Meeting report

Kennedy Institute of Rheumatology Division, Imperial College London, 12–13 November 2003: Towards a molecular toolkit for studying lymphocyte function in inflammatory arthritis

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Abstract

T lymphocytes have been implicated in the pathogenesis of inflammatory arthritis for approximately 30 years. Over that period a vast literature has described the phenotype, location and behaviour of T cells derived from patients with inflammatory rheumatological disease. The arthritiogenic roles of MHC class I and class II molecules, which present antigen to T cells, have been hotly debated. The T cell has been variously conceived as a central or peripheral (or even incidental) component in the arthritogenic response. Rapid developments in genomics and use of biological therapeutic agents coupled with recent insights in the field of T cell differentiation and immunoregulation together offer novel methods of investigating the pathogenesis of chronic inflammatory disease. A number of UK researchers, with diverse interests within the field of synovitis, met recently at the Kennedy Institute of Rheumatology. Presentations on T cell memory, cytokines of homeostasis and inflammation, unconventional behaviour of MHC molecules and immunoregulation in murine models, rheumatoid and spondyloarthritis reflected the breadth of the discussion.

Keywords: cytokines, HLA-B27, immunoregulation, migration, rheumatoid arthritis, spondyloarthritis

Introduction

Despite many years of study, the aetiology of inflammatory arthritis remains poorly understood. A growing body of data describing leukocyte differentiation, migration and cellular interactions has put us in a promising position to further dissect the molecular basis of inflammatory arthritis. A recent meeting brought together more than 60 researchers from across the UK at the Kennedy Institute of Rheumatology, Imperial College, London. The informal atmosphere of the meeting encouraged the presentation of recent results and novel ideas by 20 speakers covering four themes.

T cell activation and differentiation

Professor M Salmon (Birmingham University, UK) outlined recent changes in the model of T cell differentiation in which activation turns naive CD45RA$^+$ T cells into CD45RO$^+$ primed/memory T cells, which divide periodically until they die. It is now clear that both CD4$^+$ and CD8$^+$ subsets contain CD45RA$^+$ memory cells. Detailed study of CD8 memory using MHC class I/viral peptide tetramers has defined several new models of CD8 differentiation according to the changing expression of numerous cell surface markers. Memory CD45RA$^+$ cells are now widely accepted; their function, particularly proliferative potential, is currently under debate. Professor Salmon showed proliferation in CD8CD45RA$^+$ memory cells, but only under stringent stimulation conditions; this may explain the poor responses reported for these cells. These new concepts of differentiation have prompted re-examination of T cells in arthritis. Lymphocyte function-associated antigen-1 (LFA-1) and the chemokine receptor CCR7

β_2m = β_2 microglobulin; IFN-γ = interferon-γ; IL = interleukin; LFA-1 = lymphocyte function-associated antigen-1; NK = natural killer cells; PPD = purified protein derivative; RA = rheumatoid arthritis; SCID = severe combined immunodeficiency; SF = synovial fluid; TCR = T cell receptor; Th = T helper cells; TNF = tumour necrosis factor; TREC = T cell receptor excision circle.

discriminate the two CD45RA$^+$ populations in healthy subjects; naive cells are LFA-1^{low}CCR7^{high}, memory cells LFA-1^{high}CCR7^{low} [1]. Dr J Faint (Birmingham University, UK) has characterised CD8$^+$CD45RA$^+$ cells found in rheumatoid synovial infiltrates. Synovial CD8CD45RA$^+$ cells are LFA-1^{high} memory cells, containing Epstein–Barr virus tetramer binding cells in seropositive subjects. Some synovial, but not blood, CD8CD45RA$^+$ memory cells expressed CCR7, which could be induced by culture in rheumatoid synovial fluid (SF). CCR7 directs migration to lymph nodes, with naive T cells migrating through high endothelial venules, and maturing tissue dendritic cells to afferent lymphatics. These data suggest that tissue infiltrating T cells might operate a similar mechanism to return to draining lymph nodes.

T cell differentiation in arthritis was also examined by Dr F Ponchel (Leeds University, UK), using differential expression of CD45 isoforms and T cell receptor excision circle (TREC) analysis [2]. TRECs are not replicated during division and provide an indication of the replicative history of cell populations. Patients with rheumatoid arthritis (RA) had reduced frequencies of naive and 'conventional' memory cells compared with healthy donors, yet expressed additional populations not evident in controls. This might result from lymphopoenia, which is a feature common to many diseases. Reduced bone marrow stromal cell production of interleukin (IL)-7 in rheumatoid patients leads to a lack of circulating cytokine, which was restored in some patients by therapy with anti-tumour necrosis factor-α (anti-TNF-α) antibodies.

In addition to the alterations in subset frequencies, T cells in rheumatoid patients are hyporesponsive to stimulation through the T-cell receptor (TCR). Dr A Cope (Kennedy Institute, Imperial College, London, UK) demonstrated that TCR triggering leads to transient internalisation and subsequent re-expression of TCR/CD3. Chronically stimulated cells, particularly in the presence of TNF-α, show sustained low-level expression of the ζ signalling chain of the CD3 complex, impairing signal transduction in these cells [3]. TCRζ^{dim} cells express many markers typical of highly differentiated, senescent effector cells, and respond poorly to stimulation by CD3/CD28. The rheumatoid synovium is highly enriched in TCRζ^{dim} cells, which might explain their hyporesponsiveness, while also suggesting that effector responses of these cells are relatively independent of antigen signals. Tissue-infiltrating cells in arthritis seem to be chronically stimulated, yet it is unclear how they compare with cells during an evolving immune response. Professor A Akbar (University College London, UK) has modelled a cutaneous inflammatory response to purified protein derivative (PPD), showing the T cell infiltrate to be oligoclonal and extensively proliferating; 80% of clones were maintained between days 7 and 19 of the response. T cell proliferation is affected by telomere shortening (repeating sequences of DNA found at the chromosome ends) with each cell division, eventually leading to replicative senescence; the enzyme telomerase can also lengthen telomeres. Shortened telomeres were seen from day 14, yet little telomerase activity was evident *in vivo*. Cells stimulated *in vitro* upregulated telomerase. This was inhibited by fluid obtained from inflammatory blisters and might explain the limitation of expansion of specific T cells at sites of inflammation.

Synovial infiltrating T cells have been extensively studied, yet they are far outnumbered by neutrophils. Professor R Moots (Liverpool University, UK) has been dissecting the interactions between these cells. Joint neutrophils are long lived and produce many inflammatory mediators. Neutrophils from healthy subjects express MHC class II after culture in a cytokine cocktail, whereas rheumatoid joint neutrophils spontaneously express MHC class II after overnight culture [4]. These neutrophils can present superantigen to T cells *in vitro*, suggesting a possible antigen-presenting function for neutrophils; however, this activity has yet to be demonstrated *in vivo*.

Lymphocyte recognition of HLA-B27

The association of ankylosing spondylitis with HLA-B27 is among the strongest described for an HLA locus, giving an odds ratio of about 161. HLA-B27 also exhibits associations with other spondyloarthropathies, including reactive arthritis, psoriatic spondyloarthritis and spondyloarthritis associated with inflammatory bowel disease.

Dr P Bowness (Weatherall Institute of Molecular Medicine, Oxford, UK) discussed the unusual biology of HLA-B27 molecules and the implications of this for recognition by T cells and natural killer (NK) cells. Classically the heavy chain of MHC class I molecules folds in association with β_2 microglobulin (β_2m) and a nonamer peptide. This monomer presents the peptide to TCRs on CD8$^+$ T cells. Indeed, the HLA-B27 monomer is capable of presenting several peptides derived from bacterial triggers of reactive arthritis. HLA-B27 is unusually (although not uniquely) able to form heavy-chain homodimers, via a disulphide bridge at Cys 67 [5]. Modelling this complex suggests that the α1 helix unfolds and that the peptide-binding groove can accommodate a peptide much longer than a nonamer. Expression of HLA-B27 heavy chain in cells deficient in TAP (transporter associated with antigen processing) results in homodimer formation, demonstrated by binding of homodimer-specific monoclonal antibody. Mice transgenic for HLA-B27, but deficient for β_2m, express large quantities of surface homodimer. HLA-B27$^+$ β_2m$^+$ mice express the conventional monomeric molecule but low levels of homodimer are also detectable. HLA-B27 homodimer tetramers have revealed that about 5% of peripheral CD8$^+$ T cells are capable of recognising the HLA-B27 in this conformation. Of great interest is the

observation that homodimer tetramers also bind to B cells, monocytes and NK cells. Further work is under way to characterise the profile of receptors that recognise the homodimer, and the relationship of homodimer formation to the development and activity of spondyloarthritis.

Conventionally, HLA-B27/peptide is recognised by $CD8^+$ T cells. The hypothesis that some $CD4^+$ T cells might recognise HLA-B27 arose from observations in spondyloarthritic HLA-B27-transgenic mice [6]. Despite the apparent importance of HLA-B27 in the pathogenesis, $CD8^+$ T cells were shown not to be essential for the development of arthritis; indeed, disease was transferable with $CD4^+$ T cells. Professor JSH Gaston (University of Cambridge School of Clinical Medicine, UK) discussed the characterisation of a variety of unconventional T cell clones from patients with spondyloarthritis. Several patterns of specificity in these $CD4^+$ clones have emerged: first, proliferation in response to HLA-B27, either empty monomers or, possibly, homodimers; second, proliferation in response to an HLA-B27-derived peptide presented by HLA-Cw1; and third, proliferation in response to an HLA-B27-derived peptide presented by alloantigens, including HLA-B51 and HLA-A2.

Dr H Bodmer (Edward Jenner Institute for Vaccine Research, Newbury, UK) continued the discussion on the nature of HLA-B27-restricted $CD4^+$ T cells. A double transgenic mouse model expressing GRb (TCR specific for HLA-B27 presenting influenza nucleoprotein 383–391) and HLA-B27/β_2m revealed the presence of HLA-B27-restricted $CD4^+$ as well as $CD8^+$ T cells. The $CD4^+$ cell required the presence of MHC class II to be selected, presumably by dual α-chain expression [7]. However, the selected $CD4^+$ T cells responded to HLA-B27/NP(383–391) with similar sensitivity to the $CD8^+$ T cells. *In vitro*, both Th1 and Th2 differentiation of HLA-B27-restricted $CD4^+$ T cells could be achieved. The expression of MHC class II molecules is generally restricted to professional antigen-presenting cells, so as to regulate the initiation of responses by $CD4^+$ T helper cells. Recognition of MHC class I molecules by $CD4^+$ T cells could therefore have important implications for the initiation of autoimmunity. Professor Gaston also discussed the isolation of unconventional $CD8^+$ T cells from patients with spondyloarthropathy. These autoreactive $CD8^+$ T cell lines/clones produce IL-4 but not interferon-γ (IFN-γ). They constitutively express the $\alpha\beta$ TCR, CD69, CD25 and CD30 and express CD40L on activation; they are perforin-negative and express only low levels of granzyme. These clones are restricted by autologous MHC class I molecules and are dependent on an unidentified (ubiquitous?) peptide. The possibility that they are $CD8^+$ T regulatory cells is under investigation.

Immunoregulation

Immunoregulation is an integral part of normal innate and adaptive immune responses. Recently, the literature has burgeoned with the reports of the regulatory properties of $CD4^+$ T cells which constitutively express CD25. It is clear that regulation does not reside exclusively within this group but has also been attributed to subsets of $CD8^+$ T cells (see above), dendritic cells, NK cells, neutrophils and eosinophils, as well as to the plethora of soluble regulatory molecules. A tangential but germane observation is that pathogenic microbes have developed an astonishing capacity for host immunointerference. Many multicellular parasites exemplify the delicate balance of immunomodulation without global immunosuppression; this permits chronic infection of the host, often over decades.

Dr L Taams (Kings College, London, UK) presented comparisons of the phenotype and function of $CD4^+$ $CD25^+$ T cells in healthy controls and in both peripheral blood and SF of patients with RA. *In vitro*, the $CD4^+CD25^+$ T cells from RA and control peripheral blood were equally potent at inhibiting the proliferation of $CD4^+CD25^-$ T cells. The $CD4^+CD25^+$ T cells derived from RA SF were more potent suppressors but, curiously, the $CD4^+CD25^-$ T cells from SF were more resistant to proliferative suppression. Peripheral blood $CD4^+CD25^+$ cells modified cytokine release in an antigen-specific stimulation of $CD4^+CD25^-$ cells. TNF-α production was reduced in $CD4^+CD25^-$ cells from peripheral blood, whereas IFN-γ and IL-10 were both decreased in $CD25^-$ cells from SF. Continuing the theme of regulatory T cells in RA, Michael Ehrenstein (Centre for Rheumatology Research, London, UK) discussed the change in function of $CD4^+CD25^+$ T cells in the peripheral blood of patients with RA before and after TNF-α blocking therapy. In comparison with healthy controls, patients with RA demonstrated a defect in the ability of their $CD4^+CD25^+$ T cells to inhibit the production of TNF-α by monocytes. This defect was unchanged by treatment with methotrexate but was restored in patients who were successfully treated with TNF-α blockade.

Dr L Wedderburn (Institute of Child Health, London, UK) advanced the hypothesis that immune regulation contributes to the mild phenotype of children with oligoarticular juvenile idiopathic arthritis compared with patients with extended oligoarthritis. The $CD25^+$ and particularly $CD25^{hi}$ T cells in the SF of patients with juvenile idiopathic arthritis exhibited a regulatory phenotype ($GITR^+$, $CTLA\text{-}4^+$, $[FoxP3\ mRNA]^{high}$). The regulatory activity of this subset was also supported by the demonstration that depletion of SF $CD25^+$ cells releases the proliferation of $CD25^-$ cells. T cells in SF from children with oligoarthritis expressed an approximately ten-fold higher CD25 expression than T cells from patients with extended oligoarthritis, which is of particular interest because the most potent regulatory activity has been shown to reside in the $CD4^+CD25^{hi}$ subset. Children with mild disease also had higher overall numbers of $CD4^+CD25^+$ cells in the SF. Although

suppressive potency per cell seemed comparable in cells from patients with mild and severe disease, these observations are consistent with the hypothesis that the $CD4^+CD25^+$ regulatory subset is less effective in the children who develop extended oligoarticular arthritis.

Frances Hall (University of Cambridge School of Clinical Medicine, UK) presented data from a murine model of inflammatory arthritis that suggested a role for regulatory T cells in the repression of arthritis and dermatitis. Adult DBA/1 males were thymectomised and then treated with CD25-depleting antibody, which depleted both $CD4^+CD25^{med}$ and $CD4^+CD25^{hi}$ cells. Although a low level of inflammatory arthritis is usually evident in elderly DBA/1 males, the CD25-depleted mice developed more severe arthritis. Histologically, this was associated with pannus formation and erosions. In addition, about 60% of the CD25-depleted mice also developed an ulcerating rash, characterised by a dense neutrophilic infiltrate. The nature of the emerging inflammatory disease and the influence of the genetic background of the mice are currently under investigation.

Dr L Pazmany (Clinical Sciences Centre, Liverpool, UK) broadened the immunoregulatory discussion by considering the role of NK cells. NK cells are present at autoimmune sites of pathology, including the rheumatoid joint [8]. In the murine model of extrinsic allergic encephalitis, depletion of NK cells resulted in an exacerbation of disease. For other models of autoimmune disease, results of NK depletion have been variable. The effect of addition of autologous NK cells to a PPD-pulsed culture of T cells and autologous antigen-presenting cells was investigated, using proliferation as a read-out. The effect of freshly isolated NK cells was dependent on the donor. However, NK cells incubated with IL-12 reproducibly inhibited T cell proliferation, even at NK:T ratios of 1:10. The significance of these observations for the susceptibility of individuals to RA and for the severity of disease is being investigated.

Professor I McInnes (University of Glasgow, UK) demonstrated how the immunoregulatory prowess of multicellular parasites might inform our search for the optimal disease-modifying agent. The filarial protein ES62 promotes a Th2 response in the BALB/c mouse. It seems to bind to TLR4 and promotes the development of type 2 dendritic cells, thereby decreasing IL-12 and TNF-α production by macrophages. In the murine collagen-induced arthritis model, serial subcutaneous ES62 administration decreases the severity, although not the incidence, of disease [9]. This is associated with a decrease in the proliferation and production of IL-6, IFN-γ and TNF-α by draining lymph node cells from bovine collagen type II immunised mice. This effect of ES62 is mirrored by a decrease in the production of TNF-α and IL-6 in primary synovial membrane cultures. Because parasitic products such as ES62 might be well tolerated for decades, without global immunosuppression, they seem a promising therapeutic strategy for Th1-dominant inflammatory diseases, such as RA.

Homing and effector function

Tissue-specific 'address codes' have been defined for skin and gut homing lymphocytes by using the pattern of chemokine receptors and adhesion molecules that they express. Isolating specific microvascular vessels has proved difficult and has prevented the definition of a synovial tissue code. Professor C Pitzalis (Guy's Hospital, London, UK) outlined a novel approach to this problem by using phage display libraries constructed from blood vessels formed in human synovial tissue transplanted into SCID (severe combined immunodeficiency) mice [10]. Initial results show promise in isolating joint-specific homing markers. He also showed that the rheumatoid joint has a similar spatial organisation of lymphocytes and chemokines to that seen in lymph nodes. Lymph node development requires careful and coordinated interactions between different cell types; these results indicate that similar interactions might occur in the joint. The theme of cell–cell interactions was continued by Professor F Brennan (Kennedy Institute, Imperial College, London, UK). T cells prolong spontaneous TNF-α release from cultured rheumatoid synovial membrane cells through cell contact with macrophages. Using an *in vitro* model, T cells activated by TCR crosslinking induced monocytes to produce TNF-α and IL-10, whereas T cells stimulated with a cytokine cocktail (analogous to bystander activation) induced predominantly TNF-α production. By using pharmacological inhibitors it now seems that these distinct responses arise through the recruitment of different signal transduction pathways in responder macrophages. Thus, phosphoinositide 3-kinase inhibitors reduced cytokine production induced by TCR-stimulated T cells, yet NFκB inhibitors enhanced the response. The opposite responses to inhibitors were seen if bystander T cells were used. Importantly, rheumatoid synovial T cells behave like bystander activated cells, providing further clues to the mechanisms of T cell effector responses in the joint. $CD3^-$ $CD56^+$ NK cells present in bystander activated populations also induced TNF-α from synovial membrane cells in a contact-dependent manner. Joint-infiltrating $CD56^+$ cells were shown by Professor M Callan (Imperial College, London, UK) to enhance TNF-α production by monocytes. About 10% of NK cells from peripheral blood express higher levels of CD56 than the majority of cells, yet $CD56^{bright}$ cells were the predominant population infiltrating the joint [11]. Attempts to differentiate $CD56^{int}$ into $CD56^{bright}$ cells have failed, indicating that they represent two separate lineages. $CD56^{bright}$ NK cells express low levels of perforin, mount poor cytotoxic responses and produce many proinflammatory cytokines on stimulation. These activities might further exacerbate responses in inflammatory sites.

Professor J Isaacs (Newcastle University, UK) concluded with some words of caution about our ability to develop novel antibody therapies, which have generally proved less effective in humans than in the mouse. This might be due to targeting the wrong molecules or the wrong epitopes of those molecules, or to incorrect doses and duration of therapy. Tolerance might take time to develop, and concomitant therapy might actually hinder its development. A further complication is that some therapies might initially exacerbate symptoms before providing benefits. This might partly account for the clinical improvement seen in some patients in antibody trials after treatment was stopped owing to disease flares. The challenge for the future will lie in recognising what are side effects of treatment and what are potentially dangerous turns in disease.

Conclusions

The overwhelming message from this meeting was the appreciation of not only the diversity of cell types present in the inflamed joint but also the diversity of their interactions. No cell type seems to be solely responsible for the maintenance of inflammation; rather, it is the interactions between the multiple cell types present. This presents a number of potential targets for future therapies, yet suggests that an effective cure will require multiple interventions targeting multiple pathways.

Competing interests

None declared.

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