Abstracts

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Genetics of Rheumatoid Arthritis

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Genetic associations between rheumatoid arthritis and specific HLA class II genes provide clues to understanding the molecular basis for disease susceptibility. There is a remarkable structural relationship among different rheumatoid arthritis (RA) susceptibility genes, in which each of the associated class II alleles encodes a sequence of key amino acids termed the 'shared epitope.' Mechanistic models to account for the shared epitope association with RA can be interpreted in the context of an HLA-directed pathway for the development of disease. We suggest that altered T cell activation results from recognition of the shared epitope, providing a potential mechanism by which the shared epitope may be involved in the generation or modulation of self-recognition during antigen presentation and processing. We propose that the shared epitope association with RA is not solely based on a specific peptide binding motif and peptide determinant selection but rather is influenced by a strongly biased direct recognition of shared epitope residues by direct T cell contact.

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Immunogenetic Aspects of Disease Progression in Rheumatoid Arthritis

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In an ongoing collaborative prospective study aimed at the identification of prognostic factors for the development of erosive disease and clinical severity of disease in early rheumatoid arthritis (RA) [1], 48 patients were followed for more than 4 years, and 87 patients were seen for 2 years. Significant associations with progressive joint destruction, measured by the Larsen index, were observed after 2 and 4 years for three parameters: 1) the presence of rheumatoid factor IgM; 2) bony erosions present at study entry, and 3) HLA DRB1 markers. Patients who expressed the shared epitope on a DR4 allele had significantly higher Larsen indices after 2 years (0.86 *vs* 0.12; $P=0.0015$) and after 4 years (1.22 *vs* 0.53; $P=0.002$) of disease duration. Similarly, the presence of the epitope sequence on either DR1 or DR4 also resulted in higher Larsen indices for epitope-positive patients (0.59 *vs* 0.06; $P=0.006$ after 2 years, and 1.0 *vs* 0.69; $P=0.03$ after 4 years). A more severe radiologic outcome after 2 years (Larsen index >0.7) was detected with a sensitivity of 0.7, 0.61, and 0.58 and a specificity of 0.42, 0.84 and 0.75 using RF IgM, erosiveness at initial presentation, and presence of the shared epitope on a DR4 as prognostic parameters. Most useful, however, was the combination of DR4 positivity and erosiveness at study entry as prognostic indicators of a more severe course of joint destruction (sensitivity 0.68; specificity 0.77).

In summary, seropositivity, early erosiveness, and RA-associated HLA-DRB1 markers are useful prognostic indicators of the progression of joint destruction. Moreover, this influence is sustained during the first four years of the course of the disease.

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Rheumatoid Arthritis: A Polygenic Disease with Multiple Phenotypes

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Rheumatoid arthritis (RA) is recognized as a multigene disorder with a number of genetic polymorphisms contributing to disease pathogenesis. Here, we propose that the diagnostic category of RA includes multiple subtypes of disease and that the different phenotypes of RA correlate to different genotypes.

Support for this concept has come from a reappraisal of the clinical heterogeneity of RA and the observation that HLA-DRB1 polymorphisms are useful in describing genetic heterogeneity of RA phenotypes. A series of HLA-DRB1 genes has been identified as RA-associated and, in recent years, emphasis has been put on the sequence similarities of these alleles. An alternative view focuses on the amino acid variations found with different alleles being enriched in distinct subtypes of RA. Rheumatoid factor positive destructive joint disease is predominantly associated with the HLA-DRB1*0401 allele while HLA-DRB1*0404 and B1*0101 predisposes for milder and often seronegative disease. Expression of disease-associated alleles on both haplotypes carries a high risk for extra-articular manifestations. Besides HLA gene polymorphisms, emergence of CD28-deficient CD4 T cells identifies RA patients with extra-articular manifestations. These cells undergo clonal expansion *in vivo*, produce high amounts of IFN-γ and exhibit autoreactivity. Concordance of monozygotic twins for the expression of $CD4^+CD28^-$ T cells suggests a role for genetic factors in the generation of these unusual T cells. Evidence for heterogeneity of the synovial component of RA comes from studies describing three distinct patterns of lymphoid organizations in the synovium. Each pattern of lymphoid organization correlates with a unique profile of tissue cytokines, demonstrating that several pathways of immune deviation modulate disease expression in RA. Defining RA variants has major implications on how the disease is studied, treated, and managed. Identifying combinations of RA risk genes that correlate with disease variants could become an important diagnostic tool.

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T Cells in Rheumatoid Arthritis

Redox Balance Alterations and Hyporesponsiveness of Synovial T Cells in Rheumatoid Arthritis

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In rheumatoid arthritis (RA), the functional status of T lymphocytes is incompletely understood. Synovial fluid (SF) T lymphocytes display phenotypic evidence of former activation, but there is hardly any production of T cell derived cytokines in the synovium. Moreover, the *in vitro* proliferative responsiveness of SF T lymphocytes is decreased compared with that of peripheral blood (PB) T lymphocytes.

Previous studies have revealed reduced intracellular Ca^{2+} responses and a decreased overall tyrosine phosphorylation pattern in SF T lymphocytes upon TCR/CD3 simulation [1]. Specifically, the tyrosine phosphorylation of the TCR zeta chain, one of the most proximal events in TCR signaling, was clearly diminished in RA SF T lymphocytes [2]. Moreover, the phosphorylation of a 36 kDa protein was virtually absent in RA SF T lymphocytes. Here we report that the 36 kDa protein is identified as LAT (linker for activation of T cells). In healthy T lymphocytes, LAT is heavily phosphorylated on tyrosine residues by ZAP-70 (zeta-associated protein of 70 kDa) upon TCR engagement, which is required for the activation of PLCg1 and the subsequent influx of Ca^{2+} [3]. Using FACS analysis, we show that the expression of LAT is reduced in both SF and PB T lymphocytes from RA patients compared with T lymphocytes from healthy controls.

LAT is normally a membrane-bound protein due to the presence of a short α-helical structure. Using immunofluorescence staining and microscopy, we found that LAT is displaced from the membrane in SF but not PB T lymphocytes from RA patients. The synovium provides an environment of oxidative stress for the SF T lymphocytes, which are severely depleted in their intracellular levels of the antioxidant glutathione (GSH). The replenishment of GSH in SF T lymphocytes by treatment with NAC (*N*-acetyl-L-cysteine) restores the membrane localization of LAT, and also the phosphorylation of LAT and the expression of IL-2 after TCR stimulation.

Conclusively, it is demonstrated that the hyporesponsiveness of synovial T lymphocytes in RA is associated with the membrane-displacement of LAT. The membrane localization of LAT is sensitive to intracellular GSH alterations [4]. The displacement of LAT fails to bring ZAP-70 in its proximity after TCR stimulation, whereby LAT remains unphosphorylated and the TCR-mediated signaling pathway is abrogated. Hence, these results suggest a role for the membrane-displacement of LAT in the hyporesponsiveness of SF T lymphocytes as a consequence of local oxidative stress.

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T Cell Homeostasis and Repertoire Contraction in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is not limited to chronic articular inflammation, but patients have alterations in their global immune system in line with the clinical experience of significant systemic disease. Specifically, RA patients frequently have clonal T cell populations in the circulation that characteristically lack the expression of the CD28 molecule. Here, we propose that RA patients have a defect in T cell generation that results in the contraction of the T cell repertoire and eventually in the emergence of $CD28^{null}$ clonal T cells. We have determined the diversity of the peripheral CD4 T cell repertoire by determining the frequencies of arbitrarily selected T cell receptor (TCR) β-chain sequences. Healthy individuals displayed a highly diverse repertoire, with a median frequency of individual TCR β-chain sequences of 1 in 2.4×10^7 CD4 T cells. In RA patients, the median TCR β-chain frequency was 10-fold increased. The loss in TCR diversity was not limited to CD4 memory T cells but also involved naive T cells, suggesting an abnormality in T cell repertoire formation and not a consequence of antigen recognition in the synovium. Also, control patients with chronic inflammatory disease such as hepatitis C expressed a diverse repertoire. In further support for this concept, telomere length studies indicated an increased replicative history of peripheral CD4 T cells in RA patients. Lymphocyte telomeres were age-inappropriately shortened with almost complete telomere erosion at an age of less than 40 years. Again, naive T cells were predominantly affected, and their capacity to undergo clonal burst after stimulation was about 10-fold reduced. These data are consistent with a premature exhaustion of lymphopoiesis in RA that may contribute to the autoimmune response as well as to the immunodeficiency in these patients and has important implications for their clinical management.

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Contribution of T Cell Subsets to Joint Degradation

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The role of T cells in the pathogenesis of rheumatoid arthritis (RA) has been and remains a matter of debate. One of the arguments for discussing such contribution has been the difficulty to measure the production of cytokines described as characteristic of T cells such as IFNγ. However, when the technology used to raise antigen specific T cell clones was applied to T cells from RA synovium, such cells were found to be fully able to produce IFNγ. In addition, RA synovium immunostaining revealed the presence of IFNγ producing cells. Conversely, the production of IL-4, another T cell cytokine, was found to be low and, to some extent, defective. Such findings led to the classification of RA as a Th1-associated disease. More recently, IL-17 has been described as a proinflammatory cytokine specifically produced by T cells. Studies with supernatants of RA synovium explants showed a high production of bioactive IL-17. Such an effect was linked to a synergistic interaction between IL-17 and the major monocyte-derived cytokines IL-1 and TNFα. Similarly, staining of RA synovium showed the presence of IL-17-producing T cells. Extension of studies with T cell clones from RA synovium indicated that a subset of IFNγ, but not of IL-4-producing T cells, was able to produce IL-17, allowing the classification of IL-17 as a Th1 cytokine.

The addition of blocking anti-IL-17 antibody to culture supernatants was able to reduce by approximately 50% the production of IL-6 LIF as well as that of MMP-1. Such an effect resulted in an important decrease of extracellular matrix destruction when IL-17 was inhibited. Conversely, when the anti-inflammatory cytokines IL-4, IL-13, and, to a lesser extent, IL-10 were added, production of proinflammatory cytokines was inhibited, including that of IL-17. Similarly, this resulted in an increase of TIMP production associated with reduced destruction. In the same conditions, increased repair as indicated by collagen synthesis was observed.

Through their production of cytokines, a subset of Th1 T cells can aggravate the proinflammatory and destructive pattern associated with monocyte activation. Manipulation of the cytokine profile of such a subset may control the destructive pattern, which remains a therapeutic target difficult to control.

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The p78 Putative Rheumatoid Arthritis T Cell Autoantigen

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Abstract not available

Altered T Cell Differentiation in Patients with Early Rheumatoid Arthritis

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Chronic inflammation in rheumatoid arthritis (RA) is likely to be driven by activated Th1 cells without sufficient Th2 cell differentiation to down-modulate inflammation [1,2]. To test whether, in RA, Th cells express an alteration in their ability to differentiate into effector cells, we investigated Th cell differentiation in patients with early untreated RA and in age- and sex-matched controls *in vitro*. All patients had active RA with symptoms of the disease for 6 weeks to 12 months. A cell culture system was used that permitted the differentiation of Th effectors from resting memory T cells by short-term priming [3]. No difference in the cytokine secretion profile of freshly isolated T cells was detected between patients and controls. However, marked differences were found in the response to priming. Th2 cells could be induced in all healthy individuals by priming with anti-CD28 in the absence of TCR ligation. By contrast, priming under those conditions resulted in Th2 differentiation in only 9/24 RA patients. The addition of exogenous IL-4 could overcome the apparent Th2 differentiation defect in seven patients but was without effect in the remaining eight patients. In all patients, a marked decrease in IL-2 producing cells and a significant increase in well-differentiated Th1 cells that produced IFN-γ but no IL-2 was evident after priming with anti-CD3 and anti-CD28 [4]. The data suggest that CD4+ memory T cells from patients with early untreated RA manifest an intrinsic abnormality in their ability to differentiate into specific cytokine producing effector cells, which might contribute to the characteristic Th1 dominated chronic (auto)immune inflammation in RA.

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B Cells in Rheumatoid Arthritis

Evidence for an antigen-driven immune response in the chronically inflamed synovium

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Recently it has been shown that proinflammatory cytokines, like TNF-alpha and lymphotoxin play a crucial role in the organogenesis of the lymphoid tissue. Thus, during chronic inflammation, in the affected tissue, these cytokines may promote the development of a micro-environment which supports a local immune response. In patients with rheumatoid arthritis large lymphocytic infiltrates are often seen in the synovial tissue. These cell clusters have a characteristic arrangement of T, B and plasma cells. Proliferating B cells are found only centrally in a network of follicular dendritic cells. Using micro-manipulation $CD20^+$ B cells and plasma cells were isolated separately from different parts of single infiltrates. DNA was extracted and the V_H/V_L gene repertoires determined. The data show that within the network of follicular dendritic cells there is an oligoclonal expansion of B cells. During proliferation V-gene variants are generated by the hypermutation mechanism. The pattern of somatic mutations suggests that both naive and memory B cells become activated in the synovial tissue. Within single infiltrates we did not find

identical rearrangements between CD20+ B and plasma cells. Nevertheless, the finding of clonally related plasma cells suggests that these cells underwent terminal differentiation in the synovial tissue. The analysis of individual B cells recovered from synovial tissue opens a new way to determine the specificity of those cells which take part in the local immune reaction.

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Expression of RAG1, RAG2, and TdT in Rheumatoid Arthritis Synovia: Evidence for Receptor Revision of Immunoglobulin Light Chains

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Some rheumatoid arthritis (RA) synovia contain structures similar to germinal centers (GC), the site of affinity maturation of B lymphocytes [1,2]. Previous analyses of immunoglobulin (Ig) kappa and lambda light chains expressed in RA synovia showed clonally related sequences with frequent N region addition and unusually long CDR3s [3,4]. The presence of clonally related Ig heavy chain sequences in GC-like structures from RA synovia suggests *in situ* antigen-dependent B cell maturation. RAG1 and 2, enzymes that mediate V(D)J recombination during B cell development, are expressed in a subset of GC B cells in normal peripheral lymphoid organs [5,6]. RAG expression allows secondary Ig rearrangements, which salvages B cells with undesirable specificities or low antigen affinity (receptor revision). We sought to determine whether RAG and TdT (the enzyme responsible for N region addition) are expressed in RA synovia and whether secondary Ig rearrangements occur. Using nested RT-PCR, we detected RAG1, RAG2, and TdT mRNA in 8, 9, and 6 of 12 synovial samples (11 RA, 1 JRA), respectively. RAG1 was expressed in B cells (5/8 samples) and T cells (4/8). RAG2 was expressed in B cells (4/8) more often than in T cells (1/8). TdT was expressed in B cells only (2/8). Immunohistochemical staining indicated that RAG proteins were distributed in lymphoid aggregates. In some synovia, secondary rearrangement products (ds-DNA breaks at recombination signal sequences in the Jkappa region) were detected by ligation-mediated PCR. We speculate that receptor revision in nonlymphoid tissues such as RA synovia may generate autoreactive antibodies, which has important implications for chronic inflammatory diseases.

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Role of Rheumatoid Factor B Cells in Normal and Pathologic Antigen Presentation

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The pathogenesis of rheumatoid arthritis (RA) is a major focus of my research group. We have used molecular and immunologic techniques to attack three main problems: 1) the role of anti-IgG autoantibodies (rheumatoid factors) in normal immune responses to environmental antigens, and in RA, 2) the mechanism by which HLA-DR4 contributes to the pathogenesis of RA, and 3) the role of the synovial microenvironment in RA.

The results of our investigations have shown that genes encoding rheumatoid factor autoantibodies are present in the germ line of most people. Their immunoglobulin products greatly potentiate the presentation of tiny amounts of antigen during secondary immune responses, and also may provide bystander help for T cell activation. The development of high-affinity pathogenic IgG rheumatoid factors is normally prevented by an antigen-specific deletion mechanism. This mechanism fails in RA.

HLA-DR4 molecules contain a short peptide sequence, 'the shared epitope,' that is duplicated in the dnaJ class of heat shock proteins in bacteria and in the gp 120 capsid protein of EBV. Patients with early RA, but not normal subjects, have circulating Th1-type T lymphocytes that respond to these exogenous antigens but not to autologous HLA-DR4 molecules.

The synovium has a rich blood supply and macrophage lining. Consequently, exogenous antigens easily become trapped there. Although rheumatoid factors and HLA-DR4 molecules normally protect the host from infection, they may promote the

conversion of a low-grade, nonspecific synovitis into the chronic granulation tissue characteristic of RA.

Synovial B Cells in Rheumatoid Arthritis: Clonal Expansion, Diversification, and Persistence

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The extent of B cell clonal diversity in the blood, synovial fluid (SF) and synovial tissue (ST) of rheumatoid arthritis (RA) patients was studied. We measured diversity using an Ig V_H gene fingerprinting assay that identifies B cell clones based on V_H CDR3 length. By comparing fingerprints from genomic (g)DNA vs cDNA, clones that were expanded in number could be distinguished from those that were activated and contained increased amounts of Ig VH gene mRNA (but were not necessarily numerically-expanded). These assays were performed on total B cells and on naïve, germinal center, and memory B cells and plasma cells. The data were confirmed by V_H gene sequence analyses.

Comparisons of the gDNA and cDNA assays indicated that certain clones were expanded at the DNA but not at the RNA level, suggesting increased numbers of resting memory cell-like B cells. By contract, some clones were less expanded at the DNA level and much expanded at the RNA level, indicating activated B cells.

Studies of paired blood and SF or ST samples identified clones that were joint-specific, blood-specific and blood-joint common. When B cell clones in the SF from the same joint of the same patient were studied over time, most of the B cell clones changed. However, a few remained constant. The persisting clones exhibited V_H gene diversification with time. We also compared the B cell clones present in the ST of two hip joints surgically removed from the same RA patient at the same time. These expanded clones also exhibited intraclonal sequence diversity.

Finally, ST B cells were sorted into memory and plasma cell subsets and analyzed. Certain B cell clones were unique to the memory compartment, while others were also expressed as plasma cells. Some of these B cell clones may play a role in the synovitis of RA.

Can IgG Rheumatoid Factors Explain Everything?

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The stochastic pattern of onset of rheumatoid arthritis (RA) suggests that it is initiated not by an external stimulus but by random genesis of new antibody species by immunoglobulin gene mutation [1]. Most autoantibody-committed B cells probably die from a lack of T cell help and from a follicle-centre suicide signal from uncomplexed self antigen. IgG rheumatoid factor (RF)-committed B cells may, in contrast, self-perpetuate, using their antibody product both to obtain nonspecific T cell help and, as self-associated complexes, to provide a positive survival signal in follicle centres. They should also facilitate survival of clones committed to production of RF of other classes.

Molecular modelling indicates that IgG1 RF-based complexes, comprising not more than two or three IgG molecules, should evade complement clearance, cross endothelium, and access tissue macrophages [2]. Recent work indicates that, of the three IgG Fc receptors on macrophages only FcγRIIIa will induce TNFα release in response to complexes of this size [3]. FcγRIIIa is expressed at high level only on macrophages in tissues targeted by rheumatoid arthritis and, in dermis, only at sites where nodules form [4]. The dominant involvement of synovium probably reflects the sensitivity of synovial fibroblasts to TNFα and the resulting tendency to synovial lymphoid metaplasia with local antibody production [5].

If IgG RFs are responsible both for their own production and for clinical disease, deletion of IgG RF-committed B cells should produce long term-remission. Initial results from a phase-I therapeutic trial of B cell depletion will be presented.

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Plenary Lecture

Overview: Destruction of the Extracellular Matrix in Rheumatoid Arthritis

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A major cause of morbidity in rheumatoid arthritis (RA) is the destruction of the extracellular matrix (ECM) of cartilage, bone, and soft tissues of the joint. Proteolytic enzymes, such as the matrix metalloproteinases (MMPs) and the recently characterized and cloned aggrecanases with disintegrin domains, have a role in these destructive processes that result in loss of fibrillar (collagens) and nonfibrillar components of the ECM. MMPs, eg collagenases, cannot degrade the ECM of mineralized bone, but mechanisms require action of specialized cells (osteoclasts) generated from hemopoietic precursors in marrow and in the inflammatory cell mass. Ligands, which increase bone resorption, initiate osteoclast generation by acting on mesenchymal cells (fibroblasts, stromal cells, and osteoblasts) to induce cell-bound osteoclast differentiation factor (ODF). ODF in turn binds to a receptor (RANK) on osteoclast precursors and, with M-CSF, generates active osteoclasts. Another factor, osteoprotegerin (OPG), binds to ODF (also known as OPGL[ligand]) and inhibits osteoclastogenesis. A potent inducer of ODF is parathyroid hormone-related peptide (PTHrP); receptors for PTH/PTHrP are found on RA synovial fibroblasts, in culture, and by *in situ* hybridization, and PTH is produced by RA synovium through the action of inflammatory cytokines. There is evidence derived from studies in animal models that the action of collagenase produced by mesenchymal cells is required for PTH/PTHrP-induced osteoclast generation. Delineation of the precise function of the ligands and proteinases described would help in designing therapy to prevent or retard the focal bone erosions and more diffuse bone loss of RA.

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Antigen-Presenting Cells, Macrophages, and Mast Cells in Rheumatoid Arthritis

Monocytes and Macrophages in Synovitis: Villains or Victims?

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Rheumatoid arthritis (RA) is characterized by cartilage and bone destruction via pannus formation. It is also a systemic disease, where monocytes are known to play an important role in the inflammatory processes. To further investigate mechanisms of monocyte activation, differential gene analysis appears to be a powerful tool. Thus, we have differentially analyzed gene expression in activated monocytes from first (activated) and third (nonactivated) leukapheresis preparations from an RA patient. Typical genes identified were IL-1α, IL-1β, IL-6, and TNF-α. Furthermore, GRO-α/melanoma growth-stimulatory activity, MIP-2/GRO-β, ferritin, α-1 antitrypsin, lysozyme, transaldolase, EBER-1/EBER-2 associated protein, thrombospondin-1, an angiotensin receptor-II C-terminal homologue, RNA polymerase-III elongation factor (elongin), three unknown (BSK-66, -80, -89), and homologues of functionally undefined genes (BSK-67, -83) were differentially expressed. Overexpression was confirmed for selected genes by semiquantitative PCR analysis in monocytes from RA patients versus healthy donors, including IL-1β, the ATR-11 homologue, BSK 66, –67 and –89. To investigate the importance of monocytes in the synovial tissue and to take other cells in the synovial pannus into account, especially

fibroblasts, which may modulate the destructive process essentially, a representational difference analysis has been performed in whole synovial tissue between RA and osteoarthritis (OA). Furthermore, a modified technique of semiquantitative PCR was used to verify these data and to analyze a group of genes possibly involved in the tissue homeostasis of cartilage. A preliminary analysis of 151 clones revealed that genes other than regulatory cytokines dominate the difference. Especially genes of effector functions like immunoglobulin and collagen type 1 are over-represented. Furthermore, receptors with immunoregulatory function like HLA-DR, natural killer cell receptor p58, and a homologue to the cytokine receptor EB13 as well as genes involved in global cell activity are upregulated in RA. Many clones with unknown sequences need further investigation. Using semiquantitative PCR, a group of genes not yet considered for RA, the bone morphogenetic proteins (BMP), were differentially studied between RA, OA, traumatic joint diseases, and normal tissue. Specific members of this huge family of genes are selectively down-regulated in synovial inflammation and possibly differentially regulated depending on the type or stage of inflammation.

In summary, monocytes are certainly global players in the inflammatory process in RA. However, in the synovial tissue, modulating factors from fibroblasts, natural killer cells, or T- and B-cells play an essential role. Especially in chronic cartilage degradation, the decrease of differentiation factors most likely derived from cartilage nursing synovial fibroblasts may contribute to joint destruction.

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Dendritic Cells: What Is Their True Role in Rheumatoid Arthritis?

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The use of arthroscopic biopsy for examination of rheumatoid arthritis (RA) synovial tissue (ST) for disease activity and prognostic indices is of major interest for improving patient outcome and for assessing clinical trial efficacy. Dendritic cells (DCs), the professional antigen-presenting cells of the immune system, have been proposed to play a central role in the initiation and perpetuation of the immune response in RA. We have previously demonstrated that differentiated DCs can be specifically identified in tissues by double immunohistochemical staining, which includes the identification of the NFκB family member, RelB, in the nucleus. Differentiated DCs were thus identified in the T cell areas of normal or reactive human lymph nodes and in a perivascular location in ST from patients with untreated active RA. Double immunohistochemical staining on 13 ST biopsies demonstrated that 88% of the nRelB$^+$ cells present are differentiated dendritic cells, the remaining 12% comprising activated B cells, sub-lining monocytes, and occasional follicular DCs. To examine the relationship between the presence of differentiated DCs in the ST and disease activity, serial ST biopsies from seven patients with RA were obtained before and after either successful or unsuccessful therapy, as determined by the ACR response criteria. RA treatment-associated clinical improvement correlated with a reduction in infiltrating differentiated DCs, infiltrating lymphocytes, tissue vascularity, expression of TNF-α in the lining and sub-lining layers, and CD68+ lining layer cellularity. In contrast, IL-1β expression was not correlated. These data suggest that clinical joint inflammation, tissue vascularity, local TNF-α production, DC and lymphocyte infiltration, and lining-layer hypertrophy are interdependent events. Furthermore, taken together with published data, they suggest that the synovial immune response and joint destruction are linked pathogenetic processes.

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Rheumatoid Arthritis is a Lining Cell Disease: An Evolving Concept

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Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting predominantly lining structures, including synovial tissue, tendon sheaths, bursae, pleura, and pericardium. Characteristically, these tissues contain large numbers of macrophages, suggesting that these cells are crucial in the pathogenesis of the disease. Data obtained by us and by others have indicated that phagocytic lining cells are essential for disease onset and expression. Removal of synovial lining cells by intra-articular injection of apoptosis-inducing clodronate liposomes prevents joint inflammation in murine arthritis models, and pilot data suggest that these liposomes can be succesfully used in humans. The degree of monocyte/macrophage (but not of T-cell) infiltration in rheumatoid joints has been reported to correlate with both disease activity and progression of joint destruction in rheumatoid arthritis (RA). Recent studies have shown that intrinsic macrophage characteristics may determine an individual's susceptibility to develop arthritis. Murine collagen-induced arthritis-susceptible mouse strains were shown to have markedly higher expression of FcK RII/III by macrophages than nonsusceptible strains (Blom, 1999). In human RA, we found that activated monocyte-derived macrophages produce significantly higher IL-8 mRNA levels than those in controls, pointing to an innate hyper-responsiveness of these cells in this disease. The central role of lining macrophages in the pathogenesis of RA has therapeutic consequences in terms of targeting these cells and/or their proinflammatory products.

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Mast Cells and the Rheumatoid Lesion

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The distribution and activation of mast cells (MCs) in the rheumatoid lesion has been examined by tryptase immunolocalisation. MCs were observed in all specimens examined, but their distribution and local concentrations varied, both within and between specimens. MC activation was observed at sites of cartilage erosion and was associated with localised oedema and matrix disruption [1]. Dual immunolocalisation studies often demonstrated co-distributions of MCs with the matrix metalloproteinases (MMPs) collagenase 1 and stromelysin 1, as well as the proinflammatory cytokines TNFα and IL-1β [2]. Although IL-15 is purported to induce TNFα and IL-1β expression by cells *in vitro* [3,4], its production *in situ* was not associated with MC activation, as judged by IHC.

The potent mediators of MCs, especially histamine, heparin, and TNFα, are all likely to modify the phenotype of both synoviocytes and chondrocytes. The addition of soluble MC products to synovial fibroblast cultures was shown not only to stimulate MMP production but also to activate the MMP precursors via processing by the MC serine proteinases tryptase and chymase [5]. Experiments using rheumatoid synovial explants and MC secretagogue, rabbit antibody to human IgE, have shown that MC activation induced tryptase release, increased PGE_2 production, and brought about changes in both MMP [6] and cytokine production. Subsequent experiments have assessed the effects of added histamine or heparin on enzymically dissociated rheumatoid synovial cell cultures with respect to cytokine production. Collectively, the data demonstrate that MC activation in the rheumatoid lesion modulates MMP and proinflammatory cytokine expression by neighbouring cells, thereby suggesting an important contributory role in mediating matrix degradation and oedematous changes within microfoci of the rheumatoid lesion.

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Synoviocytes, Stromal Cells, and Endothelial Cells in Rheumatoid Arthritis

Activation of Synovial Fibroblasts in Rheumatoid Arthritis

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Activation of synovial fibroblasts (SF) by upregulation of proto-oncogenes is thought to play a major role in rheumatoid joint destruction. To explore distinct signaling pathways in this activation we used retroviral gene transfer in the SCID mouse model. Specifically, we transferred dominant negative (dn) mutants, such as dn Raf-1 and dn c-myc to inhibit the Ras-Raf-MAPK cascade as well as the cascade involving myc. FLAG-tagged constructs used in this study were cloned into the retroviral vector LXSN. The data show that both Raf- as well as myc-dependent pathways contribute to the activation of synovial fibroblasts in RA.

Since mutations in PTEN have been described in cancer and associated with their invasiveness we studied the expression of this novel tumor-suppressor in RA. Although, no mutations of PTEN could be detected in RA synovium, only 40% of cultured RA-SF expressed PTEN, and a down-regulation was observed at sites of invasion into cartilage. These findings suggest that endogenous or autocrine down-regulation of this tumor-suppressor may contribute to the invasive behavior of RA-SF by maintaining their aggressive phenotype at sites of cartilage destruction in RA. Most interestingly, the same SF found at these sites are also the major producers of interleukin-16, a strong chemoattractant for CD4+ cells.

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Biologic Aspects of Nurse-Like Cells Found in Bone Marrow and Synovial Tissue of Rheumatoid Arthritis Patients

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Using collagen-induced arthritis (CIA) rats, we studied the origin of synovial stromal cells, which rapidly appeared and proliferated in joints at the onset of inflammatory polyarthritis, we partially labelled the bone marrow stromal cells (BMSC) with fluorescent dye or 3H-Tdr,and analyzed the migration of labelled BMSC after the immunization with collagen. At the onset of CIA, BMSC migrated through small canals from the bone marrow into the affected joint cavities and seemed to contribute to synovial proliferation in joints [4].

Based on the data above, we were interested in establishing and characterizing the nurse cell-like stromal cells (NCs) in bone marrow (RA-BMNC) as well as those in synovial tissue of rheumatoid arthritis (RA) patients (RA-SNC). RA-BMNC showed the characteristic cell–cell contact with lymphocytes (pseudoempeliporesis), resulting in mutual biologic activation, such as maintaining infiltrating lymphocytes and producing large amount of cytokines. Those were very similar to the reactions of RA-SNC reported in our latest paper [2].

Another point of interest was whether NCs could invade the bone, resulting in erosive changes characteristically observed in RA patients. Although NCs (both RA-SNC and RA-BMNC) were shown to produce IL-6, IL-8, and other cytokines, these were not thought to contribute directly to bone erosion. By co-culturing NCs with lymphocytes, we found activation in the production of MMP-1 and MMP-3, and in the expression of mRNA of both MMP-9 and cathepsin-K. Thus, NCs could be thought to contribute directly to bone erosion in RA patients.

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Effects of 1α,25dihydroxyvitamin D_3 on matrix metalloproteinase and prostaglandin E_2 production by cells of the rheumatoid lesion

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1α,25dihydroxyvitamin D_3 (1α,25D_3), the active form of vitamin D_3, through its interaction with intracellular vitamin D receptors

(VDRs), is reported to effect a variety of anabolic and catabolic events, especially in bone and cartilage tissues [1–3]. VDRs were demonstrated in most specimens of cartilage–pannus junction, within synovial pannus tissue, and by chondrocytes often close to the erosive lesion [4]. *In vitro* studies have examined the effects of $1\alpha,25D_3$ on rheumatoid synovial explants and monolayer cultures of rheumatoid synovial fibroblasts (RSFs) and human articular chondrocytes (HACs) with respect to MMP and prostaglandin E_2 (PGE_2) production. Explant cultures exposed to $1\alpha 25D_3$ showed little change in the production of matrix metalloproteinase (MMP)-1 and MMP-3, but MMP-9 was markedly suppressed. The expression of MMP-1, -3, and -9 by RSFs was unaffected by $1\alpha,25D_3$ alone, but when stimulated with IL-1β the resultant increase was signficantly inhibited (~60%) by $1\alpha,25D_3$. Conversely, although treatment of HACs with $1\alpha,25D_3$ alone had little effect on MMP production, the increase in MMP-1 and -3 seen with IL-1β stimulation was further enhanced by the simultaneous addition of $1\alpha,25D_3$. Such observations demonstrate that IL-1-activated RSFs and HACs respond differently to $1\alpha,25D_3$ exposure, and that the latter has different effects on the regulatory pathways of specific MMPs. PGE_2 production by RSFs stimulated with IL-1β was also significantly reduced (by up to 70%) following the simultaneous addition of $1\alpha,25D_3$, a suppression not observed in IL-1β-stimulated HAC cultures. Moreover, when RSFs were preincubated with $1\alpha,25D_3$ prior to the addition of IL-1β, subsequent PGE_2 production was more markedly suppressed – an observation suggesting either that $1\alpha,25D_3$ interferes with the IL-1β signalling pathway or by its recognised immunosuppressive functions reported for other cells. Immunohistochemical studies of the rheumatoid lesion have demonstrated similar distributions for the expression of VDRs and specific MMPs *in situ* – observations that further suggest that $1\alpha,25D_3$ may be involved in the modulation of MMP and prostanoid expression of the rheumatoid lesion.

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Cytokines and Chemokines in Rheumatoid Arthritis

Interleukin-1 Receptor Antagonist Isoforms in Collagen-Induced Arthritis

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Interleukin-1 receptor antagonist (IL-1Ra) is a natural IL-1 inhibitor that possesses anti-inflammatory properties *in vivo*. IL-1Ra refers to three different proteins, one secreted (sIL-1Ra) and two intracellular (icIL-1RaI and icIL-1RaII). Levels of both sIL-1Ra and icIL-1RaI are increased in the rheumatoid synovium, although icIL-1RaI predominates, particularly in cultured rheumatoid synovial fibroblasts. Since collagen-induced arthritis (CIA) reproduces some of the pathologic characteristics of rheumatoid arthritis, we investigated the expression of IL-1Ra isoforms in the joints of mice with CIA. Total RNA and proteins were extracted from whole joints of immunized mice prior to the development or during the course of CIA, as well as from synovial tissues obtained from normal mice, immunized mice before the appearance of arthritis, and at different time points during the course of CIA (1 to 20 days). The presence of IL-1Ra mRNA and protein isoforms was examined by RT-PCR, RNase protection assay, and Western blot analysis. The results showed that sIL-1Ra mRNA was present at low levels in normal whole joints, as assessed by RT-PCR, and was further stimulated during arthritis, whereas icIL-1RaI mRNA was detected only in joints during CIA. By Western blot analysis, sIL-1Ra protein was detected in whole joint extracts of immunized mice prior to clinical signs of CIA, whereas icIL-1RaI was detected only in the joints of mice with CIA. icIL-1RaII protein followed the production of sIL-1Ra. icIL-1RaI was the predominant isoform in the isolated inflamed synovium, with levels increasing during the course of CIA. In conclusion, sIL-1Ra mRNA was present in low amounts in normal joints and its levels increased early during arthritis. In a similar fashion, sIL-1Ra protein was present in the joints at early stages of CIA. In contrast, icIL-1RaI mRNA and protein was produced in the joints of mice with CIA in a delayed manner and was the predominant isoform in the inflamed synovium.

Chemokines in Rheumatoid Arthritis

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We examined the role of chemokines, which recruit a variety of cells, including leukocytes and endothelial cells, in rheumatoid

arthritis (RA). We determined the role of the angiogenic chemokines IL-8 and epithelial neutrophil activating peptide-78 (ENA-78) in RA using a whole synovial tissue (ST) homogenate model. RA ST homogenates produced greater levels of these chemokines than did normal ST homogenates. Angiogenic chemokines often immunolocalized in proximity to ST blood vessels. RA ST homogenates contained more angiogenic activity *in vitro* and angiogenic activity *in vivo* than did normal ST extracts. Hence, IL-8 and ENA-78 are major contributors to the RA ST angiogenic activity *in vitro* and *in vivo*. To determine the role of chemokines in the development of arthritis, we examined inflammation in rats. Anti-ENA-78 diminished peritoneal neutrophil recruitment in response to rhENA-78 or lipopolysaccaride. In adjuvant-induced arthritis (AIA), a model for RA, rats produced serum ENA-78 from day 7 post adjuvant induction, even prior to the onset of clinical AIA. Joint ENA-78 was elevated in AIA rats from the onset of clinical arthritis. Prophylactic therapy of rat AIA with anti-ENA-78 prior to clinical disease onset resulted in a reduction of arthritis and a decrease in joint IL-1β and tumor necrosis factor (TNF)-α. These results suggest a role for ENA-78 in the early stages of AIA development. Efforts aimed at eliminating these angiogenic chemokines may be of benefit in the therapy of RA.

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Chemokines and Chemokine Receptors in Lymphocyte Traffic

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Since the discovery of IL-8, about 40 human chemokines and 16 chemokine receptors were characterized. Early on chemokines were considered as mediators of inflammation, but with time more and more chemokines were found that function mainly in the basal traffic of leukocytes, lymphocytes in particular. Inflammatory chemokines are induced by bacterial toxins and a number of cytokines, eg IL-1 and TNF, and can be produced locally in virtually all types of tissues as a consequence of inflammation or infection. The chemokines involved in the physiologic traffic of lymphocytes, by contrast, are produced constitutively in restricted regions. T lymphocytes express the widest variety of chemokine receptors and the expression is highly regulated. CXCR3, CCR1, CCR2 and CCR5, receptors for inflammatory chemokines, are upregulated by IL-2 in effector/memory T cells and decay rapidly when IL-2 is withdrawn or after stimulation with anti-CD3 and anti-CD28. Different stimulatory conditions modulate the expression of other receptors, eg CCR6, CCR7, or CXCR4, which have homeostatic rather than inflammatory functions. CCR7, for instance, is present at low levels in resting naive and memory T cells and is rapidly and transiently upregulated by stimulation with IL-2 and/or PHA. SLC and ELC, the ligands for CCR7, are constitutively expressed in secondary lymphoid tissues. The expression of different receptors on Th1 and Th2 cells provides a mechanism for their selective recruitment to inflammatory sites. For instance, the lymphocyte infiltrates in the pannus and synovial fluid of rheumatoid arthritis joints, which are rich in Th1 cells, are strongly positive for CCR5 and CXCR3.

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T Cell Maturation in Inflammatory Synovitis

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Functional maturation of T cells towards a Th1 or Th2 phenotype has important implications in the generation and perpetuation of autoimmune responses. Those factors that promote and sustain Th1 responses in rheumatoid arthritis (RA) are unclear. We are currently exploring the functional effects of the predominantly macrophage-derived cytokines, IL-12, IL-15 and IL-18, on synovial T cells. We have previously shown a role for IL-15 in synovial T cell chemokinesis and in TNFα production through promotion of cytokine-mediated T cell/macrophage cell contact. We have now extended these studies to include IL-12 and IL-18. IL-18 mRNA and protein may be detected by RT-PCR, ELISA, and immunohistochemistry in RA synovial membrane. The absolute levels present vary considerably between patients. In synergy with IL-12 and IL-15, IL-18 induces high levels of IFNγ, TNFα, and GM-CSF production. IL-18 mediates effects directly on

synovial T cells and macrophages. IL-18 and IL-15 expression is itself enhanced by IL-1β/TNFα *in vitro*, suggesting the existence of feedback loops that provide reciprocal amplification of Th1 cells and monokine production within the synovial membrane. These data clearly indicate that synergistic combinations of T cell activatory cytokines can promote synovial inflammation. This has important implications for the choice of therapeutic targets designed to suppress Th1 cell activity in autoimmune lesions.

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Autoimmunity in Rheumatoid Arthritis

Citrullinated Peptides as a Substrate for the Detection of Rheumatoid Arthritis-Specific Autoantibodies

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The diagnosis of rheumatoid arthritis (RA) depends primarily on the clinical manifestation of the disease. The only serologic test routinely used is the determination of the presence of rheumatoid factors in the serum. However, these antibodies are also present in relatively high percentages in other autoimmune and infectious diseases and in up to 15% of healthy individuals. Antibodies of a more specific nature were first described by Nienhuis and Mandema [1], who discovered that RA sera specifically label the 'perinuclear factor,' a component of the keratohyaline granules in buccal mucosa cells. These antiperinuclear factor (APF) antibodies are reported to be present in 49–91% of RA patients, with a specificity of over 70%. Due to technical reasons, the APF test never became very popular.

We discovered that APF-directed autoantibodies in RA specifically recognise citrullinated residues in polypeptides [3] and developed an ELISA procedure using a single cyclic citrullinated peptide (CCP) as substrate. Our studies showed that the anti-CCP ELISA assay was positive in about 70% of RA sera, with a specificity of more than 98% against disease controls (n>700). Other studies, which will be presented, corroborate the extreme diagnostic specificity of this autoantibody for RA. Therefore, we expect that a serologic test based on our peptides will be a valuable addition to RA diagnostics and a help in the decisions to be made concerning the treatment of early RA patients.

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Mechanism of Arthritis

Collagen Type II-Specific Autoantibodies in Chronic Inflammatory Joint Diseases

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Cartilage components are considered a source of antigens that could continously fuel tissue-specific immune reactions directed to the joints. One of the structural components is collagen type II (CII), the predominant collagen type in cartilage. Immunization with CII is associated with the development of an experimental autoimmune arthritis in mice and rats [1,2]. Collagen-induced arthritis (CIA) is a relevant experimental model for rheumatoid arthritis (RA) [3]. The induction of CIA is critically dependent on immunization with CII in its native conformation, and the onset of arthritis is preceded by an early rise in IgG autoantibodies (Abs) that are specific for triple helical structures on CII [4]. The preparation of a large panel of recombinant chimeric collagen molecules enabled us to determine precisely the epitopes on CII that are recognized by monoclonal antibodies established from arthritis prone DBA/1 mice [5]. The insertion of a series of CII cassettes into the triple helical recombinant collagen X allowed the identification of 5 triple helical immunodominant domains of 5–11 amino acid length, to which the majority of the mAbs bound [5]. In addition, a separate epitope has been identified that seems to be a major target of the B-cell response in the rat model of CIA. Interestingly, some of the epitopes that have been characterized in the rodent arthritis models are also target structures of the CII-specific IgG response in patients suffering from severe RA. The results indicate that the arthritogenic B-cell response is directed to evolutionary conserved CII-structures.

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Genetic Studies in Experimental Arthritis

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The genetic influence on rheumatoid arthritis (RA) is very complex with both polygenicity and heterogeneity and most likely with variable penetrance due to a strong influence by environmental factors. To make it possible to analyse which genes are of importance for controlling the disease and to further understand the basic pathogenesis, we use animal models in mice and rats. With gene segregation F2 experiments we have identified the major gene regions controlling the collagen induced arthritis in the mouse and pristane induced arthritis in the rat. In the rat we have found that different genes are controlling different phases of the disease, such as arthritis onset, erosions of the joints and the chronic development of arthritis. The most important gene region in both rats and mice is located in the major histocompatibility complex (MHC). In the mouse we have proved that the gene in the MHC region, which is responsible for the effect, is a class II gene called Aq. Interestingly, the protein encoded by this gene is structurally very similar to the RA-associated DR4B1*0401/DRA gene product, and mice expressing the human molecule are susceptible to collagen-induced arthritis. The function of these molecules is to bind peptides derived from different infectious agents and then present them to T lymphocytes. Both Aq and DR4 molecules bind peptides derived from position 260-273 in type II collagen. Studies using this structural knowledge show that 1) T cells recognize predominantly carbohydrate modifications of the peptide, and 2) the T cells are partially tolerized but can nevertheless participate in the induction of arthritis. In conclusion, these studies show that animal models are highly useful for specific analysis of the pathogenesis of RA by identifying genes and humanizing mouse strain.

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Role of Cytokines in Cartilage Destruction in Experimental Arthritis

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Rheumatoid arthritis (RA) is characterized by major destruction of the articular cartilage. The mediators TNFα and IL-1 probably play a major role in RA. Following studies in experimental murine arthritis models, using neutralising antibodies and scavenging receptors, it became clear that TNF is a major inflammatory cytokine, whereas IL-1 is the main destructive cytokine. These observations were recently confirmed using TNF and IL-1 knock-out mice. Remarkably, arthritis in TNF-deficient mice was strongly reduced, and yet ongoing cartilage destruction was still noted. In contrast, cartilage destruction was minimal in IL-1β knock-out mice.

Apart from inhibition of IL-1, major therapeutic benefit can be achieved with IL-4 and IL-10 treatment. These cytokines reduce IL-1 levels and relatively enhance IL-ra. Local gene transfer with adenoviral IL-4 constructs revealed major protection against cartilage and bone erosions in experimental arthritis despite marked inflammation.

Stromelysin seems a pivotal enzyme involved in cartilage breakdown. It is not an essential MMP in the early proteoglycan loss, and a major role for aggrecanase seems obvious. However, stromelysin seems crucial in collagenase activation and collagen breakdown, and irreversible cartilage erosion is markedly reduced in arthritis in stromelysin knock-out mice. Induction of stromelysin-induced neoepitopes and late destruction can be blocked with IL-1 neutralization.

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Synovial Pathophysiology

The Role of COX-2 in Rheumatoid Arthritis Synovial Tissues

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Synovial tissues of patients with rheumatoid arthritis (RA) have increased expression of COX-2. COX-2 expression in most tissues is highly regulated with rapid induction in response to inflammatory stimuli. Anti-inflammatory mediators, particularly glucocorticoids, inhibit up-regulation of COX-2 expression. Increased COX-2 expression in synovial tissues is driven by the pro-inflammatory cytokines IL-1 and TNF-α. These cytokines stimulate COX-2 transcription by activating transcription factors including NF-κB and c/EBP. IL-1 also increases mRNA stability. Modulating influences by other cytokines and growth factors utilize the STAT family of transcription factors. In addition to cytokine networks, signalling via cell surface integrins can increase expression of COX-2. The intracellular pathways triggered by stimulation of integrins include phosphorylation of the ERK kinases.

PGE_2, the major PG product of COX-2 in synoviocytes, has been shown to alter matrix metalloproteinase balance and to increase expression of the angiogenic factor, VEGF. In some cell types, constitutive over-expression of COX-2 is associated with phenotypic changes including increased resistance to apoptosis and production of angiogenic factors. To analyze the effect of COX-2 on synovial fibroblast-like cells, we have developed a method for highly efficient constitutive over-expression of COX-2 using a retroviral vector system. Early-passage synoviocytes stably transduced to express high levels of COX-2 can be evaluated for phenotypic changes that contribute to the pathogenesis of rheumatoid arthritis.

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Interfering With Inflammation During Cell–Cell Interaction

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In chronic inflammatory diseases, immunocompetent infiltrating cells are in the vicinity of or in direct contact with resident cells, representing the principal pathway for stimulating proinflammatory and prodestructive cytokines and metalloproteinases (MMPs). During the direct contact between activated T lymphocytes and fibroblasts or mesenchymal-derived cells, such as synovial cells, membrane-bound TNFα and IL-1 are the principal cytokines involved in MMP production by fibroblasts. Therefore, therapies involving Ab to TNFα (ie soluble receptors or antibodies) and to IL-1 (ie IL-1Ra or soluble IL-1 receptor type II) are perfectly rational. In the direct contact between activated TL and monocyte-macrophages (Mϕ), TL stimulated by mAb to CD3 favor the production of MMP-1 over that of TIMP-1, with Th1 cell clones inducing preferentially the expression of IL-1β and TNFα, and Th2 that of IL-1Ra. The induction of cytokines and MMPs during TL/Mϕ contact is partially inhibited (30–50%) by mAb to CD11 (b>c>a) and CD69. Contrary to the interaction between TL and fibroblasts, the blockade of membrane-bound TNFα and IL-1 has no effect on TL/Mϕ interaction. The identification of distinct cell-surface molecules on TL driving either proinflammatory cytokines and MMP or anti-inflammatory cytokines and TIMP-1 allows the design of drugs that ensure more precise targeting of therapeutic intervention. Some of these recent drugs decrease cell-surface molecules on activated T cells that are involved in the induction of IL-1β (but not of IL-1Ra) on monocytes.

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Life and Death in the Synovium

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Studies of the p53 tumor suppressor gene in rheumatoid arthritis (RA) have demonstrated somatic mutations in rheumatoid synovium and synoviocytes but not in RA skin or OA synovium [1]. Most of the mutations identified in RA samples are also present in various neoplastic diseases, suggesting that they are dominant negative. To determine if RA mutations are dominant negative, site directed mutagenesis was used to produced two RA mutants: Asparagine>Serine at codon 239 (N239S) and Arginine>Stop at codon 213 (R213*) [2]. Co-transfection experiments were performed using a construct containing the p53-responsive bax promoter construct with a luciferase reporter gene (bax-luc). Low levels of bax promoter activity were detected in HS68 cells co-transfected with bax-luc and empty vector, N239S, or R213*, indicating that the RA mutant lacked transcriptional activity. Transfection with wt and bax-luc led to a 10-fold increase in luciferase expression. When the wt gene was co-transfected with either of the mutants, there was a dose dependent inhibition of bax promoter activity. These data indicate that at least 2 of the p53 mutants identified in RA joint samples are dominant negative and suppress endogenous wild type p53 function.

Greater than 80% of the p53 mutations identified in synovium and cultured synoviocytes were G/A and T/C transitions. Such mutations are characteristic of oxidative deamination by nitric oxide and suggest that the mutagenic environment in chronically inflamed synovium contributes to alterations in the p53 gene. Mutations accumulate over time and specific alterations in the p53 gene, ultimately contributing to synoviocytes autonomy and perpetuation of disease. Other genes might also be altered in arthritis, and mutations in the H-ras gene were also recently reported in RA and OA synovium. The occurrence of somatic mutations secondary to inflammation indicates that a window of opportunity exists in RA after the initiation of disease, but before establishment of aggressive pannus.

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Apoptosis in Rheumatoid Synoviocytes

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Rheumatoid arthritis (RA) is systemic autoimmune disease characterized by synovial proliferation and infiltration of various

inflammatory cells. The process of disease progression, characterized by hyperplasia of synoviocytes, mainly of synovial fibroblasts, results in cartilage and bone destruction. Proliferation of synoviocytes is, however, not limitless, and spontaneous arrest and remission of synovial proliferation are occasionally observed. Previous studies from our laboratories as well as by other investigators have demonstrated that RA synoviocytes express functional Fas antigen (CD95/APO-1) and that these cells undergo Fas-mediated apoptosis both *in vivo* and *in vitro*. These findings suggest that Fas-mediated apoptosis may play a critical role in the regression of synovial hyperplasia in RA. Identification of regulatory mechanisms in RA synoviocytes may provide important insights and may lead us to establish novel strategies for RA therapy. We describe a current study by our laboratory on the regulatory mechanisms of apoptosis in RA, especially FasL and FADD gene transfer into inflamed synovium to induce remission stage and propose a novel strategy termed 'apomodulation' in the treatment of RA.

Synovial Histopathology

Digital Image Analysis to Quantify Inflammation in Synovial Tissue

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Computer-based assessment of images acquired in a microscope requires an attached video camera transferring the images to a computer and a screen. The analytical decisions made by the computer equipped with a special software program will be displayed on the screen in discriminating pseudocolors, enabling the operator to monitor and accept or discard the results of a given field. Quantification of cells expressing cell surface antigens or intracellular antigens can be readily done in immunohistochemically stained tissue sections from synovial tissue. Conventional microscopy is a good tool for counting but poor at measuring areas, staining intensity, or distances, which can be handled in a superior way by computer-assisted analysis. We have successfully applied this technology to quantify the incidence of various cell types, intimal layer thickness, and cytokine-producing cells in rheumatoid synovial tissue specimens. Computerized image analysis offers a major advantage in sequential studies to evaluate changes in response to therapy.

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Synovial Tissue Analysis in Clinical Studies

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Synovial tissue analysis may provide new insights in studies of the clinical course and outcome, response to treatment and disease mechanisms [1]. In a prospective clinical study of acute phase serum amyloid A (A-SAA) (the principle component of amyloid deposition) in early rheumatoid arthritis (RA), strong correlations with disease activity and response to therapy were observed [2]. A-SAA production by the liver is regulated by the pro-inflammatory cytokines IL-1, TNF-α and IL-6 [3]. Little is known about A-SAA production at peripheral sites of inflammation. This study was undertaken to investigate if A-SAA is produced by inflamed synovium in RA and other arthropathies.

Synoyiocytes were cultured following arthroscopic biopsy from patients with early arthritis. Total RNA was analysed for A-SAA mRNA using Northern blot analysis and RT-PCR. Immunohistochemistry was performed on frozen tissue sections using a polyclonal rabbit anti-human antibody. Using immunohistochemistry, A-SAA protein was demonstrated in the superficial layer of the synovial intima and in vascular endothelial cells. A-SAA mRNA was not detectable by Northern blot analysis in cultured synoviocytes. The RT-PCR technique, which is 100–1000 times more sensitive, resulted in the detection of constitutive expression of A-SAA. A fragment of 335 bp nucleotides was identified by RT-PCR and was verified by Southern blot analysis. A-SAA mRNA expression was investigated in cultured synoviocytes in response to inflammatory cytokines. Initial data indicates that IL-1, TNF-α and IL-6 are important regulators of A-SAA expression in these cells.

This is the first study to demonstrate A-SAA gene expression in human synovium. Local production of A-SAA, demonstrated by both RT-PCR and immunohistochemistry, suggests a physiological role for A-SAA at sites of inflammation.

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Predictors of Early Bone Erosion in Patients With Synovitis of Recent Onset

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To identify risk factors for the development of radiographic erosions early in the course of inflammatory arthropathies, an inception cohort of 238 patients with peripheral joint synovitis of less than one year duration was evaluated. Of these patients, 70 (28%) had early RF+RA, 36 (15%) had RF-RA, 43 (18%) had spondylarthropathy, and 89 (37%) had undifferentiated arthritis. Patients underwent clinical, serological and radiographic evaluation, HLA typing, and closed synovial biopsy of an affected joint. A total of 23% of patients had multiple radiographic erosions, with RA patients having the highest frequency (37%). The presence of multiple erosions was associated with the presence of the shared epitope only in the RF-RA patients (OR 9.9, $P<0.01$). Anti-Sa antibodies were associated with the presence of multiple erosions in both RF+ and RF– patients (odds ratios 2.0 and 6.3 respectively), but numbers were too small to reach statistical significance. The presence of either SE or anti-Sa was highly associated with erosions in the RF-RA patients (odds ratio of 25.7, $P<0.05$).

As elevated tissue and serum levels of the gelatinases (MMP-2 and MMP-9) have been associated with enhanced invasiveness in cancer, we tested the hypothesis that elevated levels and activity of the gelatinases were associated with the presence erosions. Serum levels of active MMP-2 and -9 were measured in a subset of 45 RA, 21 non-RA patients, and 4 normal volunteers. Tissue expression of MMP-2,-9,-14 and TIMP-1, 2 were assessed using immunohistology and synovial activity of MMP-2 and -9 were measured using gel zymography.

Serum levels of total and active MMP-2 and -9 did not predict erosions. Patients with erosions had higher synovial tissue levels of active MMP-2 than patients with no erosions (3.5 ± 2.1 ng/mg vs 1.9 ± 2.5 ng/mg, $P=0.04$). MMP-9 levels tended to be higher in the patients with erosive disease, although the range of expression was very wide. No other immunohistologic parameter correlated with the presence of early erosions.

In this cohort of patients with early synovitis, the presence of either shared epitope or anti-Sa antibodies was associated with early erosion formation, particularly in patients with RF-RA. In the synovial tissues of these patients, high levels of MMP-2 activity are the most closely associated with erosive disease.

Histopathology of Bone Erosions in Rheumatoid Arthritis

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Focal bone erosions in areas of pannus invasion are a hallmark of established rheumatoid arthritis (RA). We have utilized histochemical techniques and *in situ* hybridization to demonstrate that the cells responsible for these bone erosions express an osteoclast phenotype. Our results indicate that the multinucleated cells in resorption lacunae in continuity with pannus express abundant tartrate resistant acid phosphatase (TRAP), cathepsin K and calcitonin receptor (CTR) mRNA. Although CTR expression is restricted to multinucleated and mononuclear cells on the mineralized tissue surfaces, numerous TRAP positive mono- and multinucleated cells are present within the pannus remote from bone surfaces. We speculate that these TRAP positive cells represent osteoclast precursors that are recruited to the pannus tissue. Interaction of these cells with bone and calcified cartilage provides the additional signals that induce these cells to differentiate into resorbing cells with a definitive osteoclast phenotype. To further investigate the specific factors involved in the differentiation of osteoclasts in RA we have initiated studies to examine the expression of a recently described regulator of osteoclastogenesis, osteoclast differentiation factor (ODF), a member of the membrane associated TNF-ligand family, and its putative receptor on osteoclast precursors, the receptor activator of NF-κB (RANK). Analysis of synovial tissues by RT-PCR from eight patients with RA, five with juvenile arthritis and three with normal synovium reveals the expression of mRNA for ODF in 7/8 RA, 5/5 juvenile arthritis, and 0/3 normal cases. These data provide preliminary evidence that ODF may be an important factor in the pathogenesis of osteoclast-mediated bone resorption in RA.

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Synovial Histopathology in Early Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by a progressive destruction of joints. Previous studies identified capillary damage, edema, vascular congestion, and cellular infiltrates as earlier pathological changes in the rheumatoid synovium of disease of less than 6 weeks duration. However, it is possible that even 6 weeks is too late to identify some important early changes, since recent studies have suggested that the debut of RA already represents a chronic phase of the disease. In this report, we show the histological features of the synovium in early RA. Synovial tissues were obtained by arthroscopic synovectomy from three patients who had had episodes of monoarthritis for one or two years prior to the development of typical RA, satisfying the American Rheumatism Association 1987 criteria. As controls, four patients with osteoarthritis in the knee joints and, two patients with systemic lupus erythematosus and aseptic necrosis of the knee joints were additionally studied. The common histologic findings of the synovium of the early RA patients on the light microscopy revealed very mild proliferation of lining cells with capillary congestion and mild mononuclear cell infiltration. However, similar changes were also detected in the control patients. Of note, a proportion of the biopsied synovium showed only angioneogenesis without either lining cell proliferation or mononuclear cell infiltration, which was not detected in the control patients. The results thus suggest that angioneogenesis may precede any other features, such as lining cell proliferation and cellular infiltration in early RA. Moreover, it is most likely that angioneogenesis might be the feature that is most proximal to the etiology in early RA.

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Synovial Tissue Response to Treatment: The Effects of IFN-β Treatment in Rheumatoid Arthritis Patients

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Analysis of serial synovial biopsy specimens may provide useful surrogate end points for clinical studies. This approach could lead to a rapid screening method requiring relatively low numbers of patients for predicting the effects of novel anti-rheumatic drugs [1]. Before the therapeutic effects on synovial tissue (ST) could be properly analyzed, studies were conducted to establish the methods for synovial biopsy [2], microscopic examination [3], the relationship between the immunohistologic characteristics and disease activity [4], and the features of serial biopsies after placebo treatment [5].

This approach was used to evaluate the effects of IFN-β therapy on ST from RA patients [6]. Eleven patients were treated for 12 weeks with purified natural fibroblast IFN-β (Frone®, Ares-Serono) s.c. 3 times weekly with the following dosages: 6 million units (MIU) (n=4), 12 MIU (n=3), and 18 MIU (n=4). Synovial biopsy specimens were obtained by needle arthroscopy at 3 time points: directly before and at 1 month and 3 months after initiation of treatment. Immunohistologic analysis was performed to detect CD3, CD38, CD68, CD55, TNF-α, IL-1β, IL-6, MMP-1, and TIMP-1. A significant reduction in the mean immunohistologic scores for CD3+ T cells, CD38+ plasma cells, and expression of IL-1β, IL-6, MMP-1, and TIMP-1 was observed in ST after treatment. The scores for CD68+ macrophages and TNF-α expression also tended to decrease, but these differences did not reach statistical significance. The inhibitory effects of IFN-β on MMP-1 production by fibroblast-like synoviocytes were confirmed by *in vitro* studies.

The significant changes in synovial tissue after IFN-β treatment support the view that IFN-β therapy has immunomodulating effects on rheumatoid synovium and might help to diminish both joint inflammation and destruction.

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Fibroblastoid Synoviocytes: Their Intrinsic and Disease-Modified Phenotype as Revealed by a Differential Subtraction Library Approach

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Through their unique and increasingly defined pattern of gene expression, the fibroblastoid intimal synoviocytes appear to be

differentiated to perform a series of functions critical to the biologic function of the normal joint, including responsibility for its developmental patterning and the maintenance of its structural integrity. This phenotype also appears to confer the potential for fostering the development and synovial localization of the autoimmune response underlying rheumatoid arthritis, and to participate in joint destruction. The use of synoviocyte lines cultured from either rheumatoid arthritis or osteoarthritis synovia has facilitated progress in the process of gene discovery, although it remains to be determined the extent to which the distinctive phenotype of cells from rheumatoid arthritis reflects persistent modulation by exposure to the inflammatory microenvironment and the extent to which lines from osteoarthritis synovia include large proportions of more conventional fibroblasts from deeper layers of the joint rather than intimal synoviocytes. The possible explanations of the distinctive phenotype include: being intrinsic to the fibroblastoid intimal synoviocyte lineage, persistent modulation by inflammation, or that the over expression phenotype reflects a genetically determined increased expression due to a disease predisposing regulatory polymorphism. Other genes that are identified as equivalently expressed in both lines cultured from either rheumatoid arthritis or osteoarthritis synovia are likely to be related to the more general role of members of the fibroblastoid lineage, or at least those developing in the synovial milieu.

The profile of genes being identified by us and in other laboratories are relevant to novel functions exhibited by this lineage in the normal joint. Fibroblastoid intimal synoviocytes differ from other fibroblastoid lineage cells in that the structure of the intimal lining involves both homotypic cell–cell interactions and heterotypic interactions with monocytes. Genes encoding receptors selectively expressed in synoviocytes are being identified that appear to be involved in these interactions as well as mediating cell–connective tissue matrix interactions. Additional genes are being recognized that appear responsible for the localization and guidance clues that result in the entrance and differentiation of monocytes in the intima, including certain chemokines such as SDF-1. An exaggeration of this mechanism is likely relevant to nonantigen-specific entry of lymphocytes and monocytes into the joint in inflammation. Genes implicated in the patterning of the central nervous system are expressed constitutively at high levels in synoviocytes, perhaps reflecting a role in the development of the joint architecture. A last group of genes is thought to be induced by cellular activation pathways and their expression could relate to the modulation of the synoviocyte by inflammation.

The Effects of 'Pulse' Corticosteroids on Mediators of Inflammation and Joint Damage in the Rheumatoid Synovium

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We have demonstrated that the dramatic reduction in clinical inflammation in rheumatoid arthritis (RA) joints caused by high-dose 'pulse' methylprednisolone (MP) is associated with a rapid reduction in neutrophil ingress. MP also causes a reduction in L-selectin and β_2 integrin expression in synovial fluid neutrophils. To determine the mechanisms of these effects, arthroscopic synovial biopsies were taken before and 24 hours after MP and at disease relapse, and the expression of various cytokines and cell adhesion molecules were determined using standard immunohistochemical techniques. It was observed that MP caused a marked reduction in TNFα, IL-8, E-selectin, and ICAM-1 expression in the synovial membrane with 24 hours, thereby reducing the chemotactic gradient for neutrophils as well as neutrophil activation and adhesion in the synovial membrane. Disease relapse was associated with re-expression of these molecules. There was no effect on IL-1β, IL-1Ra, P-selectin, or PECAM expression.

Although no effect on macrohage infiltration was observed, there was a reduction in the expression of MCP-1 and MIP-1α expression in the synovial membrane within 24 hours of MP.

The effects of glucocorticoids on joint damage remain controversial. We have observed that MP significantly reduces MMP-1 and TIMP-1 expression but not MMP-3 expression in the rheumatoid synovium.

The role of the S100 proteins in RA remains unclear. We have observed that the expression of S100A8 and -A9 (MRP8 and -14) is increased by MP. The significance of these findings has yet to be determined.

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Novel Therapies of Rheumatoid Arthritis

Assessment of Innovative Therapies: A Mode-of-Action Approach

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A better understanding of the pathogenesis of rheumatoid arthritis (RA) has led to a number of new therapeutic possibilities. The clinical introduction of such novel pharmaceutical molecules is usually delayed because of difficulty in assessing their *in vivo* potential. Furthermore there is considerable wastage in early development as phase I and II studies do not formally assess true disease modification, concentrating instead on symptomatic efficacy and toxicity. The availability of targeted therapies, such as blockade of TNF, highlight the inadequacy of such an approach.

The rapid advances in imaging techniques have revolutionised the possibilities for drug development. The modalities include small needle arthroscopy with synovial biopsy, dynamic enhanced high-field magnetic resonance imaging (MRI), fan-beam dual energy X-ray absorptionometry (DXA) and high resolution ultrasound (HRUS). Instead of large numbers of patients being studied using insensitive end points, proof of concept/mode of action studies undertake intensive investigation of highly selected patients, using predetermined end points usually involving the primary target organ.

Multidimentional imaging studies have been undertaken to directly examine novel therapies, which include intra-articular anti-CD4, combination monoclonal antibodies, stem-cell transplantation, and comparisons of novel pharmaceutical agents, eg, the direct effect of corticosteroids on the synovium. These studies, which are scored in a blinded fashion, have shown significant changes in small numbers of patients. Depending on the type of disease, the imaging can focus on either the synovium (knee) or the synovial/bone interface (hand). The value of individual techniques will be discussed and a developmental plan for therapies in arthritic disease proposed.

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Lessons Learned From Gene Therapy Approaches

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Our ability to transfer genes to the synovial lining of joints allows the roles of various gene products in synovitis to be studied in novel ways. One strategy uses adenoviral vectors to transfer genes of interest to the synovial lining of normal and inflamed rabbits' knee joints. Rabbits are used because their knee joints are sufficiently large for reproducible intra-articular injection and lavage. Moreover, it is possible to study the responses of articular cartilage to disturbances occurring in the synovium. These studies need to be carefully performed as the adenoviral vector itself has inflammatory properties. Nevertheless, it is possible to gain important new information in this way.

When introduced into naive joints, vectors carrying IL-1β provoke a dramatic synovitis and pannus, which severely erode the adjacent cartilage. They also trigger systemic disturbances, including fever and weight loss. Oddly, the introduction of similar vectors carrying TNF produces only mild inflammatory and chondrodestructive changes. The TGF-β gene, in contrast, generates a massive fibrosis. The IGF-I and BMP-2 genes, on the other hand, produce few synovial changes and modestly elevate the synthesis of proteoglycans by cartilage.

When introduced into joints with antigen-induced arthritis, adenoviral vectors carrying genes encoding bivalent IL-1sR type I protect the articular cartilage and reduce the influx of leukocytes into the joint space. They do not, however, reduce synovitis or swelling. Similar vectors carrying bivalent TNFsR type I have no effect on cartilage and are only very weakly anti-inflammatory. Co-introduction of both vectors is strongly anti-inflammatory and chondroprotective. In animals with bivalent disease, there is also amelioration of disease in the contralateral knee joint that was not injected with the therapeutic genes. An even stronger effect on synovitis and associated joint pathologies was obtained with the viral IL-10 gene, which also produced a marked contralateral response. This effect has been reproduced in collagen-induced arthritis in mice.

New Vectors and New Targets for Gene Therapy in Rheumatoid Arthritis

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The immunopathology of rheumatoid arthritis (RA) is associated with the production of inflammatory cytokines (IL-1, TNF-α, IL-6), synovial proliferation, and cartilage invasion. A sustained

increase in the amounts of cytokine antagonists obtained through gene therapy should inhibit the process. Targeting of a single molecule, however, is unlikely to be sufficient for the reversal of the complex molecular and cellular events that lead to the progressive destruction of cartilage and bone in RA. Several groups have developed efficient gene transfer of therapeutic molecules in experimental arthritis (IRAP, TNF-R, IL-10, TGF-β) through retrovirus (*ex vivo* procedure) or intra articular (i.a) or systemic adenoviral delivery (*in vivo* procedure).

To increase the efficiency of gene transfer, new targets have been identified. They include transduction signal inhibitors (super repressor IκBa), synovial-cell activation cascade (c-Jun, Ras antagonist), and synovial apoptosis (fas ligand, p53 or Rb gene transfer). Suicide gene (HSV tk) may also be administered i.a. and induces a 'genetic synovectomy' after IV gancyclovir treatment. Angiogenesis may also be inhibited after gene transfer (antagonist of αVβ3 or plasminogen activator [PA], PF4, angiostatin). We will present new data showing a decrease in arthritic severity after adenoviral transfer of PA antagonist. All of these targets may be combined with the cytokine approach.

Progress in the development of safe nonviral gene delivery has been made in recent years. Liposome HVJ is efficient to deliver DNA in chondrocytes and synoviocytes without systemic diffusion. Efficient HSV tk gene transfer has been achieved in the synovium by local injection of naked DNA plasmids. Plasmid injection in the muscle combined with electroporation increases by 1000 the serum concentration of cytokine.

AAV vectors are parvoviruses designed to be gutless and efficient for direct gene transfer *in vivo*. Interestingly, only a weak immune response against the transgene product is detected in animals following AAV-mediated gene transfer, allowing long-term expression (>18 months). These vectors are suitable to transfer genes in the synovial tissue. Using the SCID mouse model, we showed the feasibility of gene transfer in human tissue with AAV recombinant vectors.

For gene therapy to be an effective and safe approach for the clinical management of disease, gene expression must be highly regulated. The design of safe vectors to enhance the duration of transgene expression and to co-transfer regulatory genes is an active area of research.

Obstacles to Anti-CD4 Therapy in Rheumatoid Arthritis

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Monoclonal antibodies (mAbs) to CD4 have been employed in a variety of studies to patients with active rheumatoid arthritis (RA) in an attempt to control disease progression not only by preventing T cell activation but also by inducing tolerance to the putative autoantigen(s) [1,2,5]. Initial open labeled clinical trials have provided encouraging clinical results [1,2,4]. However, randomized controlled studies with chimeric immunoglobulins have largely failed to demonstrate a significant benefit of anti-CD4 mAbs over placebo [1,2,5]. Following those disappointing trials, favorable results of several dose-finding studies with high dosages of nondepleting CD4 mAbs were recently presented [1,2,6]. Whereas the mechanisms underlying the clinical benefit remain to be elucidated, it appears to be clear that depletion of CD4 T cells cannot explain clinical efficacy. Moreover, the data are consistent with the hypothesis that long lasting successful treatment of RA with mAb to CD4 might require long-term inhibition of peripheral T cell functions by non-depleting mAbs to CD4. Future studies are necessary to delineate precisely the dosing requirements for clinical benefit and to elucidate the mode of actions of anti-CD4 mAbs. Exciting new data that are indicative of a modulation of the detrimental Th1-dominated immune response in RA by anti-CD4 mAbs [6] warrant further investigation. Finally, data from animal studies suggest that new promising therapeutic perspectives in man might result from combining anti-CD4 mAbs with other biologic therapeutics [1,7,8].

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The Role of TNFα in the Pathogenesis of Rheumatoid Synovitis

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In this presentation we summarise the insights that have been gained into the role of TNFα in the pathophysiology of

rheumatoid synovitis from observations in patients receiving infliximab (Remicade™), an anti-TNFα specific, neutralising, monoclonal antibody [1–3].

Serial synovial biopsies and serum samples before and after anti-TNF therapy show a reduction in the density of infiltrating lymphocytes and macrophages associated with a reduction in the expression of adhesion molecules E-selectin, ICAM-1 and VCAM-1 [4]. Circulating lymphocyte count is increased in a dose-dependent manner. Further studies have shown a reduction in chemokine expression [5]. Taken together these data have suggested that anti-TNF therapy alters leukocyte trafficking patterns with a net reduction in immune and inflammatory cell mass in the synovium.

Direct evidence of reduced retention of indium^{111}-labelled polymorphonuclear cells in RA joints has recently been obtained following infliximab therapy [6]. This is likely to be due to effects on cell margination and migration but may also reflect a reduction in angiogenesis, a conclusion supported by the finding of a reduction in circulating VEGF concentrations and vessel density in serial synovial biopsies [7].

Neoangiogenesis not only increases delivery of inflammatory cells to the joint, but plays a part in cartilage and bone destruction in RA. It is not yet known whether anti-TNF treatment prevents structural damage but reduced serum levels of complexed (inactive) MMP-1 and MMP-3 are observed in treated patients [8].

Clinical trials have demonstrated that ~30% of patients do not appear to respond to anti-TNF therapy. Clearly other molecular mechanisms are involved in this sub-population. In the majority of RA patients, however, TNFα plays a key role in the expression of synovitis. The need for continuing therapy to control disease expression implies that other, as yet unidentified, factors are involved in maintaining the chronicity of disease.

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Etanercept for Treating Rheumatoid Arthritis

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Etanercept (Enbrel™) has specifically been developed to achieve therapeutic neutralization of TNF. DNA encoding the soluble portion of the human p75 TNFR was linked to DNA encoding the Fc portion of the human IgG molecules and expressed in a mammalian cell line. The resultant protein binds two TNF molecules and has increased circulating half-life due to the Fc moiety. In the first placebo-controlled trial, 75% of patients receiving the highest dose of etanercept (16 mg/m^2 subcutaneously twice a week) achieved at least a 20% ACR clinical response after 3 months of therapy. This improvement was associated with significant reductions in pain and morning stiffness, as well as in the biochemical markers (ESR and CRP) of disease activity. In a subsequent placebo-controlled phase III study, using a dosing schedule of 10 mg or 25 mg of etanercept subcutaneously biweekly, sustained clinical efficacy and safety were both verified over a period of 3 months. The 25-mg dose proved superior to the 10-mg dose in terms of effectiveness. No adverse events other than injection site reactions were more commonly noted with either dosage compared to the placebo group. Rheumatoid arthritis (RA) patients with suboptimal clinical responses on DMARD therapy may be the most optimal candidates for the addition of the TNF antagonist etanercept to the treatment regimen. Results of a phase II/III study of combination therapy with etanercept and methotrexate in RA patients refractory to moderate doses of methotrexate have recently been reported. The combination of etanercept and methotrexate resulted in 71% of patients achieving a 20% ACR response, compared with only 27% of patients who received methotrexate alone. The encouraging clinical results observed in short-term trials using etanercept clearly warrant further studies to determine whether TNF inhibitors are capable of modifying the destructive component of the disease and to assess safety in long-term use. A pivotal trial with etanercept is now in progress comparing etanercept with methotrexate in early RA. Radiographic changes will be measured in this trial.

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Stem Cell Transplantation in the Treatment of Rheumatoid Arthritis and Other Autoimmune Diseases

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The concept of hematoimmunoablation followed by autologous hemopoietic stem cell transplantation (HSCT) as a treatment for severe autoimmune disease has gained momentum in the past several years. Almost from the beginning, international consensus was reached concerning principles of patient selection and treatment regimens, and several phase-I and -II pilot studies were commenced. It was also agreed to collect as much data as possible following mobilisation in order to develop a databased risk assessment of the procedure and later to balance this against benefit. Under the auspices of the EBMT and EULAR, a database was established, which was later extended to include Australian and US cases. The current database includes over 150 cases and has been presented in several forums. In addition, the IBMTR contains similar cases and case reports, and small series have been published. The majority of cases have been MS, followed by scleroderma (systemic sclerosis or SSc), RA, juvenile arthritis, SLE, and others.

A preliminary analysis of the international data base showed a transplant-related mortality of 9%, comparable to that seen in a group of lymphoma patients treated in the EBMT centres over the same period (6%). So far, no fatalities have been published in the literature.

Several issues have emerged as the experience grows. The most important is patient selection and exclusion. The original loose guidelines of 'potentially sick enough to justify the risk but not too severe to be either irreversible or too ill for the treatment' have proven difficult to quantify. Potential disease-specific aspects contribute to transplant-related mortality (eg cardiac involvement in SSc and cyclophosphamide toxicity) and to transplant-related problems (eg age of patient and general medical condition). The unwanted events have been generally consistent with previous BMT experience, with the possible exception of mobilisation complications in SSc.

Several subgroups have been formed under the auspices of the EBMT to look at disease-specific aspects in SSc, RAS/JCA, MS, and SLE/vasculitis, and these findings will be published.

Concerning mobilisation-related events, a suggestion of growth factor-related autoimmune disease (AD) flare is subject to a separate ongoing study. The current feeling is that in systematically active AD, such as Still's disease, SLE, and vasculitis, cyclophosphamide should be given prior to growth factor until more data are available. Only a few patients have experienced failed mobilisation, and this was successful at second mobilisation in most.

No conditioning regimen has so far proven superior with respect to remission induction and/or maintenance. Regimens have mostly followed published guidelines, with a suggestion of more infectious complications following heavy T-cell purging.

Efficacy has ranged from dramatic to none and death from disease progression. In general, outcome measurement has not been standardised between groups but is well defined within the individual centres. Around two thirds have been reported as 'improved or stabilized,' and more data are required, including relapse rates and severity.

Data collection is critical, with a current liaison group working toward standardising MED A and MED B equivalent data collection in Europe and the US. In particular, standardised measurements of immune or B-cell phenotype in peripheral blood has been associated with successful or unsuccessful outcome. As predicted, heavy T-cell purging was associated with more prolonged T-cell CD4-penia and increased infection. This is also subject to a subgroup working party. Eventually, the final place of such a treatment, if it exists, will be established through prospective comparative trials.

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