with disturbance in cell physiology reflecting the impairment in the endocytic machinery. uRBP can identify the patients with higher risk for the development of chronic allograft dysfunction that have no abnormalities in renal function or no outstanding changes in histology but a modification in renal gene profile. Our data demonstrated that although PTD is associated with normal renal function and no specific histological abnormalities, it is characterized by a set of genes that might unveil its link with development of chronic allograft dysfunction.
Financial support: CNPq and FAPESP.
Disclosure of interest: None declared.

P-504
THE ROLE OF IL-1B AND PARTS OF INFLAMMASOME COMPLEX (CASPASE-1 AND ASC) IN ACUTE RENAL INJURY CAUSED BY ISCHEMIA/REPERFUSION INJURY

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Introduction: Acute kidney injury (AKI) constitutes important clinical problem, leading to high rates of morbidity and mortality. The most important etiology of AKI involves ischemia/reperfusion injury (IRI), an inflammatory response related to IL1-β participation. These data prompted us to evaluate the participation of the inflammasome complex, which is responsible for secretion of active IL-1β, in the development of IRI.
Methods: C57BL6 WT animals and KO for caspase-1, ASC and MyD88 were subjected to 45 min of ischemia and 24 h of reperfusion. Blood samples and kidney tissues were collected for analysis.
Results: Serum creatinine and histological data showed that KO animals for caspase-1, ASC and mainly MyD88 had a significant protection of the renal function compared to WT animals 24 h after IRI. The protected animals showed lower areas of hypoxia and gene expression of HIF-1α. In addition, protected animals had higher expression of anti-apoptotic gene BCL-2. Regarding gene and protein expression of pro-inflammatory cytokines, we found that WT animals had higher expression of these inflammatory factors. Some of these results are presented here: IL-6 (WT: 6.78, casp-1 KO: 1.17; Asc KO: 1.47; MyD88 KO: 1.74) and TNF-α(WT: 6.96; casp-1 KO: 0.91, Asc KO: 2.14; MyD88 KO: 0.99), and the chemokine MCP-1 (WT: 5.47; casp-1 KO: 0.77; Asc KO: 1.1; MyD88 KO: 0.57).
Conclusions: The inflammasome complex is involved in the inflammatory response of AKI related to IRI. The mechanism related to this response seems to involve caspase-1 and the adapter molecule ASC, which are capable to trigger the inflammatory response. This mechanism represents important therapeutic target for clinical practice.
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Disclosure of interest: None declared.

P-505
THE AMPLIFICATION OF ENDOTHELIAL STRESS BY POORLY REGULATED COMPLEMENT CONTRIBUTE TO THE PATHOGENESIS OF THE ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Glomerular endothelial cell damages are a hallmark for atypical hemolytic uremic syndrome (aHUS) and involve an uncontrolled activation of complement alternative pathway (AP), as suggested by the association with mutations of complement proteins. Starting from the hypothesis that endothelial cells themselves trigger such complement activation, we investigated conditions which could transform quiescent cells into AP activators, and the effect of a poorly regulated complement on these cells. Human quiescent umbilical vein HUVEC and glomerular GEnC endothelial cells were treated with hemolysis-derived or inflammation stimuli (free heme, neuraminidase, *E. coli* LPS, TNFα/IFNγ/IL1β). Cells were, then, incubated with human serum and analyzed for complement membrane deposition, by flow cytometry after cells detachment. Blocking anti-MCP mAb, factor H depleted serum or aHUS sera with genetic complement abnormalities were used as models for poorly regulated complement. With normal human serum, minimal C3 deposits were detected on quiescent adherent cells. Among tested stimuli, maximal levels of cell-bound C3 were observed on cells pre-incubated with heme or TNFα/IFNγ/IL1β, but these levels remained limited. In vitro functional inhibition of MCP or aHUS sera resulted in a modest increase of C3 on quiescent adherent cells. This C3 deposition was, however, further enhanced on cells pre-treated with heme or pro-inflammatory cytokines and became significantly higher than that observed with normal serum, emphasizing the amplifying effect of defective complement control. In conclusion, quiescent endothelial cells are well protected against autologous complement activation on their surface. Common agonists released during inflammation or hemolysis trigger low level complement activation on endothelial cell membrane. This is amplified by uncontrolled or over-reactive AP components and could result in the release of C5a and C5b-9, with known pro-inflammatory and pro-coagulant effects.
Disclosure of interest: None declared.

P-506
BRADYKININ RECEPTORS ROLE ON INFLAMMATORY REACTION INDUCED DURING ADRIAMYCIN NEPHROPATHY

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Introduction: Adriamycin (ADM) nephropathy (AN) is a disease characterized by glomerular sclerosis, proteinuria and up regulation of proinflammatory cytokines. Many works pointed for a possible role of kinin receptors in inflammatory studies. On renal diseases models the blockage of B1 receptor (B1RBK) seems to diminish the inflammatory markers on the other hand the role of B2 receptor (B2RBK) has not been unveiled yet. In this way we studied the role of these kinin receptors in the inflammatory reaction induced by ADM injection using specific antagonists for these receptors.
Material and methods: The AN was induced by a single injection of ADM in the tail vein of Balb/c mice. The mice were treated with the kinin antagonists DALBK (B1RBK antagonist) in for 3 days, DABK (B1RBK agonist) for 3 days and with HOE-140 B2RBK (antagonist) for 3 or 10 days. The renal function parameters were analyzed by levels of albuminuria, Western Blot and PCR real time expression of podocyte proteins. The levels of cytokines like TGFβ1 were evaluated by ELISA, the macrophage infiltration by Flow cytometry and the levels of proinflammatory cytokines were analyzed by a mouse cytokine Bioplex assay kit.
Results: The use of DALBK protected and revert the signs of mice albuminuria and podocyte dysfunction. This result was associated with the downregulation of proinflammatory markers such as TNF-α, TGF-β, IL-1β, MCP-1 and microphage infiltration, a mirrored result was observed with the Treatment with DABK (B1RBK antagonist) showing a positive effect of B1RBK blockage in AN. Differently the treatment with HOE-140 (B2RBK antagonist) did not protected animals from AN signs at the beginning of the AN on the other hand in an established disease downregulated the levels of proinflammatory cytokines, suggesting that the role of this receptor depends of the stage of AN progression. Differently the B1RBK blockade that at all phases of the disease was showed to be an important target to AN.
Disclosure of interest: None declared.

P-507
THE ROLE OF DENDRITIC CELLS IN CD4+ T CELLS MODULATION IN KIDNEY ISCHEMIA AND REPERFUSION INJURY

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Ischemia and reperfusion injury (IRI) is an acute inflammatory response considered to be the main cause of acute renal injury in kidneys. Dendritic cells (DC) have been recently associated to have a role in IRI, although a few is known about their functionality and their possible role in T cell activation in this model. In this way, we investigated whether DC are involved in IRI and their participation in T cell modulation in this model. We used C57Bl/6 and C57Bl/6-CD11c-DTR mice to perform the ischemia and sham group as control. We observed that after 24 h of reperfusion, urea (>250 mg/dl) and creatinine (>1 mg/dl) levels were increased in ischemic group, indicating the lesion. No difference of DC numbers were observed in the kidney and draining lymph nodes, however, DC from ischemic group were more activated, presenting more pronounced expression of CD86. We then used two models of depletion of dendritic cells: clodronate depletion of phagocytes and C57B/l6-CD11c-DTR mice with diphtheric toxin (4 ng/g of mice). Both protocols resulted in increased levels of urea (>300 mg/dl) and creatinine (>2 mg/dl) in the serum of DC depleted mice. In order to investigate the influence of DC in CD4+ T cell modulation in IRI, we analysed the phenotype of CD4+ T cells in DC depleted mice after ischemia and reperfusion induction and we observed that the population of CD4+ CD69+ (activated) T cells was increased, while no differences were observed in CD4+ CD25+ FOXP3+ T cells (Tregs), indicating that DC might be important for the regulation of T cell response in IRI. We conclude that DC are involved in IRI and they modulate CD4+ T cells in order to control the injury.
Support: FAPESP.
Disclosure of interest: None declared.

P-508
EVALUATION OF PRO-RESOLVING MEDIATORS DURING URINARY BLADDER INFLAMMATION: EFFECT OF ASPIRIN, IBUPROFEN AND MORPHINE

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Introduction: The objective of this study was to highlight the effects of aspirin, ibuprofen and morphine on the production of pro-resolving lipid mediators during a cyclophosphamide (CYP)-induced bladder inflammation.
Methods: For this, female rats were dosed with aspirin, ibuprofen, morphine or vehicle prior to a single injection of CYP. Urines were then collected over 4 h and mediators analyzed using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) methodology to quantitatively evaluate bioactive lipids production in urine.
Results: During these experiments, we showed that CYP induced the production of lipid mediators derived from the cyclooxygenase (COX) and lipoxygenases (LOX) pathways. Moreover, CYP treatment induced bladder pain, which was inhibited by aspirin, ibuprofen and morphine treatments. The use of aspirin and ibuprofen completely abrogated the mediators depending on COX pathways while morphine had no effects on those. On the other hand, aspirin boosted the LOX pathways and the production of the precursors of resolvins, maresin and protectins. In contrast, the use of ibuprofen and morphine inhibited the production of these same precursors.
Conclusions: Taking together the results suggest that aspirin may act on bladder pain through the induction of key pro-resolving mediators, while ibuprofen and morphine may promote the desensitization of bladder nociceptors by other pathways.
Disclosure of interest: None declared.

Skin and sensory organs diseases

P-509
INFLAMMATION CORRELATES TO CELLS AND VESSELS IN HUMAN DISSEMINATED LEISHMANIASIS

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Background: Skin infection by *Leishmania braziliensis* results in cutaneous and mucosal leishmaniasis. In between this clinical spectrum, disseminated leishmaniasis (DL) aside primary ulcer and ulcerated secondary lesions differs from others by clinical manifestation of multiple papules and non ulcerated lesions. In this study, we describe the histopathology of DL papular lesions, correlating them with inflamed area, CD4, CD20, CD68, CD31 cells and von Willebrand factor (vW) amount characterizing immune inflammatory aspects.
Methods: Eighteen biopsies from patients diagnosed by clinical criteria, positive skin test and fine needle aspiration in vitro cultivation were analyzed. Histopathological aspects were obtained by analysis of Hematoxylin and Eosin (H&E) stained slides. To quantify the inflamed area and cells positivity for CD4, CD20, CD 68, CD31 and vW the Image-Pro (Media Cybernetics) software was used.
Results: Diffuse lymphoplasmocytic perivascular infiltrate and granulomas not well organized were found in dermal skin. Histopathological patterns were graded from I to III [39% (I), 11% (II) and 50% (III)]. Inflammation extent varied from 3 to 73%, presenting a significant linear correlation with vW+ vessels and with graded patterns. The most frequent inflammatory cells were macrophages (CD68+) followed by B cells (CD20+) and T cells (CD4+). Significant linear correlation between CD4+ and CD20+ cells and inflamed area was also found. In more inflamed lesions we found grater amount of CD31+ cells. Amastigotes were visualized or immunostained in 8 biopsies.
Conclusions: Our findings suggest that chronic inflammation was present in all papules predominantly formed by macrophages plasmocytes, T and B cells. As inflammation area expands, granulomas and vessel framework increases proportionally. Thus, the present study highlights the possible role small vessels may have in clinical evolution and spectrum from acneiform papules to larger nodular skin lesions.
Disclosure of interest: None declared.

P-510
ESTIMATION OF (IGA) ANTIGLIADIN, (IGA) ANTIENDOMYSIUM AND (IGA) TISSUE TRANSGLUTAMINASE ANTIBODIES IN THE SERUM OF PSORIATIC PATIENTS

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Background: Studies have shown the association between psoriasis and celiac disease (CD), an immune-mediated gluten-dependent enteropathy due to inappropriate T cell mediated response against ingested gluten, and also improvement of psoriasis after gluten-free diet. However, the exact relationship between psoriasis and CD remains controversial.
Aim: We aimed to assess the prevalence of IgA anti gliadin (AGA) antibodies and other celiac disease associated antibodies as IgA tissue transglutaminase (tTG) antibodies and IgA antiendomysium antibodies (EMA) in psoriatic patients, and their possible role in the pathogenesis of psoriasis.
Patients and methods: Forty-one psoriasis patients and 41 healthy controls were included in this study. Blood samples were taken from all subjects and screened for AGA, tTG, measured by ELISA, and EMA assessed by standard immunofluorescence with cryo section to monkey oesophagus.
Results: There was a significant difference between patients and controls as regards AGA but no significant difference between the 2 groups as regards tTG and EMA was detected.
Conclusion: Finally, the significant increase in the level of some CD antibodies detected in this study indicates that their serological evaluation should be considered in psoriasis patients. Also, gluten-free diet could lead to better management and decreased morbidity of psoriasis.
Disclosure of interest: None declared.

P-511
IL-33, MAST CELLS AND KERATINOCYTES: A TRAIT D'UNION IN INNATE IMMUNITY MECHANISMS OF PSORIASIS

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IL-33 is a pro-inflammatory cytokine recently identified as a ligand for the orphan receptor ST2. IL-33 constitutive nuclear expression has been found in cells of tissues exposed to the environment. Moreover, endothelial cells have been shown to abundantly express IL-33. Many of the inflammatory effects of IL-33 define its ability to induce Th2-type mediated responses but recently IL-33 was found to have a pro-inflammatory role in arthritis, which is Th1 and/or Th17 mediated. It can be hypothesized that IL-33 has a pro-inflammatory role in psoriasis too. In the present study we aimed to assess the role of IL-33 in psoriasis, investigating its property to promote inflammation via mast cell (MC) and keratinocyte (KC) activation. Here we report that IL-33 is elevated in the skin of psoriasis patients. Furthermore, IL-33 upregulates IL-4 and IL-13 and is able to induce a higher increase of MCP-1 and VEGF respect to TNF-α in MCs. In presence of TNF-α, IL-33 induces MCP-1 and IL-6, whereas in association with IL-17, is able to induce IL-20 in KCs. In conclusion, our study provides evidence that IL-33 is involved in psoriasis biology. Furthermore, our results reinforce the IL-33 activity in driving Th1 response too, contributing to the maintenance of psoriasis pathogenesis.
Disclosure of interest: None declared.

P-512
DIMETHYLFUMARATE INHIBITS MIF INDUCED PROLIFERATION OF KERATINOCYTES BY INHIBITING MSK1 AND RSK1 ACTIVATION AND BY INDUCING NUCLEAR P–C-JUN(S63) AND P-P53(S15) EXPRESSION

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Objective: Dimethylfumarate (DMF) is used in the treatment of psoriasis. Macrophage migration inhibitory factor (MIF) is elevated in patients with severe psoriasis. We studied the effect of DMF on the MIF induced activation of the Mitogen and Stress-Activated Kinase 1 (MSK1) and p90 kDa Ribosomal S6 kinase (RSK1) signaling pathways which regulate the proliferation of human keratinocytes via transcription factors.
Methods: The effect of DMF on the MIF induced activation of MSK1, RSK1, CREB, Cox-2 and c-Jun, JunB and p53 were studied by Western Blotting using phospho-specific antibodies.
Results: DMF inhibited the MIF induced phosphorylation of MSK1, RSK1, CREB and JunB, and reduced Cox-2 expression and the proliferation of cultured human keratinocytes. The expression of p-p53 (S15) was induced, simultaneously with the inhibition of Cox-2. Addition of DMF before MIF induced nuclear expression of p-c-Jun (S63) and c-Jun. Transfection with small interfering MSK1 and RSK1 RNA before MIF incubation, stimulated p-p53 (S15) and nuclear p–c-Jun (S63) similar to DMF.
Conclusion: Our results indicate that the specific inhibitory effects of DMF on RSK1 and MSK1 activation together with the induction of p-c-Jun (S63) and p-p53 (S15) lead to the inhibition of keratinocyte proliferation, partly explaining the anti-psoriatic effect of DMF.
Disclosure of interest: None declared.

P-513
IN VIVO PARTICIPATION OF NITRIC OXIDE IN HYPERPROLIFERATIVE EPIDERMAL PHENOMENA IN MICE

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Many authors reported a significant involvement of nitric oxide (NO) in the process of keratinocytes proliferation with many divergences. In order to determine the involvement of NO in hyperproliferative process of epidermis in vivo, chronic inflammatory process was induced by multiple applications of croton oil (0.4 mg/ear) for 9 days on alternate days. Mice were topically treated with the NG-nitro-L-arginine-methyl ester (L-NAME, 10 μmol/ear), Aminoguanidine (AG, 10 μmol/ear), Sodium nitroprusside (SNP, 2.5 μmol/ear), 7-nitroindazole (7-NI, 10 μmol/ear), or Dexamethasone (0.1 mg/ear), starting on the 5th day. Oedema was measured daily as increase in ear thickness and on the last day 6 mm sample of ear tissue was collected, weighed and analyzed by histology and immunohistochemistry (PCNA). Procedures were approved by Institutional Ethics Committee (130). L-NAME, 7-NI, and dexamethasone treatments were able to decrease, while SNP increased ear oedema during treatment. However, the weight of ears at the end of experiment was reduced in the groups that received L-NAME (16.9%) and 7-NI (44.3%), while the AG (24.9%) and SNP (22.0%) groups presented an increment in the ear weight compared to control (24.3 ± 1.4 mg). The evaluation of epidermis thickness show that L-NAME (28.2%), AG (20.7%) and 7-NI (42.0%) reduce, while SNP application contributed to enlarge in 35.6% when compared with control group (133.8 ± 8.1 μm). Similar results were observed in the PCNA immunohistochemical staining, where treatments with L-NAME, AG and 7-NI caused reduction of 34.2, 15.9 and 70.2%, respectively, while SNP enhanced in 15.6% when compared with control group (87.2 ± 6.9 cell/field). Therefore, in the skin NO must be produced by iNOS and nNOS, and is probably involved in the control of keratinocytes hyperproliferation in the epidermis, also with a contribution to the oedema formation.
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Disclosure of interest: None declared.

P-514
SIMVASTATIN: TOPICAL ANTI-INFLAMMATORY ACTIVITY IN ANIMAL MODELS

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Statins represent a class of drugs that effectively lowers cholesterol, but have shown to possess also pleiotropic effects, like promotion of vasculogenesis, prevention of bone loss, immunomodulating and anti-inflammatory effects. Thus, the aim of this study was to investigate the activity of simvastatin in acute and chronic inflammatory models through topical treatment. Skin inflammation was induced in the ear of female Swiss mice (25–30 g) by topical application of 12-*O*-tetradecanoylphorbol acetate (TPA). In the acute model, ear oedema was measured by the increase of ear thickness 6 h after TPA (2.5 μg/ear). The chronic inflammatory process was induced by multiple applications of TPA (2 μg/ear) for 9 days on alternate days and the oedema was measured daily as the increase in ear thickness. Topical treatment with simvastatin (0.24–2.40 μmol) or dexamethasone (0.13 μmol) started on the 5th day of experiment. Samples of ear tissue (6 mm) from acute and chronic model were collected, weighed and analyzed: ear weigh, histology, and myeloperoxidase (MPO) enzymatic activity. Procedures were approved by Institutional Ethics Committee (390). Simvastatin application was able to reduce the ear oedema induced by a single TPA treatment in a concentration-dependent manner [ID_{50} of 0.48 (0.22–1.13) μmol], with maximal inhibition of 70 ± 4% (2.4 μmol). The activity of MPO was reduced by simvastatin with maximum inhibition of 53 ± 4%. In the chronic model, simvastatin (1%) was able to reduce ear oedema in 16 ± 3% and ear weight in 11 ± 2%, at the end of experiment. Histological analysis showed a reduction of swelling and decrease in leukocyte infiltration. The results confirm the anti-inflammatory activity of simvastatin when applied topically in acute and chronic models of skin inflammation.
Support: CAPES, CNPq and REUNI.
Disclosure of interest: None declared.

Inflammation and metabolic diseases

P-515
NF3, A FORMULATION OF TRADITIONAL CHINESE MEDICINE, ON DISEASE STATES OF ARTHRITIC RATS WITH DIABETES

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Introduction: NF3 is a formulation of traditional Chinese medicine composed of *Rehmanniae radix* and *Astragali radix* in 1:2 ratio. It is developed by the Institute of Chinese Medicine of The Chinese University of Hong Kong to promote healing of foot ulcer in diabetic patients. In this study, we have investigated the anti-arthritic and anti-diabetic potentials of NF3 in arthritic rats with diabetes.
Methods: Neonatal streptozotocin (70 mg/kg) (STZ)-induced diabetic rats were randomized into three groups. The first group received daily oral administration of human equivalent dose (HED) of NF3, i.e., 0.98 g/kg/day for 2 weeks (standard dose). The second group received daily oral administration of 5× HED of NF3, i.e., 4.91 g/kg/day for 2 weeks (high dose). The third group received parallel vehicle (water) administration. After 1 week of oral treatment, all rats were injected with 125 μl Freund's complete adjuvant (FCA) into one knee to induce monoarthritis (noted as day 0). Symptoms of arthritis were assessed daily until termination of the experiment one day after the last oral administration. A parallel study was performed in age-matched normal rats.
Results: FCA treatment produced similar arthritis symptoms in normal and diabetic rats. Compared to the vehicle treated group, neither standard dose (HED) nor high dose NF3 (5× HED) affected body weight or joint hyperaemia in normal rats, but high dose NF3 slightly attenuated joint swelling, allodynia, and plasma glucose level. Symptoms of arthritis and diabetes in diabetic rats were not affected by treatment with either standard or high dose NF3.
Conclusions: Diabetes does not affect the development of arthritis. High-dose NF3 may have minor hypoglycaemic, anti-inflammatory and analgesic effects in normal rats, but not in diabetic rats. The active components in NF3 may have to be identified and extracted to yield more efficacious anti-arthritic and anti-diabetic actions.
Disclosure of interest: None declared.

P-516
VITAMIN D PRODUCTION BY OCULAR BARRIERS

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Many cells and tissues in the body including those at barrier sites express the vitamin D receptor (VDR) and 1-alpha-hydroxylase (CYP27B1), the enzyme required to convert inactive $25(OH)D_3$ into active $1,25(OH)_2D_3$. These can activate and locally produce $1,25(OH)_2D_3$, which acts as an immunomodulatory hormone. The eye is an immune privileged site maintained by blood–aqueous (ciliary body and iris) and blood–retinal barriers. Corneal endothelium contributes to barrier function between corneal stroma and aqueous humor. This research aims to study the role of $1,25(OH)_2D_3$ in ocular barrier function. To investigate this, human adult retinal pigment epithelial (ARPE-19), non-pigmented ciliary body epithelium (ODM2), and human corneal endothelial (HCEC-12) cell lines were cultured, and gene expression was examined by conventional and real-time polymerase chain reaction (RT-PCR) both in the absence and presence of TLR ligands and vitamin D substrate ($25(OH)D_3$). Protein expression was analysed by immunohistochemistry. Production of cytokine and $1,25(OH)_2D_3$ was measured by enzyme immunoassay. ARPE-19, ODM2, HCEC-12 cells expressed mRNA for VDR, CYP27B1, and the catabolizing enzyme, 24-hydoxylase (CYP24A1). This was confirmed by immunohistochemistry in non-pigmented ciliary body epithelium and corneal endothelium tissue sections and by immunofluorescence in ARPE-19 cells. ARPE-19, ODM2, and HCEC-12 converted $25(OH)D_3$ into active $1,25(OH)_2D_3$ with HCEC-12 showing higher conversion rates. ARPE-19 cells responded to TLR ligand stimulation but this did not influence elements of vitamin D gene expression. Conversely, the addition of $25(OH)D_3$ did not affect cytokine production by these cells. This novel research has identified that ocular barrier cells can convert inactive $25(OH)D_3$ into active $1,25(OH)_2D_3$ and this is not influenced by TLR ligand stimulation. Vitamin D_3 produced by ocular barrier cells may possibly play a role in ocular immune privilege.
Disclosure of interest: None declared.

P-517
IMMUNE SIGNALING MOLECULES AS A TARGET AGAINST OBESITY-INDUCED INFLAMMATION AND METABOLIC DISORDER

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Chronic inflammation is an important factor in the development of insulin resistance, type 2 diabetes mellitus and fatty liver disease. Recent progress has led to the identification of several adipose tissue inflammatory components such as immune cells (e.g. macrophages, T cells) and cytokines/chemokines that account for obesity-induced inflammatory responses and insulin resistance. However, the intrinsic factors that trigger adipose tissue inflammation are not yet completely understood. 4-1BB/CD137 (TNFRSF9), a member of the tumor necrosis factor receptor superfamily expressed on immune cells, provides a bidirectional inflammatory signal through binding to its ligand 4-1BBL. 4-1BB and 4-1BBL have been shown to play an important role in the pathogenesis of various inflammatory diseases. However, the role of 4-1BB/4-1BBL in obesity-induced inflammation has not been established. Here we demonstrate that 4-1BB deficiency protects against high fat diet (HFD)-induced obesity, glucose intolerance and fatty liver disease. 4-1BB-deficient mice fed an HFD for 9 weeks showed less body weight gain, adiposity, adipose infiltration of macrophages/T cells, and tissue levels of inflammatory cytokines (e.g., TNFα, IL-6, MCP-1) compared to HFD-fed control

mice. HFD-induced glucose intolerance/insulin resistance and fatty liver were also markedly attenuated in the 4-1BB-deficient mice. These findings suggest that 4-1BB and 4-1BBL may be useful therapeutic targets for combating obesity-induced inflammation and metabolic disorders.
Disclosure of interest: None declared.

P-518
INDUCTION OF HEME OXYGENASE-1 EXPRESSION BY CILOSTAZOL CONTRIBUTES TO ITS ANTI-INFLAMMATORY EFFECTS IN J774 MURINE MACROPHAGES

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The effects of cilostazol on stimulating heme oxygenase (HO)-1 expression including signal pathways and suppression of inflammatory cytokines and molecules, were studied. Cilostazol stimulation time- and concentration-dependently increased the HO-1 mRNA and protein expression associated with increased HO-1 activity, as did cobalt protoporphyrin IX in J774A.1 macrophages. In addition, cilostazol concentration-dependently reduced lipopolysaccharide (LPS)-mediated nitrite and TNF-a production, in accordance with the inhibition of LPS-stimulated inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in the J774 macrophages, as did CoPP. In parallel with these results, LPS-induced IkBa degradation and NF-κB nuclear translocation were significantly decreased after treatment with cilostazol as well as with CoPP. These effects of cilostazol and CoPP were significantly reversed by Zn protoporphyrin IX. The effects of cilostazol on IkBa expression and nitrite production were not manifested in the cells transfected with HO-1 small interfering RNA. In the J774 macrophages, cilostazol time- and concentration-dependently increased the nuclear expression of NF-E2 related factor (Nrf2) and antioxidant response element (ARE) activity. PI3-kinase and Akt play a role in the major signal pathways with cilostazol-induced HO-1 expression. In summary, cilostazol suppressed production of anti-inflammatory cytokines and molecules via inhibition of NF-κB activation, through a mechanism involving up-regulation of cyclic AMP-dependent protein kinase activation-coupled Nrf2-linked HO-1 expression in J774A.1 macrophages.
Disclosure of interest: None declared.

P-519
RELEASE OF INFLAMMATORY FACTORS BY HUMAN ADIPOSE TISSUE EXPLANTS AND RELATION WITH MACROPHAGES: IMPACT OF DRASTIC WEIGHT LOSS

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In obesity, white adipose tissue (WAT) is considered a site of production of inflammatory factors and is characterized by macrophage infiltration. The contribution of WAT secretion and macrophages to weight loss-induced amelioration of systemic inflammation in obese subjects is not clear. Subcutaneous WAT was obtained from 20 morbidly obese patients before, 3 and 6 months after gastric bypass (GP). Luminex technology was used to measure the release of 27 factors in WAT explants over 24 h incubation. Adipocyte diameters and CD68+ macrophages content were determined in WAT slides obtained at the same time points. Following GP, BMI and adipocyte diameters regularly decreased, accompanied by an overall amelioration of patients' systemic inflammation. In WAT explants, leptin release steadily dropped while adiponectin remained roughly stable. The secretion rate of the anti-inflammatory cytokines IL-10 and IL-13 increased up to 2-fold the initial values after 3 months and remained stable thereafter. Unexpectedly, a similar kinetic profile was observed for pro-inflammatory factors, including TNFα, IL-12 and RANTES. WAT release of three major angiogenic factors, bFGF, PDGF-BB and VEGF, increased regularly, reaching 5-fold the pre-surgical values at month 6. Different patient's kinetic profiles were found for WAT CD68+ macrophages, with increased, stable or decreased counts. Early variations in WAT macrophage content were positively correlated with changes in the release of pro-inflammatory factors in paired explants. These variable secretion profiles in WAT do not directly account for amelioration of systemic inflammation but might be related to variations in macrophage content. Secretion of angiogenic factors suggests a local vascular response to sustained weight reduction. Change in WAT macrophage phenotype is under study to further explore their contribution to the dynamics of WAT secretion, tissue remodeling and bioclinical phenotypes during GP-induced weight loss.
Disclosure of interest: None declared.

P-521
EFFECTS OF METHOTREXATE IN THE INFLAMMATORY ALTERATIONS ASSOCIATED TO OBESITY: AN IN VIVO AND IN VITRO STUDY

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Obesity is associated to a systemic inflammatory process characterized by proinflammatory cytokine production and macrophage infiltration in adipose tissue (AT). Methotrexate (MTX) is an anti-TNF-α drug and it could be a role in inflammation associated to obesity. The aim of this project was to evaluate MTX effects in inflammatory alterations induced by obesity. Swiss mice were feed with high-fat diet (HFD) during 12 weeks and received MTX 2 (MTX2) or 4 (MTX4) mg/kg/week during the last 2 weeks. Body weight, food intake, glucose blood levels and insulin tolerance were evaluated. Visceral AT depots were quantified and biopsies were obtained for protein expression analysis, immunohistochemistry, adipokines production and basal lipolysis measurement. MTX actions were evaluated in vitro using 3T3-L1 adipocytes or Raw 264.7 macrophage/3T3-L1 adipocytes co-cultures. There was no reduction in body weight, food intake or adiposity in obese mice after MTX treatment. However, glucose blood levels were reduced and insulin sensitivity was increased in MTX2 and MTX4. Ex vivo, the basal lipolysis, leptin, TNF-α and IL-6 production were reduced while

adiponectin and IL-10 were increased in AT from MTX4 mice. The MTX also reduced iNOS expression, JNK phosphorylation, macrophage infiltration ($F4/80^+$ cells) and MCP-1 expression in visceral AT. In vitro, MTX inhibited lipolysis through adenosine receptor A1 activation in 3T3-L1 adipocytes. IL-6 in LPS-stimulated co-cultures was also inhibited by MTX. Our results demonstrate that MTX improves glucose homeostasis in obese mice. This improvement seems to be associated with a reduced production of proinflammatory and increased production of anti-inflammatory adipokines by adipose tissue. The effect anti-lipolysis could also contribute to glucose homeostasis, suggesting that drugs with anti-inflammatory associated to anti-lipolytic action could be a tool for controlling inflammatory alterations associated to obesity.
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Disclosure of interest: None declared.

P-522
OLEIC ACID INDUCES DEGRANULATION, REACTIVE OXYGEN SPECIES RELEASE AND CD11B EXPRESSION DEPENDENT OF INTRACELLULAR CALCIUM IN NEUTROPHILS

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Oleic acid (OA) is a nonesterified fatty acid (NEFA) released during lipomobilization around calving in cows or following traumatic bone injury in humans. OA induces well known effects on pancreatic cells via binding to GPR40 receptor, however, recent evidence suggests a possible role on innate immune response. In this study, we assessed the effect of oleic acid on bovine neutrophils activation and the role of calcium on reactive oxygen species (ROS) production, degranulation, CD11b expression and MAPK phosphorylation. Neutrophils isolated from blood samples of healthy heifers were incubated with 100-500 μM OA, in presence or absence of calcium chelating agents. Intracellular calcium mobilization was measured by spectrofluorimetry using Fura-2AM. Superoxide production and CD11b expression, using hydroethidine and CD11b-APC antibody, respectively, were assessed by flow cytometry. Metalloproteinase-9 (MMP-9) release was assessed by zymography gel and MAPK phosphorylation by immunoblot. Oleic acid, in a dose-dependent manner, raised the intracellular calcium, superoxide release and CD11b expression. The intracellular calcium mobilization was partially reduced by EGTA, an extracellular calcium chelating, and inhibited by BAPTA-AM, an intracellular calcium chelating. Superoxide release and CD11b expression induced by 250 μM OA were reduced by BAPTA-AM. OA induced degranulation of neutrophils, as was demonstrated by MMP-9 release. A dose-dependent increase in MMP-9 activity was observed. OA also induced, in a dose-dependent manner, the ERK1/2 phosphorylation between 2 and 5 min of stimulation. In conclusion, we show that oleic acid induces CD11b expression, superoxide production, degranulation and ERK1/2 phosphorylation, dependent of intracellular calcium release. Thus, these results provide evidence about the role of OA in blood neutrophils activation that could affect the innate immune response.
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P-523
ROLE OF IL-6 IN GENE EXPRESSION OF J774 CELLS AFTER ADIPOCYTE COCULTIVE

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Background: The obese state is characterized by inflammation of low intensity and this increased production of pro-inflammatory molecules by adipose tissue has been associated with the accumulation of macrophages in this tissue. The molecular signals that initiate macrophages activation in obese adipose tissue are not yet known, but there are several candidates.
Objective: In order to determine the role of adipocyte released from IL-6 in this activation process, we used a model of co-cultivation between adipocytes and monocytes.
Procedure: J774 cells were maintained in DMEM containing 10% fetal calf serum. We extract the adipocytes of epididymal adipose tissue of mice C57 black using collagenase type II and incubated with J774 cells for different periods of time (1 and 3 h) in the presence or not of IL-6 neutralizing antibody. We then evaluated J774 cells gene expression of TNF-α, IL-1β, IL-6, IL-10, MRS-1 and MIP-1 by real-time PCR. Total RNA was extracted using Trizol reagent. The expression of genes was compared with that of the constitutive Hprt1 gene whose expression was not modulated by treatment.
Results: The co-culture with adipocytes stimulated the expression of TNF-α, IL-1β, IL-6, IL-10 and MRS-1 cells by J774, at specific periods of incubation. The expression of TNF-α increased about 4 times after 1 h of incubation. All other genes had their expression increased after longer periods of co-cultivation besides MIP-1. In cells incubated with adipocytes and anti-IL-6 antibody we noticed a further increase in mRNA expression of these inflammatory mediators.
Conclusion: The results suggest that, at least locally, IL-6 has an anti-inflammatory effect in monocytes exposed to adipocytes.
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Disclosure of interest: None declared.

P-524
CHANGES OF EICOSANOID AND INFLAMMATORY CYTOKINES IN ODUS/ODU

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When periodontal disease develops, many inflammatory cells, mainly lymphocytes, can be observed infiltrating the lesion. It is believed that chemokines like MCP-1 and IL-8, which are produced by gingival epithelial cells undergoing stimulation by bacterial cell components from plaque bacteria through toll-like receptor-2, are involved in the migration of these immunocompetent cells to the lesion site. It has been confirmed that the expression of these inflammatory cytokines is elevated in the gingival tissue and gingival sulcus exudates of periodontal disease patients. When we investigated the dynamics of the inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ, and MCP-1 and anti-inflammatory cytokine IL-10 in rats with spontaneous periodontal disease (ODUS/Odu), we identified them to be an animal model of

periodontal disease, and maintained them in our laboratory. In this study, we measured the plasma levels of the PGE_2 and Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) and the anti-inflammatory cytokine IL-4 in ODUS/Odu. Plasma cytokine levels were measured in these and control rats (Res) at 0 (5 weeks old), 1,3,6,9, and 12 months after the start of the experiment using an enzyme-linked immunosorbent assay (ELISA) kit. The plasma levels of the PGE_2 and CINC-1 were greater in ODUS/Odu than in Res throughout the experimental period. However, the plasma level of the anti-inflammatory cytokine IL-4 was lower in ODUS/Odu than in Res. These results, similar to those obtained in patients with periodontal disease, suggest that ODUS/Odu is a useful animal model of human periodontal disease.
Disclosure of interest: None declared.

P-525
HYPOXIA INCREASES THE EXPRESSION OF SAA IN DIFFERENTIATED 3T3-L1 AND HUMAN ADIPOCYTES

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In obesity, adipocyte hypertrophy and hyperplasia might generate hypoxic areas within the tissue. In a close relationship with hypoxia, the adipose tissue produces inflammatory molecules involved in obesity-related complications, such as cardiovascular disease and metabolic disorders. Besides the hypoxia-inducible factor 1α (HIF-1α), several proteins have their stability or expression modulated by hypoxia. It was also recognized that adipocyte size was correlated with the expression and production of the serum amyloid A (SAA). This protein acts as a potent stimulus for the production of cytokines in immune cells. Thus, the aim of the present study was to assess the influence of hypoxia on the expression of adipocyte-derived protein SAA isoforms, and investigate, in normoxia, the effect of SAA on the production of proinflammatory cytokines by 3T3-L1 adipocytes and human adipocytes. Western blotting was used to measure HIF-1α and SAA; for the last RT-PCR analyses was also performed. The production of the cytokines TNF-α, IL-6, IL-1β and IL-8 in cell culture supernatants was determined by ELISA assays. Ambient hypoxia (1% O_2) caused a two and threefold increase in SAA3 mRNA expression in differentiated 3T3-L1 adipocytes under 12 and 24 h of induction, respectively. For mature human adipocytes, the low oxygen pressure caused an approximately twofold increase in SAA1, SAA2 and SAA4 mRNA expression on 12, 6 and 24 h, respectively. During the differentiation of 3T3-L1, the presence of SAA markedly increased the release of TNF-α and IL-6. The same effect was observed only for TNF-α when human adipocytes were stimulated with SAA. At 24 h, it was observed a sevenfold increase in the TNF-α production. Our data show that the *SAA* gene is responsive to hypoxia, and that SAA possibly favors the improvement of the inflammatory profile of the adipose tissue that may contribute to the overall health complications related to obesity.
Financing: FAPESP; CAPES; CNPq.
Disclosure of interest: None declared.

P-526
PROPIONIC ACID AND D-LACTIC ACID PRODUCED DURING METABOLIC ACIDOSIS INCREASES THE BOVINE BLOOD NEUTROPHILS ACTIVITY

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During acute ruminal acidosis, the animals show a clinical pattern similar to septic shock. The ingestion of excessive amounts of highly fermentable concentrates is followed by an increase in propionic acid production, in this environment, cellulolytic and lactate-using bacteria and protozoa are destroyed, while the growth of lactobacilli is promoted, increasing D-lactic acid production. During overload of oligofructose, we observed an increase of metalloproteinase-9 (MMP-9) release and L-selectin shedding in bovine neutrophils. We assessed if neutrophil response increase during ruminal acidosis by effect of propionate or D-lactic acid. Neutrophils isolated from venous blood of healthy heifers were incubated with 0.3–30 mM propionate or 0.2–10 mM D-lactic acid. The intracellular calcium release was measured by spectrofluorimetry with Fluo-4AM. Reactive oxygen species (ROS) production was assessed by chemiluminescence. CD11b and L-selectin expression was detected by flow cytometry. Metalloproteinase-9 (MMP-9) release was assessed by zymography and the myeloperoxidase enzyme activity with a microplate reader. The presence of GPR41 and GPR43 was evaluated using RT-PCR, western blot and confocal microscopy. 0.3 mM propionate induced MMP-9 secretion, meanwhile 30 mM increased the myeloperoxidase release. Also we demonstrated that bovine neutrophils expressed the putative receptors of propionate GPR41 and GPR43. On the other hand, D-lactic acid induced MMP-9 release, and increased the CD11b expression and L-selectin shedding. Additionally, we observed that D-lactic acid interfered with the ROS production, CD11b expression, L-selectin shedding and MMP-9 release induced by platelet activating factor. We concluded that propionic and D-lactic acid active the blood neutrophils and could contribute with the septic shock signs observed during acute ruminal acidosis.
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Disclosure of interest: None declared.

P-527
ANTI-INFLAMMATORY EFFECTS OF PROCYANIDINS AND DOCOSAHEXANOIC ACID IN HUMAN MACROPHAGES THP-1

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The long chain ω-3 polyunsaturated fatty acid of fish oil, docosahexanoic acid (DHA) and polyphenols like procyanidins have been found to inhibit the inflammatory process. Our study investigated the anti-inflammatory effects of pure DHA, B_1, B_2, C_1 procyanidins and the combination between procyanidins-DHA, on interleukin-6 (IL-6) and prostaglandin E_2 (PGE_2) secretion, cyclooxygenase activity and in the nuclear factor NF-κB signaling in lipopolysaccharide (LPS)-stimulated THP-1 monocyte-derived macrophages. Pretreatment with 10 μg/mL of B_1, B_2 and C_1 procyanidins with or without 25 μM of DHA for 48 h, significantly decreased proinflammatory IL-6 secretion, nevertheless any of the pretreatments had a significantly effect on PGE_2 secretion in (LPS)-stimulated THP-1 macrophages. The pretreatments DHA, B_1, B_2 and C_1, decreased cyclooxygenases inducible (COX_2) and housekeeping (COX_1) in vitro activity. The pretreatment with B_2 were significantly more selective in inhibition of COX_2 than COX_1 activity. The nuclear factor NF-κB activation was significantly reduced by DHA, B_1, B_2, C_1 procyanidins and the combination procyanidins-DHA pretreatments. The IkB kinase activity was decreased through the block of the ρIκB-α phosphorylation by the combination of DHA-procyanidin pretreatments. B_1, B_2, C_1 with or without DHA pretreatments blocks, both p65 and p50, nuclear translocation and the retention of both subunits in the cytoplasm. The nuclear factor NF-κB binding activity was dramatically decreased as a response of the pretreatments with DHA with or without B_1, B_2 and C_1 procyanidins. The results indicate that the anti-inflammatory effects of DHA polyunsaturated fatty acid and B_1, B_2, C_1 procyanidins, alone or together are due to inhibition of IL-6 secretion and cyclooxygenase activity, as well as the inhibition of NF-κB activation by blocking NF-κB p65 translocation and nuclear binding.

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P-528
MONOSODIUM URATE CRYSTALS PROMOTE TH17 CELL DIFFERENTIATION THROUGH AN IL-1- AND IL-18-DEPENDENT MECHANISM

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In the last years, studies have clearly demonstrated that non-microbial molecules released upon cellular damage have the ability to alert immune system through activation of macrophages and dendritic cells (DC). Among these signals, monosodium urate (MSU), the etiological agent of gout, has been found to trigger inflammation through the activation of NLRP3 inflammasome in macrophages, leading to secretion of the proinflammatory cytokines IL-1β and IL-18. Although the inflammasome is currently under investigation for its role in the activation of innate cells, less is known about its ability to program DC to prime a specific T cell response. In the present study, we investigated the response elicited by MSU in DC and how this could promote adaptive immune response. Bone marrow-derived DC (BMDC) stimulated with MSU combined with the NOD2 agonist muramyldipeptide (MDP), strongly produced IL-1β, IL-6, TNFα, and IL-23. Co-culture of BMDC with naïve $CD4^+$ T cells in the presence of MDP/MSU revealed a specific T cell polarization. We show that MDP/MSU stimulation induced the maturation of DC into stably committed Th17-promoting effector DC, as assessed by the presence of IL-17-secreting T-cells both in vitro and in vivo. Similar effects were seen using MSU-activated BMDCs primed with non-microbial stimuli, such as CD40L, GM-CSF or TNFα. In vitro, the MSU-driven skewing of $CD4^+$ T cell polarization towards the Th17 lineage was dependent on IL-1, IL-18 and NLRP3. In vivo antigen-specific Th17 polarization was similarly dependent on IL-1 and IL-18 signaling, but not on NLRP3 activation. Collectively these data show for the first time that a sterile danger signal, acting in synergy with pro-inflammatory priming signals, induces Th17 cell differentiation in vitro and in vivo through an IL-1- and IL-18-dependent mechanism.

Disclosure of interest: None declared.

P-529
C-REACTIVE PROTEIN, FIBRINOGEN AND FERRITIN VALUES IN OBESE CHILDREN WITH NORMAL WHITE BLOOD CELL COUNT IN THE ABSENCE OF ANEMIA

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Obesity is often described as low-grade systemic inflammation and several proinflammatory cytokines are involved in this complex process. Due to the limited studies performed on children, the relationships between obesity and some inflammatory parameters as well as acute phase reactants were investigated in children with normal blood count and iron status parameters. 153 children aged 6.0–17.9 years were recruited for this study. They were divided into two groups; 63 constituted the control group (BMI 16.1 $\pm$ 1.6 kg/m^2). Based on WHO criteria, those defined as having a BMI above the gender-specific 95th percentile were included in obese group (BMI 25.6 $\pm$ 3.9 kg/m^2). Complete blood count, serum iron, total iron binding capacity (TIBC), ferritin, C-reactive protein (CRP), fibrinogen analyses were performed. Data were analyzed by SPSS. $p \leq 0.05$ was accepted as the degree of statistical significance. There was not any statistically significant difference between the groups in terms of mean age, male to female ratio, serum iron, TIBC, white blood cell (WBC), red blood cell, platelet counts as well as hemoglobin, hematocrit, mean corpuscular volume values. No gender difference was noted for these parameters, either. In comparison with those of the control group, CRP values were significantly higher ($p \leq 0.001$) in obese children with normal WBC count. Furthermore, in obese group, the concentrations of the other acute phase proteins such as fibrinogen and ferritin were also higher ($p \leq 0.01$) than those obtained for non-obese children, which confirms the hypothesis of low-grade systemic inflammation in childhood obesity. Even in the absence of elevated WBC count as well as anemia indicators, inflammatory parameters are elevated in obesity during childhood. These may be the pre-indicators of the development of atherosclerosis in the future. Therefore, the importance of the understanding of early life-style interventions is essential for the prevention of cardiovascular diseases.

Disclosure of interest: None declared.

P-530
DOWN-REGULATION OF THE TUMOR SUPPRESSOR P16INK4A CONTRIBUTES TO THE POLARIZATION OF HUMAN MACROPHAGES TOWARDS AN ATM-LIKE PHENOTYPE

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Macrophage recruitment into adipose tissue (AT) plays an important role in the pathogenesis of obesity and insulin resistance. Human adipose tissue macrophages (ATMs) display an alternatively activated M2 phenotype but capable of producing inflammatory mediators. However, the processes driving macrophage polarization and phenotype in AT are not fully understood. Recently, wide-association studies have associated a single-nucleotide polymorphism on chromosome 9p, in the vicinity of the CDKN2A locus encoding also the tumour suppressor $p16^{INK4a}$, with the development of type 2 diabetes (T2D).

Here, we determined the expression levels of $p16^{INK4a}$ in human ATMs and in monocyte-differentiated macrophages (MDMs) from obese patients and we further assessed its role in the macrophage response to inflammation. Our results show that primary ATMs from obese patients express lower levels of $p16^{INK4a}$ than MDMs from the same donors. In line, monocytes from healthy donors in vitro differentiated to M2-polarized macrophages, do not express $p16^{INK4a}$. Furthermore, inhibition of $p16^{INK4a}$ expression in MDMs differentiated also from healthy monocytes, simultaneously increased the expression of M2 related genes and enhanced LPS-induced gene expression. Adenovirus-mediated overexpression of $p16^{INK4a}$ in these MDMs resulted in the opposite phenotype. Thus, decreasing $p16^{INK4a}$ expression in MDMs from healthy donors results in a phenotype similar to ATMs from obese patients, identifying a role for $p16^{INK4a}$ in determining the "M2 like - ATM inflammatory" phenotype.

Disclosure of interest: None declared.

P-531
THE EFFECTS OF METFORMIN AND INSULIN ON MANAGEMENT OF INFLAMMATORY STATE OF DIABETIC PATIENTS

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Type 2 diabetes mellitus is characterized with lipid, carbohydrate and metabolism disorders which caused by lack or resistance of insulin. Metformin is an antihyperglycemic drug used to treat non-insulin dependent diabetes mellitus. Cardiovascular diseases, obesity and type 2 diabetes mellitus are associated with endothelial activation and elevated vascular inflammation. This study aimed to determine whether the serum levels of high-sensitivity C-reactive protein (hsCRP) and myeloperoxidase (MPO) were influenced by metformin and insulin treatment in type 2 diabetes mellitus patients.

Material and methods: The study included 59 patients (35 female and 24 male; mean age 59 $\pm$ 14 years) who were diagnosed as type 2 diabetes mellitus and used either metformin or insulin. Control group was composed of healthy individuals (n = 30). Blood samples were collected after overnight fast. hsCRP levels were determined in immunoturbidimetric methods. The plasma concentrations of myeloperoxidase levels were measured by spectrophotometric method.

Results: Plasma concentrations of MPO for control group, metformin group and insulin group were respectively (mean $\pm$ SD) 0.85 $\pm$ 0.13, 1.43 $\pm$ 0.16, 1.37 $\pm$ 0.21 U/L. Control myeloperoxidase levels were significantly different from insulin and metformin groups ($p < 0.05$). Also hsCRP levels of control, metformin and insulin groups were given respectively (mean $\pm$ SD) 1.5 $\pm$ 1.1, 3.7 $\pm$ 2.05 3 $\pm$ 1.55 mg/dl. Control group hsCRP levels were significantly lower than metformin and insulin groups ($p < 0.05$).

Conclusion: In conclusion, we have demonstrated that both insulin and metformin treatment have similar effects on inflammatory markers of type 2 diabetes mellitus patients.

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P-532
INCREASED LEPTIN ACTIVITY INDUCED BY MATERNAL PROTEIN DEPRIVATION DURING LACTATION INHIBITS APOPTOSIS OF THYMIC CELLS OF YOUNG OFFSPRING

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The consequences of maternal malnutrition in early life on thymocyte responses and the role of leptin were investigated in rats. The young progeny (PD) of dams submitted to protein deprivation during lactation showed at 30 days of age lower body and thymus weights, with no alteration in the thymocyte number or any difference in the profile of T cell subsets, or in their proliferative response. Similar circulating levels of corticosterone and of GR nuclear contents were detected in thymic cells of PD or control groups. In contrast, despite the rats from PD group did not present alterations in leptin circulating levels, the expression of leptin receptor ObRb was enhanced in their thymocytes. This change was accompanied by amplification in leptin signaling response of thymocytes from PD rats, which showed an increase in JAK2 and STAT3 phosphorylation after stimulation with leptin. Moreover, the thymic cells from PD rats presented a decreased rate of spontaneous apoptosis when compared to controls. Accordingly, higher expression of anti-apoptotic protein Bcl-2, and lower of pro-apoptotic protein Bax, with no change of pro-apoptotic Bad, and higher pro-caspase 3 content were detected in PD thymocytes. Moreover, thymocytes from PD group exhibited a constitutive higher nuclear content of p65 NF-kB associated to a lower IkB content in the cytoplasm. Finally, although there was no change in ObR genic expression in PD thymocytes, a higher expression of mRNA for Ob gene was observed in the thymic microenvironment from PD animals. Taken together, the results show that maternal protein deprivation during lactation affects thymic homeostasis, inducing leptin activity, which protect thymocytes from apoptosis in young progeny and, perhaps, may prone these animals for alterations in immune response in adult life.

Disclosure of interest: None declared.

P-533
EXPRESSION OF ANNEXIN A1 IN ADIPOSE TISSUE IS DISSOCIATED FROM MACROPHAGE INFILTRATION DURING DEVELOPMENT OF OBESITY IN MICE

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Obesity is associated with chronic inflammation that increases the risk of type II diabetes and cardiovascular disease. Accumulation of visceral adipose tissue (VSAT) is more proinflammatory and carries a higher risk for disease compared with an increase in subcutaneous adipose tissue (SCAT). Annexin A1 (AnxA1) is an antiinflammatory proresolving mediator whose regulation has not been investigated in obesity. Expression of AnxA1 was evaluated by RT-PCR in VSAT and SCAT of male C57BL6 mice fed either a 60% kcal/fat diet (HFD) or chow diet. A significant upregulation of AnxA1 was observed in VSAT ($p < 0.006$), but not SCAT, of mice fed a HFD for 5 weeks compared with chow groups. Upregulation of AnxA1 by HFD was an early event that preceded both significant weight gain and macrophage infiltration in VSAT, as measured by expression of CD68. When HFD was prolonged for 13 weeks, mice developed obesity and insulin resistance. At this time point, a significant induction of AnxA1 was observed in both VSAT and SCAT ($p < 0.0001$) compared with chow groups. Despite equivalent induction of AnxA1 in VSAT and SCAT, expression of CD68 was significantly lower in SCAT (2-fold increase compared to chow) vs VSAT (100-fold increase) of obese mice, indicating a dissociation between upregulation of AnxA1 and degree of adipose tissue inflammation. Comparable results were obtained in *ob/ob* mice, a model of genetic obesity. A significant induction of AnxA1 was observed in both VSAT ($p < 0.001$) and SCAT ($p < 0.007$) of *ob/ob* mice compared to their lean littermates, even though upregulation of CD68 was significantly higher in VSAT *vs* SCAT. These results indicate that modulation of AnxA1 in adipose tissue is an early event and is dissociated from the process of macrophage infiltration. Upregulation of AnxA1 likely represents a frustrated attempt at controlling adipose tissue inflammation, a response that might be exploited therapeutically to reduce the risk of obesity-associated pathologies.

Disclosure of interest: None declared.

P-534
MATERNAL PERIODONTAL DISEASE PROMOTES DECREASE OF INSULIN SENSITIVITY AND INSULIN SIGNAL IN ADULT OFFSPRING

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The periodontal disease (PD) during pregnancy has been referred as one of the causal factors for preterm birth and low weight at birth. And several other studies have shown that low body weight at birth babies are more likely to develop insulin resistance (IR) in adult life. Studies have shown that both PD as the increase in adipose tissue raises the level of plasmatic cytokines. And cytokines such as TNF-α cause IR. Several studies have shown that both the decrease in "tyrosine phosphorylation status of insulin receptor substrates" (TyP-IRS) and the increase in "serine phosphorylation insulin receptor substrate" (SP-IRS) status are present in experimental models of IR. This study aimed to evaluate in adult offspring of female rats with PD: (1) glycemia (G) and insulinemia (I), cholesterolemia (CH); (2) Ty-IRS and SP-IRS status, in white adipose tissue (WAT). Therefore, 20 female Wistar rats (210 g bw) were divided into two groups: (1) with PD, that was induced by a suture ligature placed around teeth; (2) control rats (CN). After 7 days of the placement of the ligature in the PD group, the rats of both groups were placed for mating. After the offspring birth, male offspring were divided into two groups: (1) PDO group, offspring from female rats with PD; (2) CNO group, offspring of CN female rats. When these rats completed 75 days of age, the evaluation of G, I, CH, and Ty-IRS and SP-IRS status in WAT was performed. The PDO showed significant ($p < 0,05$) increase in plasma concentrations of: CH, G and I compared to CNO. From these values (G and I), the ratio G/I was calculated, by which it was found IR at group PDO. And the PDO group had significant ($p < 0,05$) reduction in Ty-IRS status, in WAT, in addition there was a significant increase ($p < 0.05$) in IRS SP-IRS status in PDO group. Maternal periodontal disease during pregnancy promotes insulin resistance, decreases insulin signal and changes in cholesterol, insulin and glucose levels in the adulthood offspring.

F. S.: FAPESP.

Disclosure of interest: None declared.

P-535
SUPPRESSION NITRODATIVE STRESS & INFLAMMATION BY EPIGALLOCATECHIN GALLATE AMELIORATES DIABETIC NEUROPATHIC PAIN

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Nitrodative stress and inflammation it is implicated as a final common pathway in the development of diabetic neuropathy. The study was designed to evaluate the impact of epigallocatechin-3-gallate alone and in combination with insulin on neuropathic pain and neuroinflammatory cascade in streptozotocin-induced diabetic rats. After 4 weeks, streptozotocin induced diabetic rats developed neuropathy which was evident from decreased tail-flick latency in tail-immersion test (thermal hyperalgesia) and decreased paw withdrawal threshold both in Randall sellitto (mechanical hyperalgesia) and von-Frey hair test (mechanical allodynia). Decrease in nociceptive threshold was accompanied by significant increase in lipid peroxidation and nitrite levels in sciatic nerve of diabetic rats along with marked decrease in reduced glutathione and superoxide dismutase activities. Decrease in pain threshold was coupled with marked increase in TNF-α, IL-1β, caspase-3 activities in cytoplasmic lysate and active p65 subunit of NF$\kappa\beta$ in sciatic nerves of diabetic rats. Administration of epigallocatechin-3-gallate (25, 50, 100 mg/kg) significantly and dose-dependently attenuated all the behavioral and biochemical alterations. Insulin alone corrected the hyperglycemia and partially reversed the pain response in diabetic rats. However, combination with epigallocatechin-3-gallate not only attenuated the diabetic condition but also reversed neuropathic pain through modulation of functional, biochemical and molecular deficits associated with diabetic neuropathy. Collectively, the data reveal that activation of nitrodative stress and

inflammation induced NF$\kappa\beta$ signaling pathway is associated with diabetes induced neuropathic pain and point towards the therapeutic potential of epigallocatechin-3-gallate in diabetic neuropathy.
Disclosure of interest: None declared.

P-536
EXPRESSION OF TH17 AND RELATED CYTOKINES ACCORDING TO CLINICAL ACTIVITY OF BEHÇET'S DISEASE

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The etiology and pathogenesis of Behçet's disease (BD) have remained unclear, however, it has been assumed that genetic factors, infectious, and immune mechanisms are involved in the onset of this disease. A recent study has shown elevated production of IL-17, IL-23, and IFN-γ by peripheral blood mononuclear cells (PBMCs) besides increased frequencies of IL-17 and IFN-γ producing T cells in BD patients with active uveitis. It was also reported that increased expression of IL-23 p19 mRNA in erythema nodosum-like lesions in patients with active BD. In this study, we investigated the expression Th17 and related cytokines according to clinical activity of BD. A total of 22 patients with active and inactive BD were enrolled in this study. The disease control and healthy control (HC) group consisted of age- and sex-matched 10 recurrent aphthous ulcer (RAU) patients without any other evident disease and 10 healthy volunteers, respectively. PBMCs of subjects were cultured and stained with the appropriate fluorescent antibody for analysis by flow cytometry. To measure the IL-17, IL-23 and IFN-γ concentrations in serum and in culture supernatants, ELISA was performed. To investigate the mRNA expression level of IL-12p35, IL-12/23p40 and IL-23p19 in PBMCs, real time polymerase chain reaction (RT-PCR) was performed. In the active BD group, CD4+ and CD8+ T cells from patients expressed significantly higher level of IL-17 and IFN-γ, compared with the cells from the control group. High serum and supernatant concentration of IL-17 and IFN-γ was in active BD group compared with HC and RAU groups. In addition, mRNA expression of IL-23p19, IL-12p35 and IL-12/23p40 in PBMCs was also up-regulated in BD group. Expression of IL-23p19 mRNA was significantly higher in the active BD group compared with HC group. Increased expression of the IL-17 and IFN-γ was observed in this study and these results suggest that Th17 cells have an important implication in immune modulation of BD.
Disclosure of interest: None declared.

Genetics and inflammation

P-538
GENE EXPRESSION PATTERN IN ACUTE GRAFT VERSUS HOST DISEASE IN TARGET AND NON TARGET ORGANS AFTER CONDITIONING

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Objectives: Graft versus host disease (GVHD) is a major complication that limits the use of allogeneic hematopoietic stem cell transplantation. In the present study we investigated the gene expression patterns among different organs (target and non target organs) in GVHD mouse model based on chemotherapy conditioning regimen.
Methods: Female BALB/c (H-2K^{d}) mice were conditioned with busulfan (80 mg/kg) for 4 days followed by cyclophosphamide (200 mg/kg) for 2 days. Allogeneic and syngeneic bone marrow transplantation (BMT) were carried out at day 0 by infusing bone marrow and spleen cells from C57BL/6 (H-2K^{b}) or BALB/c (H-2K^{d}) mice, respectively. Snap frozen samples of liver, intestine, kidney and muscle were collected from control (day -7), conditioned (day 0), allogeneic and syngeneic (day +7) transplanted mice. After extraction of total RNA, samples were analyzed using Affymetrix Mouse Genome 430 2.0 GeneChip micro array, data analyzed by Gene Spring™ software version GX 10.
Results: Gene expression pattern after conditioning (day 0) compared to control mice (day -7) showed down regulation of gene expression in all tissues. After BMT gene expression pattern were extremely different in allogeneic transplanted mice compared to syngeneic. Moreover, we found a unique pattern of CD274 or programmed cell death ligand-1 that is known to be a negative regulator of T cell activation in GVHD mice. The expression of CD274 was significantly up regulated in the muscle and kidney by 66 and 52-fold, respectively, while much lower increment was observed in the liver (15-fold) and intestine (threefold). This could be a potential protective mechanism that renders muscle and kidney less affected organs by acute GVHD.
Conclusions: Chemotherapy conditioning influences the gene expression pattern in all the tissues. Expression of CD274 might have protective effects on tissue injury in muscle and kidney of GVHD pathophysiology.
Disclosure of interest: None declared.

P-539
SKIN TRANSCRIPTOME SHOWS DISTINCT GENE EXPRESSION PROFILES IN LOCI REGULATING HIGH OR LOW INFLAMMATION INTENSITY

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Two mouse lines were phenotype-selected for maximum (AIRmax) or minimum (AIRmin) acute inflammation responses to polyacrylamide bead (Biogel) subcutaneous injection. These lines differ in terms of bone marrow granulopoiesis, neutrophil resistance to apoptosis, and inflammatory cytokine production. Genome wide scan using SNP markers detected two significant quantitative trait loci (QTL) on chromosomes 5 and 7 ($p < 10^{-6}$), as well as suggestive ones on chromosomes 2, 4, 6 11, 12 and 17 ($p < 10^{-3}$). We compared gene expression profiles of epidermal tissues of AIRmax and AIRmin mice submitted to acute inflammatory reactions, in order to correlate with QTL found. Epidermal tissues were recovered 48 h after Biogel subcutaneous injections. Global gene expression analysis was performed on Affymetrix bioarrays (27 k genes) using individual RNA from both control and Biogel-treated AIRmax and AIRmin mice.

Differentially expressed genes were statistically established and the over-represented gene ontology biological process categories were identified. Up-regulations of about 479 and 121 genes were observed in Biogel-treated AIRmax and AIRmin mice, respectively, but only 1 gene were found to be down-regulated in AIRmin, as compared to 167 genes in AIRmax mice. The over-represented biological themes of the differently expressed genes among AIRmax and AIRmin mice represent inflammatory response, signal transduction and cell adhesion. We were able to demonstrate a distinct gene usage between both lines during acute inflammation, and significant differentially expressed genes co-localized with mapped regions on chromosomes 2, 4, 5, 6, 7, 11, 12 and 17.
Disclosure of interest: None declared.

P-540
CYTOKINE GENES EXPRESSION PROFILE AND THEIR ASSOCIATION WITH CARDIOVASCULAR EVENTS IN HYPERTENSIVE PATIENTS

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Inflammation and impaired function of endothelium are crucial factors of initiation, progression and clinical implications of essential hypertension (EH). Cytokines are signaling molecules, modulating cellular cooperation during inflammation process. The aim of the present study was to investigate the expression pattern of cytokine genes and their association with cardiovascular disease, and to study possible interactions between genes involved in the blood pressure maintenance. Study group consisted of 355 EH patients and was divided into three subgroups: first included 219 patients with non-complicated EH, second—95 hypertensive patients with myocardial infarction, third—41 hypertensive patients with ischemic stroke. Control group included 273 unrelated healthy subjects. In the experiment, we used SNPs in 5 cytokine genes and 5 genes encoding for renin–angiotensin system (RAS) and endothelial relaxation factor synthesis components, and performed an association study between EH and control subjects. 84 cytokines and cytokine receptor genes were screened for their expression profile with the RT^{2}Profiler^{TM} PCR Array (SuperArray Bioscience Corporation, USA). An association was detected between *IL1B T511C* ($P = 0.029$) and *IL10 C627A* ($P = 0.016$) SNPs and EH. *IL1B, IL6, IL10, IL12B* and *TNFA* gene variants were found to be associated with cardiovascular complications of EH. Evidence for association with stroke was observed for *IL1B T511*C ($P = 0.022$), *IL-6 G572C* ($P = 0.03$), *IL12B A1159C* ($P = 0.017$) and *TNFA G308A*. *VNTR*-polymorphism in 4-th intron of endothelial nitric oxide synthase gene (*NOS3*) was associated with left ventricular hypertrophy in hypertensive patients ($P = 0.01$). Patients with EH were demonstrated to have altered transcriptional activity of *CCL16, CCL17, CCL18, CCL19, CCL23, CCL8, CCR6, CCR8, CX3CR1, CXCL1, CXCL13, ICEBERG, IL13, IL17C, IL1F10, IL1F6, ILF9, SPP1, CD40LG, XCR* and *CCL2* genes.
Disclosure of interest: None declared.

P-541
INCORPORATION OF THE DONOR DNA INTO THE RECIPIENT LYMPHOID CELLS AFTER ALLOGENEIC TRANSPLANTATION

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Background: Passenger cells, normal constituents of whole organs, migrate from the graft create microchimerism which is suggested to be essential for sustained survival of allografts. The process of DNA transfer between mammalian cells remains not well understood. In our previous study we found donor Sry-PCR product in recipient tissues. This product was detected in splenic macrophages and DC's, but was absent from hepatocytes and parenchymal cells. It has been suggested that donor DNA may play a role in rejection having influence on tolerance.
Aim: In our study we tried to assess the amount of *Sry* gene in different recipient tissues after allogeneic heart transplantation with and without immunosuppression (FK506).
Material/methods: In allogeneic combination male rats BN served as donors and female rats LEW as recipients. Genomic DNA was extracted from the recipient blood and tissues at different times after grafting. *Sry* was detected using Real-time PCR. Relative amount levels in the different samples were calculated by using the comparative C_T method with *Gapdh* as internal control.
Results: The relative amount of *Sry* gene was high in all female tissues 30 days after heart transplantation with immunosuppression, with highest values in blood, liver, spleen and lymph node. Thirty days after heart transplantation without immunosuppression the relative amount of *Sry* gene was in all tissues lower than with immunosuppression.
Conclusion: Detection of donor male DNA isolated from female blood and tissues suggests its spontaneous transport into recipients. Its higher levels in immunosuppressed recipients point to cytotoxicity of anti-rejection drugs. The question remains open whether long-lasting presence of donor DNA may have any relevance to the rejection or tolerance process.
Disclosure of interest: None declared.

P-542
DONOR DNA PERSIST FOR YEARS AFTER KIDNEY TRANSPLANTATION IN RECIPIENT BLOOD

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Background: Organ allograft releases a large amount of genetic material to the recipient circulation. The sources of donor DNA are

the passenger cells and debris of damaged organ endothelial and parenchymal cells. Immunosuppression may cause additional release of genetic material due to its cytotoxic effects.
Aim: The question arises whether the level of released donor DNA may be helpful in assessment of the intensity of the rejection process. In our previous studies, we looked for the presence of donor-specific STR loci (phospholipase *A2-HUMPLA2A1(AAT)$_n$*, cytochrome P-450 *A2-HUMCYARO(AAAT)$_n$* and locus *D1S80*) in the recipient blood.
Material/methods: In this study we have examined the plasma and blood mononuclear cells of recipients to assess the amount of *SRY* gene in sex- mismatched combinations and phospholipase A2-*HUMPLA2A1(AAT)$_n$* in the same sex recipients after kidney transplantation, in relation to the patients probes before grafting by the use of the Real-time PCR method. Recipient's blood and donors spleen samples were collected before kidney transplantation and at different times after grafting. Genomic DNA was isolated from plasma and mononuclear cells. The amount of DNA in different samples after tx was calculated by using the comparative Ct method with *GAPDH* as internal control.
Results: We observed increase in donor DNA level in recipient's blood mononuclear cells already on day 1 after grafting. The relative amount of *SRY* gene was much higher in female's lymphocytes 21 days after transplantation than in plasma. The amount of *HUMPLA* gene was also higher in blood lymphocytes than in plasma 14 days after kidney transplantation.
Conclusions: Donor DNA is present either in "passenger cells" or recipient's phagocytes. An open question remains whether it may be incorporated into recipient cell genome.
Disclosure of interest: None declared.

P-543
PREDISPOSITION TO SEPSIS, ACUTE TISSUE INFECTIONS AND DELAYED INFECTED WOUND HEALING MAY DEPEND ON THE SAME GENETIC POLYMORPHISMS AT TNFA G308A, TNFB G252A, CCR2 G190A, CD14 C159T, TLR2 G2259A AND C2029T AND TLR4 A1036G AND T1336C

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Objectives: Most published studies on infections and genetic polymorphisms are dealing with sepsis.
Aim: We studied polymorphisms of selected allele of cytokines and TLRs at 9 polymorphic sites in patients with sepsis, acute tissue infections and prolonged wound suppuration as we hypothesize with same genetic predilection.
Results: (1) in entire group of patients with systemic and local infections, higher frequency of TNFαG308A GG, TNFβ G525A mutated homozygote AA, and CCR2 G190A mutated homozygote AA than in controls (all $p < 0.0001$) was found. At TGFβ G25C site there was a low expression of GG compared with controls ($p < 0.001$). (2) comparison of sepsis, acute tissue infections and delayed infected wound revealed more of CD14 C-159T CT, TLR1,2 C2259A GA and C2029T CT in sepsis than other infections but differences were not significant. There was lack of differences in subgroups in expression of TNFαG308A GG, TNFβ G525A heterozygote GA, CCR2 G190A AA, TLR4 1 A1036G AA and TLR4 2 C1336T CC.
Conclusions: Polymorphism of TNFα and β, CD14, TLR2,1, CCR2 and TGFβ genes at certain mutation points may be predisposing to surgical type of infections. No significant differences in investigated polymorphisms were found between sepsis, acute local tissue infections and delayed infected wound healing.
Disclosure of interest: None declared.

P-544
GENETIC FACTORS PREDISPOSING TO BONE FRACTURE NON-UNION. A ROLE OF SINGLE POINT MUTATION ASP299GLY TLR4 ON PATHOGEN—EVOKED DELAYED HEALING

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Objective: We reported previously on the presence of viable pathogens and their genetic material (16SrRNA) in gaps of closed non-healing lower limb bone fractures. This may suggest impaired pathogen recognition and/or elimination at the genetic level.
Aim: To validate hypothesis that patients with delayed fracture healing express the higher frequency of TLR4 mutations.
Methods: Observations were performed in 151 patients with delayed (Group A) and 144 with uneventfully healing fractures of lower extremities (Group B). 125 healthy blood donors served as controls. Blood leukocyte DNA was used for analysis of mutations of TLR4 gene at Asp299Gly (1/W) and Thr399Ile (2/W). Fracture gaps biopsies provided material for microbiological studies.
Results: Microbiological studies revealed positive isolates in 31.5% gaps in group A and 16.4% in B ($p < 0.05$). The most frequent isolates were staphylococci (*epidermidis, aureus*, *warneri*, *capitis*, *sciuri* and *lentus)* and less frequent micrococci and enterococci. 16S rRNA was detected in 56.8 and 65.2%, respectively. The frequency of 1/W mutation was significantly higher ($p < 0.05$) in groups of patients with non-healing infected versus sterile fractures. In subgroup with isolated pathogens frequency of 1/W allele was higher compared with those with sterile fracture gaps.
Conclusion: The obtained data suggest predisposal to infection in subjects with single TLR4 mutation. This coincided with delayed bone fracture union.
Disclosure of interest: None declared.

P-545
IL10 POLYMORPHISMS IN ACUTE ANTERIOR UVEITIS

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Acute anterior uveitis (AAU) is a rare disease but the most common form of uveitis with proposed autoinflammatory rather than autoimmune background. At the moment, defined genetic component responsible for this sight threatening disease manifestation is unknown. There is, however, growing number of evidence that single nucleotide polymorphisms (SNPs) in cytokine genes can influence the development of ocular inflammation through their effect on cytokine level production. The gene encoding IL-10, an anti-inflammatory cytokine, was broadly investigated and several SNPs were proved to be implicated in various forms of uveitis and outcome (Stanford et al. 2005; Mizuki et al. 2010; Remmers et al. 2010; Atan et al. 2010). Data obtained with idiopathic intermediate uveitis patients revealed that homozygotes for rs1800896A (−1082) in the *IL10* promoter region were strongly associated with bad outcome. Functional studies also reported that the −1082A, −819T and −592A haplotype is associated with low IL-10 production whereas GCC haplotype formed by the same set of SNPs is linked with IL-10 upregulation and is protective against disease recurrence. For this study, DNA from 137 patients with AAU and 92 healthy controls recruited through Oregon Health and Science University were analyzed for polymorphisms in the promoter region of the *IL10* gene at the established -1082A/G, −819C/T and −592A/C positions by SSP-PCR. Data analysis revealed no significant association of haplotypes with AAU. Four additional markers of interest were analyzed by TaqMan SNP genotyping assays: rs1518111, rs3024490, rs2222202 and rs3024505. HaploView4.2 software was used to define haplotypes based on SSP-PCR and TaqMan results. Joint analysis released six haplotypes. None reached the significance level for the association with the AAU however, the addition of subsequent markers to the established ATA haplotype showed a trend towards association. Therefore further haplotype extending will be of interest.
Disclosure of interest: None declared.

P-547
AN IN VITRO MODEL TO STUDY GENDER DIFFERENCES IN INFLAMMATION

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Gender influences the severity and evolution of various inflammatory conditions. Recent clinical studies showed significant gender differences in inflammatory markers of prepubertal children with very low sex steroid levels, suggesting a genetic contribution. We studied in 24 healthy adults of both sexes some main leukocyte functions of the innate immunity depending on X-linked genes. The respiratory burst measured by chemiluminescence of purified neutrophils stimulated with fMLP was not different in males and females ($p > 0.05$) although the main NADPH oxidase subunit gp91phox is linked to the X chromosome. Percentage of monocytes expressing CD99 was higher in men than in women in the basal state and after stimulation with LPS and fMLP in whole blood ($p < 0.05$). This confirms the higher CD99 protein expression reported in males using RT-PCR and probably due to the partial silencing of the CD99 gene in the inactivated X chromosome of female cells. But using flow cytometry, we were unable to observe any difference in the level of CD99 expression ($p > 0.05$). Using Cytometric Bead Array, we measured inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α) produced in whole blood after a 24 h incubation with and without LPS at 1 ng/ml and 1 μg/ml. Only TNF-α showed a significant difference with a higher production in men than in women, either with LPS at 1 ng/ml ($p < 0.05$) or with LPS at 1 μg/ml ($p < 0.05$). TNF-α production was significantly correlated with the monocyte count ($r = 0.57$, $p < 0.01$) and men had a higher monocyte count than women ($p < 0.01$). Normalization of cytokine levels to monocyte counts suppressed differences in TNF-α production but showed a higher IL-8 production in women than in men after stimulation with both LPS concentrations ($p < 0.05$). IL-8 being the main neutrophil chemoattractant, this difference could explain the higher neutrophil count observed in girls with acute inflammatory disease.
Disclosure of interest: None declared.

P-548
NAKAJO-NISHIMURA SYNDROME (FAMILIAL JAPANESE FEVER) AND RELATED AUTOINFLAMMATORY DISORDERS ACCOMPANIED WITH LIPODYSTROPHY

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Nakajo-Nishimura syndrome (ORPHA2615, MIM256040), also designated as familial Japanese fever, is a particular autosomal recessively-inherited inflammatory and wasting disease which onsets with perniolike eruptions in early infancy and gradually develops emaciation and long-clubbed fingers resulting in joint contracture, occasionally accompanied with periodic spiking fever and nodular erythema-like eruptions. This syndrome had originally been reported as "secondary hypertrophic periostosis with pernio" by Japanese dermatologists Nakajo (Tohoku University) and Nishimura (Wakayama Medical University) in 1939 and 1950, respectively. Afterwards, a group of neurologists described the disease as "hereditary lipo-muscular atrophy with joint contracture, skin eruptions and hyper-gammaglobulinemia" in 1993. So far about 20 cases have been reported as this syndrome from Japan, most of which arise in consanguineous parents and are concentrated in Tohoku and Kansai areas. Therefore, the concept is being accepted that this syndrome represents a new hereditary autoinflammatory disorder accompanied with lipodystrophy. Here we report the summary of Japanese cases, including one infant case, as the result of the national surveillance of this syndrome. Furthermore, comparison to the recently-reported related disorders, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome and an autosomal recessive syndrome of joint contractures, muscular atrophy, microcytic anemia, and panniculitis-associated lipodystrophy (JMP syndrome), is discussed. Identification of the responsible gene mutation will be presented in another poster.
Disclosure of interest: None declared.

P-549
SLC11A1 GENE POLYMORPHISM EFFECT IN MACROPHAGE ACTIVATION AFTER THIOGLYCOLLATE OR BCG STIMULATION IN AIRMAX AND AIRMIN MOUSE LINES

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Polymorphism in *Slc11a1* (formerly *Nramp1*) gene is a major determinant of resistance to *Salmonella*, *BCG* and *Leishmania* infections in mice, due to interference in macrophage (Mφ) activation, inflammatory cytokine production and nitric oxide (NO) release. Mice genetically selected for maximal (AIRmax) and minimal (AIRmin) acute inflammatory reaction are, respectively resistant and susceptible to *S. Typhimurium* infection. The aim of this work was to investigate the activation of thioglycollate-induced and BCG-infected Mφ in the peritoneal exudates from mice homozygous for resistance (R) or susceptibility (S) *Slc11a1* alleles (AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS}). Adherent peritoneal cells harvested at 4 days after thioglycollate ip injection or 14 days after BCG ip infection were cultured for 48 h in the presence or not of LPS. NO was measured with Griess reagent, and cytokines were determined in culture supernatants by ELISA. In thioglycollate-induced Mφ, NO release after in vitro LPS stimulation was higher in AIRmax^{RR} followed by AIRmin^{RR}, AIRmax^{SS} and AIRmin^{SS} the higher production been attributed to the R allele of *Slc11a1* gene. However, IL-1beta, IL-12 and TNFα production was higher in AIRmax^{RR} and AIRmax^{SS} than in cells from AIRmin^{RR} and AIRmin^{SS} pointing out the effect of AIRmax genetic background in this phenotype. When mice were infected with BCG, LPS stimulated Mφ from AIRmax mice bearing the *Slc11a1* R allele produced high levels of IL-1beta, IL-12, IL-6 and TNFα whereas low levels of these cytokines were found in the other mouse lines. On the other hand, NO production was higher in AIRmin^{SS} which, accordingly to *Slc11a1* genotype, is the most susceptible strain to BCG infection, evaluated by bacterial growth in the spleen. In this model the genetic background relevant to acute inflammatory response regulation and *Slc11a1* polymorphism interfere differentially in cytokine or NO production by thioglycollate or BCG induced peritoneal macrophages.

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P-550
ROLE OF IL-1B IN NLRP12-ASSOCIATED AUTO-INFLAMMATORY DISORDERS AND RESISTANCE TO ANTI-IL1 THERAPY

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A new class of auto-inflammatory syndromes called NLRP12-associated disorders (NLRP12AD) has been associated with mutations in NLRP12. Conflicting data have been generated in vitro on the putative role of NLRP12 on IL-1B signaling. This prospective study assesses the secretion of IL-1B and of three IL-1B-induced cytokines (IL-1Ra, IL-6 and TNFa) in patients' PBMC cultured ex vivo and evaluates the patients' response to IL-1 receptor antagonist (IL-1Ra, anakinra), a major drug in the treatment of auto-inflammatory disorders. Recording of patients' manifestations and cytokine measurements were performed before onset of anakinra treatment, during 14 months of therapy, and after anakinra withdrawal. Spontaneous secretion of IL-1B by patients' PBMC was found to be dramatically increased (80- to 175-fold) compared to controls. Consistently, anakinra initially led to a marked clinical improvement and to a rapid near-normalization of IL-1B secretion. However, a progressive clinical relapse occurred secondarily, associated with an increase in TNFa secretion, a persistence of elevated levels of IL-1Ra and IL-6 and a reincrease in IL-1B secretion. Anakinra was withdrawn after 14 months of therapy. This study, which provides in vivo evidence for the crucial role of IL-1B in the pathophysiology of NLRP12AD, reports the first use of anakinra in this disorder and one of the few examples of resistance to anti-IL1 therapy in auto-inflammatory syndromes. Our data also point to the potential interest of cytokine ex vivo measurements in predicting response to treatment and provide new insights into the cellular mechanisms underlying resistance to anti-IL1 therapy.

Disclosure of interest: None declared.

P-551
EFFECT OF RETINOIC ACID RECEPTOR BETA ABLATION ON CELLS INVOLVED IN INFLAMMATION IN A CONDITIONAL MOUSE LINE

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Vitamin A and its active derivatives (referred to as retinoids) play critical roles in cell differentiation, proliferation and apoptosis. Retinoids are ligands that bind and activate retinoic acid receptors (RARs) and retinoid X receptors (RXRs) each consisting of three isotypes (α, β and γ) and these, in turn, function as transcription factors that regulate the expression of target genes. Several studies have shown that vitamin A and their receptors have crucial effects on cells involved in the inflammatory response. However, RARβ effect in these cells has not been studied. On the other hand, RARβ act as a

tumor suppressor and is required for the anti-proliferative effect exerted by retinoids and its expression is frequently lost in many neoplastic tissues like cervical cancer. Conditional mice generated by floxing RARβ gene and expressing Cre recombinase (RAR$\beta^{L-/L-}$) represent a useful model to analyze the function of this receptor. This work examined the role of RARβ on DCs and macrophages in spleen as well as the effect of RARβ ablation in cervix of RAR$\beta^{L-/L-}$ conditional mice. Our results showed that RARβ is expressed mainly in the splenic white pulp zone of wild type mice. In addition, low levels of RARβ expression were detected in the spleen of RAR$\beta^{L-/L-}$ mice, as determined by immunohistochemistry and Western blot analyses. These results are consistent with a decrease in the population of splenic CD11c+MHC-II+ white cells. Histopathology analyses of conditional mice spleen showed a reduction of macrophage-like cells and defects in cell organization and structure. These results correlated with RARβ ablation, histological alterations and a neutrophil-morphology-cell inflammatory infiltrate in cervix of RAR$\beta^{L-/L-}$ mice as detected by Western Blot and histopathology analyses. Our results suggest that RARβ is involved in splenic and cervical cell organization as well as the homeostatic maintenance of cells implicated in inflammation like macrophages and DCs.
Disclosure of interest: None declared.

P-552
THE RELATION BETWEEN THE SEVERE INFLAMMATORY BURN PROCESS AND HUMAN LEUKOCYTE ANTIGEN

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Introduction: Human Leukocyte Antigen (HLA) is significantly variable in the inflammatory process of the burn trauma. HLA-Dr antigens expressed several days after stimulation by burn injury and remains expressed for several weeks. This work aims to detect if there is a relation between the inflammatory process of burned patients and HLA as an important immunological marker, and HLA-Dr expression on peripheral T lymphocytes.
Patients and Methods: Twenty six patients suffering from major burn versus 100 apparently healthy controls were included. Five milliliters of whole blood were collected from control and patient groups at 24 h, 1 week and 2 weeks after burn injury. HLA-DRB1 was tested using sequence specific oligonucleotide (SSO) technique. HLA-Dr expression in T lymphocytes was also tested using flowcytometry.
Results: Comparisons between patients and controls in the frequency of HLA-DRB1 different alleles were performed revealing that HLA-DR4, DR11 and DR13 were significantly decreased in patients group with the P values of (<0.046, <0.04 and <0.04, respectively). In addition, DR13 frequency was significantly increased in patients with gram negative bacilli infection ($p < 0.05$). The absolute number of HLA− Dr^+ T lymphocyte subsets all over the time of the study are lower than controls except that of HLA− Dr^+ T lymphocytes after 2 weeks ($p = 0.009$) in patient compared to control groups. No correlations were found between the HLA-DRB1 different alleles and the expression level of HLA-Dr.
Conclusions: Significant associations of the polymorphism of HLA-DR13 with gram negative bacilli occurring in severe burn inflammation must be considered. Therefore, it seems possible that DR13 may influence the occurrence of severe infection in burned patients and explained that as an evidence of natural selection by microorganisms. HLA-Dr expression on T lymphocytes occurred independently with no relation to different inflammatory conditions accompanying burn injury.
Disclosure of interest: None declared.

Resolution of inflammation and tissue repair

P-553
THERAPEUTIC EFFECT OF LOW LEVEL LASER THERAPY ON REPAIR OF RADIUS FRACTURE IN RABBIT

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Introduction: Studies on the effects of low level laser therapy (LLLT) on fracture bone repair have shown different results: some of them have reported positive effects of LLLT on bone healing, whereas others did not obtain any significant results. The aim of this study was evaluation of therapeutic effect of LLLT on the rate of callus formation and bone healing time in the radius of rabbit.
Methods: 30 white male rabbits of Dutch race (2 ± 0.2 kg weight) were used. After applying anesthesia, 3 mm in diameter lesion was induced in the craniomedial aspect of radius bone by orthopedic saw. Then rabbits were randomly divided into control and experimental groups. In the experimental group a laser ($\lambda = 830$ nm, $F = 1500$ Hz, $P = 50$ mW, $D = 4$) was applied transcutaneously on 3 points at 9 sessions during 21 days. The first irradiation was performed 24 h after surgery. The control defects were allowed to heal spontaneously. To assess the callus formed, 4 series of radiographic images from 2 views (lat and AP) with 1 week interval were taken. Data were analyzed using independent sample t-test and repeated measurement.
Results: Statistical analysis of X-ray data show that LLLT can increase the average amount of callus in comparison to control group, but the difference is not significant ($P > 0.5$).
Conclusion: Our finding indicates that LLLT does not accelerate healing of radius fracture in rabbit.
Keywords: LLLT, radiographic image, bone healing, rabbit, radius.
Disclosure of interest: None declared.

P-554
EVALUATION OF HYPERTENSION AND ATENOLOL EFFECT IN ALVEOLAR HEALING PROCESS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Hypertension is an unfavorable condition for dental treatments that require bone healing. The purpose of this study was to evaluate the alveolar wound healing in spontaneously hypertensive rats (SHR) and atenolol effect on this process. Wistar rats and SHR treated with atenolol (100 mg/kg/day) were submitted to the extraction of the upper right incisive tooth and sacrificed at different days after surgery. The hemi-jaws were extracted and the radiographic images were obtained and analysed. Histological, histomorphometric and immunohistochemical reactions were done in histological sections, 5 μm thick, stained with hematoxylin and eosin or subjected to immunolabeling to RANK, RANKL, OPG and MMP-9 proteins. The results were analyzed by two-way ANOVA. In immunohistochemical analysis, scores were assigned to the images and the results were analyzed by Kruskal–Wallis and Mann–Whitney statistical tests ($p < 0.05$). Reduced bone mineral density (BMD), lower bone percentage and less thickness of trabecular bone was observed in the final periods of alveolar bone healing in SHR. Increased RANKL, RANK and MMP-9 immunolabeling were observed at 28 days after surgery in SHR alveolus. Atenolol increased the BMD observed in most of the periods analyzed and increased trabecular bone thickness at 28 and 42 days in SHR alveolus. Increased OPG immunolabeling at 7 and 42 days of alveolar healing were observed in treated SHR. Increased expression of RANKL and MMP-9 at 21 and 42 days, and increased RANK expression at 21 day was also observed in alveolus of SHR treated with atenolol. Our results suggested that the delay in alveolar healing observed in SHR was associated with increased peripheral sympathetic tone described in these animals. Changes in alveolar bone formation and remodeling would be associated with differential expression of proteins in bone metabolism OPG, RANKL, RANK and MMP-9, which is modulated by the effect of atenolol.
Disclosure of interest: None declared.

P-555
PHAGOCYTOSIS OF APOPTOTIC CELLS LEADS TO DIMINISHED PROINFLAMMATORY FUNCTIONS OF NEUTROPHIL GRANULOCYTES

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Neutrophil granulocytes (PMN) are rapidly recruited from the bloodstream to the site of acute inflammation where they die in large numbers. Since release of toxic substances from dead neutrophils can propagate the inflammatory response leading to tissue destruction, clearance of dying inflammatory neutrophils has a critical function in the resolution of the inflammatory response. Apoptotic neutrophils are phagocytosed primarily by macrophages, provided these cells are present in adequate numbers. However, macrophages are rare at sites of acute inflammation while the number of neutrophils can be extremely high. In the present study in vitro experiments with human neutrophils were carried out to investigate whether neutrophils can ingest apoptotic neutrophils. We show that "naïve" granulocytes isolated from venous blood have a limited capacity to phagocytose apoptotic cells. However, exposure to activating stimuli such as LPS, GM-CSF and/or IFN-gamma results in enhanced phagocytosis of apoptotic cells. The efficient uptake of apoptotic cells by PMN was found to depend on the presence of heat labile serum factors. Importantly, the contact to or uptake of apoptotic cells inhibited neutrophil functions such as respiratory burst and the release of the pro-inflammatory cytokines TNF and IP-10. The data suggest that activated neutrophils participate in the clearance of apoptotic cells. In addition, since apoptotic cells inhibit pro-inflammatory functions of neutrophils, uptake of apoptotic cells by PMN contributes to the resolution of inflammation.
Disclosure of interest: None declared.

P-556
INHIBITORY ACTIVITY AGAINST TACE (TUMOR NECROSIS FACTOR-ALPHA CONVERTING ENZYME) BY FOOD-DERIVED PEPTIDES OCCURRING IN HUMAN PERIPHERAL BLOOD

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Some food-derived peptides have inhibitory activities against various peptidases such as ACE (Angiotensin I-converting enzyme), which are involved in processing of cytokines and hormones. TACE (tumor necrosis factor-α converting enzyme) is currently being explored as a good target for anti-inflammatory drugs. Our previous studies demonstrated that the occurrence of food-derived di-peptides in human blood after oral ingestion of some peptides. Pro-Hyp, Hyp-Gly, Gly-Pro, Pro-Gly, pGlu-Leu, and Val-Ala have been identified in human blood at relatively higher level (>μM) after ingestion of food peptides. The objective of the present study was to investigate the inhibitory activity against TACE of the food-derived peptides (Pro-Hyp, Hyp-Gly, Gly-Pro, Pro-Gly, pGlu-Leu, and Val-Ala) occurring in human peripheral blood. The enzymatic activity of TACE was measured by using the quenched fluorogenic peptide substrate Mac-P-L-A-Q-A-V-Dpa-R-S-S-S-R-NH_2. TAPI-2 (TNF-α processing inhibitor) was used as a positive control. Assays were performed in 100 μl of reaction buffer (50 mM Tris, 5 μM $ZnCl_2$, 0.01% Brij35, pH 9.0) containing 112 μM substrate at 37°C in the absence or presence of test compounds. Substrate and products were separated by HPLC using C18 reversed phase column and detected by fluorescence detector at 320 nm excitation and 405 nm emission, respectively. Significant inhibition of TACE was observed only for pGlu-Leu in a dose-dependent manner. Whereas, other di-peptides did not have significant effects on TACE activity. pGlu-Leu occurs after ingestion of wheat gluten hydrolysate, which has been demonstrated to moderate acute hepatitis in rat model. Further studies on TACE inhibitory activity of food-derived tri-peptides in human peripheral blood, are now in progress.
Disclosure of interest: None declared.

P-557
CHONDROCYTES IN TISSUE-ENGINEERED CARTILAGE USING SCAFFOLDS COULD SUPPRESS TISSUE REACTIONS THROUGH FORMATION OF IMMUNE PRIVILEGE

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Tissue reaction after transplantation of engineered tissues could hamper their regeneration. The understanding of those biological events provides valuable information to inhibit severe inflammation and could improve the clinical results of tissue engineering. This study was conducted to elucidate the interaction between tissue-engineered cartilage using biodegradable polymer scaffolds and host cells. Tissue-engineered cartilage consisting of C57BL/6 mice chondrocytes and poly-L-lactic acid (PLLA) scaffolds were transplanted in EGFP transgenic mice of the same genetic background. Host-derived EGFP-positive cells were mostly F4/80-positive macrophages, which were drastically decreased and excluded to non-cartilage areas after transplantation. Meanwhile, IL-1β was significantly lower in the tissue-engineered cartilage than PLLA scaffolds alone. Fas ligand (FasL) was immunolocalized on the chondrocytes in tissue-engineered cartilage, indicating that immune privilege was formed in tissue-engineered cartilage, suppressing tissue reactions. We then used FasL-hypomorphic mice (gld) to prove the function of FasL in the formation of immune privilege. Chondrocytes of wild-type induced apoptosis of macrophage with higher incidence, than those of gld. In the transplantation of tissue-engineered cartilage, the constructs of wild-type underwent more accumulation of cartilage matrix than those of gld. To examine the trigger of FasL expression on chondrocytes interacted with macrophages, the co-culture medium of both cells were exhaustively analyzed, which detected G-CSF. The G-CSF-treated constructs underwent less infiltration of macrophages with increased formation of cartilage after transplantation. The G-CSF secreted from macrophages could increase the expression of FasL on chondrocytes and render immune privilege, which may in turn suppress the viability and localization of macrophages, promoting the maturation of tissue-engineered cartilage.
Disclosure of interest: None declared.

P-558
MC3 AGONISTS AS NOVEL PRO-RESOLVING THERAPEUTICS

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Inflammation is a defensive response of the body against pathogens and injury, which is tightly controlled by endogenous pro-resolving factors (such as lipids, e.g. lipoxins and resolvins, and peptides, e.g. Annexin A1) operative in restoring homeostasis after tissue injury. Melanocortin (MC) peptides are emerging as another group of anti-inflammatory mediators, though their ability to activate resolving circuits is yet not investigated. Here we studied an analogue of αMSH, compound AP214, currently in clinical development for cardiac surgery (http://clinicaltrials.gov). In acute peritonitis, AP214 (400 μg/kg) afforded ~30% reduction of 4 h neutrophil infiltration promoted by zymosan. In vitro, zymosan-stimulated cytokine (IL-1β, IL-6 and TNFα) release from macrophages was inhibited by AP214 (1–100 nM). At these concentrations, a potent pro-resolving action of the phagocytosis of human apoptotic neutrophils (process of efferocytosis) was observed: this occurred in vitro as well as in vivo. To identify the receptor target for the anti-inflammatory and pro-resolving properties of AP214, cells and mice lacking the MC_3 receptor were employed observing that inhibitory effects of the compound on neutrophil infiltration, IL-1β release and efferocytosis were no longer measured. Similar observations were obtained with another compound, AP1189: when AP1189 was given at the peak of inflammation (12 h after injection with zymosan) in the peritonitis model, it was able to increase resolution compared to vehicle treated mice, an effect independent on increased leukocyte apoptosis. In summary, MC-related drugs are anti-inflammatory therapeutics endowed with genuine pro-resolving properties, thus adding this family of compounds (peptides or small molecules) to the successful exploitation of the resolution of inflammation paradigm for innovative drug discovery programmes.
Disclosure of interest: T. Montero-Melendez: None declared, T. Jonassen Other: Co-founder of Action Pharma, M. Seed: None declared, M. Perretti: None declared.

P-559
TROPHIC ACTIVITIES OF POLARIZED MACROPHAGES DURING MYOGENESIS AND MUSCLE REPAIR

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Skeletal muscle regeneration is associated with macrophage infiltration. We have previously shown that macrophages exert dual and beneficial role during skeletal muscle repair. Soon after injury, infiltrating monocytes/macrophages are classically activated and phagocyte tissue debris while preventing too early myogenic differentiation. Then macrophages switch their phenotype to resolve inflammation: the second phase of muscle repair is characterized by the presence of so called M2 macrophages that, while dampening environmental inflammatory signals, directly support myogenesis and myofibre growth. We aimed at exploring the effects of these different types of macrophages at the cellular level on the sequential steps of in vitro human myogenesis, namely expression of the differentiation (myogenic) programme, prefusional migration of the cells, and eventually fusion of the cells to form myotubes. We found that pro-inflammatory (M1), alternatively activated (M2a) and anti-inflammatory (M2c) macrophages exert distinct, sometimes opposite effects on the steps of in vitro myogenesis, essentially through secreted factors. As a whole, M1 macrophages inhibit myogenesis while M2 macrophages stimulate each step of myogenesis. Secretome of human polarized macrophages was performed to identify putative candidates involved in these trophic functions. Using blocking antibodies against various cytokines and growth factors (IL-1beta, IL-4, VEGF, TGFbeta, TWEAK, etc.) in functional tests helped to define the nature of

these factors, that depends on the polarization of the cells. Our results show that macrophages exert specific trophic effects on myogenic cells. These effects vary according to their state of polarization, indicating that macrophages finely regulate the sequential steps of myogenesis.
Disclosure of interest: None declared.

P-560
EVALUATION OF THE MECHANISMS OF ACTION OF FATTY ACIDS FROM VEGETABLES OILS ON WOUND HEALING

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Introduction: Wounds of difficult healing affect 2% of population in developed countries. Despite of its clinical relevance, in Brazil there are not statistical data yet. Researchers have been showing that fatty acid from vegetal origin improves the wound healing. Vegetables oils for wound treatment have been used in Brazil currently, but do not have scientific endorsement. Therefore, our aim was to describe the mechanism of action of Curatec AGE® (commercial product rich with oleic acid, linoleic acid and medium chain triglycerides) in skin wound healing in mice.
Methods: The wound was surgically induced and treated daily with Curatec AGE®. Mineral oil was used as control.
Results: The Curatec AGE® induced an increase of neutrophil accumulation in the wound at days 1, 4, 7 and 14, which has a peak at day 4. A high level of IL-13 in wound homogenate was observed at day 7 but no difference was found in IL-10, IL-6, TNFα and TGFβ levels. Curatec AGE® showed an increase of new blood vessels and predominance of fibroblasts in newly formed tissue at day 7 after wound. However the wound closure in treated and control groups was similar throughout the 14 days of evaluation.
Conclusions: Taken together, Curatec AGE® promoted pro-inflammatory effects only in early days after injury. The presence of new blood vessels and fibroblasts indicate granulation tissue formation, which was not evident in control group. Data also suggest that fatty acids from Curatec AGE® affect the wound microenvironments leading to an improvement of new tissue formation after injury. We intend to evaluated the Curatec AGE® influence on the total collagen production at the wound, as well as parameters of oxidative stress and deposit of matrix proteins in the wound bed.
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P-561
IL-24 PLAYS A KEY ROLE IN CUTANEOUS WOUND HEALING VIA SIGNALING THROUGH IL-22R1/IL-20R2 RECEPTOR COMPLEX

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Cutaneous wound healing is a complex regenerative and immunological process. Its disturbance leads to chronic wounds and represents a great medical problem. In the past, we demonstrated that the novel IL-10 cytokine family members IL-20, IL-22, and IL-24 play a major role in inflammatory skin diseases like psoriasis. Here, we studied the significance of these cytokines in cutaneous tissue repair. Surprisingly, using an in vivo mouse model, IL-20 was almost constantly expressed upon wounding and following healing process, whereas IL-22 was not expressed at all. In contrast, IL-24 was highly upregulated in the inflammatory phase of wound repair. The major sources of IL-24 appeared to be T cells and keratinocytes. IL-24 acts through a transmembrane receptor complex, which is composed of IL-22R1 and IL-20R2. Importantly, mice lacking IL-22R1 (IL-22R1$^{-/-}$) compared to corresponding wild-type mice (WT) showed a delayed wound closure starting in the early phase of the healing process. Further studies identified keratinocytes, but not dermal fibroblasts, endothelial cells, melanocytes, or subcutaneous adipocytes as being targets of IL-24 action. The IL-24 treatment of human keratinocytes regulates the expression of antimicrobial proteins, chemokines and keratinocyte differentiation-associated genes. This study suggests that the IL-24/IL-22R1 system plays a key role in the inflammatory phase of cutaneous wound healing.
Disclosure of interest: None declared.

P-563
INCREASED EXPRESSION OF GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) IS ASSOCIATED WITH RESOLUTION OF ACUTE INFLAMMATION

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The induction of neutrophil apoptosis could be of potential benefit in the control of inflammatory diseases. Glucocorticoid (GC)-induced leucine zipper (GILZ) has been shown to mediate several GC functions, such as, apoptosis, anti-proliferative and anti-inflammatory

activities. In this study, we investigated the role of GILZ on the spontaneous and pharmacologically-induced resolution of neutrophilic inflammation in the pleural cavity of LPS-challenged mice. The injection of LPS induced a time-dependent influx of neutrophils into the pleural cavity of mice which was maximal at 8–24 h, diminished at 48 h, with complete resolution occurring at 72 h. The spontaneous resolution of neutrophilic inflammation was accompanied by an increase of mononuclear cells at the pleural cavity and was associated with an increase in the number of apoptotic cells at 8–48 h, interval of time that precedes complete resolution, as demonstrated by morphological and biochemical criteria (increase of Bax and caspase-3 cleavage). The expression of GILZ was detected in PBS challenged mice and its levels decreased markedly at the peak of inflammation and increased during the resolution phase of the response. Pharmacological treatment of mice with drugs that cause resolution of inflammation, including rolipram, wortmannin and dexamethasone, reduced the number of neutrophil in the pleural cavity and increased expression of GILZ, which was associated with activation of a pro-apoptotic program (increase of Bax and caspase-3 cleavage and decrease of Bcl-xL). In conclusion, our results showed a positive correlation between expression of GILZ, increased expression of pro-apoptotic pathway and resolution of inflammation, suggesting GILZ plays an important role in the signaling events leading to resolution of neutrophilic inflammation.

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P-564
ALTERED RESOLUTION OF PERITONEAL INFLAMMATION IN MMP-9-DEFICIENT MICE

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Matrix metalloproteinase-9 (MMP-9) degrades extracellular matrix (ECM) substrates and plays an important role in neutrophil infiltration, e.g. during zymosan peritonitis. In other inflammatory models, similar roles were documented for heparanase that degrades ECM heparan sulphate proteoglycans. We report here that synthesis of heparanase is, surprisingly, increased only during the late phase of zymosan peritonitis (at 24 h). An anti-inflammatory role of heparanase is revealed by finding that specific inhibition of heparanase does not decrease neutrophil influx, and in contrast, neutrophil numbers are increased but without affecting neutrophil apoptosis. For MMP-9, we showed previously that during zymosan peritonitis, early neutrophil infiltration in MMP-9-deficient (MMP-9−/−) mice was significantly weaker during maximal neutrophil influx (6 h) than in wild-type mice. However, during the late stages of peritonitis (24 h) an extended accumulation of neutrophils was observed in MMP-9−/− mice. Recently, we reported that this was due to increased release of COX-1-derived PGE_2 that inhibited apoptosis of inflammatory neutrophils. The aim of the current study was to investigate the production of mediators/enzymes in the resolution of inflammation and evaluate whether these are altered in mice lacking MMP-9. mRNA expression and release of IL-10 and TGF-β were significantly decreased at 6 h in MMP-9−/− mice, whereas in late inflammation (24 h) they were significantly augmented. Moreover, we detected that intracellular and extracellular heparanase contents were increased in leukocytes from MMP-9−/− mice. This phenomenon was also observed in the early stage of peritonitis. Overall, we reveal that the resolution of zymosan peritonitis is enhanced in MMP-9−/− mice as the production of IL-10, TGF-β and heparanase is increased. This suggests that MMP-9 has unexpected impacts on the termination of inflammation.

Disclosure of interest: None declared.

P-565
IMPLANT OVERLOADING AND PARAFUNCTIONS: AVOIDING AND MANAGING COMPLICATIONS

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Aim: The aim of this study was to show the destructive effects of abnormal occlusal forces on implant supported prostheses in patients with bruxism, abnormal habits and other parafunctions, and to focus on concepts and the clinical procedures to reduce the potential risk factors for implant failure.

Material and methods: 40 TMD patients were compared to 40 no-TMD patients in which were inserted 430 implants with the same features as number, size, position, design. Another experimental group of 50 TMD patients treated by prevention protocol was assessed. Besides, were considered type of restoration, cemented or screwed, malocclusion type, smoking, load timing. The heavy force of compression, clenching and grinding, as in bruxism, simultaneously applied strong pressures to the implants, crestal bone, restorations and temporomandibular joints. This was a potential risk factor for crestal bone loss, loss of integration before and after restoration, abutment screw loosening and fracture, implant fracture, decementation of restorations and fracture of the porcelain.

Results: The 5 years follow-up showed a 58% of soft tissues, bone and prosthetic complications in TMD patients versus a 11% in non TMD patients ($P < 0.01$). When TMD patients were undergone to occlusal overload prevention protocol, the complications were diminished to 13% ($P < 0.01$). Increasing the number of implants and reducing cantilevers decreases the stress; using the longest and widest implant possible increases implant/bone surface area and reduces also strain. Also implant design, occlusal table size, the direction, duration and magnification of the forces influences the stress at the crestal bone/implant surface.

Conclusion: Developing treatment plan that control the chronic bruxism through night-guards and an occlusal adjustment protocol to modify the occlusal forces on implants and their restorations, patients with temporomandibular disorders and bruxism can be candidates for implants.

Disclosure of interest: None declared.

P-566
BONE TISSUE REPAIR AND BILATERAL SINUS AUGMENTATION GRAFTING: AUTOGENOUS BONE VS. BIOMATERIALS

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Aim: Two different graft materials, Beta-tricalcium phosphate (Beta-TCP) and autogenous bone, were used in the same patient. The aim of this study was to determine whether donor site morbidity could be avoided by using pure-phase Beta-TCP.
Materials and methods: Bilateral sinus grafting was performed on 20 selected patients; Beta-tricalcium phosphate was used on the experimental side, and autogenous bone was used on the control side. In addition to routine panoramic radiographs, 2- and 3-dimensional computerized tomographic examinations were performed pre- and postoperatively and after implantation. Eighty bone biopsy specimens were taken at the time of implant placement.
Results: Histologically and histomorphometrically, there was no significant difference between the experimental and control grafts in terms of the quantity and rate of ossification. For each histologic sample, the total surface area, the surface area that consisted of bone, and the surface area that consisted of graft material were measured in mm^2, and bone and graft material were analyzed as percentages of the total. The mean percentage bone areas were 36.47 ± 6.9 and $38.34 \pm 7.4\%$, respectively; the difference was not significant ($P = .25$). The autogenous bone was initially less visible than the Beta-TCP, but new bone formation was clearly observed for both materials. The rate of graft resorption was lower on the experimental side than on the control side. The mean graft area percentages were 13.95 ± 5.38 and $8.47 \pm 3.17\%$, respectively, and the difference was highly significant ($P < .001$). The mean areas of the biopsy samples taken from the 2 sides were quite similar: 9.18 ± 2.42 mm^2 on the experimental side and 8.98 ± 1.76 on the control side.
Conclusion: Comparisons with other studies reveal that Beta-TCP is a satisfactory graft material for maxillary sinus floor augmentation, even without autogeneous bone.
Disclosure of interest: None declared.

P-567
COMPARISON OF DIFFERENT TISSUE-DERIVED STEM CELL SHEETS FOR PERIODONTAL REGENERATION IN A CANINE 1-WALL DEFECT MODEL

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Cytotherapeutic approaches have been investigated to overcome the limitation of existing procedures for periodontal regeneration. In this study, cell sheet transplantation was performed using three kinds of mesenchymal tissue (periodontal ligament, alveolar periosteum, and bone marrow)-derived cells to compare the differences of cell source in a canine severe defect model (one-wall infrabony defect). Periodontal ligament cells (PDLCs), iliac bone marrow mesenchymal stromal cells (BMMSCs), and alveolar periosteal cells (APCs) were obtained from 4 beagle dogs, respectively. Three layered cell sheets of each cell source supported with woven polyglycolic acid were transplanted to the denuded root surface autologously. One-wall intrabony defects were filled with the mixture of β-tricalcium phosphate (β-TCP)/collagen. Eight weeks after the transplantation, periodontal regeneration was significantly observed with both newly formed cementum and well-oriented PDL fibers in the PDLCs group compared to other groups. In addition, nerve filament was observed in the regenerated PDL tissue only in the PDLCs group. Alveolar bone regeneration was observed in all groups. These results indicate that PDLCs combined with β-TCP/collagen scaffold is the promising tool for periodontal regeneration.
Disclosure of interest: None declared.

P-568
IDENTIFICATION OF PEPTIDE RESPONSIBLE FOR MODERATION OF HEPATITIS IN WHEAT GLUTEN HYDROLYSATE BY IN VIVO ACTIVITY-GUIDED FRACTIONATION

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Glutamine is an non-essential amino acid, but it becomes essential under surgical trauma and some pathological conditions. Gln has been supplemented to patients suffering from hepatitis and other illness. However, Gln is easily degraded into pyroglutamic acid in solution and lost its beneficial nutritional value. Wheat gluten is rich in glutaminyl residue. Then, the enzymatic hydrolysate of wheat gluten has been considered as good glutamine source. There is an episode suggesting that ingestion of wheat gluten hydrolysate (WHG) could moderate symptom of hepatitis (Horiguchi et al. 2004). The objective of the present study was to identify the active compound in WHG. Ingestion of WHG can moderate galactosamin-induced acute hepatitis in rat model. To identify the active component in WHG, in vivo activity-guided fractionation was carried out. Peptides in WHG were fractionated on the basis of amphoteric nature of sample peptide by the method of Hashimoto et al. (2005). The acidic peptide fractions showed significant moderation of the hepatitis. Peptides in active fraction predominantly consisted of free pyroglutamic acid (pyroGlu), pyroGlu-Leu, pyroGlu-Ile, pyroGlu-Gln, and pyroGlu-Gln-Gln. Among them, pyroGlu-Leu significantly suppressed the hepatitis. On the basis of the present results, pyroGlu-Leu can at least partially be responsible for the beneficial effects of WHG. The pyroGlu-Leu is absorbed into portal blood of rat after oral ingestion and suppress induction of proinflammatory cytokines and NO. Then it could be concluded that pyroGlu-Leu moderates acute hepatitis by suppressing inflammatory response.
Disclosure of interest: None declared.

P-569
MELANOCORTIN RECEPTOR AGONISTS ARE NOVEL CHONDROPROTECTIVE THERAPEUTICS

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The possibility that homeostatic mediators, effectors of the resolution of inflammation, might impact on chondrocyte biology has been poorly explored. Here we tested the potential effects of anti-inflammatory and tissue-protective peptides discovered in the melanocortin biology arena. The human chondrocytic cell line C-28/I2 was cultured in high-density micromass (MM) cultures. Peptides AP214 (10–1,000 nM; under clinical development http://clinicaltrials.gov) and NDP-MSH (1–100 nM) were tested both alone and/or upon IL-1β stimulated cells. qRT-PCR allowed quantification of anabolic (COL2A1, SOX9, ACAN) and catabolic (MMP-1, ADAMTS5) gene products, to reveal the chondrocyte phenotype. Production of matrix glycosaminoglycans (GAG) was quantified by staining the MM with Alcian blue (AB) dye. Data are from 3 to 4 independent experiments. In resting cells, AP214 produced a concentration dependent anabolic response (+250% on COL2A1). Upon IL-1β stimulation, AP214 was predominantly effective in offsetting the catabolic response, with 30–50% reduction in IL-1β-induced metalloproteinase MMP1 and ADAMTS5. This effect was not restricted to AP214, and NDP-MSH also potently inhibited the IL-1 response. Detection of melanocortin receptor MC1 and MC5 mRNA in these cells confirmed recently published data (Grassel S et al A&R 10:3017, 2009). In summary, applying a novel predictive protocol using C-28/I2 chondrocyte cell line cultured in MM, we report the chondroprotective properties of AP214. These findings may impact on the development of melanocortin receptor agonists and, more in general, may become paradigmatic for exploiting the resolution of inflammation area of research for novel chondroprotective therapeutics. We thank Dr M Goldring (Cornell Medical College, New York) for the C-28/I2 cells.
Disclosure of interest: None declared.

P-570
EXPRESSION OF WNT RELATED GENES IN HUMAN PERIODONTAL LIGAMENT CELLS DURING OSTEOGENIC DIFFERENTIATION

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Background: Periodontitis is a bacterial infection and can lead to tooth loss. Periodontal ligament (PDL) is a connective tissue that exists between tooth and alveolar bone, and considered to contain mesenchymal stromal cells. So PDL is suggested to be useful for periodontal regeneration. When PDL cells are cultured with osteoinductive medium (OIM), alkaline phosphatase (ALP) activity and osteogenic gene expressions are enhanced and PDL cells form mineralized nodules after the long-term cultivation. The aim of this study was to investigate the involvement of Wnt signaling during osteogenic differentiation in human PDL cells.
Methods: To investigate the multipotency of hPDL cells, colony forming assay (CFA) and differentiation assay were performed. hPDL cells were cultured in OIM, and ALP activity was measured with various reagents. To analyze the Wnt-related genes during osteoblastic differentiation, mRNAs were quantitatively analyzed using real-time RT-PCR. About interested genes, loss and gain of function experiments were performed.
Results: hPDL cells in this study showed mesenchymal stromal cell-like properties. hPDL cells grown in OIM showed high ALP activity, however it was decreased by Wnt inhibitor, XAV939. From the analysis of PCR array, secreted Frizzled-related protein 3 (SFRP3) was up-regulated, on the other hand, that of SFRP4 was down-regulated during the osteoinduction. TaqMan PCR assay also confirm the gene expressions, and time course studies revealed the expression of SFRP3 was gradually increased, whereas that of SFRP4 was decreased. SFRP3 siRNA and recombinant human SFRP4 decreased ALP activity.
Conclusions: These results suggest that Wnt signaling, especially SFRPs, involves in the osteogenic differentiation of hPDL cells. SFRPs, which are extracellular antagonists of Wnt signaling, may be differentially regulated during osteoinduction and can be a new therapeutic reagent for preventing inflammatory periodontal bone loss.
Disclosure of interest: None declared.

P-571
DUAL EFFECT OF HYALURONAN ON LIPOPOLYSACCHARIDE CYTOTOXICITY IN HUMAN MACROPHAGES: ROLE IN INFLAMMATION MODULATION

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Hyaluronan the major component of the extracellular matrix is distributed ubiquitously in vertebrate tissues. Hyaluronan binds to cell via several receptors such CD44, this interaction induces many physiological events. The aim of our study was to investigate cytoprotective effects of low and high molecular weight hyaluronans (19,000 Da, 1,270,000 Da) against LPS, known as a P2X7/inflammasome activator.
Methods: We used macrophages derived from U937 cell line using Phorbol-myristate-acétate 16 nM for 48 h to study cell viability (Alamar blue test), oxidative stress (DCF-DA test), P2X7 cell death receptor activation (YO-PRO-1 test) and cytokines release (ELISA). Cells were preincubated with hyaluronan for 2 h then stimulated and incubated with LPS for 24 h.
Results and discussion: We observed that high molecular weight hyaluronan protected macrophages against LPS cytotoxicity (cell viability, oxidative stress) whereas low molecular weight hyaluronan increased LPS cytotoxicity. Low molecular weight hyaluronan (< 20,000 Da) was already known to be proinflammatory but we demonstrated here that high molecular weight hyaluronan has protective effects against LPS which is known to induce P2X7/inflammasome.
Disclosure of interest: None declared.

P-572
IL-6 MODULATES PANCREATIC INFLAMMATORY INFILTRATE BUT DOES NOT AFFECT OVERALL DISEASE SEVERITY IN OBESE MICE WITH ACUTE PANCREATITIS

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Obesity exacerbates severity in acute pancreatitis (AP) and is associated with elevated levels of IL-6 in both humans and experimental animals, including the murine model of AP induced by co-administration of IL-12 + IL-18. To study the role of IL-6 in AP exacerbation by obesity, WT and IL-6 KO C57BL6 male mice were fed either a high-fat diet (HFD) or chow diet (lean) for 13 weeks. IL-6 deficiency did not affect the degree of obesity or insulin resistance induced by HFD. Lean and HFD WT and IL-6 KO mice received two injections of IL-12 + IL-18 and were euthanized at 1, 3, 7 or 15 days. Acinar cell and fat necrosis were both significantly more severe and prolonged in HFD compared with lean groups, irrespective of genotype. The degree of inflammatory infiltrate was comparable in each group at day 1. However, a significant delay in the resolution of inflammation was observed in the pancreas of HFD WT mice compared with both HFD IL-6 KO and lean mice. Specifically, resolution of the neutrophil and lymphocyte infiltrate followed a comparable kinetics in HFD IL-6 KO and lean mice, whereas a significant delayed resolution was observed in HFD WT mice. In contrast, no effect of genotype was observed for resolution of macrophage infiltration, which was delayed in both HFD groups compared with lean mice. The pattern of chemokine production was in agreement with histologic data, with significantly higher levels of CXCL1 and CXL2 in HFD WT compared with HFD IL-6 KO mice, whereas levels of CCL1 were not significantly different between the two groups. Despite the important role of IL-6 in augmenting the inflammatory infiltrate, overall the exacerbated pathological response and associated lethality was not affected by IL-6 deficiency in HFD mice. Therefore, elevated production of IL-6 cannot by itself explain the increased AP severity of obese mice receiving IL-12 + IL-18, although IL-6 mediates the increased pancreatic inflammatory infiltrate of obese mice.

Disclosure of interest: None declared.

P-573
HYDROGEN PEROXIDE RESOLVES NEUTROPHILIC INFLAMMATION IN A MODEL OF ANTIGEN-INDUCED ARTHRITIS IN MICE

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Neutrophil accumulation contributes to the pathogenesis of rheumatoid arthritis. The aim of this study was to examine the ability of hydrogen peroxide (H_2O_2) to influence neutrophilic inflammation in a model of antigen-induced arthritis (AIA) in mice. AIA was induced by administration of antigen into the knee joint of previously immunized mice. Neutrophil accumulation was measured by counting neutrophils in the synovial cavity and assaying myeloperoxidase activity in tissues surrounding the knee joint. Apoptosis was determined by morphological and molecular techniques. The role of H_2O_2 was studied by using mice that do not produce reactive oxygen species (gp91$^{phox-/-}$ mice) and drugs which enhance H_2O_2 generation (superoxide dismutase) or its degradation (catalase). Antigen challenge of immunized mice induced neutrophil accumulation that peaked at 12–24 h after challenge. H_2O_2 production peaked at 24 h, concomitant with the resolution of the joint inflammatory process. Neutrophil recruitment was similar in WT and gp91$^{phox-/-}$ mice; however we observed a delayed resolution in gp91$^{phox-/-}$ mice or after administration of catalase. In contrast, administration of H_2O_2 or SOD accelerated the resolution of neutrophilic inflammation. Resolution of inflammation induced by SOD or H_2O_2 was accompanied by increase in number of apoptotic neutrophils. Apoptosis was caspase-3- and bax-activation-dependent and secondary to PI3K/AKT activation. Levels of H_2O_2 increase during neutrophil influx and are necessary for natural resolution of neutrophilic inflammation. Mechanistically, enhanced levels of H_2O_2 (endogenous or exogenous) decreased p-AKT and induced apoptosis of migrated neutrophils. Modulation of H_2O_2 production may represent a novel strategy to control neutrophilic inflammation in the joints.

Disclosure of interest: None declared.

P-574
EXPRESSION OF INFLAMMATORY MEDIATORS FOR TRANSFORMING GROWTH FACTOR-BETA INDUCED ENDOTHELIAL TO MESENCHYMAL TRANSITION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Introduction and aims: The tubule-interstitial fibrosis is a major event of chronic kidney disease and is also an important predictor of renal dysfunction. The fibrous tissue is caused by the accumulation of fibroblasts, myofibroblasts and extracellular matrix. Recently it has been demonstrated that fibroblasts can also be derived from endothelial cells by the process of endothelial to mesenchymal transition (EndMT). Transforming growth factor-beta protein (TGF-β1), a master cytokine acts as a stimulator of EndMT. Our aim was to demonstrate EndMT in vitro using human umbilical vein endothelial cells (HUVECs) stimulated with TGF-β1 and verify the expression of inflammatory mediators.

Methods: The HUVECS were stimulated TGF-β1 (10 ng/mL) at 72 h. The phenotypical changes of HUVECs were analyzed by confocal imaging, protein expression (western blot) and gene expression (real time PCR) of endothelial cells related molecules such as Von Willebrand factor (VWF) and VE-cadherin, and mesenchymal cells markers such as α-smooth muscle actin (α-SMA) and vimetin protein.

Results: TGF-β1 stimulated EndMT in HUVECs showed expression of α-SMA and vimentin protein, decrease in the expression of VE-cadherin, VWF and TGF-β1 protein by western blot. It was demonstrated that mesenchymal cells markers appear after 72 h when HUVECs are stimulated with TGF-β1 (10 ng/mL). It was also positive to antibodies specifics from mesenchymal cells, capture confocal imaging indicate that the cells were undergo to EndMT.

Conclusions: TGF-β1 was able to induce EndMT in HUVECs being a suitable model to study in vitro and these new findings suggest that targeting EndMT may be a novel therapeutic strategy, which is broadly applicable not only to chronic kidney disease but also to various other disease states.
Disclosure of interest: None declared.

P-575
CORTICOSTEROID ENHANCEMENT OF MACROPHAGE CLEARANCE OF APOPTOTIC CELLS VARIES WITH MACROPHAGE PHENOTYPE AND THE INFLAMMATORY MEDIATOR MILIEU

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Macrophage phagocytosis and clearance of apoptotic inflammatory cells is a key mechanism in resolution of inflammation. The interaction of macrophages with apoptotic cells has also been demonstrated to suppress pro-inflammatory cytokine release by macrophages. In disease states such as severe asthma and COPD resolution is defective, resulting in chronic inflammation. It has been demonstrated that macrophage phagocytosis is impaired in these diseases. Glucocorticosteroids are powerful anti-inflammatory drugs used in the treatment of these respiratory diseases and have been shown to augment phagocytosis of apoptotic cells by macrophages. Here, different phenotypes of human macrophage were characterised and the effects of corticosteroids on phagocytosis of apoptotic neutrophils examined. Human blood monocyte derived macrophages were cultured by differentiating with GM-CSF or M-CSF. Responses were contrasted with those of macrophages from human lung. Conditions were identified that mimic the phagocytic defects observed in chronic inflammation and the effect of corticosteroids examined. GM-CSF differentiated macrophages demonstrated poor phagocytosis which was greatly enhanced by corticosteroids. In contrast, M-CSF differentiated macrophages were found to be efficient at phagocytosis and corticosteroids showed little enhancement. Lung macrophages showed similar phagocytic levels to the M-CSF differentiated phenotype, with good basal phagocytosis and little enhancement with corticosteroids. Pre-treatment of all subsets of macrophages with TNFα or LPS/IFNγ was found to induce a phagocytic-defective state. Under these conditions, the enhancing effect of corticosteroid treatment was also impaired. The efficacy of corticosteroids in clearance of apoptotic cells by lung macrophages under inflammatory conditions was found to be suppressed, thus providing an opportunity for alternative therapeutic intervention in the resolution of respiratory inflammation.
Disclosure of interest: A. Gibson Employee of: GlaxoSmithKline, Stock ownership or royalties of: Participant in GlaxoSmithKline sharescheme, E. Gower Employee of: GlaxoSmithKline, Stock ownership or royalties of: Participant in GlaxoSmithKline sharescheme, M. Foster Employee of: GlaxoSmithKline, Stock ownership or royalties of: Participant in GlaxoSmithKline sharescheme, S. Farrow Employee of: GlaxoSmithKline, Stock ownership or royalties of: Participant in GlaxoSmithKline sharescheme.

P-576
EARLY INFLAMMATORY EVENTS DURING EAR TISSUE REGENERATION IN MICE

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Introduction: The biological response to skin injury can be subdivided into two distinct categories, regenerative and non-regenerative types of wound healing. Homozygous AIRmax and AIRmin sublines for *Slc11a1 S* alleles, produced by genotype-assisted breeding, differ in ability to completely repair ear holes. AIRmax^{SS} mice showed fast ear tissue regeneration while AIRmin^{SS} mice did not show regeneration after ear punch.
Objectives: Our aim in this work was to evaluate the influence of the early inflammatory response in the determination of those distinct phenotypes.
Methods: Two-millimeter ear holes were punches in mice of each subline and the inflammatory reaction was characterized by histomorphometric analysis and MPO activity. Global gene expression analysis was used to identify sets of differentially expressed genes during the inflammatory stage of regeneration and quantitative PCR experiments were performed to validate microarray results.
Results: The local inflammatory response was slightly more intense in AIRmin^{SS} than AIRmax^{SS} mice 24 and 48 h after ear punch, which was demonstrated by histomorphometric analysis and MPO levels. Global gene expression analysis demonstrated over-represented distinct biological themes between AIRmax^{SS} and AIRmin^{SS} control mice. Inflammatory response biological theme was observed only in AIRmin^{SS}. At 24 h after punch, both AIRmax^{SS} and AIRmin^{SS} mice showed significant ($P < 0.001$) up-regulated genes related to inflammation. However, angiogenesis, regulation of myeloid cell differentiation and epidermal growth factor receptor signaling pathway related genes were expressed only in AIRmax^{SS}. Microarray results were validated by quantitative PCR.
Conclusions: These results suggest that distinct group of genes related to inflammatory response in the early events after injury modulates the quality of regeneration or wound healing.
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Disclosure of interest: None declared.

P-577
MESENCHYMAL STEM CELL ROLE IN MURINE FOLIC ACID-INDUCED ACUTE KIDNEY INJURY MODEL

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Introduction: Upon the lack of more efficient therapies, great interest has been shown in the therapeutic use of mesenchymal stem cells (MSC) towards Acute Kidney Injury (AKI) treatment. In this work, we aimed to study the effect of mesenchymal stem cell (MSC) administration in mice with nephrotoxic AKI induced by high doses of folic acid (FA).
Methods: MSC were obtained from bone marrow of male C57Bl/6 mice (6 weeks) and cell population was expanded in Mesencult cell media® (Stem Cell Technologies). Before administration of these cells, MSC were characterized by immunophenotyping assays for CD34, CD45, CD73 and CD105, and also by differentiation assays for adipocyte and osteocyte. The experimental model was achieved by administration of FA (200 mg/kg body mass) and animals were sacrificed after 48 h. After 24 h of FA administration, 5.105. MSC were administered for each animal intraperitoneally. Serum and kidney tissue samples were collected 48 h after FA treatment for kidney function evaluation, morphologic study and PCR analysis (TNF-a, IL-1b and IL-6).
Results: MSC-treated animals demonstrated reduced serum creatinine (MSC treated animals 0.96 ± 0.38 mg/dL vs. untreated animals 1.29 ± 0.35 mg/dL, $p < 0.05$) and urea levels (MSC treated animals 76.67 ± 27.14 mg/dL vs. untreated animals 119.53 ± 30.84 mg/dL, $p < 0.05$). In addition, pathologist evaluated kidney tissue necrosis is reduced in MSC treated animals. This functional amelioration was accompanied by reduction of IL-6 (MSC-treated animals 2.45 ± 1.88 vs. untreated animals 27.30 ± 10.24, $p < 0.05$), IL-1b (MSC treated animals 0.64 ± 0.50 vs. untreated animals 2.62 ± 1.14, $p < 0.05$) and TNF-a (MSC treated animals 1.75 ± 0.27 vs. untreated animals 8.45 ± 0.25, $p < 0.05$) mRNA expression at kidney tissue in MSC-treated animals when compared to untreated animals.
Conclusions: This study demonstrated that MSC have immunomodulatory and morphological protective potential that correlate with functional improvement in AKI achieved by AF.
Disclosure of interest: None declared.

P-578
RESOLUTION PHASE MACROPHAGES AND THEIR CONTROL OF MYELOID DERIVED SUPPRESSOR CELLS IN ACUTE INFLAMMATION

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Introduction: Previous work by our group has investigated the mechanisms of resolution of innate inflammation. Recently, we identified a unique phenotype of macrophage during resolution. Termed resolution phase macrophage (rM), they possess a phenotype that is a hybrid of both classically activated M1 and alternatively activated M2 macrophages, expressing both iNOS and COX-2. This unique phenotype is controlled by cAMP and mediates the repopulation of protective lymphocytes (rLØ) post inflammation and the restoration of tissue homeostasis. Having identified the unexpected expression of iNOS in rM, we sought to further investigate its role during resolution using iNOS knockout (iNOS−/−) mice.
Methods: Wild type mice (WT) and iNOS−/− were injected intraperitoneally with 0.1 mg zymosan. At various time points throughout inflammation cells were analysed and sorted by FACS, as well as protein expression of iNOS, and cytospins of sorted samples. Cell free inflammatory exudates were analysed for NOx, cytokines and lipids.
Results: In iNOS−/− mice we identified a population of cells, which occupied a discreet region on size versus granularity plot. These were identified as ring cells by their characteristic nuclear morphology following sorting and rapid Romanowsky staining of cytospins. We have further characterised the phenotype and potential function of ring cells during resolution. FACS analysis showed that ring cells can be subdivided into ring neutrophils (RPMN) and ring monocytes (RMon). RPMNs are F480low/Gr1low, whilst RMons are F480hi/Gr1low. These cells have a morphological phenotype similar to myeloid suppressor cells. There is a higher occurrence of ring cells in iNOS−/− compared to WT. This coincides with a decrease of 12-Lipoxygenase (12-LOX) protein expression, as well as 12-LOX derived lipids in iNOS−/− inflammatory exudates.
Conclusions: We propose that these 12-LOX derived lipid mediators are produced by rM, at least in part under the control of iNOS, may have a role to play in the appearance of ring cells.
Disclosure of interest: None declared.

P-580
RESOLVING-PHASE MACROPHAGES: A DISTINCT SUBSET IN MICE

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Introduction: Depending on the activating stimuli, macrophages (Mφ) can develop into different subsets, broadly referred as either M1 or M2. So far, however, most of the characterisation of subsets has been carried out using mφ in vitro. As the environment in which mφ develop in vivo is complex, it is likely that mφ phenotype is tissue-, stimuli- and inflammatory phase-specific. Therefore it was the aim of this work to catalogue the inflammatory status of mφ isolated from the resolution-phase of a murine zymosan-induced peritonitis model and determine their post-inflammation tissue homeostatic role.
Methods: Resolution-phase Mφ (rMφ) were isolated from the peritoneum 72 h after intra-peritoneal injection of a very mild dose of zymosan (0.1 mg). Its transcriptome was analysed using Affymetrix microarray technology and genes that were found to be exclusively upregulated in the rMφ compared to 'inflammatory' and/or naive resident peritoneal mφ were validated at the transcriptional level by quantitative RT-PCR.
Results: We have found that the transcriptome of rMφ has a distinct molecular signature to that of both 'inflammatory' and naïve resident mφ. Some of the genes exclusively upregulated have been previously shown to be important for resolution functions such as efferocytosis (Alox15, Tgfb2 and Timd4), and in initiating the adaptive response, i.e. antigen presentation (5 subunits of the class II, major histocompatibility complex) and chemoattraction, activation and proliferation of B- and T-lymphocytes (Cxcl13, Ccr2, Ccl5, Tnfsf4, Clec2i and Clcf1).
Conclusions: Transcriptome profiling not only reveals novel molecules and signatures associated with murine rMφ but has also helped to determine their role post-inflammation. We find that in addition to having a crucial resolution role, these Mφ function to develop the

adaptive immune response by initiating the repopulation of B and T cells into the cavity and by presenting antigen to these cells.
Disclosure of interest: None declared.

New targets for drug development

P-581
CARDAMONIN (2′,4′-DIHYDROXY-6′-METHOXYCHALCONE) ATTENUATED PMA-STIMULATED MMP ACTIVITIES AND INFLAMMATORY GENES EXPRESSION THROUGH BLOCKAGE OF NF-KB PATHWAY IN SYNOVIAL FIBROBLAST CELLS

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In anti-arthritic drug development, non-steroidal anti-inflammatory drug (NSAID), disease modifying anti-rheumatic drug (DMARD), steroidal agents, immunosuppressants have been widely used to control and suppress the syndrome of arthritis patient. However, these drugs are known to produce various adverse effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. On the natural end of the treatment spectrum, our group has shown that cardamonin (2′,4′-dihydroxy-6′-methoxychalcone) isolated from *Alpinia rafflesiana* exert promising anti-inflammatory activities in activated macrophage cells. Therefore, we further explored the anti-arthritic effects of cardamonin as well as investigated its underlying mechanism action on PMA-stimulated synovial fibroblast (SF) in vitro. Zymography results reveal that cardamoin treatment significantly inhibited the enzymatic activities of gelatinase B (MMP-9) in PMA-stimulated SF. In addition, cardamonin suppressed collagenase activities of PMA-stimulated SF which mediated by inhibition of MMP-1. Beside, cardamonin strongly suppressed *MMP-3* and *IL-6* gene expression as well as inhibited *COX-2* gene expression albeit in moderate level. We also demonstrated that the cardamonin abolished the p65 subunit of NF-κB translocation from cytosol to nucleus and DNA binding activity of NF-κB in dose-dependent manner. Collectively, these findings suggested that cardamonin exerts an anti-arthritic effect through immunomodulatory action on MMP production/activity and pro-inflammatory genes at least partly via NF-κB pathway in PMA-stimulated SF. This study provides a new evidence of cardamonin used as potent inhibitor of joint destruction in rheumatoid arthritis patient.
Keywords: Anti-arthritic, cardamonin, MMP, NF-kB, synovial fibroblast.
Disclosure of interest: None declared.

P-582
ANTI-INFLAMMATORY EFFECTS OF LUTEOLIN, AN ACTIVE PRINCIPLE OF VITEX NEGUNDO LINN

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Luteolin, the flavonoids that were isolated from the active fraction of *Vitex negundo* leaf. The purpose of this study is to clarify the role of luteolin as anti-inflammatory agent in *V. negundo*. Anti-inflammatory actions were assessed using 4 different in vitro bioassays techniques. Nitric oxide (NO) synthesis by inducible nitric oxide synthase (iNOS) is increased in inflammatory diseases and leads to cellular injury. Luteolin concentration-dependently (1.95–125 μg/ml) inhibited NO production upon stimulation by lipopolysaccharide/interferon-g (IFN-g) in RAW 264.7, a murine macrophage cell line. 5-LOX is the initial key enzyme for the production of leukotrienes (LTs) from arachidonic acid, inhibitors of LTs biosynthesis are potential candidates for further development into novel therapies for inflammation, in particular for asthma and allergic diseases. Luteolin also concentration-dependently (1.56–100 μg/ml) inhibited activity of soybean lipoxygenase (LOX) and the IC_{50} value is lower than standard control for LOX inhibitor Nordihydroguaiaretic acid (NDGA). It was also shown to contain anti-gout activity by reducing the activity of xanthine oxidase, the key enzyme that catalyses the oxidation of hypoxanthine to xanthine and then uric acid, which plays a crucial role in gout. This study also showed that luteolin are relatively strong platelet activating factor (PAF) receptor binding inhibitor. PAF is known to be a biologically active phospolipid, involved in many pathological conditions such as allergy, inflammation, asthma, cardiac anaphylaxis, gastrointestinal ulceration, endotoxin shock, transplanted organ rejection and thrombosis. In summary, we demonstrated that luteolin possesses anti-inflammatory properties in various mechanism of action. It is crucial to study one of mechanism in deep in order to further validate the actual mechanism of action.
Keywords: Luteolin, V*itex negundo*, nitric oxide, lipoxygenase, xanthine oxidase, platelet activating factor.
Disclosure of interest: None declared.

P-583
NANOPARTICLES OF LDE: A POTENTIAL DELIVERY ROUTE FOR MTX IN SINOVIAL TISSUE OF ANTIGEN-INDUCED ARTHRITIS

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Methotrexate (MTX) remains the first choice in the management of rheumatoid arthritis (RA). Resistance to this drug is largely associated to MTX entry into the cell. Lipidic nanoemulsion has emerged as a new strategy since it carries drugs directed to affected tissue. Our aim was to evaluate the anti-inflammatory efficacy of a cholesterol-rich nanoemulsion coupled MTX (MTX-LDE) in antigen induced arthritis (AIA). Toxicity, pharmacokinetics and biodistribution assays were performed. The dose–effect curve of the complex was also determined. Arthritis was induced in the knee joint of methylated serum albumin sensitized NZW rabbits. Twenty four hours after AIA induction animals were intravenously (I.V.) or intraarticularly (I.A.) injected with a single dose of commercial MTX (0.25 mg/kg), LDE or saline. To give an equivalent dose of MTX-LDE, the amount of drug was evaluated by HPLC. Animals were sacrificed 48 h after AIA induction when synovial fluid (SF) and membrane (SM) were collected for total and differential leukocyte count,

protein leakage and histopathological evaluation. Our data shows that MTX-LDE did not promote any hematological toxicity. The uptake of this complex was preferentially to liver, spleen and inflamed SM. MTX-LDE I.V. promoted a reduction of 65% of leukocyte infiltrate, 41% in protein leakage and a significant reduction in the SM cell infiltrate. Likewise, the treatment with MTX-LDE I.A. also reduced the inflammatory parameters. In contrast, a single dose of commercial MTX, a DMARDs that requires several weeks to have a clinical effect, did not alter any evaluated parameter. LDE alone or saline were also ineffective. Our study suggests that MTX-LDE is effective, well tolerated and exhibit a rapid effect, probably as a result of their good uptake thus representing a novel therapeutic strategy to treat RA patients.
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Disclosure of interest: None declared.

P-584
VALIDATION OF A SWINE SYSTEMIC MODEL OF INFLAMMATION AND THE IMPACT OF A NON-STEROIDAL ANTI-INFLAMMATORY DRUG ON THE EXPRESSION OF MRNA BIOMARKERS

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Non-steroidal anti-inflammatory drugs (NSAID) function to reduce pain, fever, and inflammation, and are commonly used in people and animals for this purpose. Currently there are no NSAIDs approved for the control of inflammation in swine due to a lack of validated animal models and suitable biomarkers to assess efficacy. A swine model based on inflammation biomarkers could prove very useful in the drug approval process. The study was designed to accomplish the following objectives: to verify expression patterns of in vitro candidate markers in an in vivo model and to determine the utility of the biomarkers through simultaneous administration of LPS and flunixin meglumine in swine. A previous in vitro study investigated the differential expression of genes altered in response to endotoxin-induced inflammation. Based on that study five of the genes previously identified were chosen for in vivo verification. Two groups of healthy swine were tested: group one was stimulated with only LPS while group two received both flunixin meglumine and LPS. Five genes (MCP-1, Serum Amyloid A2 (SAA2), CD4, CD1, and Caspase 1) were investigated in this system. After LPS stimulation, blood was retrieved at four time points (0, 3, 24, and 48 h). Total RNA was extracted from the blood and analyzed via quantitative RT-PCR (qRT-PCR). Following the analysis, two genes (SAA2 and CD1) were found to have statistically significant (p-value <0.05) temporal differences in gene expression. In addition to these findings, the animals that received both flunixin meglumine and LPS demonstrated reduced mRNA expression relative to the group stimulated with LPS alone. These results demonstrate that two out of five genes (SAA2 and CD1) may serve as biomarkers of inflammation as well as indicators of NSAID efficacy. As swine are also used for human drug development, the results of this study represent the development of an alternative model for NSAID drug development.
Disclosure of interest: None declared.

P-585
AN6414, A NOVEL BENZOXABOROLE PDE4 INHIBITOR, DEMONSTRATES OPTIMAL EFFICACY AND SAFETY FOR DEVELOPMENT AS A THERAPEUTIC FOR ARTHRITIS

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AN6414(5-Chloro-6-(1-hydroxy-1,3 dihydrobenzo[c][1,2]oxaborol-5-yloxy)-2-(2-isopropoxyethoxy) nicotinonitrile)is a novel benzoxaborole under investigation for the treatment of inflammatory diseases. AN6414 inhibits PDE4 with an IC_{50} of 5.2 nM, and inhibits TNFα secretion from human leukocytes with an IC_{50} of 2.5 nM. AN6414 binds to the active site of PDE4 in a different mode from catechol PDE4 inhibitors making contact with the activated water in the bimetal center. In an in vivo mouse model measuring inhibition of LPS-induced TNFa levels in mouse plasma, AN6414 demonstrates an ED_{50} of 0.15 mg/kg, Roflumilast exhibits an ED_{50} of 7.5 mg/kg. In a mouse collagen induced arthritis (CIA) model AN6414 reduces disease burden by 90% when dosed orally BID for 21 days at 10 mg/kg. At the same dose and duration reduction by Roflumilast is 80%. Once a day dosing of AN6414 also demonstrates activity in the mouse CIA model, with doses as low as 5 mg/kg showing significant arthritis suppressive activity. Histologic examination of the feet and ankles of AN6414 and Roflumilast treated animals show a strong suppression of inflammation, bone erosion, cartilage erosion and extra pannus formation. In a mouse 5-day toxicity study, AN6414 demonstrates lesser GI toxicity compared to Roflumilast, (NOAEL of 20 mg/kg/day compared to 10 mg/kg/day). In the monkey AN6414 is less emetic than Roflumilast, since it requires a higher plasma concentration of AN6414 to cause emesis. The in vivo pharmacological effect in both a 4 h monkey LPS-induced TNFa secretion test and a 21 day mouse CIA joint damage test can be well explained for both AN6414 and Roflumilast by free plasma drug level and enzyme affinity. AN6414 shows good in vivo efficacy, safety, and pharmacokinetics, and thus provides a new candidate therapeutic for autoimmune diseases.
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P-586
S-PROPARGYL-CYSTEINE PROTECTS MICE AGAINST CAERULEIN-INDUCED ACUTE PANCREATITIS

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Hydrogen sulfide (H_2S) is synthesized endogenously from L-cysteine by two pyridoxal-5′-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). S-propargyl-cysteine (SPRC), an H_2S releasing drug, may modulate the endogenous H_2S production. The present study was aimed to investigate the effects of SPRC in an in vivo model of acute pancreatitis (AP) in mice. AP was induced in mice by hourly caerulein injections (50 μg/kg) for 10 h. Mice were treated with SPRC (10 mg/kg) or vehicle (distilled water). SPRC was administered 3 h before the induction of pancreatitis. Mice were sacrificed 1 h after the last caerulein injection. Blood, pancreas and lung tissues were collected and processed to measure the plasma amylase, plasma H_2S, myeloperoxidase (MPO) activities and cytokine levels in pancreas and lung. The results revealed that significant reduction of inflammation, both in pancreas and lung was associated with SPRC treatment. Furthermore, the anti-inflammatory effects of SPRC were associated with reduction of pancreatic and pulmonary pro-inflammatory cytokines and increase of anti-inflammatory cytokine. Plasma H_2S concentration showed significant difference in H_2S levels between control, vehicle and SPRC treatment groups. In conclusion, these data provide evidence for anti-inflammatory effects of SPRC by modulating endogenous H_2S production.

Disclosure of interest: None declared.

P-587
DISCOVERY OF CC-930, AN ORALLY ACTIVE ANTI-FIBROTIC JNK INHIBITOR

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Idiopathic pulmonary fibrosis (IPF) is a fatal disease manifested by a gradual loss of lung function. Jun N-terminal kinase (JNK) has been implicated in multiple processes that promote fibrosis pathology including the production of cytokines such as TNF-alpha, TGF-beta, and CTGF, myofibroblast transformation, and matrix deposition. In an accompanying abstract, we describe the identification of potent aminopurine-based JNK inhibitors which showed efficacy in animal models of ischemia reperfusion injury following i.v. administration. In this paper, we present the chemical optimization of CC-930, a potent, selective, and orally available aminopurine JNK inhibitor, and its pharmacological profile in animal models of inflammation and fibrosis.

Disclosure of interest: None declared.

P-588
THE EFFECT OF THE ALARMIN HMGB1 ON POLYMORPHONUCLEAR NEUTROPHIL MIGRATION DEPENDS ON ITS CONCENTRATION

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Polymorphonuclear neutrophils (PMN) play a key role in host defenses against invading microorganisms but also potentiate inflammatory reactions in case of excessive or misdirected responses. Release of the alarmin HMGB1 by cells that die at an inflammatory site may act as an alert signal for the immune system. We studied the effect of HMGB1 on human PMN migration, using whole-blood samples to avoid cell activation associated with isolation procedures. HMGB1 50–100 ng/ml reduced baseline PMN migration as well as fMLP- and IL-8-induced PMN chemotaxis. This inhibitory effect was mediated by the RAGE receptor and was reversed by preincubation with ERK1/2 inhibitor. It was also associated with a decrease in PMN phospho-p38MAPK content and a concomitant increase in phospho-ERK1/2. In contrast, a higher HMGB1 concentration (5,000 ng/ml) had a chemoattractant effect on PMN, through IL-8 production. This effect required the engagement of TLR2 and TLR4 in addition to the RAGE receptor. Finally, the A Box component of HMGB1, which antagonizes the endogenous protein, reduced chemotaxis and also strongly inhibited the enhancement of PMN migration observed with the highest HMGB1 concentration. These results strongly point to a key regulatory role of HMGB1 in PMN recruitment to inflammatory tissues. The A Box component could potentially serve to inhibit inappropriate PMN recruitment during chronic inflammatory disorders associated with excessive HMGB1 release.

Disclosure of interest: None declared.

P-589
A-MSH AND DTRP8-G-MSH INHIBIT PRO-INFLAMMATORY CYTOKINE RELEASE FROM STIMULATED PRIMARY BOVINE CHONDROCYTES

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Melanocortin peptides exert anti-inflammatory effects via activation of melanocortin receptors (MC) of which five have been identified-MC_1 and $_3$ bring about the anti-inflammatory effect of melanocortins (Getting et al. 2006). Here, the effect of melanocortins was determined by utilizing stimulated primary bovine articular chondrocytes. Western blotting was used to identify expression of MC on chondrocytes. Chondrocytes were treated with TNFα (0–80 pg/ml). In separate experiments cells were treated with PBS, αMSH or selective MC_3 agonist DTRP^8-γ-MSH (0–10 mg/ml), or in combination with the $MC_{3/4}$ antagonist SHU9119 for 30 min prior to 6 h stimulation with TNFα (60 pg/ml). Cell-free supernatants were collected and analysed by cytokine ELISAs. Data is presented as mean ± SEM of $n = 4$ determinations vs. control. Western blot showed expression of $MC_{1,3,5}$ on chondrocytes; TNFα caused concentration-dependent increase in IL1β, IL6, IL8, whereas IL10 levels were not altered. Maximal release of cytokines was caused by 60 pg/ml TNFα with 27- and 2-fold increase in IL1β and IL8, respectively ($p < 0.05$) compared to controls, whilst maximal 48-fold increase of IL6 occurred at 80 pg/ml. αMSH and DTRP^8-γ-MSH suppressed TNFα-stimulated production of proinflammatory cytokines causing significant increase in IL10 secretion. αMSH (1 μg/ml) caused 42% decrease in IL1β and DTRP^8-γ-MSH (10 μg/ml) caused 76%, $p < 0.05$. Similar reductions in IL6 and IL8 were observed following peptide treatment. SHU9119 failed to inhibit αMSH, but attenuated the effect of DTRP^8-γ-MSH. TNFα upregulates pro-inflammatory cytokine release in bovine articular chondrocytes and this was abrogated by αMSH and DTRP^8-γ-MSH, whilst treatment of cells with these peptides led to

increase in anti-inflammatory cytokine IL10. The $MC_{3/4}$ antagonist SHU9119 blocked the effects of $DTRP^{8-}\gamma$-MSH but had no effect on αMSH activity. These data suggest that both $MC_{1/3}$ mediate the anti-inflammatory effect of melanocortins in bovine chondrocytes.
Disclosure of interest: None declared.

P-590
MIMETIC PEPTIDES OF THE SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)1 KINASE INHIBITORY REGION (KIR) INHIBITS IFN-GAMMA-DEPENDENT IMMUNE FUNCTIONS OF EPIDERMAL KERATINOCYTES IN VITRO AND IN A MOUSE MODEL OF SKIN INFLAMMATION

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Suppressor of cytokine signalling (SOCS)1 negatively regulates the molecular cascades triggered by IFN-γ by disabling Jak2 and STAT1 phosphorylation and, thus, impeding the activation of IFN-γ-dependent genes. In human keratinocytes, the inhibitory activity of SOCS1 inhibits the IFN-γ signalling by binding Jak2 through its kinase inhibitory region (KIR) and, thus, inhibiting the IFN-γ receptor (IFN-γR) and STAT1 phosphorylation. As consequence of STAT1 lack, keratinocytes over-expressing SOCS1 cannot longer express ICAM1 and MHC class II as well as CXCL10, CXCL9, and CCL2 molecules. Aimed to potentiate the inhibitory function of SOCS1 in IFN-γ-activated keratinocytes, we developed a sets of peptides mimicking the SOCS1 KIR domain. The effects of two cell-permeable mimetic peptides, termed TAT-KIR and TAT-P5, were evaluated on cultured keratinocytes and in a mouse model of allergic contact dermatitis. We found that IFN-γ-activated keratinocytes treated with TAT-KIR and, to a lesser extent with TAT-P5, exhibited a reduced Jak2, IFN-γRα and STAT1 phosphorylation, compared to cells treated with a control peptide. The expression of IRF-1, a late IFN-γ-inducible transcription factor, and of STAT1-dependent genes, such as ICAM-1, HLA-DR, CXCL10 and CCL2, were quite totally abrogated. Also, the reduced expression of ICAM-1 determined by treatment with peptides, resulted in an impaired adhesiveness of keratinocytes to autologous T lymphocytes. Consistently, the migratory responses of T helper (Th)1 lymphocytes towards supernatants from keratinocytes treated with peptides were drastically reduced due to their impaired release of the CXCL10 and CCL2. Finally, we found that the topical administration of peptides on mouse ears during skin challenge with the hapten TNCB led to a significant reduction of the contact hypersensitivity responses. Our data suggest that TAT-KIR and TAT-P5 could be effective for the treatment of Th1-dependent inflammatory skin diseases in humans.
Disclosure of interest: None declared.

P-591
FUNCTIONAL ANALYSIS OF MICRORNAS UPREGULATED IN A CELL CULTURE MODEL OF SEPSIS

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Objectives: MicroRNAs (miRNAs) are under intensive research in the last years. Nevertheless we are still at a very early stage in understanding regulation mechanisms and functions of miRNAs in sepsis.
Methods: In a first step a conditioned medium was produced by the stimulation of THP-1 monocytes with 10 ng/ml LSP [4 h]. This conditioned medium was used in a second step for the stimulation of HUVECs or HPMEC [16 h] and changes in miRNA expression were analyzed by miRNA array analysis. MiR-146a, miR-146b, and miR-155 were selected for further analysis due to their different expression pattern after stimulation. Altered expression of proinflammatory cytokines were investigated after transfection of HUVECs with inhibitory miRNAs.
Results: The expression of up to 1,900 microRNAs was investigated after stimulation of HUVECs and HPMECs by a conditioned medium. MiRNA array analysis identified among others a twofold upregulation of miR-146a, miR-146b, and miR-155. MiRNA inhibitors for the selected miRNAs were used in order to investigate the functional properties of miR-146a, miR-146b, and miR-155. Inhibition of miR-146a resulted in a 68% diminished expression of Interleukin (IL)6 and a 64% reduced expression of IL8. Further, expression of IL6 and IL8 was reduced by inhibition of miRNA-146b at 49 and 26%, respectively. Additionally, inhibition of miR-155 resulted in a reduced expression of IL6 of 31% after stimulation of HUVEC miR-155 inhibited cells.
Conclusions: In conclusion, miR-146a, miR-146b, and miR-155 as well, seem to be highly involved in the regulation of inflammatory cytokines, especially IL6 and IL8.
Disclosure of interest: None declared.

P-592
PRECLINICAL PROOF OF CONCEPT OF THERAPEUTIC BLOCKING ANTIBODIES TARGETING TLR3

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Toll like receptors (TLRs) have emerged as key players in the detection of pathogens and the induction of anti-microbial and anti-viral immune response, shaping both innate and adaptive immune response. To date, 10 TLRs have been identified in humans. Toll-like receptor (TLR) agonists have attracted a lot of attention from the pharmaceutical industry. Two TLR agonist are approved either for treatment of cancer or as vaccine adjuvant, while numerous clinical trials are currently evaluating the efficacy of TLR agonists in vaccine, cancer and viral infection, as well as in allergy/asthma. An emerging field in TLR targeting drug development is the use of TLR antagonists for use in inflammation, including nucleic acid, phospholipids but few monoclonal antibodies. Indeed, there is accumulating evidences that while TLR activate the immune systems to fight infections or cancer, TLR may also respond to endogenous ligands and maintain unwanted inflammation, leading to tissue damage and inflammatory pathologies. TLR3, a receptor for dsRNA, is widely expressed by immune cells but also epithelial, endothelial and fibroblast, and TLR3 stimulation induces IFN type I and inflammatory cytokines. TLR3 over-expression has been demonstrated in several inflammatory pathologies, and TLR3 ligands can mimic inflammatory diseases in vivo in mice. In the present study we generated and selected antibodies targeting

specifically the human TLR3 receptor and demonstrating antagonist activity in vitro. We further characterized their specificity and bioactivity in vitro. Using a surrogate anti-mouse TLR3 antagonist antibody, we established proof of concept of inhibiting TLR3 in vivo, in mice models of inflammatory pathologies.
Disclosure of interest: C. Paturel Employee of: Innate Pharma, I. Perrot Employee of: Innate Pharma, C. Bonnafous Employee of: Innate Pharma.

P-593
NEW EVIDENCE FOR THE ROLE OF MCP-1/CCL2 IN COPD. SPIEGELMER® MNOX-E36 IS EFFECTIVE IN A SUBCHRONIC SMOKE MOUSE MODEL

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Chronic obstructive pulmonary disease (COPD) is characterized by incompletely reversible airway obstruction associated with inflammation in which macrophages are the predominant inflammatory cells. Monocyte chemoattractant protein-1 (MCP-1, CCL2) is the ligand of the chemokine receptor 2 (CCR2) and a mediator of inflammation in COPD. In order to assess the therapeutic potential of MCP-1 inhibition in murine models we generated a polyethylene glycol conjugated, mirror image oligonucleotide (Spiegelmer®), mNOX-E36, with high specific binding affinity to mouse MCP-1. The mNOX-E36 was characterized in the LPS induced neutrophilia rat model and the sub chronic (11 days) tobacco smoke induced inflammation mouse model. Anti-inflammatory effects were determined based on differential cell counts of the broncheo-alveolar lavage fluid (BALF).

In the acute rat model intravenous administration of mNOX-E36 prior to LPS challenge significantly reduced the number of infiltrating neutrophils in the BALF at a dose of 2 mg/kg body weight (bw). In the sub-chronic tobacco smoke mouse model mNOX-E36 (0.2 mg/kg bw), given subcutaneously every other day, starting just prior to first smoke exposure, resulted in a significant decrease of macrophages (41%), lymphocytes (52%), eosinophils (86%), and neutrophils (57%) in the BALF compared to vehicle treated control animals. The effects were comparable to those obtained in the positive control arm with the reference compound roflumilast. These preliminary results demonstrate potent effects of MCP-1 inhibition in an animal model of COPD. Spiegelmer mNOX-E36 administration significantly prevented the recruitment of different inflammatory cells, involved in both the innate and the adaptive immune response, into the broncheo-alveolar space. Preventing the recruitment of these inflammatory cells with the human MCP-1 specific NOX-E36 into the lung might translate into a benefit for patients with COPD.
Disclosure of interest: None declared.

P-594
EFFECTS OF THE HEPCIDIN BINDING SPIEGELMER NOX-H94 IN A MODEL OF IL-6 INDUCED ANEMIA

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Hepcidin is a central regulator of systemic iron homeostasis. Increased expression results in functional iron deficiency and is a potential reason for the genesis of anemia of inflammation. With the objective to neutralise the biological activity of hepcidin, the Spiegelmer NOX-H94 was developed. This compound has a high affinity and specificity for human and cynomolgus hepcidin. The aim of this study was to further characterize NOX-H94 in in vitro and in vivo models. In vitro the expression of the iron exporter and hepcidin receptor ferroportin was assessed in the cell line J774A.1 after stimulation with hepcidin. Western blot analysis was used to analyse ferroportin expression. After binding to its receptor the ferroportin–hepcidin complex is degraded and intracellular iron cannot be exported. Acute models of serum iron reduction induced by human hepcidin (mouse) and interleukin-6 (cynomolgus monkey) were established to characterize the efficacy of NOX-H94 in in vivo models. NOX-H94 inhibited hepcidin-induced ferroportin degradation in J774A.1 cells and also a decrease of the serum iron concentration in two in vivo models. These results demonstrate the efficacy of NOX-H94 in in vitro and in acute in vivo models. This ferroportin protecting and iron liberating strategy may supply sufficient iron for erythropoiesis and avoid treatment with intravenous iron preparations. The potential harmful increase of intracellular iron is prevented. Based on these preliminary data, NOX-H94 may be used in patients to influence one reason for the genesis of anemia of inflammation.
Disclosure of interest: None declared.

P-595
ENDOTHELIN RECEPTOR ANTAGONIST BOSENTAN AMELIORATES COLLAGEN-INDUCED ARTHRITIS IN MICE AND MODULATES GENE EXPRESSION IN LYMPH NODES

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Endothelins (ETs) are involved in inflammatory events, including pain, fever, edema, and cell migration. The present study investigated the involvement of ETs in arthritis using the most reasonable experimental model of this disease, collagen-induced arthritis (CIA). CIA was induced in DBA/1 mice, and on the day that CIA became clinically detectable, the mice were treated with a dual ETA/ETB receptor antagonist (Bosentan). Disease progression was assessed by measurements of visual clinic score, paw swelling and hypernociception. Gene expression in lymph nodes was evaluated by microarray technology. Validation of microarray genes and ET1-prepro expression in sham and immunized mice and in control and AR human patients treated with Methotrexate or anti-TNF, were evaluated by real time PCR. We observed that the oral administration of Bosentan markedly ameliorated CIA. Gene expression in the lymph nodes of ill mice showed a characteristic profile during Bosentan administration that allowed us to distinguish the treatment from disease state. Prepro-ETs mRNA expression was increased in the lymph nodes of arthritic mice compared with that of sham mice. From a clinical perspective, prepro-ETs mRNA expression was also increased in the PBMCs of RA patients during

conventional treatment when compared with anti-TNF therapy and healthy individuals. The findings suggest a critical role of ETs in CIA and RA, through the modulation of important genes. This study offers new advances concerning the role of endothelin in arthritis mechanisms and further supports the potential of endothelin receptor antagonists in controlling inflammation.
Disclosure of interest: None declared.

P-596
ANTI-INFLAMMATORY EFFECTS OF CONIFERALDEHYDE VIA THE SUPPRESSION OF NF-KB PATHWAY IN RAW 264.7 MACROPHAGES

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It has been reported that coniferaldehyde isolated from *Fraxinus rhynchophylla* exhibits inducible nitric oxide synthesis inhibitory activity. But, it is not clear how coniferaldehyde down-regulate inducible nitric oxide synthesis. In the present study, we investigated the anti-inflammatory effect and the underlying molecular mechanisms of coniferaldehyde in lipopolysaccharide (LPS)-induced RAW 264.7 cells. Coniferaldehyde dose-dependently reduced the productions of nitric oxide (NO) and prostaglandin E_2 (PGE_2) induced by LPS. Consistent with these findings, coniferaldehyde significantly suppressed the LPS induced expressions of inducible nitric oxide synthase (iNOS) and microsomal prostaglandin E_2 synthase (mPGES-1). In addition, coniferaldehyde attenuated LPS-induced DNA binding and the transcriptional activities of nuclear factor-kappa B (NF-κB), which was accompanied by a parallel reduction of degradation and phosphorylation of inhibitory kappa B-α (IκB-α) and consequently by decreased nuclear translocation of p65 subunit of NF-κB. Taken together, these results suggest that the anti-inflammatory effect of coniferaldehyde in LPS treated RAW 264.7 macrophages is associated with the suppression of NF-κB transcriptional activity.
Disclosure of interest: None declared.

P-597
ANTI-INFLAMMATORY EFFECTS OF FUCOSTEROL VIA THE SUPPRESSION OF NF-KB PATHWAY AND P38 PATHWAY IN LPS-INDUCED RAW264.7 MACROPHAGE CELLS

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It has been already reported that fucosterol, found in several brown algae, have anti-diabetic and anti-oxidant effects. However, the anti-inflammatory effects of fucosterol are not widely known. In this study, we investigated the anti-inflammatory effects of fucosterol in lipopolysaccharide (LPS)-stimulated murine macrophage RAW 264.7 cells. Fucosterol was found to inhibit LPS-induced nitric oxide (NO), prostaglandin E_2, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) production in a dose-dependent manner. In addition, fucosterol suppressed the LPS-induced expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) at the mRNA and protein levels. Moreover, fucosterol attenuated LPS-stimulated DNA binding activity and the transcription activity of nuclear factor-kappa B (NF-kappa B) and decreased phosphorylation of inhibitor kappa B (Ikappa B)-alpha which is regulating NF-kappa B. Furthermore, fucosterol attenuated the phosphorylations of p38 mitogen-activated protein kinase (p38) in LPS-stimulated RAW 264.7 cells. Taken together, these results suggest that the anti-inflammatory effect of fucosterol in LPS-treated RAW 264.7 cells is associated with the suppression of NF-kappa B transcriptional activity via the inhibitory regulation of Ikappa B and p38 phosphorylation.
Disclosure of interest: None declared.

P-598
INHIBITION OF NEUROTOXIC SECRETORY PHOSPHOLIPASE A2 ENZYMATIC, INFLAMMATORY AND MYOTOXIC ACTIVITIES BY HARPALYCIN 2, AN ISOFLAVONE ISOLATED FROM HARPALYCE BRASILIANA BENTH

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The secretory phospholipase A_2 ($sPLA_2$, EC 3.1.1.4) family of enzymes catalyze the hydrolysis of the *sn*-2 ester of glycerophospholipids to produce free fatty acids and lysophospholipids, and may exert pro-inflammatory actions through augmenting lipid mediator production, or through other mechanisms such as ligand-like effects. These enzymes were found elevated in many inflammatory disorders such as rheumatoid arthritis, sepsis and atherosclerosis. Recently reported studies using various plant species have shown that flavonoids induce a dose-dependent inhibition of in vitro phospholipid hydrolysis in both secretory and cytosolic PLA_2. Moreover, exogenous administration of $sPLA_2$, such as snake venom $sPLA_2$, can induce or exacerbate inflammatory response in animals. The aim of this study was evaluate the effect of harpalycin 2, an isoflavone isolated from *Harpalyce brasiliana* Benth., in the enzymatic, inflammatory and myotoxic activities of neurotoxic secretory phospholipases A_2 from *Bothrops pirajai*, *Crotalus durissus terrificus*, *Apis mellifera* and *Naja naja* venoms. Harpalycin 2 inhibits all $sPLA_2$ tested when the treatment was done before the substrate addiction, with percentages of inhibition about 58.7% for PrTX-III (Asp49 sPLAs from *B. pirajai* venom), 78.8% for Cdt F15 (Crotoxin B from *C. d. terrificus* venom),

87.7% for Apis (from bee venom, without mellitin) and 88.1% for Naja (from *N. naja* venom) secretory phospholipases A_2. Edema induced by exogenous $sPLA_2$ administration performed in mice paw by harpalycin 2 showed significant inhibition of the edema initial step induced by all sPLA2. In addition, harpalycin 2 also inhibited the myotoxic activity of these sPLA2. In order to understand how harpalycin 2 interacts with these enzymes, docking calculation were performed, showing strong interaction (hydrogen bond) with His48 and Asp49 residues in the active-site of these enzymes. These results pointed to a possible new anti-inflammatory drug.
Disclosure of interest: None declared.

P-599
3D CULTURES OF C-28/I2 CHONDROCYTES TO STUDY CHONDROCYTE BIOLOGY AND THERAPEUTIC DEVELOPMENT

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Availability of rapid and robust assays to determine chondrocyte reactivity can help in the identification of novel treatments and mechanisms for osteoarthritis. We tested here the C-28/I2 chondrocytic cell line developing its culture in micromasses (MM). MM using C-28/I2 cells were obtained by pipetting 20 μl droplets of a cell suspension (density of 2.5×10^7 cells/ml) into 48-well plates. Medium was changed after 24 h and stimuli [IL-1β (20 ng/ml), TGFβ1 (10 ng/ml) and/or Naproxen (0.1–10 μM)] added 24 h later for further 48 h culture. Human fibroblasts were used as negative control. MM were harvested for staining with Alcian blue (AB; 1% in HCl 0.1 N). Anabolic (COL2A1, SOX9, ACAN) and catabolic genes (MMP1, ADAMTS5) mRNA levels were analyzed by qRT-PCR. Data are from 3 to 4 independent experiments in triplicate. IL-1β promoted a catabolic response characterized by $\sim$40% induction in MMP1 and ADAMTS5 mRNA associated with reduction ($-$55%) in the production of matrix glycosaminoglycan (GAG) measured by AB staining. TGFβ1 (10 ng/mL) had an important anabolic effect on gene expression complemented by +47% in GAG production. As a proof of concept for therapeutic development, we tested Naproxen; this compound abolished the catabolic effect of IL-1β. Such pharmacological actions were paralleled by inhibition of PGE_2 release in the MM supernatant. We describe a novel medium-throughput protocol for studying inflammatory processes, candidate compounds and gene expression in chondrocytes. The system is also amenable to different levels of analysis, from simple and rapid quantitative measurement of matrix GAG, to more complex investigations including multiple gene expression analysis at the mRNA level. We thank Dr M Goldring (Cornell Medical College, New York) for the C-28/I2 cells.
Disclosure of interest: None declared.

P-600
HYPERFORIN, A CONSTITUENT OF ST. JOHN'S WORT, INDUCES APOPTOSIS BY TRIGGERING THE INTRINSIC CELL DEATH PATHWAY IN HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Hyperforin (HF) is a natural phloroglucinol isolated from St John's wort (*Hypericum perforatum*) with antibiotic and antidepressive activities. Acute myeloid leukemia (AML) is a deadly disease which results from the clonal expansion and accumulation of hematopoietic stem cells arrested at various stages of development. AML cells are characterized by abnormal proliferation and resistance to apoptotic signals, and Akt and NF-kB pathways are activated in AML. We studied here the potential antitumor efficacy of HF in AML cell lines and AML patients' cells ex vivo. HF inhibited the growth of AML cell lines U937, NB4, HL-60 and OCI-AML3 differing in phenotype and p53 status. The antiproliferative effects were associated with apoptosis induction as indicated by sub-G1 cell-cycle accumulation, phosphatidylserine externalization and DNA fragmentation. HF also triggered mitochondrial membrane depolarization, down-regulation of Bcl-2 anti-apoptotic protein that controls mitochondrial permeability, activation of caspases-3, -8, -9 and cleavage of the caspase substrate PARP-1. Pan-caspase inhibitor Z-VAD-fmk and caspase-3 inhibitor, but not caspase-8 inhibitor, prevented apoptosis indicating a mitochondrial caspase-dependent cell death (intrinsic pathway). HF-elicited apoptosis was also associated with Akt inactivation and activation of pro-apoptotic dephosphorylated Bad which is downstream target of Akt. In contrast, using a specific IKK inhibitor and a specific repressor of NF-kB activation, we showed that NF-kB signaling pathway did not interfere with the proapoptotic effect of HF**.** Furthermore, HF induced apoptosis of cultured AML patients' cells while normal peripheral blood mononuclear cells were not affected. These results indicate that HF may be of interest in the development of new therapies for AML based on the induction of apoptosis warranting further in vivo investigation.
Disclosure of interest: None declared.

P-601
EFFECT OF CROTOXIN ON SECRETORY ACTIVITY OF PERITONEAL MACROPHAGES CO-CULTIVATED WITH TUMOR CELLS. INVOLVEMENT OF FORMYL PEPTIDE RECEPTORS

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Crotoxin (CTX) inhibits tumor growth and modulates the function of macrophages. Despite these evidence, the contribution of macrophage inhibition to the decrease in tumor growth, caused by CTX, was not determined yet. Macrophages provide a defense mechanism against tumor cells and two distinct polarization states, M1 and M2, have been described for these cells. In the beginning of tumor progression, M1 macrophages release reactive nitrogen/oxygen intermediates and the cytokines TNF-α, IL-1β and IL-6. In contrast, during tumor development, the release of these mediators by tumor-associated macrophages (M2 cells) is inhibited, contributing to tumor development. In the present study the effect of CTX on the activity (nitric

oxide-NO) of macrophages co-cultivated with LLC WRC 256 tumor cells was evaluated. Macrophages were obtained from peritoneal cavity and cells (2×10^5) were incubated with CTX (0.3 μg/mL) for 2 h at 37°C. After this time, the macrophages were co-cultivated in presence of LLC WRC 256 tumor cells (2×10^4), previously plated in 96-well culture dishes. The results showed that macrophages previously incubated in the presence of CTX and co-cultivated with tumor cells generated a greater quantity of NO (35%) than control cells. Tumor cells co-cultivated with macrophages pre-incubated with CTX showed reduction (25%) of proliferation. Boc-2 reversed the stimulatory effect of CTX on secretory activity of macrophages and the inhibitory effect these macrophages on tumor cells proliferation. Taken together, the results indicate that CTX modifies the secretory activity of M2 cells, which may contribute to the inhibitory action of the toxin on tumor growth and activation of formyl peptide receptors seems to play a major role in this effect. These data reinforce the actions of CTX on defence mechanisms and bring new perspectives for the development of a new substance with therapeutic properties.

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P-602
POTENT, SELECTIVE, AND ORALLY ACTIVE CRTH2 ANTAGONISTS FOR ALLERGIC DISEASE

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Prostaglandin D_2 (PGD_2) is a potent prostanoid released from mast cells in response to allergens. The subsequent actions of PGD_2 are not controlled by existing therapies. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2, also known as DP2) is a G-protein coupled receptor specific for PGD_2 and related metabolites. Activation of CRTh2 mediates chemotaxis and activation of basophils, eosinophils and Th2 lymphocytes. It is also involved in the production of IL-4, IL-5 and IL-13 from this subset of lymphocytes. Selective antagonism of CRTh2 offers a therapeutic approach for the treatment of allergic disease and, accordingly, drug discovery efforts were initiated. A set of substituted phenyl acetic acids were prepared and evaluated for binding affinity to the CRTh2 receptor. Selectivity over related prostanoid receptors was also evaluated. In the event, compound **2x** (2-(4-(4-(4-chlorophenethylcarbamoyl)phenoxy)-3-cyanophenyl)acetic acid) was identified as a potent, selective, functionally active CRTh2 antagonist. Moreover, compound **2x** demonstrated good plasma exposure in rodents upon oral dosing and provided protection from inflammation in a murine model of delayed-type hypersensitivity.

Disclosure of interest: None declared.

P-603
MTOR-TARGETING DRUG, CUCURBITACIN D INHIBITS CELL GROWTH OF PROSTATE CANCER CELLS

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Prostate cancer is one of most prevalent cancers among men in western world. Despite several hundred clinical studies of both experimental and approved anti-tumor agent, chemotherapy has limited activity due to chemo-resistance and up-regulated anti-apoptotic mechanisms. Therefore, development of new drugs for prostate cancer is urgently needed. It was reported that PI3K/AKT signaling plays an important role in the maintenance and viability of prostate cancer progenitors, suggesting that inhibition of the PI3K/AKT and mTOR pathways in prostate cancer cells together with conventional chemotherapy may provide improved therapeutic outcomes. Cucurbitacins are a group of triterpenoids plant families such as cucurbitaceae and cruciferae, which have been shown to have anti-inflammatory activities and anti-cancer effects on various tumor types. Recently, we reported that cucurbitacin D, which is isolated from Trichosanthes kirilowii, has anti-tumor activity through suppression of proteasome activity. Here, we demonstrate that cucurbitacin D leads to inhibit cell proliferation of prostate cancer cells with inhibition of mTOR activation. Western blotting showed that cucurbitacin D induced suppression of p62/SQSTM1 protein level and mTOR phosphorylation which are known as markers for autophagy in PC3 cells, DU145 cells and LNCaP cells. However, cucurbitacin D did not induced typical autophagic event such as LC-3II fragment. In addition, apoptosis inhibitor, z-VAD did not affect cucurbitacin D-induced cell death. These indicate cucurbitacin D induced new type cell death but neither typical apoptotic nor autophagic cell death in prostate cancer cells. Taken together, our results provide a new insight of cell death induced by cucurbitacin D, which was targeted to mTOR. It emerged as a promising candidate for cancer therapy.

Disclosure of interest: None declared.

P-604
MAP KINASE PHOSPHATASE-1 REGULATES THE EXPRESSION OF INFLAMMATORY GENES TNF, IL-6, IL-8 AND COX-2

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Mitogen-activated protein kinases (MAPKs), namely ERK½, p38 MAPK and JNK, are important signaling pathways regulating several cellular functions, including inflammatory response. MAPK phosphatase-1 (MKP-1) dephosphorylates and thereby negatively regulates the activity of p38 MAPK and JNK. In the present study, the effect of MKP-1 on inflammatory gene expression in human A549 cells, mouse J774 macrophages and primary mouse bone marrow-derived macrophages (BMMs) from MKP-1 deficient and wild type mice were investigated. Stimulation with cytokines or LPS activated p38 MAPK and JNK in human and mouse cells and induced IL-6, TNF, IL-8 and COX-2 mRNA and protein expression. IL-6, IL-8 and COX-2 mRNA and protein expression were inhibited by p38 inhibitors SB202190 and BIRB 796 in a dose-dependent manner but not with a JNK inhibitor VIII. Down-regulation of MKP-1 with siRNA decreased MKP-1 expression and increased p38 phosphorylation in A549 cells and J774 macrophages. MKP-1 siRNA increased the expression of IL-6, IL-8, and COX-2 in A549 cells and J774 macrophages. Also, LPS-induced expression of IL-6, TNF and COX-2 was further enhanced in BMMs from MKP-1 deficient mice as compared to that seen in wild-type mice.

In conclusion, our result suggests that MKP-1 regulates the expression of inflammatory genes by limiting the activity of p38 MAPK. Compounds that increase MKP-1 expression or modulate its function are potential candidates as anti-inflammatory drugs.
Disclosure of interest: None declared.

P-605
KCA1.1 POTASSIUM CHANNELS REGULATE KEY PRO-INFLAMMATORY AND INVASIVE PROPERTIES OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory disease with unclear etiology that affects approximately 1% of the world population. It is a systemic disease but mainly targets freely moveable joints, such as the wrists or the knees, and can cause severe disability. Resident synovial cells, such as fibroblast-like synoviocytes (FLS), acquire an altered phenotype during RA and present characteristic of transformed cells. They play an important role in the disease by directly destroying the cartilage, but also by inducing angiogenesis, inflammation, bone damage, and by transferring disease from affected to healthy joints. We have found that FLS from patients with RA (RA-FLS) express large numbers of functional KCa1.1 (BK, Maxi-K, Slo1) channels at their plasma membrane. Blocking KCa1.1 channels inhibited the proliferation, the motility, and the invasive properties of RA-FLS, and may therefore prevent disease progression. Blockers of KCa1.1 channels also inhibited the production of vascular endothelial growth factor (VEGF), interleukin-8, and matrix metalloproteinase-2 by RA-FLS, without affecting the production of interleukin-6 or inducing cell toxicity. These blockers therefore have the potential of reducing angiogenesis, inflammation, and joint destruction. KCa1.1 channels expressed by RA-FLS are attractive targets for the treatment of RA, and possibly other arthritic diseases.
Disclosure of interest: None declared.

Innovative methodologies

P-606
BIOMAP® SYSTEMS INTEGRATE HUMAN BIOLOGY AND DRUG DISCOVERY ENABLING THE DEVELOPMENT OF SAFER AND MORE EFFECTIVE ANTI-INFLAMMATORY THERAPIES

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Although biologics have demonstrated utility for treatment of rheumatoid arthritis, a significant proportion of patients fail to respond or develop dose-limiting side effects. This, together with the cost and inconvenient parenteral mode of administration, has prompted renewed efforts to develop a broader armamentarium to include safe, efficacious and orally available small-molecule drugs. Inflammation-associated protein kinases fulfill these requirements and remain an attractive target for therapeutic development. However, despite promising performance in the discovery phase, several compounds have failed to deliver on efficacy and/or safety criteria in the clinical setting. BioSeek has pioneered the development of an in vitro human cell-based technology to integrate human biology into drug discovery. BioMAP® Systems model the complex signals and phenotypic biology of specific therapeutic areas including inflammation/auto-immunity. BioMAP® profiling generates a unique signature of human biological responses for every active compound. We have profiled a number of pathway-associated kinase inhibitors, JAK, p38MAPK or PI3K family members. BioMAP® profilingreveals both common and unique anti-inflammatory/mechanism of action effects (**efficacy**), secondary and off-target activities (**safety**), and clinical insights (**therapeutic guidance**) for each of these target classes. Comparison of the resulting profiles using proprietary algorithms to BioSeek's database containing profiles of thousands of experimental compounds and approved therapeutics provides an enhanced understanding of the mechanism of action of these target classes. Such actionable data can guide lead selection, lead optimization (SAR), mechanistic understanding of compound action. BioMAP® profiling yields critical human cell-based information to guide nomination of candidates for preclinical development in the areas of inflammatory and autoimmune disease and enable develop safe and effective therapies.
Disclosure of interest: None declared.

P-607
NON-INVASIVE IMAGING OF ARTHRITIC INFLAMMATION AND THERAPEUTIC RESPONSE BY 19F MRI

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Non-invasive imaging of inflammation to measure the progression of autoimmune diseases such as rheumatoid arthritis (RA) and to monitor responses to therapy is critically needed. V-Sense, a perfluorocarbon tracer agent that preferentially labels inflammatory cells, which are then recruited out of systemic circulation to sites of inflammation, enables detection by 19F MRI. With no 19F background in the host, detection is highly specific, yielding a quantitative marker of the degree of inflammation present. Collagen-induced arthritis in rats, a model with many similarities to human RA, was used to study the ability of V-Sense to measure the accumulation of inflammation over time. Disease progression in the rat hind limbs was monitored by caliper measurements and 19F MRI on days 14, 21 and 28, including the height of clinically symptomatic disease. Naïve rats served as controls. The capacity of V-Sense to assess the effectiveness of therapy was studied in a cohort of rats administered oral prednisolone on days 14–28. This study may support the use of V-Sense to clinically quantify and monitor the severity of inflammation, and to assess the effectiveness of treatments in RA and other diseases with an inflammatory component.
Disclosure of interest: A. Balducci Employee of: Celsense, Inc., Other: stock options, B. Helfer Employee of: Celsense, Inc., Other: stock options, E. Ahrens Grant/Research Support from: Celsense, Inc., Employee of: Celsense, Inc. (CSO), Stock ownership or royalties of: Celsense, Inc., C. O'Hanlon Employee of: Celsense, Inc. (CEO), Stock ownership or royalties of: Celsense, Inc., A. Wesa Employee of: Celsense, Inc., Other: stock options.

P-608
METHODOLOGY OF STUDY OF SYSTEMIC INFLAMMATION AS THE TYPICAL PATHOLOGICAL PROCESS

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Objective: Systemic Inflammation (SI) is considered as a key pathogenic base of critical states including sepsis. In our opinion, the main problem consists in absence of both of fundamental image of SI as the typical pathological process and it's specialities as compared to "Classical" (local) inflammation. It is significant because of one or another signs of SIR are observed under classical inflammation. In 2001 we defined SI as "Typical multi syndrome phase-specific pathological process caused by systemic alteration and is characterized by total inflammatory responsiveness of endotheliocytes, plasma and cell factors, and connective tissue as well as microcirculation disorders in essential organs and tissues". The main methodological approaches to study SI were suggested.
Methods: Rates of IL-6, IL-8, IL-10, TNFα, CRP, troponin I, myoglobin, cortisol; and D-dimer were determined with immunochemiluminous assay ("Immulite", Siemens Medical Solutions Diagnostics, USA). Methods of calculation of Reactivity Level (RL) to estimate intensity of SIR as well as SI scale score have been created. Integral SI scale registers the values of RL (maximum is 5 points) and occurrence of other phenomena of SI: systemic alteration, MODS, micro clottage, distress of neuroendocrine system [Patent No. 233577/Rus.].
Conclusion: SI scale intends to describe pathogenesis of shock and other critical states both of septic and aseptic origin. This scale makes it possible to define the SI phase, which could be applicable for monitoring and prognosis sever complications. Advantages of this way of estimation SIR and SI are the following: replaceability of parameters and the calculation has been based on overshooting of normal level by cytokines and CRP that makes it applicable in different method assay.
Disclosure of interest: None declared.

P-609
CYTOTOXICITY OF POLYMERIC MATERIALS ON HUMAN DENTAL PULP FIBROBLASTS

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Objectives: The aim of this in vitro study was to evaluate the cytotoxicity of different dental materials applied to human dental pulp fibroblast.
Methods: Third molar pulp was collected and cultured in DMEM 10% fetal bovine serum. Cells (50,000 cells/cm^2) were plated in 24-well plates and incubated in a humidified incubator at 37°C with 5% CO_2 and 95% air for 24 h. The materials employed were: Group 1 (G1)—Single Bond (3M ESPE), G2—Clearfil SE Bond-primer (Kuraray), G3—Clearfil SE Bond-bond (Kuraray), G4—P90 System Adhesive-primer (3M ESPE), G5—P90 System Adhesive-bond (3M ESPE), G6—Z250 (3M ESPE), G7—Z350 (3M ESPE), G8—P90 (3M ESPE) applied in the same volume and unpolymerized into transwell membranes for resin composite or added to the culture medium for adhesive systems. G9—control group (medium alone) was also evaluated. Cell metabolic activity was evaluated by succinic dehydrogenase (SDH) activity, which is a measure of the mitochondrial respiration of the cells. For such purpose, the methyltetrazolium (MTT) assay was used at times of 6 and 24 h. The data were compared by Two-way ANOVA ($p < 0.05$) and Bonferroni post-test ($p < 0.05$).
Results: The results were given in optical density. The MTT assay demonstrated the following results: G1—2.159; G2—0.7157; G3—1.928; G4—1.513; G5—1.606; G6—1.809; G7—1.679; G8—1.755 and G9—1.906 at 6 h. At 24 h the MTT assay was: G1—0.905; G2—0.606; G3—1.563; G4—1.757; G5—1.918; G6—1.483; G7—1.412; G8—1.387 and G9—1.458.
Conclusion: At 24 h, the decrease in cell metabolism was statistically significant for G1, G3, G6, G7 and G8 when compared with the same groups in 6 h.
Disclosure of interest: None declared.

P-610
BIOFABRICATION OF THREE DIMENSIONAL COMPLEX BIOLOGICAL STRUCTURES USING INKJET TECHNOLOGY FOR THREE DIMENSIONAL TISSUE MODELS

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The behaviors of individual cells are thought to be influenced with many integrated factors, such as the humoral factors from adjacent and even distant cells, the attached extracellular matrix and the mechanical stimulation. Because the environment of the cells is not two dimensional (2D) but 3D in vivo, the cells receive those stimuli not from 2D but from 3D. Therefore, the engineered 3D tissue models in vitro have great possibilities in the study of such 3D complexities of physiology and 3D pathological processes of diseases in vivo. In addition, the use of such 3D tissue models with human cells is expected much more efficient in the screening and discovery of new therapies and new drugs, more than 2D culture cells and animal experiments. Then, we have addressed to develop some effective technologies to fabricate such 3D tissue models efficiently and accurately using living cells and biological materials. Then, we have developed 3D bioprinter applying inkjet and gelation technique, which enables to position living cells at the designed 3D position arbitrary. We have succeeded to fabricate simple 3D gel structures containing living cells. Next, we have improved our 3D bioprinter with a new printing mode based on the bitmap images to fabricate more complex 2D and 3D structures. We have succeeded to fabricate some 3D structures by laminating the 2D structures based on bitmap images, however, we could not fabricate them with sufficient precision. In this study, we developed our printer by adding with Z-axis control system. Using the new version system, we successfully improved its performance, and could fabricate more complex 3D structures, such as branched tubes and the multi-layered 3D pyramid structures. In addition, we also recognized that the living fibroblasts were extended in the fabricated 3D tube with fibrin gel. We confirmed that the approach of 3D biofabrication with our 3D bioprinter has a great potential to fabricate complex 3D tissue models in vitro.
Disclosure of interest: None declared.

P-611
SYSTEMIC INFLAMMATION AS A RISK FACTOR OF CHRONIC RENAL ALLOGRAFT DYSFUNCTION

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Objective: Chronic renal allograft dysfunction is a progressive nephrosclerosis which arises under immune and nonimmune mechanisms. There is a supposition today that one of the reasons of chronic renal allograft dysfunction may be process of chronic systemic inflammation in recipients.
Study Goal: To analyze signs of the systemic inflammation as a risk factor of chronic renal allograft dysfunction in patients with chronic glomerulonephritis.
Patients and methods: There were studied three groups of patients with chronic glomerulonephritis: the group 1 is a "patients with end-stage renal disease which receive renal replacement therapy in the form of programmed hemodialysis" ($n = 22$); the group 2 is a "patients with chronic renal allograft dysfunction" ($n = 23$); the group 3 is a "patients with normal functions of allograft" ($n = 24$). The control group was a cohort of healthy persons, age 18–55 ($n = 50$, mean age 34.1 ± 10.4 years). There were measured levels of systemic inflammation markers in blood plasma such as interleukins (IL-6, IL-8, IL-10), tumor necrosis factor (TNF)α, C-reactivity protein (CRP), cortisol, myoglobin, troponin I, D-dimer. On the basis of these markers we calculated integrated criterion—scale of chronic systemic inflammation, ranging 0–8. Point ≥ 3 proves chronic systemic inflammation.
Results: Chronic systemic inflammation was detected in 82% hemodialysis-dependent patients, in 39% patients with chronic renal allograft dysfunction. Patients with normal functions of allograft had not chronic systemic inflammation. According to the results of the ROC-analysis the diagnostic accuracy of the scale of chronic systemic inflammation was quite good (AUC = 0.81).
Conclusion: Renal transplantation arrests systemic inflammation partially, but there is a strong possibility of chronic renal allograft dysfunction in recipients who have some criteria of chronic systemic inflammation.
Disclosure of interest: None declared.

P-612
INFLAMMATORY RESPONSE STUDY ON A NEW COCULTURE MODEL OF ALVEOLO-CAPILLARY BARRIER TREATED BY TIO2 AND SIO2 NANOPARTICLES

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One of the vital barriers in the human body is the air-blood interface in the lung. With an area of approximately 100–140 m^2 the alveoli of the human lung are functionally the most important element of the lung. Therefore, we developed an in vitro model that mimics the alveolo-capillary barrier in order to assess the toxicity of nanoparticles (NP). The aim of the model is to explore the toxicity, the passage and the inflammatory response of the air-blood barrier after a treatment by SiO_2 (20 and 100 nm) and TiO_2 (20 nm) NP. In order to better mimic the reality, the model of cell coculture is composed of 3 cell types which are, respectively: THP-1 macrophages, A549 or NCI-H441 epithelial cells and HUVEC-CS endothelial cells. Epithelial cells and macrophages are cultivated on the apical membrane of an insert, and endothelial cells on the basal side. Therefore, we studied toxicity, cellular inflammation and intercellular communication between the cells of the model. After 24 h of treatment with NP, IL-1β, IL-6, IL-8 and TNFα were measured in cell supernatants with a multiplex technology. SiO_2 and TiO_2 nanoparticles do not induce the same inflammatory response and the smallest nanoparticles (20 nm SiO_2 and TiO_2 NP) seem to induce more cytotoxic effects. Moreover, we demonstrate that a treatment of epithelial cells by NP-exposed-macrophage's conditioned medium does not trigger the same inflammatory response compared to direct treatment of epithelial cells by NP. Therefore, a model of air-blood barrier including macrophages is essential to assess inflammatory process close to reality. Otherwise, contrary to A549 epithelial cells, the inflammatory process in NCI-441 cells may link to the activation of the $P2X_7$ cell death receptor, functional, and known for his involvement in the inflammasome activation. Therefore, NCI-H441 cells better mimic the reality and is a better cellular model than A549 to assess the inflammatory process and damages caused by NP in the air-blood barrier.
Disclosure of interest: None declared.

P-613
JUVENILE IDIOPATHIC ARTHRITIS: CONDYLAR AND MANDIBULAR MORPHOLOGIC AND VOLUMETRIC CHANGES

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Aim: The aim of this study is to show the importance of Cone Beam Computerized Tomography to volumetrically quantify TMJ damage in patients with Juvenile Idiopathic Arthritis (JIA), measuring condylar and mandibular real volumes.
Material and methods: 30 children with temporomandibular involvement by JIA were observed by Cone Beam Computerized Tomography. The mandible was isolated from others craniofacial structures; the whole mandibular volume and its components' volumes (condyle, ramus, emibody, emisymphysis on right side and on left side) has been calculated by a 3D volume rendering technique.
Results: The results show a highly significant statistical difference between affected side volumetric values versus normal side volumetric values above all on condyle region ($P < 0.01$), while they does not show any statistical differences between right side versus left side.
Conclusion: The Cone Beam Computerized Tomography represents a huge improvement in understanding of the condyle and mandibular morphological changes, even in the early stages of the Juvenile Idiopathic Arthritis. Early initiation and optimal adjustment of aggressive therapy with disease-modifying anti-rheumatic drugs have been extremely successful in preventing irreversible joint damage. Therefore, the accurate and early diagnosis of JIA and the sensitive monitoring of the disease process are essential. Advanced imaging technology capable of identifying even the slightest trace of erosive joint damage may enable the prediction of future structural and functional deterioration.
Disclosure of interest: None declared.

P-614
DISEASE MODELING OF A NLRP3-DRIVED AUTOINFLAMMATORY DISEASE WITH INDUCED PLURIPOTENT STEM CELLS

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Background: In vitro studies for some of immunological disorders using human samples are sometimes difficult because (1) therapeutic medicines can affect the phenotype of primary immune cells, and (2) existence of somatic mosaisism interfares identifying responsible inflammatory cells. To overcome this, here we constructed an in vitro disease model for a NLRP3-drived hereditary autoinflammatory syndrome with human induced pluripotent cells (iPSCs). Chronic Infantile Neurologic Cutaneous and Articular (CINCA) syndrome is a severe autoinflammatory disease caused by gain-of-function mutations of NLRP3 gene. Mutant NLPR3 protein in macrophages causes overproduction of IL-1 beta, which leads to systemic inflammation.
Results: We obtained fibroblasts from two male patients of CINCA syndrome with somatic mosaicism. Fibroblasts were transduced with lentiviral vector encoding ecotropic receptor Slc7a1 and with retroviral vector encoding OCT4, SOX2, KLF4 and cMYC. Compact embryonic stem (ES)-cell like colonies emerged after 2 weeks and we obtained more than 20 iPSC lines. We obtained both wild-type and mutant clones from each patient's fibroblasts. After evaluating transgene silencing by quantitative RT-PCR, we selected 3 mutant and wild type iPSC clones, respectively, for further analysis. All the clones expressed human ES-cell markers. iPSCs are differentiated into CD14+ macrophages via CD34+ KDR+ hematopoietic progenitors. Mutant iPSC-derived macrophages tend to secrete higher amount of IL-1 beta when stimulated with lipopolysaccharides or infected with Listeria monocytogenes, while wild-type clones not.
Future directions: We have succeeded to recapitulate in vitro phenotypes of macrophages with NLRP3 mutations. In vitro disease modeling provides us a platform for drug screening. Now we are planning to look for novel NLRP3 inhibitors that can modulate inflammasome signaling more precisely.
Disclosure of interest: None declared.

P-615
MICROCAPILLARY IMMUNOSENSOR PLATFORM FOR DETECTING INFLAMMATORY MARKERS

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The unique properties of Quantum Dots (QDs) such as broad excitation spectra, narrow emission spectra, high resistance to photobleaching, higher photo-stability and stronger fluorescence makes them one of the most attractive fluorescent reporter molecules for a wide range of biological applications including biosensing and assay development applications. However, the high cost of QDs has limited their application in assay development where at least 50–100 μL reagents are required for performing an assay. This research capitalizes on the superior properties of QDs by utilizing just 1–2 μL of sample and antibodies conjugated to QDs for detection of biomarkers. In this work we developed a simple and inexpensive microcapillary immunosensor based on quantum dot assay for detecting myeloperoxidase (MPO) in an animal model of intestinal inflammatory disease. The biomarker MPO was detected in the stool samples collected from animal at various stages of inflammatory disease. The method utilizes commercially available polymethylmethaacrylate (PMMA) micro-capillaries (ID 250 μm, OD 500 μm) as substrates for performing a sandwich assay. The results obtained indicate that the limit of detection (LOD) of the device and the assay is 100 picomolar of MPO ($\sim$15 ng/mL) in 1–2 μL of sample. Furthermore, the assay and device robustness were tested by detecting MPO in 1–2 ml of animal stool sample and comparing the results obtained with a commercially available MPO ELISA kit that uses 100 μL of sample. In conclusion, we are reporting here a low cost microcapillary immunosensor platform for detecting inflammatory markers.
Disclosure of interest: None declared.

P-616
IN SILICO ENZYME AND RECEPTOR FIT SCREENING OF ANTIRHEUMATIC DIGLUCOPYRANOSYLAMINES: A NOVEL APPLICATION FOR DETERMINING MECHANISM OF ACTION OF NOVEL ANTI-RHEUMATIC DRUG CANDIDATES

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Diglucopyranosylamines and the oligo-SO3 salts have a confusing array of immunological properties and suppress antigen and collagen arthritis. Also, in testimonial reports, di$\beta\beta$gluco-pyransylamine (di$\beta\beta$GA) resolves herpetic lesions and pain. In silico models of active enzyme and receptor binding sites are used in ligand design for drug candidates. A novel application of this technology is in silico screening of active sites for drug binding to a wide variety of binding sites. di$\beta\beta$GA, monoglucosylamine (MGA), di$\beta\beta$GA-oligo-SO3 were screened against the Pharmacophore Database HypoDB (Inte:Ligand) with 2500 chemical feature-based 3D pharmacophore models encoding 277 targets: mammalian, bacterial and viral enzymes, GPCRs, transduction factor receptors, signalling molecules, structural proteins, toxins, and transport proteins, from protein–ligand 3D complex structures via LigandScout software (Inte:ligand). An algorithmic score of 0.7 to a maximum of 1.0 is considered highly relevant. Glucose was included to exclude its monomer binding. di$\beta\beta$GA, a bacterial glucosidase inhibitor, fitted to 0.689, and MGA to 0.741. N-acetylation and oligo-SO3 removed activity. Herpes thymidine kinase fitted to 0.7. Viral DNA polymerase and influenza neuraminidases were negative. Surprisingly, adenosine A1 receptor (A1R) fit was 0.787 for di$\beta\beta$GA, 0,571 on N-acetylation, and all others negative. The model predicted the activity of di$\beta\beta$GA and revealed potential herpes viral enzyme interference, notably herpetic thymidine kinase, relevant to DNA synthesis and cell division. Unexpected was a high A1R fit. A1R limits inflammatory pain and A1 agonists are antinociceptive in inflammation. This may explain reports that di$\beta\beta$GA

limits herpes lesions and peripheral pain. Verification of A1R and inflammatory nociceptive activity is needed. Lack of oligo-SO3-derivative binding is unsurprising since heparinoid-binding receptors likely to be involved in the anti-rheumatic activity of these agents is excluded.
Disclosure of interest: M. Seed: None declared, T. Langer Other: Owner.

P-617
CHOICE OF ANTICOAGULANT FOR LEUKOCYTE ADSORBING APHERESIS IS IMPORTANT FOR ANTI-INFLAMMATORY PROFILE OF THE APHERESIS TREATMENT

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Two leukocyte adsorbing apheresis techniques are available now for treatment of chronic inflammatory conditions: granulocyte and monocyte apheresis (GMA) (ADA®-column) using heparin as anticoagulant and leukocytapheresis (LCAP) (device Cellsorba™) using nafamostat mesilate or ACD-A as anticoagulant. We found recently that antimicrobial and immunoregulatory peptide LL-37 was released from neutrophil granulocytes within the plastic apheresis lines during LCAP using ACD-A as anticoagulant and may perform its regulatory functions when returned back into the patient. However, heparin is also known for its anti-inflammatory properties. Eight patients with chronic inflammatory diseases were treated by GMA using heparin as anticoagulant: three patients with inflammatory bowel disease, three—with sarcoidosis, one—with chronic dimyeliniserande disease and one—with pyoderma gangrenosum. Venous blood samples and samples from the apheresis lines (before and after the apheresis device) were analyzed in the same manner as we done previously in our LCAP study (ACD-A) by commercial ELISA. There was no increase of LL-37 in the lines in difference to our LCAP-study. Some slight increase of LL-37 concentration was observed over the apheresis device. We report hereby that heparin used as anticoagulant inhibits generation of LL-37. Thus, the anticoagulants have different impact on immunoregulatory molecules as LL-37 and choice of the anticoagulant influences anti-inflammatory profile of respective apheresis treatment.
Disclosure of interest: None declared.

Mini Papers for Young Investigator's Award

RETRACTED: Mini Paper 1

OC-113
ABSENCE OF MICRORNA-155 PROTECTS AGAINST ADVERSE CARDIAC INFLAMMATION AND HYPERTROPHY

Blanche Schroen[1], Maarten Corsten[1], Mathijs van de Vrie[1], Paolo Carai1[2], Wouter Verhesen[1], Rick EW van Leeuwen[1], Laura De Rijck[1], Lauran Stöger[1], Susan Coort[1], Esther E Creemers[3], Yigal M Pinto[3], Serena Zacchigna[4], Peter Carmeliet[2], Elena Vigorito[5], Ben J Janssen[1], Esther Lutgens[1], Robert Dennert[1], Menno De Winther[1], Frank Stassen[1], Anna Papageorgiou[1], Stephane Heymans[1]

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This mini paper has been retracted due to a publishing error.

Mini Paper 2

OC-043
A NOVEL CROSS-TALK IN RESOLUTION: H2S ACTIVATES THE ANNEXIN A1 PATHWAY

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Hydrogen sulphide (H2S), a gaseous mediator synthesized in several mammalian tissues by two main enzymes CBS and CSE, increases under inflammatory conditions or sepsis. Since H2S and H2S-releasing molecules afford potent inhibitory properties on the process of leukocyte trafficking, we tested whether endogenous Annexin A1 (AnxA1) could display intermediary functions. Treatment of human PMNs the H2S donor NaHS (10–100 mM) provoked prompted and intense mobilization (>50%) of AnxA1 from the cytosolic pool to the cell surface, supporting the inhibitory effects of the gas in the flow chamber assay. Such in vitro actions could be translated in analyses of the inflamed microcirculation, where NaHS (100 mmol/kg s.c., −1 h) afforded marked inhibition of IL-1-induced cell adhesion and emigration in the mesenteric vessels of wild type, but not AnxA1−/−, mice. Next, we investigated whether endogenous AnxA1 could modulate H2S synthesis, indicating existence of a positive circuit. To this end, a marked increase in CBS and/or CSE expression in a variety of tissues (aorta, kidney, spleen) tested from AnxA1−/− mice, as compared to wild type animals, was quantified by qPCR. Moreover, NaHS was able to counteract the increase in expression in iNOS and COX2 (four- and sevenfold reduction, respectively) upon LPS-stimulation of bone marrow derived macrophages (BMDM), though it was totally inactive in cells prepared from AnxA1−/− mice. Taken

together, these data strongly suggest—for the first time—the existence of a positive interlink between AnxA1 and the H2S pathway, providing a novel mechanistic explanation for the exquisite properties of H2S in the control of experimental inflammation. These finding may be relevant to innovative discovery programmes aiming at harnessing the biological properties of H2S. Supported by the Wellcome Trust (programme grant 086867/Z/08/Z).

Introduction: H2S is a gaseous mediator synthesized by two pyridoxal-5-phosphatedependent enzymes, such as CBS and CSE. L-Cysteine represents the physiological precursor, which is metabolized leading to the H2S production. Recent literature has supported the involvement of H2S in cardiovascular homoeostasis (Zhong et al. 2003; Qu et al. 2006). In particular, it has been demonstrated that H2S (a) exhibits vasodilator activity in vitro and in vivo (Ali et al. 2006), (b) is pro-apoptotic on vascular smooth muscle cells (Zhao et al. 2001; Yang et al. 2004) and (c) is involved in leukocyte-endothelium interaction (Zanardo et al. 2006). Furthermore, there is emerging evidence for a key role for H2S in cell trafficking and inflammation (Zhang et al., J Leukoc Biol, 2007). AnxA1 is also a key mediator in the inflammation and it shows prominent features in activation of the resolution process, exerting anti-inflammatory activity. Since H2S and H2S-releasing molecules potently inhibit the process of leukocyte trafficking, we tested whether endogenous AnxA1 could display intermediary functions.

Materials and methods: Human PMN isolation and membrane and cytosolic fractions preparation. Peripheral blood was collected from healthy volunteers and PMNs isolated. All healthy volunteers gave oral and written consent and cell separation was covered by ethical approval 05/Q0603/34 (East London and The City Research Ethics Committee 1). After treatment, cells (4 × 106) were re-suspended in lysis buffer and processed in order to get cytosolic and membrane fraction.

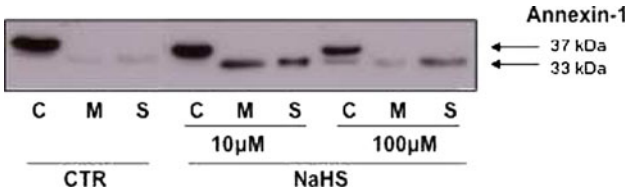
Animals and treatment: C57Bl/6 mice (Charles River, UK) were treated with LPS (10 mg/kg) and then sacrificed after 6 h. Blood, liver, aorta, kidney, lymph nodes and spleen were collected for further analysis.

Bone Marrow Derived Macrophages (BMDM) from AnxA1−/− and wild type mice: BMDM were obtained from femurs and tibias of 4–6 week old. The marrow was flushed from the bone, washed, resuspended (2–3 × 106 cells/ml) in culture medium (30% L929 conditioned medium) and incubated at 37°C for 5 days. BMDM were then treated with LPS (100 ng/ml, 6 h) alone or in presence of NaHS (100 mM, −1 h).

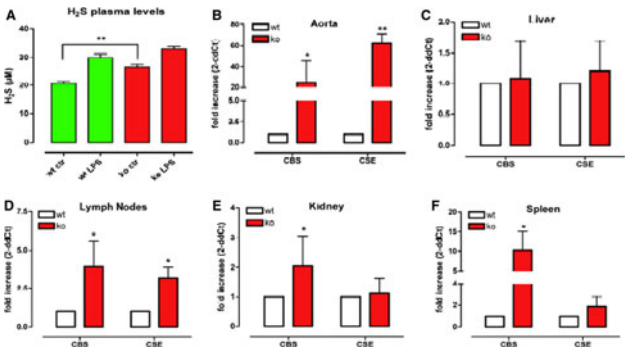
Quantitative real-time PCR: Quantization of the expression level of selected genes (CBS, CSE, COX2 and iNOS) was performed by quantitative real-time PCR. Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For realtime PCR, 10 ng template was used in combination with each primer solution (Qiagen, UK) and Fast SYBR Green Master Mix solution (Applied Biosystem, UK). All reactions were performed in a_ 7900HT Fast Real-Time PCR System instrument (Applied Biosystem). Relative expression (vs GAPDH) ±SEM was then expressed in the graphs and showed as fold-increase or decrease (n = 3).

Intravital microscopy of the mesenteric microcirculation: Following treatment with IL1b (10 ng/mouse i.p., 2 h) alone or in combination with NaHS (100 mmol/kg s.c., −1 h) or PAG (10 mg/kg i.p., −30 min) AnxA1−/− or wild type mice were mesenteries were exposed and one to three randomly selected postcapillary venules (diameter between 20 and 40 mm) were observed for each mouse (n = 5). Adherent and emigrated leukocytes were then quantified by counting the number of cells (100 × 50 mm^2). All of the animal studies were conducted in accordance with current UK. Home Office regulations and complied with local ethical and operational guidelines.

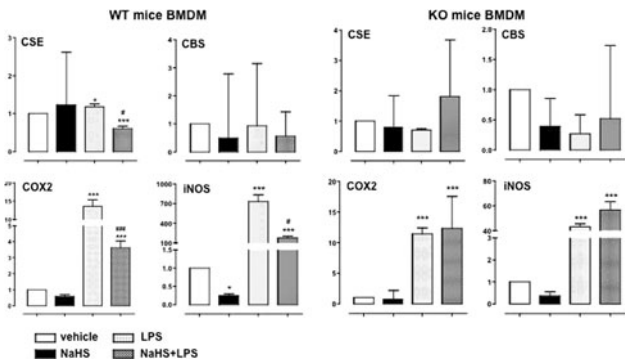
Results: Resting human PMNs retain the majority of AnxA1 in the cytosol as granular or free stores. NaHS treatment (10–100 mM, 30 min) induces mobilization of intracellular AnxA1 from cytosol towards the membrane and its further release in the culture medium (Fig. 1).



As LPS stimulation has bee reported to increase circulating H2S levels, we treated AnxA1−/− and wild type mice with LPS and monitored plasma levels for H2S. As shown in Fig. 2a, H2S levels are increased in LPS treated mice; furthermore, the gaseous mediator levels are higher in AnxA1−/− mice vs wild type even in non-treated mice. We then focused on the clear difference in H2S amount between the two animal genotypes and real time PCR data confirmed that in AnxA1−/− mice we have an increase in expression for CSE and/or CBS in all tissues analyzed (Fig. 2b–f).

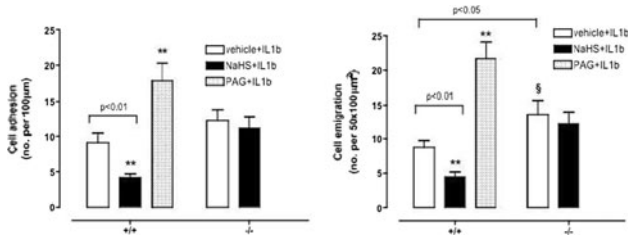


On the basis of these results we decided to investigate on possible antiinflammatory effect for H2S in a different setting such as BMDM. Preincubation with NaHS for 1 h before LPS administration could significantly prevent the increase in expression of COX2 and iNOS associated to LPS treatment. Surprisingly, this effect was occurring only in BMDM from wild type but not from AnxA1−/− mice (Fig. 3).



In order to better establish the physiological relevance for AnxA1 mobilization in human PMN we performed intravital microcopy study in a IL1b-induced inflammation model. Pre-treatment of mice with

NaHS was able to reduce the number of adherent and emigrated cells and, once again, this effect was only evident in wild type but not in wild type mice. Furthermore, pretreatment with CSE inhibitor PAG significantly exacerbated the inflammatory pattern (Fig. 4).



Discussion: The evidence that H2S is a new gaseous physiological modulator rather that a toxic and pollutant gas is now widely accepted, as many studies have shown that impairment in its production could lead to or are involved in many different pathological conditions (Brancaleone et al., Br J Pharmacol, 2008; Szabo C, Nat Rev Drug Discov 2007). The data here reported represent an interesting novelty and clearly demonstrate, for the first time, the existence of a crosslink between H2S and AnxA1 pathways, where it appears to be an evident "one-need-the other" system. However, the data this study showed are preliminary and the aspects highlighted need to be confirmed and further investigated with more targeted experiments in order to get better understanding of the mechanisms behind this circuit.

Mini Paper 3

OC-046
CATHEPSIN G HAS AN ANTI-NOCICEPTIVE EFFECT IN NORMAL RAT KNEE JOINTS

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In addition to its enzymatic properties, the serine proteinase, Cathepsin G is known to activate proteinase-activated receptor 4 (PAR4). In joints, PAR4 activation leads to inflammation and pain. Since cathepsin G is found in high concentration in arthritic joints, we hypothesised that Cathepsin G would have a pro-nociceptive effect in the joint by cleaving and activating PAR4. Electrophysiological recordings were made from knee joint afferent fibres in male Wistar rats during normal and noxious joint rotations. Fibre firing rate was recorded in naive rats during 10 s rotations before and over 15 min after local administration of Cathepsin G (1 ng/100 μl–10 μg/100 μl). Responses were compared with animals pre-treated with the PAR4 antagonist, pepducin P4pal-10 (100 μg i.p.). Cathepsin G dose-dependently decreased the firing rate of joint fibres during rotation of the knee. At doses of 1 and 10 ng, where Cathepsin G alone had no effect, PAR4 antagonism caused a significant decrease in firing rate. Thus, Cathepsin G has an anti-nociceptive effect on joint fibres in normal rats that is enhanced when PAR4 is inhibited. This suggests that Cathepsin G can activate PAR4 but the anti-nociceptive effect of Cathepsin G is via an unknown mechanism.

Introduction: Cathepsin G is a pro-inflammatory serine proteinase released from activated neutrophils that occurs in high levels in rheumatoid arthritic joints [1, 2]. This proteolytic enzyme is thought to play an important role in inflammation as evidenced by the reduced leukocyte accumulation and cytokine release observed in arthritic joints of Cathepsin G knockout mice [3]. As yet, a role for Cathepsin G in joint pain has not been examined. The intracellular signalling effects of Cathepsin G are thought to be mediated in part by the ability of this enzyme to cleave and activate members of the proteinase-activated receptor (PAR) family [2]. The PAR family currently consists of four G-protein coupled receptors. Cleavage of these receptors by serine proteinases at a specific site within the extracellular N terminus, reveals a tethered ligand sequence which can then bind to the active site and cause receptor activation [4]. PARs can also be selectively activated, without proteolytic cleavage, by synthetic activating peptides that mimic the tethered ligand sequence. PAR4 was the 4th member of the family to be discovered and we have previously shown that a PAR4 activating peptide had both pro-inflammatory and pro-nociceptive effects in the joint [5–7]. In addition, treatment with the selective PAR4 antagonist, pepducin P4pal-10 significantly reduced disease severity in a model of acute joint inflammation [5]. It is not yet known which particular serine proteinase activates PAR4 in vivo in the joint but Cathepsin G can activate PAR4 in platelets [2]. In this study we examined the effect of Cathepsin G on neuronal activity in knee joints of normal rats and determined whether this effect is mediated via PAR4.

Methods: Experiments were performed on male Wistar rats (250–400 g; Charles River Laboratories, Quebec, Canada) in accordance with the Canadian Council for Animal Care guidelines for the care and use of experimental animals. Single unit extracellular recordings were made from knee joint primary afferents as previously described [6, 7]. Briefly, small nerve fibre bundles from the saphenous nerve were placed over a platinum electrode to permit extracellular recordings. Afferent nerve fibres originating from the knee joint were identified by the elicitation of a firing response to probing of the joint with a glass rod. Conduction velocities, electrical and mechanical thresholds of fibres were determined. Four movement cycles, each consisting of 10 s of normal rotation and 10 s of noxious rotation of the knee to discrete torque levels, were performed at the beginning of the experiment. The mean afferent nerve fibre firing rate associated with these movements was the control baseline level, which was set at 100%. Then, either Cathepsin G (1 ng–10 μg in 100 μl) or boiled denatured Cathepsin G (inactive control) were administered locally to the knee via a saphenous arterial cannulation. Movement cycles were repeated 1 min post-drug administration and then every 2 min until 15 min post-drug. Rats were either naive or pre-treated with the selective PAR4 antagonist, pepducin P4pal-10 (100 μg i.p.) 30 min prior to recordings. Neuronal activity was digitized using a data acquisition system (CED1401; Cambridge Electronic Design, Cambridge, UK) and stored on a microcomputer for offline analysis. The number of action potentials per movement was determined using Spike 2 software (Cambridge Electronic Design), and the % change in afferent nerve fibre activity from baseline was calculated.

Results: One afferent joint fibre was recorded from each rat with a total of 34 fibres examined overall in this study. Table 1 summarises the electrophysiological characteristics of each fibre. All fibres tested responded to close intra-arterial administration of potassium chloride (KCl; 0.4 mM, 0.1 ml) at the conclusion of the experiment. Cathepsin G dose-dependently decreased the firing rate of joint afferent fibres during both normal and noxious rotations of the joint (Figs. 1, 2; $p < 0.05$ compared to boiled enzyme using two-way ANOVA). Boiled inactive Cathepsin G had no effect on firing rate at any time point and at any dose (Figs. 1, 2). At doses of 1 and 10 ng where Cathepsin G alone had no effect, pre-treatment with the PAR4 antagonist, pepducin, caused Cathepsin G to significantly decrease firing rate during noxious joint rotation (Fig. 3; $p < 0.05$ pepducin treated group compared to Cathepsin G alone, using two-way ANOVA.). Pepducin alone had no effect on firing rate (data not shown).

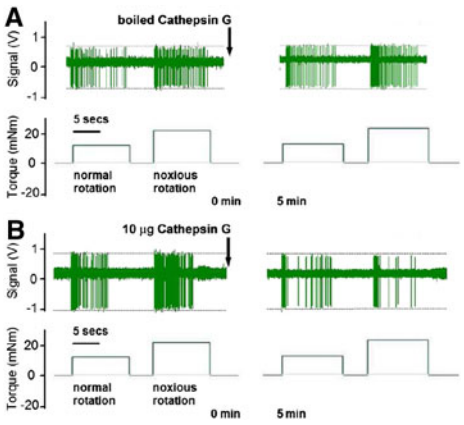

Fig. 1 Examples of recordings from single unit joint afferent fibres in two different rats before and 5 min post **a** boiled denatured 10 µg Cathepsin G and **b** 10 µg Cathepsin G. Responses to normal and noxious rotations of the knee joint are shown

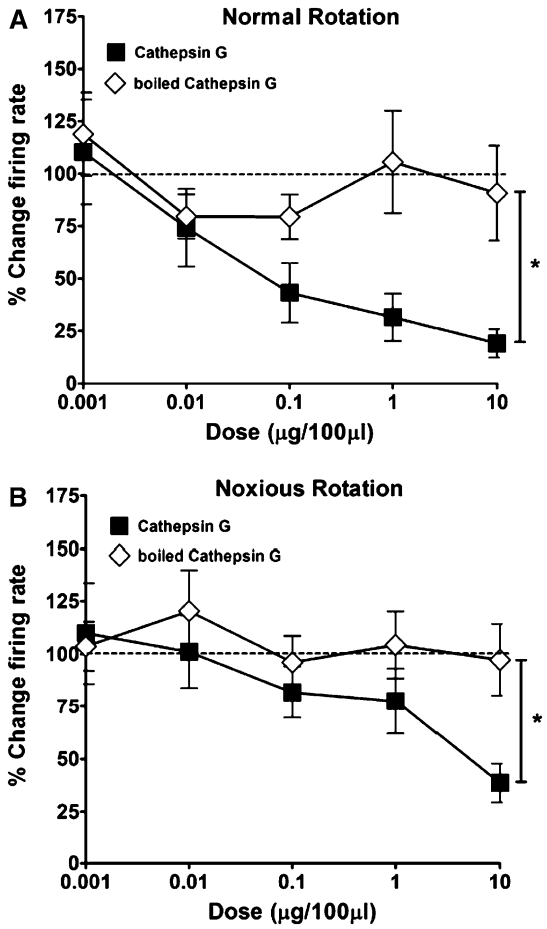

Fig. 2 Dose–response curves of Cathepsin G or boiled Cathepsin G effects on joint mechanosensitivity during **a** normal rotation and **b** noxious rotation of the knee. Results are mean ± SEM, n = 7–16 for Cathepsin G, n = 5–8 for boiled Cathepsin G. *p < 0.05 compared to boiled enzyme using two-way ANOVA

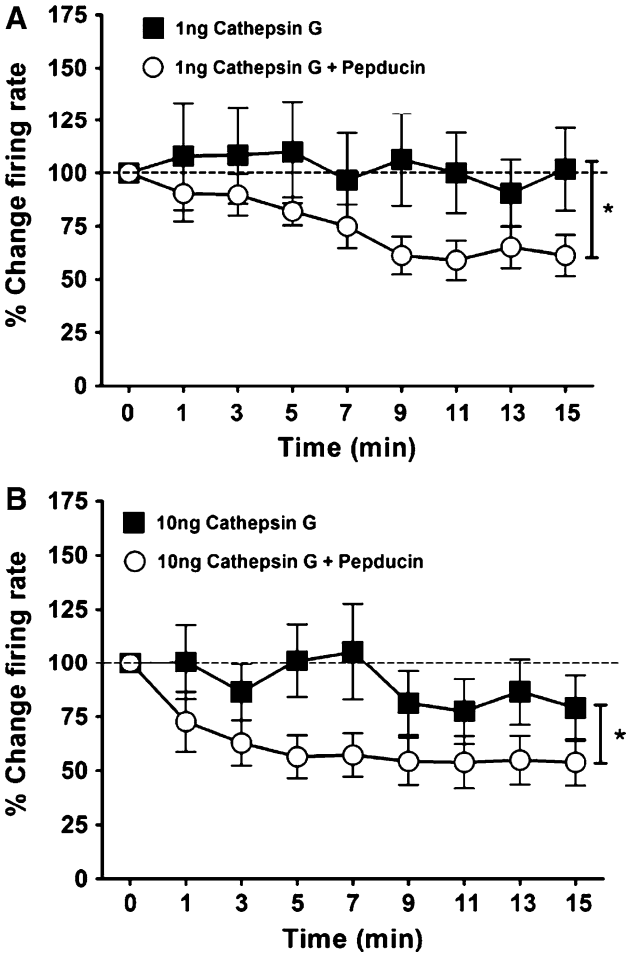

Fig. 3 Time course of % change in firing rate during noxious rotation in rats either naive or pre-treated with pepducin P4pal-10 (100 µg i.p.) after **a** 1 ng/100 µl Cathepsin G and **b** 10 ng/100 µl Cathepsin G. Results are mean ± SEM, n = 9–14. *p < 0.05 pepducin treated group compared to Cathepsin G alone using two-way ANOVA

Discussion: We have shown that in normal rat knee joints, Cathepsin G reduces the mechanosensitivity of slowly conducting afferent fibres. This is in contrast to the pro-nociceptive effect of synthetic PAR4 activating peptide in the joint [6, 7], suggesting that Cathepsin G acts via a PAR4-independent pathway. Interestingly, inhibition of PAR4 enhances this antinociceptive effect of Cathepsn G, i.e. when PAR4 activation is blocked, low doses of Cathepsin G that do not normally have an effect, now cause a decreased firing rate. Therefore, PAR4 activation by Cathepsin G may be causing a pro-nociceptive effect but this is normally masked by a stronger anti-nociceptive effect through a PAR4-independent pathway. Cathepsin G can act on other PAR members including PAR1, which has previously been shown to be anti-nociceptive [8]. Thus, further work is required to investigate the mechanisms behind this anti-nociceptive effect of Cathepsin G so perhaps this effect can be enhanced in arthritic joints where high levels of this enzyme are observed [1].

1. Miyata J, Tani K, Sato K, Otsuka S, Urata T, Lkhagvaa B et al. Cathepsin G: the significance in rheumatoid arthritis as a monocyte chemoattractant. Rheumatol Int. 2007;27(4):375–82. doi:10.1007/s00296-006-0210-8.

2. Sambrano GR, Huang W, Faruqi T, Mahrus S, Craik C, Coughlin SR. Cathepsin G activates protease-activated receptor-4 in human platelets. J Biol Chem. 2000;275(10):6819–23.

3. Adkison AM, Raptis SZ, Kelley DG, Pham CT. Dipeptidyl peptidase I activates neutrophilderived serine proteases and regulates the development of acute experimental arthritis. J Clin Invest. 2002; 109(3):363–71. doi:10.1172/JCI13462.
4. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. Pharmacol Rev. 2001;53(2):245–82.
5. McDougall JJ, Zhang C, Cellars L, Joubert E, Dixon CM, Vergnolle N. Triggering of proteinase-activated receptor 4 leads to joint pain and inflammation in mice. Arthritis Rheum. 2009;60(3):728–37. doi: 10.1002/art.24300.
6. Russell FA, Veldhoen VE, Tchitchkan D, McDougall JJ. Proteinase-activated receptor-4 (PAR4) activation leads to sensitization of rat joint primary afferents via a bradykinin B2 receptor-dependent mechanism. J Neurophysiol. 2010;103(1):155–63. doi:10.1152/jn.00486.2009.
7. Russell FA, Zhan S, Dumas A, Lagarde S, Pouliot M, McDougall JJ. The pronociceptive effect of proteinase-activated receptor-4 stimulation in rat knee joints is dependent on mast cell activation. Pain. 2011;152(2):354-60. doi:10.1016/j.pain.2010.10.038.
8. Martin L, Auge C, Boue J, Buresi MC, Chapman K, Asfaha S, et al. Thrombin receptor: An endogenous inhibitor of inflammatory pain, activating opioid pathways. Pain. 2009;146(1–2):121–9. doi:10.1016/j.pain.2009.07.016.

Table 1 Summary of the electrophysiological properties of all recorded fibres

Drug given	Fibre type	Mechanical threshold (mNm)	Electrical threshold (V)	Conduction velocity (m/s)	Normal rotation (mNm)	Noxious rotation (mNm)	n
Boiled Cathepsin	Type III	11	13	3.2	15	20	1
G	Type IV	9 ± 2	11 ± 1	0.36 ± 0.2	13 ± 3		
		20 ± 4	4				
Nd		14 ± 2	–	–	17 ± 2	23 ± 3	3
Cathepsin G	Type III	6 ± 1	10 ± 1	3 ± 1	9 ± 1	14 ± 1	2
	Type IV	8 ± 2	11 ± 1	0.5 ± 0.1	12 ± 2		
		18 ± 3	3				
Nd Cathepsin		13 ± 2	–	–	16 ± 2	23 ± 3	7

Data are means ± SEM
G + PepducinType III10 ± 29 ± 14 ± 112 ± 219 ± 34Type IV17 ± 510 ± 11 ± 0.220 ± 625 ± 52Nd13 ± 1—17 ± 224 ± 23Data are means ± SEM

Mini Paper 4

OC-126
INFLAMMATORY PROTEASES RELEASED IN A MOUSE MODEL OF INTESTINAL ISCHEMIA

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Introduction: Acute mesenteric ischemia is a potentially fatal abdominal emergency. Although mesenteric ischemia accounts only for about 1–2% of gastrointestinal illnesses, it still causes a high in-hospital mortality rate (60–80%). Interruption of blood supply to the gut results in an ischemic injury, characterized by an acute and massive inflammatory reaction [1]. Proteases represent 2% of the human genome and a number of in vitro evidences have indicated that their release is rapidly mobilized in damaged or hypo-oxygenized cells. Indeed, proteases participate to several inflammatory states [2]. In particular, activation of members of the proteinase-activated receptors (PARs) family, which are G protein-coupled receptors activated by the cleavage at an Arginin-site of their N-terminus extracellular domain, has been implicated in leukocyte recruitment at inflammatory sites [3]. Other proteases belonging to the matrix metalloprotease (MMPs) family, such as MMP-2 and MMP-9 are up-regulated in the inflamed gut [4]. Despite the strong rationale for a potential role of proteases in inflammatory response to ischemia, the profile and functions of proteases in intestinal ischemic tissues has never been investigated, nor their relationship with inflammation. The aim of our study was to investigate the proteolytic profile associated with intestinal ischemia/reperfusion injury, measuring Arginine-cleavage and MMP proteolytic activities, and to study their possible correlation with inflammatory parameters.
Methods: C57Bl6 male mice (8-week-old) were subjected to 90 min of intestinal ischemia by occlusion of the superior mesenteric artery followed by 0 or 2 h reperfusion (I/R). Sham-operated (SO) animals served as controls. Myeloperoxidase (MPO) activity was measured in intestinal tissues as an index of neutrophil tissue infiltration. Hydrolysis of tosyl-Gly-Pro-Arg-p-nitroanilide substrate was measured as an index of Arginin-cleavage proteolytic activity in plasma or intestinal tissues. MMP-2 and MMP-9 protein expression was evaluated by western-blot, while their activity was measured by gelatine zymography (0.1% gelatine).
Results:
Intestinal ischemia–reperfusion injury induces inflammation in intestinal tissues: Intestinal ischemia–reperfusion caused inflammation to ischemic tissues, as observed by macroscopic features of inflammation: redness, and swelling, but also increased MPO activity, a marker of granulocyte infiltration. This increased granulocyte infiltration was not observed after the 90-min ischemic period, but only after ischemia + reperfusion (2 h) (Fig. 1).

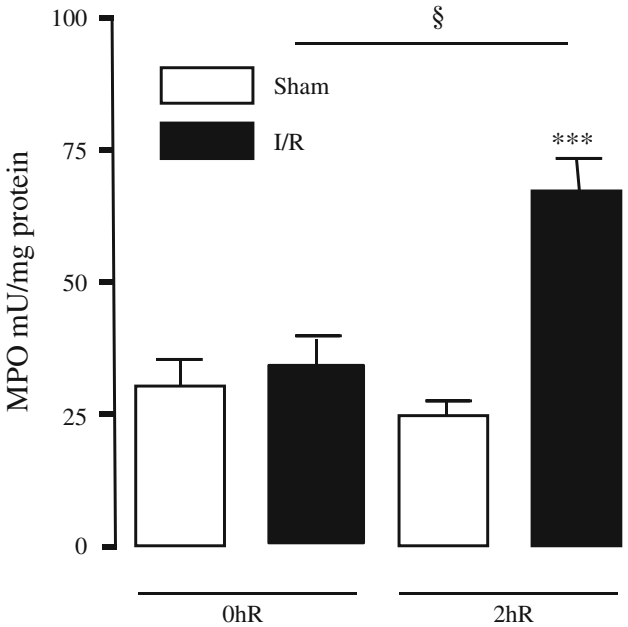

Fig. 1 Intestinal MPO activity after 90-min of ischemia only (0 h), or after 90-min of ischemia followed by 2-h reperfusion (2 h). Data are mean ± SEM of 8–10 animals per group. *Significant difference compared to the corresponding Sham-operated group (***$P \leq 0.001$). §Significant difference between I/R groups ($^{§}P \leq 0.05$)

Intestinal ischemia–reperfusion injury is associated with increased Arginin-cleavage proteolytic activity: After the ischemic period, Arginin-cleavage proteolytic activity levels were significantly increased both in plasma and in intestinal tissues, compared to the activity measured in sham-operated mice (Fig. 2). This increase was maintained in tissues, or further increased in plasma, for 2 h (Fig. 2) and up to 5-h (data not shown) after reperfusion.

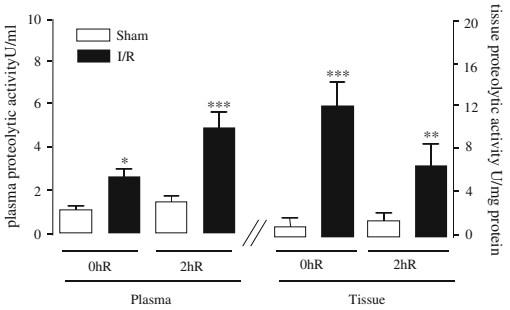

Fig. 2 Plasma and tissue Arginin-cleavage proteolytic activity levels after 90-min of ischemia only (0 h), or after 90-min of ischemia followed by 2-h reperfusion (2 h). Data represents mean ± SEM of 8–10 animals per group. *Significant difference from the corresponding Sham-operated group (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001)

Intestinal ischemia–reperfusion injury is associated with increased MMP-2 and MMP-9: As demonstrated by gelatin-zymography, intestinal ischemia followed by 2 h reperfusion induced in intestinal tissues the activation of MMP-2 with its conversion to the 66-kDa activated enzyme, but also the activation of MMP-9 with the conversion of the pro-form into the active form of 97 kDa (Fig. 3a). Quantification of this activity showed that in I/R mice, MMP-2 and MMP-9 activities were up-regulated both in intestinal tissues and in plasma, compared to Sham-operated mice (Fig. 3b). The nature of MMP-2 and MMP-9 was confirmed by Western-blot analysis (data not shown).

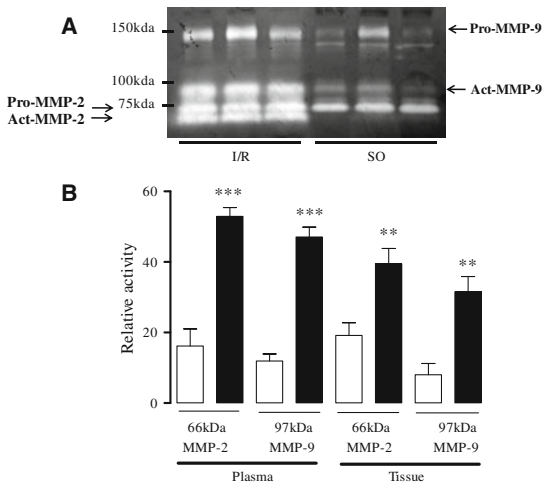

Fig. 3 *Upper panel* (**a**): Identification of gelatinolytic enzymes in intestinal tissues by SDS-polyacrylamide gel electrophoresis gelatin zymography. Zones of enzymatic activity appear as clear bands over a dark background. The three left lanes are tissues from 3 representative I/R animals (90-min of ischemia followed by 2-h reperfusion), the three right lanes are tissues from 3 representative Sham-operated mice. *Bottom panel* (***B***): the graphic represents the quantitative image analysis of the surface and intensity of the gelatinolytic bands displayed on gel zymographies. Results are expressed as arbitrary activity units related to an internal standard ($n = 6$ in each group). * Significant difference from the corresponding Sham-operated group (** P ≤ 0.01, *** P ≤ 0.001)

Correlation of proteolytic activities with granulocyte recruitment: Significant correlation between the Arginin-cleavage proteolytic activity and the granulocyte infiltration marker MPO activity was found in intestinal tissues ($F = 21.13$ for $p = 0.0003$) (Fig. 4a), while similar correlation was not observed for the Arginin-cleavage proteolytic activity in the plasma of mice submitted to ischemia–reperfusion ($F = 0.91$ for $p = 0.35$) (Fig. 4b).

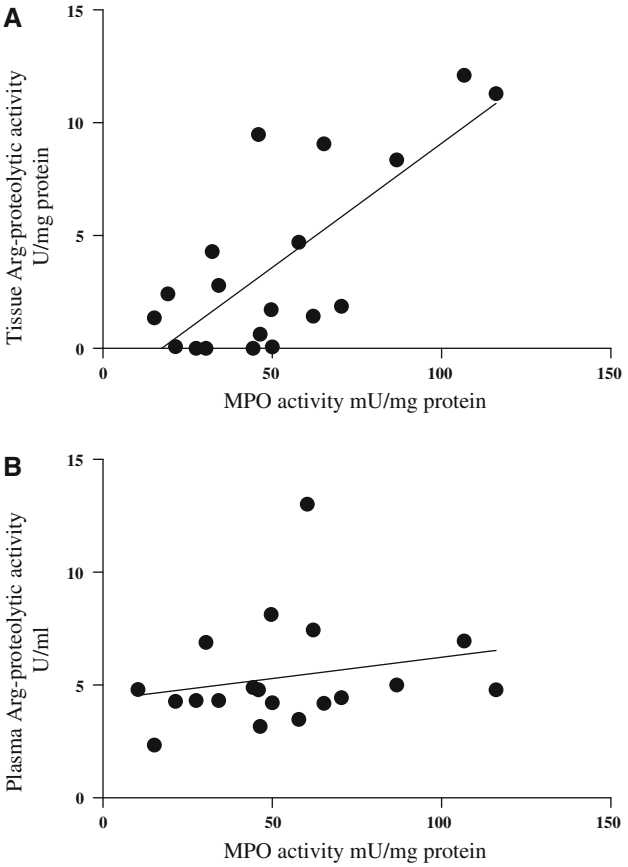

Fig. 4 Scatter graphs showing a positive correlation between tissue Arginin-cleavage proteolytic activity and MPO activity (**a**) and a lack of correlation between plasma Arginin-cleavage proteolytic activity and MPO activity (**b**)

Discussion–Conclusion: The present study shows that 90 min of ischemia followed by 2 h reperfusion causes intestinal inflammatory injury demonstrated by macroscopic inflammatory damage and an increased granulocyte recruitment. This inflammatory response is associated with the release of proteases both in tissues affected by ischemia and reperfusion (intestinal tissues), and systemically in plasma. The nature of those proteases appears to be diverse, with some proteases clearly identified as MMP-2 and MMP-9 and having a gelatinic activity, and others cleaving at an arginin site, similar to the proteases that are capable of activating PARs. The release of Arginin-cleaving proteases in intestinal tissues correlates with inflammatory parameters, but the systemic release of Arginin-cleaving proteases in the plasma does not correlate with inflammation. Considering the well known pro-inflammatory effects of a number of proteases [2], this could suggest that the proteases released in the intestinal tissues, but not the ones released in the plasma, could participate to the generation of the massive inflammatory response associated with ischemia–

reperfusion. The role of those different proteases in ischemia–reperfusion associated injury still has to be demonstrated. Contrasting roles can be suggested for MMP-2 and MMP-9. MMP-2 is constitutively expressed in almost all tissues, where it seems to play a protective role, particularly in gut diseases like colitis [5]. MMP-2 overexpression and over-activity in this model of intestinal ischemia–reperfusion could serve to limit the deleterious effects of this massive inflammatory reaction. In contrast, MMP-9 is absent from most adult tissues and was found to be mostly neutrophil-associated [4, 6]. Therefore, in our study, the increased expression of MMP-9 could be due to the recruitment of neutrophils to ischemic tissues. As for the Arginin-cleaving proteases, it is tempting to suggest that they might exert a pro-inflammatory role through the activation of PARs in the intestine, since the activation of 3 of those receptors is considered as a major pro-inflammatory signal in acute models of colitis [3]. However, those Arginin-cleaving proteases could also exert pro-inflammatory effects through other mechanisms. One of them could be the activation of MMP-9, which proform is known to be transformed into an active form by cleavage at an Arginin site [7]. The correlation between Arginin-cleavage proteolytic activity in intestinal tissues and parameters of inflammation suggests that those proteases are active players of the tissue inflammation. This increased activity could be due to the massive recruitment of inflammatory cells, which express in their granules, a number of proteases, including elastase, proteinase-3 and cathepsins. However, the lack of correlation between MPO activity and proteolytic activity in plasma demonstrates that those plasmatic proteases do not influence and are not influenced by inflammatory cell recruitment. Taken together, these results showed the massive release of different proteases upon intestinal ischemia–reperfusion and suggest that those proteases could actively participate to the associated inflammatory response.

References:
1. Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, and Burger CD. Acute mesenteric ischemia: a clinical review. Arch Intern Med. 2004;164:1054-62.
2. Ossovskaya VS, Bunnett NW. Protease-activated receptors: contribution to physiology and disease. Physiol Rev. 2004;84:579–621.
3. Vergnolle N. Proteinase-activated receptors (PARs) in infection and inflammation in the gut. Int J Biochem Cell Biol. 2008; 40:1219–27.
4. von Lampe B, Barthel B, Coupland SE, Riecken EO, Rosewicz S. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. Gut. 2000; 47:63–73.
5. Garg P, Rojas M, Ravi A, Bockbrader K, Epstein S, Vijay-Kumar M, Gewirtz AT, Merlin D, Sitaraman SV. Selective ablation of matrix metalloproteinase-2 exacerbates experimental colitis: contrasting role of gelatinases in the pathogenesis of colitis. J Immunol. 2006; 177:4103–12.
6. Garg P, Vijay-Kumar M, Wang L, Gewirtz AT, Merlin D, Sitaraman SV. Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis. Am J Physiol Gastrointest Liver Physiol. 2009;296:G175–84.
7. Rosario HS, Waldo SW, Becker SA, Schmid-Schonbein GW. Pancreatic trypsin increases matrix metalloproteinase-9 accumulation and activation during acute intestinal ischemia–reperfusion in the rat. Am J Pathol. 2004;164:1707–16.

Mini Paper 5

OC-090
ENTEROHEMORRHAGIC *ESCHERICHIA COLI*, A NON-CYTOSOLIC PATHOGEN ACTIVATING CYTOSOLIC NLRP3 AND AIM2 INFLAMMASOMES THROUGH RELEASE OF BACTERIAL PRODUCTS INTO THE CYTOSOL

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Enterohemorrhagic *Escherichia coli* (EHEC) induces elevated levels of IL-1β in infected patients. However, the molecular mechanisms involved in the activation of IL-1β by EHEC are unknown. Inflammasomes are multiprotein complexes in the cytosol that process proIL-1β to bioactive IL-1β. To identify the inflammasomes involved in IL-1β induction by EHEC, we infected macrophages derived from wild type and mice deficient in inflammasome components with EHEC and analyzed the processing and release of mature IL-1β. EHEC-induced IL-1β production was dependent on caspase-1 and the adaptor ASC. Notably, both NLRP3, a cytosolic sensor for microbial and endogenous products, and AIM2, a cytosolic sensor for DNA, were absolutely essential for EHECinduced IL-1β production. Though EHEC is a non-cytosolic pathogen, bacterial products such as DNA were observed in the cytosol of infected cells, providing a potential means by which cytosolic inflammasomes might be activated. This also indicated that phagosomal degradation of bacteria is a prerequisite for inflammasome activation by EHEC. Supporting this hypothesis, blocking lysosomal acidification and type I interferon signaling that are essential for bacterial lysis in phagosome, led to a marked decrease in EHEC-induced IL-1β secretion. Thus, our results demonstrate that EHEC potently activates multiple inflammasomes as a result of release of bacterial products from phagosomes into the cytosol.

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen linked to hemorrhagic colitis and a fatal renal complication, hemolytic uremic syndrome (HUS) [1]. An important marker for HUS is IL-1β [2], a potent proinflammatory cytokine that orchestrates antimicrobial host defense. Dysregulation of IL-1β production is linked to several autoimmune and inflammatory diseases, but pathways regulating its production are only recently becoming understood. The synthesis of pro-IL-1β is triggered by the stimulation of pattern recognition receptors (PRRs; signal 1), and its activation is mediated by cytosolic multiprotein complexes called inflammasomes (signal 2). Inflammasomes are formed when cytosolic PRRs, such as NLRP3 and AIM2 sense specific microbial or endogenous danger signals, triggering recruitment of the adaptor. ASC and procaspase-1. The autocatalytic activation of procaspase-1 generates caspase-1, which converts proIL-1β to IL-1β [3]. In this study, we investigated the mechanism by which EHEC induces IL-1β secretion and found, surprisingly, that this extracellular pathogen activates NLRP3 and AIM2 inflammasomes to elicit IL-1β production.

Materials and methods: Bone marrow-derived macrophages (BMDM) from wild type (WT) C57BL/6, ASC$-/-$, Caspase-1$-/-$, NLRP3$-/-$,

IFNR−/−, AIM2+/+, and AIM2−/− mice were infected with EHEC. IL-1β and cleaved caspase-1 in supernatants were analyzed by ELISA and immunoblotting as described [4]. Statistical analysis was conducted by two-way ANOVA and Bonferroni's post-test.

Results: EHEC infection of murine BMDM induced a robust production of pro-IL-1β and its cleavage into IL-1β by caspase-1, indicating that EHEC provides both signal 1 and signal 2 required for the production of bioactive IL-1β. EHEC-induced secretion of mature IL-1β was abrogated in ASC- and caspase-1 deficient macrophages (Fig. 1a, b). To identify the specific inflammasome pathways that sense EHEC, we infected BMDMs deficient in various inflammasome sensors, such as NLRP3 and AIM2. NLRP3 senses intracellular perturbations caused by microbial and endogenous components [5]. Release of IL-1β and cleavage of caspase-1 was completely abolished in EHEC-infected NLRP3−/− macrophages. AIM2 was recently shown to recognize DNA in the cytosolic compartment and activate inflammasome [4, 6]. In our experiments, EHEC-induced caspase-1 cleavage and IL-1β secretion were dramatically reduced in AIM2−/− cells relative to infected WT cells. These data collectively indicate that NLRP3 and AIM2 each have an essential role in inflammasome activation by EHEC (Fig. 1a, b). We and others have previously shown that AIM2 inflammasome detects the cytosolic pathogens [4]. Here, we provide evidence for the first time that AIM2 also plays an essential role in innate sensing of infection with a non-cytosolic bacterium. The role of AIM2 in sensing EHEC was unexpected because EHEC is an extracellular bacterium that is thought to be excluded from the cytosol [7]. To investigate the cellular localization of EHEC, we visualized the fate of GFP-expressing bacteria inside macrophages by confocal microscopy. EHEC was found in lysosomes within 15 min of infection. By 1 h post-infection we observed bacterial DNA but not whole bacteria in the cytosol (Fig. 2). These observations indicate that bacterial degradation products such as DNA can become localized in the cytosol, where they may trigger the activation of AIM2 and NLRP3 inflammasomes.

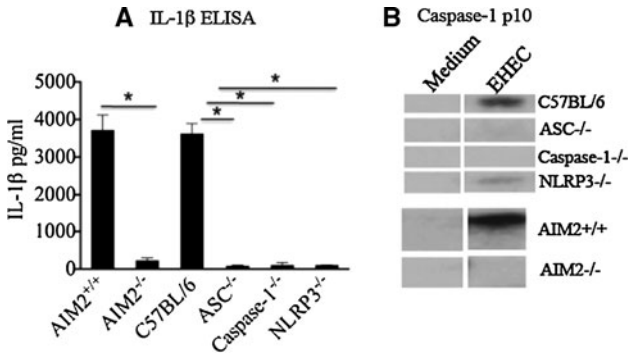

Fig. 1 ELISA for IL-1β (**a**) and immunoblot for cleaved caspase-1 (**b**) in the supernatants of wild type and inflammasome-deficient macrophages infected with 100 MOI of EHEC at 8 h post infection (*$p < 0.05$)

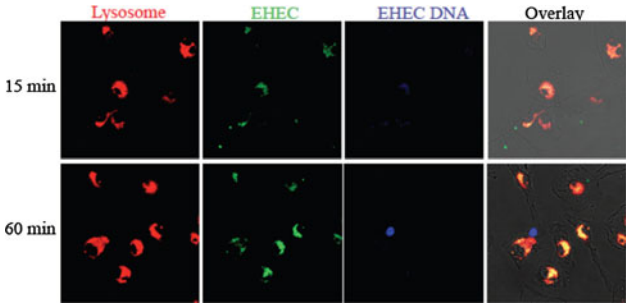

Fig. 2 Confocal microscopy of wild type macrophages infected with live GFPexpressing EHEC. EHEC DNA was stained with DAPI (*blue*) and lysosomes with fluorescent dextran (*red*)

Next, we tested whether bacterial degradation in lysosomes is essential for inflammasome activation by EHEC. Following phagocytosis, acidification of lysosomal compartments leads to activation of proteolytic enzymes in the lysosomes and subsequent bacterial degradation. Prior to infection with EHEC, we treated BMDMs with the vacuolar H + ATPase system blocker, bafilomycin A, which inhibits lysosomal acidification and thus bacterial degradation. Treatment with bafilomycin completely blocked the release of EHEC DNA into the cytosol of macrophages (Fig. 3a). Accordingly, bafilomycin treatment also markedly reduced the IL-1β secretion elicited by EHEC in a dose-dependent manner, similar to its effect on inflammasome activation by silica, which depends on lysosomal acidification (Fig. 3b). Another factor that has been implicated to contribute to bacterial lysis in lysosomes is type I interferon (IFN) signaling [8]. To test whether type I IFNs have a role in EHEC lysis in the lysosomes and thereby in inflammasome activation by EHEC, we infected WT and type I IFN receptor deficient (IFNR-/-) macrophages with EHEC and analyzed the inflammasome activation. EHEC-induced caspase-1 cleavage and IL-1β secretion were markedly decreased in IFNR−/− cells (Fig. 4a, b). Interestingly, regulation of inflammasome activation by type I IFNs is considered to be a phenomenon restricted to cytosolic pathogens [8]. Our data demonstrates that inflammasome activation by extracellular pathogens such as EHEC is also regulated by type I IFNs.

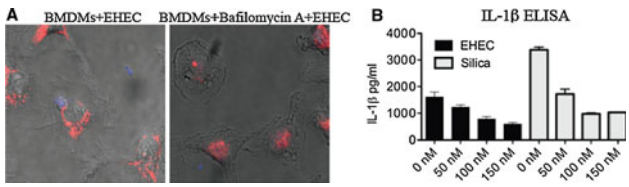

Fig. 3 Confocal microscopy of untreated cells and cells pretreated with 150 mM bafilomycin infected with GFP expressing EHEC in which DNA was tagged with DAPI (**a**). ELISA for IL-1β in supernatants of wild type BMDMs treated with 50–150 nM bafilomycin A or left untreated for 30 min before infecting with EHEC at MOI = 100 for 8 h (**b**)

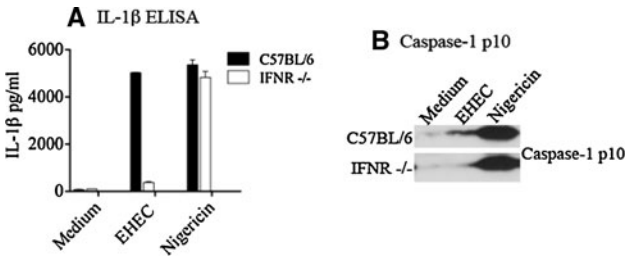

Fig. 4 ELISA for IL-1β (**a**) and immunoblot analysis for caspase-1 p10 (**b**) by wild type and IFNR−/− macrophages infected with 100 MOI of EHEC for 8 h or treated with the control nigericin for 1 h

Discussion: Collectively, our findings reveal that the host innate system employs multiple inflammasome pathways that cooperatively sense EHEC, likely upon release of bacterial components such as DNA into the cytosol. Notably, we demonstrate a role for a novel inflammasome pathway mediated by AIM2 in the detection of a non-cytosolic bacterium. We also show that bacterial lysis in the lysosomes is essential for the activation of inflammasomes by EHEC. These insights raise the prospect of potential therapeutic intervention of EHEC-induced disease by modulation of IL-1β production.

References

1. Croxen MA, Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. Nat Rev Microbiol. 2010;8:26–38.
2. Taneike I, Zhang HM, Wakisaka-Saito N, Yamamoto T. Entero-hemolysin operon of Shiga toxin-producing *Escherichia coli*: a virulence function of inflammatory cytokine production from human monocytes. FEBS Lett. 2002;524:219–24.
3. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140: 821–32.
4. Rathinam VAK, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat Immunol 2010;11:395–402.
5. Franchi L, Munoz-Planillo R, Reimer T, Eigenbrod T, Nunez G. Inflammasomes as microbial sensors. Eur J Immunol. 2010; 40: 611–5.
6. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 2009;458:514–8.
7. Shimada O, Ishikawa H, Tosaka-Shimada H, Atsumi S. Exocytotic secretion of toxins from macrophages infected with *Escherichia coli* O157. Cell Struct Funct 1999; 24:247–53.
8. Henry T, Brotcke A, Weiss DS, Thompson LJ, Monack DM. Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. J Exp Med 2007; 204:987–94.

Mini Paper 6

OC-087
AN OVERVIEW OF THE ROLE OF ANNEXIN-A1 IN ALLERGIC DISEASE

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Mast cells, basophils and eosinophils represent the first line of host defence in allergic inflammation reaction. We investigated the mechanism by which the anti-allergic drugs di-sodium cromoglycate and sodium nedocromil inhibit histamine and eicosanoid release from human mast cells (HMCs) as well as peripheral blood basophils (PBBs) using biochemical techniques. Cord derived HMCs were cultured in vitro and PBBs obtained from human donors or cardiac puncture in mice. Subsequent antigen challenged led to a prompt release of histamine and prostaglandin D_2 (PGD_2), which was inhibited by cromoglycate, nedocromil, dexamethasone and human recombinant Annexin-A1 (Anx-A1). Treatment of HMCs with cromoglycate or nedocromil induced a rapid phosphorylation on the Ser^{27} residue of Anx-A1, hence its secretion, secondary to inhibition of phosphatase activity and activation of PKC. Treatment of the HMCs or PBBs with a specific neutralising anti-Anx-A1 (but not irrelevant) monoclonal antibodies reversed the inhibitory effect of these drugs on histamine and PGD_2-released. To highlight the crucial importance of Anx-A1 in this rapid effect of these drugs we tested the effect of nedocromil in Anx-A1 null mice. Cromones did not inhibit the release of histamine nor prostaglandin D2 from basophils from Anx-A1 null mice.To translate these new findings clinically, we have analyzed Anx-A1 pattern of expression in tears from patients with Vernal KeratoConjuntivitis (VKC). While this seasonal disease is associated with an increased amount of the clipped- or inactive-form of Anx-A1, VKC patients treated with the cromoglycate-like Alomide (or Lodoxamide) show an augmentation of the total-or active-form of the protein. Al-together, the use of in vitro, in vivo investigations and the translational outcome of VKC allergic disease, suggest strongly the anti-inflammatory Anx-A1 protein to be a component of rapid cromone actions on mast cells, basophils and eosinophils.

Introduction: While the cromoglycate-like drugs can inhibit both the early and the late phase of the asthmatic reaction, their anti-asthmatic activity is attributed to their anti-inflammatory properties by most authorities [1]. Although cromoglycate, the prototype drug was developed in the 1960s the exact mechanism of action of this group of drugs has proved elusive. Early experiments [2] led to the concept that these drugs acted mainly on mast cells to suppress mediator release, but they also exert other therapeutically significant actions and mast cells are not their sole target. They are also effective in other models of inflammation and influence many facets of the inflammatory process unrelated to mast cell activation in vivo [3] or in vitro, e.g. eicosanoid generation [4]. We have recently reported that the ability of these drugs to inhibit leukocyte activation [5] and eicosanoid release by U937 cells [6] depends upon their ability to release the anti-inflammatory protein Anx-A1 secondary to an inhibitory action on the phosphatase PP2A. We demonstrate that this process is also required for the acute effect of the cromones to inhibit histamine and eicosanoid secretion by human mast cells and basophils thus supplying an important mechanistic explanation for the pharmacological action of these 50-year old drugs.

Methods: *Cord derived human mast cell culture* Commercial purified $CD34^+$ stem cells were cultured for the first 2 weeks in StemSpan (StemCell technologies) serum-free medium supplemented with 100 ng/ml human SCF, 50 ng/ml IL-6 and 1 ng/ml IL-3, and 100 μg/ml penicillin/streptomycin. After the 8 week, cells were cultured in StemSpan with 10% FCS. *Assessment of Ser^{27}-Anx-A1-P and PKC activation* The total cellular protein was determined and supernatants analysed by conventional western blotting techniques. Immunodetection was accomplished using different antibodies recognizing either the full-length Anx-A1 protein, (polyclonal anti-Anx-A1 antibody), Anx-A1 phosphorylated on Ser27 (polyclonal anti-Ser27-Anx-A1), PKC phosphorylation (polyclonal anti-phospho-PKC antibody) and α-tubulin (monoclonal anti-α-tubulin). *Measurement of Anx-A1, PGD_2 and histamine release* An enzyme immunoassay was established to detect and

quantify Anx-A1, PGD_2 or histamine-released in the supernatant. The method was conducted following the manufacturer's protocols. Results: *Phosphorylation and secretion of Anx-A1 by HMCs.* In Fig. 1a, all treatments on HMCs resulted in a significant increase (~2.5- to 3.5-fold) in the amounts of Anx-A1 and PKC phosphorylated hence an augmentation of Anx-A1 secretion in the cell culture fluid (Fig. 1b). Okadaic acid, a known inhibitor of PP2A was included here as a positive control. *Effect of human recombinant Anx-A1 protein on histamine and PGD_2 released from HMCs* We next wanted to relate the increment of Anx-A1 protein secreted after treatment with nedocromil or dexamethasone (shown in Fig. 1) with the ability of these drugs to inhibit histamine and PGD_2 release. Figure 2a shows that human recombinant Anx-A1 protein at 1 and 10 nM is able to inhibit to the same extent as nedocromil (10 nM) and dexamethasone (2 nM) the level of histamine and PGD_2 release from IgE-activated HMCs. To ascertain the role of Anx-A1 in the mechanism of action of nedocromil sodium, the effect of a specific neutralizing anti-Anx-A1 antibody was tested. Figure 2b shows the effect of the co-incubation of HMCs with neutralising anti-Anx-A1 (and irrelevant mabs) on the effect of inhibitory action of nedocromil. Under control conditions the drug inhibited histamine and PGD_2 release by approximately 50%. This effect was completely abolished in the presence of the neutralising anti Anx-A1 mab (<6% inhibition), but not the irrelevant antibody. *Effect of cromones on mediators released from human peripheral blood basophils and in Anx-A1 null mice* Nedocromil suppressed Anx-A1 released by IgE challenged PBBs by approximately 45% and this effect is evident at 10 min. There was a concomitant increase in the amount of Anx-A1 released which rose by four- to fivefold with a maximum seen at 20 min by which time approximately 90% of the intracellular store was released. To extend this concept we turned to the use of transgenic mice lacking the Anx-A1 gene. Figure 3b shows that subsequent challenge on blood with anti-IgE for 30 min releases histamine (approximately 1.5- to 3.0-fold increase above basal levels). This was strongly (~50%) inhibited in the presence of nedocromil in blood taken from wild type animals but not that taken from Anx-A1 null mice. *Anx-A1 content in tears from vernal keratoconjunctivitis patients* The seasonal allergic disease is associated with 50% increase of Anx-A1 biologically inactive clipped form measured by ELISA in tears. This is reversed following treatment with the cromoglycate-like drug Alomide, which increases both the total amount and the relative proportion of intact Anx-A1 (Fig. 4).

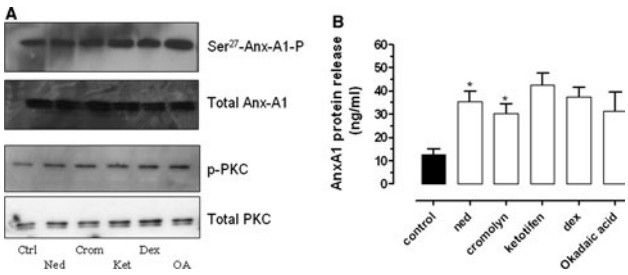

Fig. 1 Cromones promote externalisation of Anx-A1 from HMC cells. HMC cells were oultured as described and incubated with vehicle alone, nedocromil (10 nM), cromoglyoate (10 nM), ketotifen (10 nM), dexamethasone (2 nM) or okadaic acid (10 nM). Anx-A1 and PKC phosphorylation were assessed by Western Blotting (**a**). Anx-A1 released into the supernatant after 5 min incubation with the drugs was assessed using an ELISA assay and expressed as ng/ml culture fluid (**b**). * $P < 0.05$ relative to vehicle treated cells; ANOWV-bonferroni test. (Data redrawn from Yazid et al., submitted 2011)

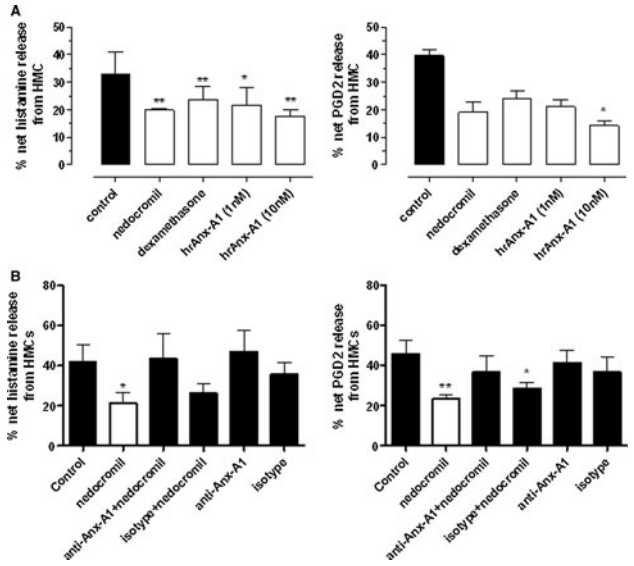

Fig. 2 Measurement of histamine and PGD, release from HMCs. **a** Human recombinant Anx-A1 protein inhibits histamine and PGD, release from HMCs. HMCs were cultured, sensitised with IgE and challenged with antigen. Vehicle or nedocromil (10 nM) or dexamethasone (2 nM) was added at the same time to the wells and their inhibitory effect compared with incubation of HMCs with human recombinant Anx-A1 protein at 1 and 10 nM. The cell culture supernatant was sampled and the net % release of histamine and PGD_2 was assessed by ELISA. *Signifies $P < 0.05$ and **$P < 0.01$ relative to vehicle treated cells. ANOVA/Bonferroni test. (Data redrawn from Yazid et al., submitted 2011). **b** The inhibitory effect of nedocromil on antigen-induced histamine and PGD, release is Anx-A1 dependent. Vehicle, nedocromil (10 nM) alone or in the presence of 10 μg/ml neutralising anti-Anx-A1 mab (or an irrelevant control) was added to wells containing HMCs and 5 min before challenge with anti-IgE. Histamine and PGD2 release into the cell culture fluid was assessed using ELISA and expressed as a percentage of net histamine released from HMCs *$P < 0.05$ relative to the appropriate controls. ANOVA/Bonferroni test. (Data redrawn from Yazid et al., submitted 2011)

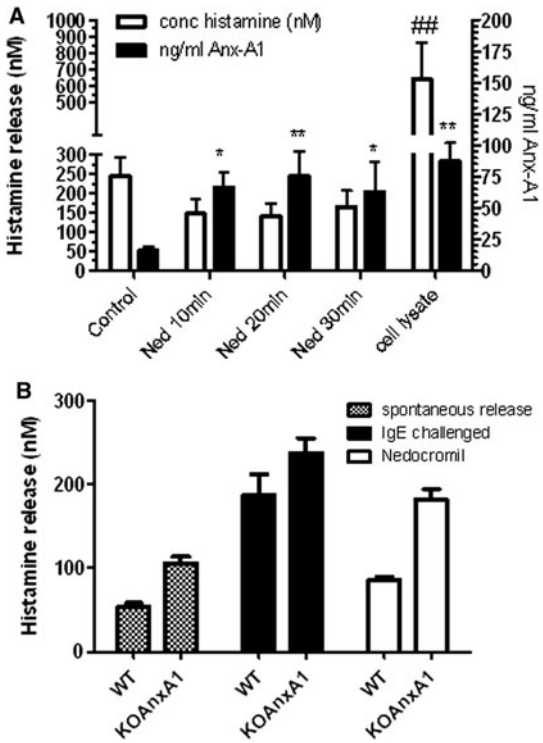

Fig. 3 Inhibition of histamine released from peripheral blood basophils is Anx-A1 mediated. **a** Vehicle or nedocromil (10 nM) was added to the wells for 5 min and the whole blood cells were challenged for 30 min with anti-IgE. At various time points (10, 20 30 min), the cell culture supernatant was sampled and the histamine concentration (expressed as nM) and the Anx-A1 concentration (expressed as ng/ml), was assessed by ELISA. The total cell lysate histamine and intracellular Anx-A1 concentrations are included for comparison purposes. *$P < 0.05$; **$P < 0.01$ relative to the challenged but untreated control cells. $^{\#\#}P < 0.01$ relative to the WBCs histamine content of untreated control WBCs. ANOVA Bonferroni test. (Data from Yazid et al., submitted 2011). **b** PBBs were obtained by cardiac puncture from wild type or Anx-A1 null mice (n = 6 mice/group). PBBs were incubated with 10 nM nedocromil for 5 min prior to challenge with anti-lgE. Histamine release from cell suspensions was assessed by ELISA and expressed as nM. PBBs from both wild type and Anx-A1 cells released histamine in response to IgE challenge. Nedocromil totally inhibited the release from the wild type but not the Anx-A1 deficient cells (data from Yazid et al., submitted 2011)

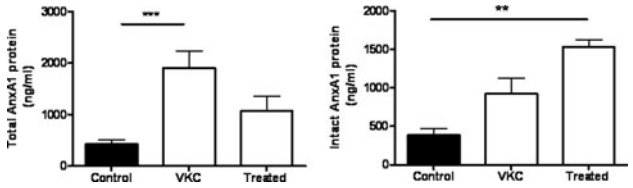

Fig. 4 Cromoglycate-like Alomide (Lodoxamide) increases Anx-A1 in tears from patients with VKC. Tears from VKC patients contain increased amount of the cleavage (inactive) form of Anx-A1 This is reversed (as a measure of intact form) when patients treated with the cromone-like Alomide. $^{**}p < 0.01$, $^{***}p < 0.001$ ANOVABonferroni test. ($n = 17$ control group, $n = 23$ VKC group and $n = 11$ Alomide treated group). Yazid et al., unpublished data, 2011

Discussion: Our data here clearly indicates that cromone drugs promote the phosphorylation, externalisation and release of Anx-A1 and that this process is mandatory for these drugs to bring about their inhibition of histamine and eicosanoid release in HMCs as well as blood basophils. That this mechanism is common to several cell types is shown by our previous observations using U937 cells and PMN, where a similar Anx-A1 dependent inhibition of cell function was observed. I propose that this mechanism underlies the acute inhibitory effect of these drugs on mast cell mediator release and accounts for the aspect of their pharmacology.

References

1. Barnes PJ. Anti-inflammatory therapy for asthma. Annu Rev Med. 1993;44:229–42.
2. Cox JS. Disodium cromoglycate (FPL 670) ('Intal'): a specific inhibitor of reaginic antibody-antigen mechanisms. Nature. 1967;216: 1328–9.
3. Kuriyama K, Hiyama Y, Nagatahira R, Okuda T, Saito K, Ito K. An antiallergic activity of disodium cromoglycate unrelated to mast cell activation. Agents Actions. 1986;18:473–8.
4. Mattoli S, Mezzetti M, Fasoli A, Patalano F, Allegra L. Nedocromil sodium prevents the release of 15-hydroxyeicosatetraenoic acid from human bronchial epithelial cells exposed to toluene diisocyanate in vitro. Int Arch Allergy Appl Immunol. 1990;92:16–22.
5. Yazid S, Leoni G, Getting SJ, et al. Antiallergic cromones inhibit neutrophil recruitment onto vascular endothelium via annexin-A1 mobilization. Arterioscler Thromb Vasc Biol. 2010;30:1718-1724.
6. Yazid S, Solito E, Christian H, McArthur S, Goulding N, Flower R. Cromoglycate drugs suppress eicosanoid generation in U937 cells by promoting the release of Anx-A1. Biochem Pharmacol. 2009;77: 1814–26.

Animal work was performed according to Home Office regulations and was approved by the Queen Mary University of London Ethics Committee. Human cells and clinical sample studies were prepared according to a protocol approved by the East London & the City Local Research Ethics Committee.

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INVITED SPEAKERS