Introduction and overview

When the First International Symposium on the Immunotherapy of the Rheumatic Diseases was held in London in February 1991, immunotherapy was an exciting but largely theoretical concept. That meeting, focusing exclusively on immune cells and mediators as therapeutic targets, was almost entirely restricted to in vitro and animal studies, as only a few small open studies had been performed in humans in vivo. Just four years later, the situation has evolved beyond all recognition. Whilst in vitro and animal experiments are still vital, an increasing variety of treatments have been tested in vivo in humans in double blind placebo controlled trials. In addition, the purview of the Symposium is now much broader as the mesenchyme, including synoviocytes, proteolytic enzymes, and angiogenesis, is now an active target for antirheumatic drug development. Inevitably, there have also been some failures over the past four years. Some initially promising treatments, such as major histocompatibility complex (MHC) blocking peptides and lymphocyte depleting antibodies, appear to have fallen by the wayside and, even more disappointingly perhaps, we are no further forward in identifying the putative autoantigens involved in triggering or perpetuating the disease. However, as can be seen from the ensuing review and abstracts, gains have far outweighed losses, setting the scene for a fascinating conference.

Somewhat paradoxically for a meeting on the Immunotherapy of the Rheumatic Diseases, the conference started with a Keynote Address by Dr L Adorini (Milan) on the non-obese diabetic (NOD) mouse as a model for developing and testing immunotherapy in human diabetes. There was method in the apparent madness. Diabetes in the NOD mouse represents one of the best spontaneously developing animal models available, as it has a similar pathology, analogous genetic susceptibility and identical putative autoantigens as obtain in human diabetes. Examination of how data from this model can be applied to the human disease may help us to interpret information from the rather less satisfactory animal models available for the rheumatic diseases.

The Symposium proper began with an overview of the pathogenesis of rheumatoid arthritis (RA) (Dr G H Kingsley), followed by five presentations on various topical aspects. Dr Panayi put forward the hypothesis that RA is an organ specific autoimmune disease in which the immune response is directed to the chondrocyte. As immune responses to two putative cartilage autoantigens, collagen type II and cartilage proteoglycan, have proved difficult to find, the group has developed a chondrocyte expression library and is screening it with RA serum to identify chondrocyte specific proteins which may have a role in RA. The identification of such candidate antigens would allow therapeutic approaches using antigen specific tolerance similar to those for diabetes discussed in the Keynote Address. Dr Natvig's group has demonstrated that rheumatoid factor in healthy individuals is subject to a controlling mechanism which limits affinity maturation. He suggested that the apparent loss of such control in RA may be of pathogenetic significance; the question of what role rheumatoid factor has in synovitis remains unanswered. Turning to T cells, Dr Amento has found, in several autoimmune animal models, that administration of TCR peptides from disease perpetuating T cells can suppress disease by upregulating counter regulatory T cells; however, as the question of whether there is restricted T cell receptor (TCR) usage in RA remains unresolved, the potential of this type of treatment in humans is as yet unknown. The collagen induced arthritis developed by Dr Bontrop and colleagues in rhesus monkeys is probably the best characterised primate inflammatory arthritis model, and should allow the testing of pathogenetic and therapeutic hypotheses in much the same way as the NOD mouse model does in diabetes. In the last presentation on immunopathogenesis, Dr David reviewed the role of human MHC class II genes in mediating susceptibility to arthritis, using a new model system. In this model, human MHC class II genes from RA susceptible or resistant haplotypes were introduced into mice from which the endogenous class II genes had been 'deleted', so that their only functional class II molecules were those coded by the human genes. The effect of these manipulations on arthritis susceptibility could then be examined in a controlled fashion which may help to explain the role of MHC class II in determining disease susceptibility in humans.

The next major theme of the meeting was the immunologist's holy grail, the induction of tolerance. Immunosuppressive approaches to the treatment of autoimmune disease have two major drawbacks—the need for long term treatment to prevent return of disease, and the risk of infection or tumour as a result of a generalised loss of immune function. In contrast, the reinduction of tolerance to the putative rheumatoid autoantigen(s) has the potential to provide a permanent and highly selective treatment. Three different approaches to induction of tolerance were discussed at the meeting. In session II, Drs Fallar, Wraith and Sieper reviewed tolerance induced by nasal or oral antigen administration. Whilst this technique has progressed the furthest in clinical terms (indeed, the results of two double blind placebo controlled clinical trials of oral collagen in RA were presented, one positive and one negative), the mechanisms underlying oral and nasal tolerance remain far from clear. Dr Cohen and Dr Sette discussed ways of interfering with the specific T cell receptors (TCR) involved in the 'autoimmune' MHC-antigen-TCR complex. Though potentially very
exciting, the work has not progressed beyond in vitro studies or animal models because the relevant elements of the trimolecular complex have not been identified in any human disease. The recognition of good candidate auto-
antigens in human autoimmune diseases would, of course, alter this situation. By contrast, it has been known for many years that RA is associated with the MHC class II antigens, HLA-DR1 and HLA-DR4. MHC antibody therapy was stopped in its tracks early on in the development of immunotherapy, partly because such antibodies produced very severe side effects when administered to certain monkeys (these effects later proved to be largely species specific), and partly because of the gold standard in MHC blocking peptides (which have failed for pharmacokinetic reasons). Now there has been a resurgence of interest, especially in using antibodies which are non-cytotoxic, such as those discussed by Dr Smith in his presentation. The final session devoted to tolerance reviewed the possibility of inducing tolerance by targeting accessory interactions involved in T cell activation such as the CD40-gp39 pathway (Dr Noelle), the CD28/CTLA-4-B7 pathway (Dr Wofsy) and the CD4 molecule (Dr Yocum). In the first two systems, inhibitory reagents (antihuman gp39 monoclonal antibodies and CTLA-4 immunoglobulin fusion protein, respectively) have been developed in vitro and tested in animal models, but in vivo data in humans are still awaited. The situation with CD40 is rather different. Anti-CD4 antibodies which deplete CD4 T cells have been extensively studied in RA, even to the point of controlled clinical trials, though with disappointing results. There was a relative lack of clinical efficacy, though this may have resulted partially from the use of inadequate doses; more importantly, the patients developed a persistently low CD4 count, leading to concern about the possibility of generalised immuno-
suppression. The new studies presented at this meeting used an antibody (IDEC-C9E.9.1) which appeared to induce only a transient CD4 T cell depletion. Early clinical results are promising, but many more data are required.

Intracellular signalling pathways are another hot target for intervention in RA. Unlike the other areas reviewed at this meeting, drugs inhibiting these pathways are in everyday clinical use though, until recently, the related basic science has lagged behind. However, as the presentations by Dr Rudd and Dr Fearon showed, over the past few years there have been enormous advances in our understanding. Not only has this enabled us to understand the mechanism of action of well known drugs, such as the anti-immunophilin cyclosporin (Dr Schreier), and of drugs just approaching human use, such as sirolimus/rapamycin, but it has also enabled the development of more specific molecules, such as the selective protein kinase C isoenzyme inhibitors (Dr Nixon). To come full circle, these new drugs have, in turn, cast doubt on some of the basic science underlying their development. In addition, well known anti-rheumatic drugs, such as leflunomide (Dr Strand) may exert their action at least partially by interfering with intracellular signalling.

The role of mesenchymal cells and molecules in the development of synovitis is receiving more and more attention. In view of the generally accepted idea that joint destruction is caused by synovial inflammation, studies from Dr Gay’s group are of particular interest. He has shown, both in a severe combined immunodeficiency disease (SCID) mouse model and in humans infected with HIV, that proliferating ‘transformed’ synoviocytes can persist in the absence of T cells or any preceding inflammation, and can produce proteases, resulting in progressive joint destruction. As erosive joint damage is the best predictor of outcome in RA, this work can be con-

strued as showing that mesenchymal rather than immuno-
logical molecules are the most appropriate therapeutic targets. Continuing with this theme, Mr K Bottomley reviewed the role of various metalloproteinases in the degradation of articular cartilage. In view of the critical role of joint erosion in the outcome of RA, metalloproteinase inhibition is likely to effect a marked improvement in the outlook for patients, even if the disease is not cured. Dr Drummond extended this argument by considering the role of metalloproteinases, not only in cartilage destruction but also in angiogenesis and in the release of tumour necrosis factor alpha (TNFalpha). His group have developed the so called TMI (TNF and Metalloproteinase Inhibitor) drugs which affect each of the three pathways in which these enzymes are involved. These are now in preclinical and clinical development for cancer and inflammatory conditions. The next two presentations brought up another method of enhancing RA treatment. Most of the drugs currently used in RA were found serendipitously and the regimens which are used may be neither the most effective nor the least toxic. Steroids are perhaps the best example of all, as they are clearly effective in inhibiting inflammation and perhaps joint damage, but their long term daily use is toxic. Dr Pitzalis demonstrated the ability of steroids to inhibit cell adhesion, whilst Dr Boumpas focused on their capacity to inhibit transcription of certain cytokine genes such as interleukin (IL)-2 and IL-8. By understanding more about these well known drugs, it is to be hoped that less toxic steroid regimens or alternative agents affecting the relevant targets can be developed. The final presentation in this session (Dr Seed) also focused on a well known drug in a new field—this time, the ability of diclofenac complexed to hyaluronan to inhibit angiogenesis.

The most explosive field in RA immunotherapeutics over the past five years has been the development of anticytokine therapy. Dr Dayer, Dr van den Berg and Dr Kollas reviewed the evidence for the role of various cytokines, most notably TNFalpha and IL-1, in the pathogenesis of RA using in vitro studies and animal models including the TNFalpha transgenic mouse. The cytokine most thoroughly explored in human therapy to date has been TNFalpha. Thus several methods of targeting this molecule were examined, including chimeric or anti-human monoclonal antibodies (Dr Feldmann and Dr Choy), soluble TNF receptors (Dr Moreland), ribozymes (Dr Forre) and gene therapy using cells transfected with soluble TNF receptors (Dr Chernajovsky). Promising clinical results have been obtained with both types of anti-TNFalpha monoclonal antibody, demonstrating the importance of this molecule in disease, though the benefit only lasts a few weeks. However, as with other monoclonal antibodies, an antilymphocyte response may prevent long term use, especially as frequent repeat dosing will be required. Clinical results with soluble TNFalpha receptor reagents are eagerly awaited, as is resolution of the controversy over whether these, too, will induce an immune response against themselves. More experimental therapeutic methods, such as ribozymes or gene therapy, may be required to allow prolonged anti-TNFalpha therapy. Alternatively, it may be more appropriate to try to inhibit TNFalpha secretion by the use of drugs such as the TMI inhibitors discussed previously by Dr Drummond although, as a disadvantage, these may also inhibit the secretion of TNFalpha receptors and will leave membrane TNFalpha intact. One question, raised by Dr Feldmann but so far only addressed in animal models, is whether combination therapy with anticytokine and antilymphocyte reagents will produce more effective inhibition than either alone. Human studies targeting other cytokines are much more limited, though some drugs, such
as Tenidap (Dr Breevd). AVOID, to exert their effect via rather broad anticytokine profiles. No monoclonal antibodies against IL-1 have yet been used in clinical practice, though they are effective in animal models, but both a recombinant form of the natural IL-1 antagonist, IL-1 receptor antagonist (IL-1ra), and a recombinant soluble IL-1 receptor (Dr Moreland) have been used in RA. The former has undergone fairly extensive clinical trials, with modest success; its major handicap is likely to be the fact that high concentrations have to be maintained at all times to prevent signalling via the IL-1 pathway. The final presentation in this session (Dr C H Divry) reviewed the possibilities for gene therapy in arthritis using IL-1ra as the antiatheritic gene. The gene has been delivered ex vivo to rabbit knees with beneficial effects on inflammation and cartilage destruction, and a human trial is about to begin.

The last session of the meeting returned us to earth with something of a bump. Despite all the scientific advances resulting in the new therapies described above, we are still very bad at assessing disease activity and outcome in RA.

Immunotherapy

Guest editor: Gabrielle H Kingsley

Abstracts

KEYNOTE ADDRESS

The NOD mouse model—the development of strategies to treat human autoimmune disease L Adorini
Roche Ricerche Milano, Via Olgettina 60, 20123 Milan, Italy

The non-obese diabetic (NOD) mouse probably represents the best spontaneous model for a human autoimmune disease, insulin dependent diabetes mellitus (IDDM). It has provided essential information in IDDM pathogenesis and, importantly, it allows testing of immunointervention strategies potentially applicable to man. IDDM is a polygenic disease, but, both in human and in NOD mouse IDDM, the primary susceptibility gene is located within the major histocompatibility complex (MHC). The unique class II MHC molecule, I-A\(\beta\), of the NOD mouse is distinguished by a Ser for Asp substitution at position 57 of its \(\beta\) chain, homologous with the non-Asp residue at position 57 of HLA-DQ \(\beta\) chain, the major IDDM susceptibility molecule in Caucasoids. The similarity extends to the putative target autoantigen(s), including glutamic acid de-carboxylase (GAD) and insulin. In both humans and NOD mice, cellular and humoral responses to GAD and insulin precede the onset of IDDM. Evidence for the pathogenicity of these autoantigens in the NOD mouse indicates that this animal model can be reliably used for testing antigen specific immunointervention strategies potentially applicable to human IDDM patients. In addition to antigen specific immunointervention, a variety of approaches have been tested in the NOD mouse. These include MHC blockade, interference with accessory molecules, administration of cytokines or anticytokines, and immunosuppressive or immunostimulating agents.

PATHOGENESIS OF INFLAMMATORY ARTHRITIS

Pathogenesis of rheumatoid arthritis: an overview
G H Kingsley
United Medical and Dental Schools, (Guy's Campus), Guy's Hospital, London, United Kingdom

Two major hypotheses on the pathogenesis of rheumatoid arthritis (RA) currently exist. In the first, T cells play only a minor initial role, the major inflammatory response being the result of interaction between monocyte-macrophages and synoviocytes. In the second, synovitis results from presentation of arthritogenic antigen(s) to specific CD4 T cells which release lymphokines, leading to monocyte and thus synoviocyte activation.

The end result is similar; the critical difference lies in the importance ascribed to T cells. There is considerable evidence that T cells are involved in RA. First, immunohistological examination of RA synovium demonstrates the presence of activated CD4 T cells. Second, there is a strong association of RA with major histocompatibility complex (MHC) class II alleles which are known to function in the presentation of antigens to the immune system. It is also known that T cells are recruited, activated, and differentiated in RA synovium; little is known, however, about the role of T cells in the initiation of the disease process.

Autoantigens in rheumatoid arthritis
G S Panayi, L Forrest
Rheumatology Unit, Division of Medicine, United Medical and Dental Schools of Guy's and St Thomas' Hospitals, London SE1 9RT, United Kingdom

Rheumatoid arthritis (RA) is linked to HLA-DR4, implying that antigen or autoantigens are involved at the initiation or perpetuation of the disease. The nature of these antigens remains unknown. Type II articular cartilage (CII) and cartilage proteoglycan (CP) have been proposed, but have not found universal support. Antibodies to CII have been found in only a minority of RA patients and T cell responses have been difficult to elicit. It would appear that immune responses to CP reported to date have been in patients with ankylosing spondylitis. Clearly a new approach is needed.

The RA antigen could be a rare protein or set of proteins which would not be intuitively obvious. We therefore screened a human chondrosarcoma expression library with IgG antibodies derived from a pool of RA sera recognising a 65 kDa protein from the human chondrosarcoma line. Sequencing clones isolated from this library showed that the encoded protein was novel. Northern blot analysis showed that this gene was not
regulated by Palo Alto and EP Immune peroxidation mediated diseases rearrangements biased when outgrown by the disease. Of individuals there is contrasts segments and related component segments, with the accumulation of mutations. However, two there factor rheumatoid arthritis is characterised of blood peripheral with RFs. The national of rheumatoid factors individuals is lost, with the expression of V regions. The heterodimeric of the T cell receptor (TCR). A subset of T cells, when outgrown from disease tissue, display rearrangements of \( \beta \) chain variable \( (\beta) \), diversity (D) and junctional \( (\delta) \) domains that are consistent among patients with disease and yet vary between disease states. T lymphocytes obtained from rheumatoid synovium are further restricted by the finding of shared V-B usage in multiple joints from the same patient. Restrictions in V-B associations, in addition to conserved CDR3 (antigen binding domain) sequences have been observed.

To aid in our understanding of these observations, similar studies have been performed in several animal models of immune mediated diseases. Assignment of relevant TCR bias was performed using the same outgrowth, polymerase chain reaction and sequencing techniques as used for analysis of human disease. To confirm the functional relevance of cells bearing these receptors on the perpetuation of disease, immunotherapy was directed specifically at these T lymphocytes. Peptides (19aa) corresponding to the CDR2 region of the TCR were administered to rats with experimental allergic neuritis (EAN) or adjuvant arthritis (AA) at the initiation of clinical activity. Clinical development of EAN or AA was halted by the administration of CDR2 peptides derived from the relevant Vβ chains, but not by peptides derived from irrelevant Vβs. The TCR is an immunoreceptor mediated by the T cell receptor-CD3 complex and upregulation of counterregulatory T cells that were present, but not sufficiently active, to regulate the function of disease perpetuating T cells in the absence of specific immunity. The presence and upregulation of T cells recognising TCR peptides was necessary for successful TCR immunotherapy.

These findings demonstrate the spontaneous occurrence of T cells in immune mediated diseases that use their recognition element sequences found in a specific region of the TCR. Similar T-T recognition may be a general consequence of immune regulation, but be inadequate in diseases such as rheumatoid arthritis or multiple sclerosis. The therapeutic utility of this approach in human disease requires that the dysregulated T cells maintaining active disease, identification of the appropriate immunogenic region of the TCR, and the presence of counter-regulatory T cells capable of responding to TCR peptides are necessary. This approach may meet in human diseases such as rheumatoid arthritis, as they have been in at least three animal models, disease specific immune regulation of rheumatic diseases may become a reality.

### Immune mediated diseases are characterised by limited T cell receptor usage: disease expression may be regulated by naturally occurring T lymphocytes that recognise T cell receptor sequences

E P Amento

Connective Therapeutics Inc and Stanford University School of Medicine, Palo Alto and Stanford, CA, USA

Immune cell activity is associated with a variety of rheumatic diseases. Therapeutic interventions have been directed toward ablating sets of cells, blocking immune cell interactions of, or, alternatively, inhibiting the function of activated T lymphocytes. T lymphocytes associated with the perpetuation of several rheumatic diseases are biased in the use of the heterodimeric \( \beta \) T cell receptor (TCR). A subset of T cells, when outgrown from disease tissue, display rearrangements of \( \beta \) chain variable \( (\beta) \), diversity (D) and junctional \( (\delta) \) domains that are consistent among patients with disease and yet vary between disease states. T lymphocytes obtained from rheumatoid synovium are further restricted by the finding of shared V-B usage in multiple joints from the same patient. Restrictions in V-B associations, in addition to conserved CDR3 (antigen binding domain) sequences have been observed.

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### Polymorphism in the HLA-DQ locus determines susceptibility and polymorphism in the HLA-DRB1 genes determine protection in collagen associated diseases in human rheumatoid arthritis

C S David, G H Nabozny, M Gonzalez-Gay, E Zanelli, M Griffiths, H Luttra

Department of Immunology and Rheumatology, Mayo Clinic, Rochester, MN, USA

In a study to determine the role of HLA-DQ and HLA-DR transgenes in collagen induced arthritis (CIA), transgenic mice that the human class II genes were generated from a rheumatoid arthritis (RA) susceptible haplotype (HLA-DQ8, DR4) and an RA resistant haplotype (HLA-DQ6, DR2). The human class II genes were introduced into mouse class II knockout mice (Ab-) such that the only class II molecules expressed in these mice were coded by the human genes. The mice were immunised with bovine type II collagen and evaluated for clinical symptoms of arthritis and autoantibodies.

Only the HLA-DQ8 transgenic mice were susceptible to CIA (80% incidence: 7-9 severity). Further, no mice between a CIA resistant HLA-DQ6 and a CIA resistant DQ6 mouse were susceptible (75%), suggesting that susceptibility is dominant. These results showed that the HLA-DQ8 molecule can present the bovine type II collagen arthritogenic epitope and activate autoreactive T cells. In order to determine whether HLA-DR genes can play a protective role, they were introduced into CIA susceptible H-2a mice. The HLA-DRB1*07 mice, which is negatively correlated with RA in Japanese patients, completely protected the H-2a mice from CIA. These studies suggest that certain DRB1 molecules can protect individuals from CIA and may affect the immunity in response to DR peptides and their predisposition in human RA.

We propose that human RA is a result of susceptibility mediated by the HLA-DQ genes and lack of protection mediated by the DRB1 genes. We also propose that the protection mediated by HLA-DRB1 molecule is...
by the binding of the DRB3 HVR3 peptides by the DQ molecules, resulting in tolerance. Studies on peptide induced treatment are in progress.

**PUBLICATIONS RELEVANT TO THE SESSION**


Natvig J B. Rheumatoid factor V genes from patients with rheumatoid arthritis are diverse and through recombination generate nonhuman primate species maps to the major histocompatibility complex 1 region. *Proc Natl Acad Sci USA* 1994;91:12917-21.

Natvig J B. Rheumatoid arthritis (RA) is an inflammatory synovial disease thought to involve T cells reacting to an antigen within the joint. Type II collagen is the major protein in articular cartilage and is a potential autoantigen in RA. Oral induction of tolerance to type II collagen suppresses the two primary models of EAE. A report of results of a randomised double blind trial involving 60 patients with severe, active RA. In subjects in whom tolerance was induced orally with chicken type II collagen for three months, there was a steady improvement in the number of swollen joints, tender joints, walk time, and global assessments compared with those that received placebo. Four patients in the collagen group had complete remission of RA. No side effects occurred. These data suggested the clinical efficacy of oral induction of tolerance with type II collagen in RA. A multicentre randomised double blind dosing trial in which chicken collagen type II has been included in the diet of patients with functional class II and III rheumatoid arthritis will be completed during the summer of 1995.

**TOLERANCE**

**Oral tolerance in autoimmune diseases**

D A Hailer

Brigham and Women’s Hospital and Harvard Medical School, Boston, USA

An emerging concept in the treatment of T cell mediated autoimmune disease is to alter the function of autoreactive T cells so that they are no longer pathogenic but instead can actively downregulate organ specific immune responses. We have examined two such models of changes in T cell function, one using oral tolerance and the other using altered peptide ligands. This report is of the former.

The function of transforming growth factor (TGF)-β secreting myelin basic protein (MBP) reactive T cells can be studied in vivo using the experimental autoimmune encephalitis (EAE) model of autoimmunity: oral administration of MBP suppresses EAE by inducing peripheral tolerance. T cell clones were isolated from the mesenteric lymph nodes of SJL mice in which tolerance to MBP had been induced orally. Though these clones were CD4+ and were structurally identical, sharing the same T cell receptor α and β chain sequence as Th1 type encephalitogenic CD4+ clones, they produced TGFβ1 with varying amounts of interleukins (IL)-4 and IL-10. When adoptively transferred to naive animals, these MBP reactive T cell clones suppressed EAE induced with either MBP or proteolipid protein (PLP). This demonstrates that autoreactive TGFβ secreting clones are capable of growing regulating immune responses in vivo by a mechanism of bystander suppression. On the basis of the oral tolerance work in mice, we examined the antigen specific secretion of cytokines by β1-21. Our clones had been induced by mouth. More than 2500 short term T cell lines were analysed for MBP and PLP reactivity as measured by H-thymidine incorporation and by secretion of the cytokines interferon gamma, IL-4 and TGFβ1 measured by enzyme linked immunosorbent assay in 13 patients with MS (seven received 500 mg bovine myelin daily for a minimum of two years and six relapsing remitting MS patients received no such diet). No difference was found in the frequency of Th1 or Th2 lines derived from the two groups of patients, but the frequency of TGFβ1 secreting T cell lines after MBP and PLP antigen stimulation in those given myelin was greater than that in the other group (p = 0.01). Thus oral induction of tolerance with bovine myelin in patients with EAE induced T cell lines that may regulate the immune response at the site of the demyelinating lesion.

Rheumatoid arthritis (RA) is an inflammatory synovial disease thought to involve T cells reacting to an antigen within the joint. Type II collagen is the major protein in articular cartilage and is a potential autoantigen in RA. Oral induction of tolerance to type II collagen suppresses the two primary models of EAE. A report of results of a randomised double blind trial involving 60 patients with severe, active RA. In subjects in whom tolerance was induced orally with chicken type II collagen for three months, there was a steady improvement in the number of swollen joints, tender joints, walk time, and global assessments compared with those that received placebo. Four patients in the collagen group had complete remission of RA. No side effects occurred. These data suggested the clinical efficacy of oral induction of tolerance with type II collagen in RA. A multicentre randomised double blind dosing trial in which chicken collagen type II has been included in the diet of patients with functional class II and III rheumatoid arthritis will be completed during the summer of 1995.

**Nasal tolerance**

B Metzler, D C Wraith

Department of Pathology, Cambridge University, Cambridge, United Kingdom

While oral tolerance boasts a long history of investigation, antigen inhalation represents a more recent approach designed to modulate systemic immune responses in an antigen specific manner. The problems faced by the immune system are, however, very similar. In common with the gastrointestinal tract, the respiratory tract is in constant contact with numerous antigenic particles, of which only a minority are potentially pathogenic. The similarities between gut associated lymphatic tissue and bronchial associated lymphatic tissue suggest that the task of selective tolerance of attacking potentially detrimental agents while sparing innocuous ones, might be solved in a similar fashion. Unlike oral tolerance, however, antigen inhalation has not yet been widely investigated as a strategy for induction of tolerance in inflammatory autoimmune diseases. One report mentioned the amelioration of experimental autoimmune encephalitis (EAE) by inhalation of myelin basic protein (MBP) in Lewis rats. Another study demonstrated that nasal administration of retinal antigens suppressed experimental autoimmune uveitis in Lewis rats. EAE in MBP reactive T cell clones is dominated by responses to the N-terminal peptide Ac1-9. Analogue of this peptide, with substitutions at position 4, bind to the major histocompatibility complex (MHC) class II molecule with higher affinity than the original peptide, with a hierarchy of affinity Ac1-9(4Y) ≪ [Ac1-9]. In contrast to results in mice, in rats, there was no evidence for oral tolerance in the H-2 mouse model of EAE when whole porcine MBP, mouse myelin, or Ac1-9K[4Y] were fed. Abrogation of T cell responses and EAE after oral administration of repeated high doses of the [4Y] analogue showed, however, that encephalitogenic T cells could be targeted by peptide feeding.

Unlike oral administration, a single intranasal dose of Ac1-9[4Y] analogues inhibited EAE induced with Ac1-9 or whole myelin, each experiment demonstrating a positive correlation between peptide-MHC affinity and the degree of protection. Administration of the single dominant epitope induced linked suppression of the response to a subdominant epitope within MBP. Intranasally administered peptides could also downregulate the functions of encephalitogenic T cells after the induction of EAE.

Results of a German study of oral tolerance

J Sieper, S Kary, N A Mitchison

Deutsches Rheumaforschungszentrum and Free University Berlin, Germany

Suppressor T cells in the mucosa of the gut are activated by absorbed antigen in order to avoid a systemic immune response to this antigen. This long known phenomenon of oral tolerance is now used in the treatment of rheumatoid arthritis (RA) with oral collagen type II, which is the most important protein of cartilage. Although the role of collagen in initiating and maintaining the immune response in the joint is not clear, these suppressor T cells (either CD8 or, possibly, also CD4 T cells) can be stimulated in a trigger specific and effector non-specific way by contact with collagen II in the joint. It is assumed that a local immunosuppression then takes place through the secretion of inhibitory cytokines such as transforming growth factor β and interleukin-4. In July 1993 a double blind study with oral collagen type II for the treatment of RA (disease duration less than three years) was started in Berlin. We used bovine collagen II, which has a high homology with the human form. Three groups of 30 patients each were treated with 1 mg or 10 mg collagen per day, or placebo, over three months. No severe side effects occurred. (Full results of this study anticipated by March 1995.)

**Mechanism of action of T cell receptor antagonist peptides**

A Sette, D Page, S Hendricks, J Alexander, J Ruppert, K Snake, M Coggeshall, H M Grey

Cytel Corporation, San Diego, CA 92121, USA

T cell receptor (TCR) antagonism induced by complexes of antigen analogue with major histocompatibility complex (MHC) class II molecules results in the inhibition of antigen dependent T cell responses. We have investigated some of the possible mechanisms
by which TCR antagonists bound to the MHC molecules of antigen presenting cells (APC) can inhibit T cell activation. Using a non-stimulatory analogue of the antigenic peptide, influenza haemagglutinin 307-319, we showed that MHC-antagonist complexes completely prevent early intracellular events of antigen dependent T cell activation, such as inositol phosphate turnover and Ca⁺⁺ influx. In a parallel series of experiments, the effect of TCR antagonist peptide on membrane related activation events was also investigated. To our surprise, TCR antagonists did not inhibit antigen induced conjugate formation. Thus our data suggest that antagonistic peptides do not interfere with the selection process that required for stable T cell-APC conjugate formation, but do inhibit early biochemical events required for T cell proliferation. We have investigated the effect of TCR peptide antagonists on thymocyte activation. First, peptide antagonists were identified for the cytochrome c specific T cell clone, AD10. These peptides were then tested for their ability to induce negative selection in an in vitro model system using thymocytes from mice transgenic for the AD10 TCR. Although unable to induce mature T cell activation, the TCR peptide antagonists induced deletion of CD4 CD8 thymocytes. These results suggest that negative selection of CD4 CD8 thymocytes can be induced by TCR interactions of a lower affinity than those required for mature T cell activation.

T cell receptor peptide vaccination
I R Cohen
The Weizmann Institute of Science, Rehovot 76100, Israel

T cell receptors (TCR) of autoimmune T cells can be used as the basis of specific vaccines to shut off undesirable autoimmune responses. Such T cell vaccines can be whole T cells or peptides from the relevant TCR. The nature of the immune response to certain TCR vaccines suggests that the procedure may activate a control mechanism inherent in the organisation of the immune system around natural autoimmunity. If we can learn to provide the immune system with the regulatory peptides it has been programmed to obey, we might succeed in curing patients physiologically. This has been demonstrated in the case of spontaneous autoimmune diabetes in NOD mice. Disease can be shut shown using whole T cell vaccination or vaccination with a shared idiotypic TCR peptide from the CDRI region of the β chain. The mechanism involves both anti-idiotypic T cells and a switch (TH₁→TH2) in the behaviour of the autoimmune disease causing T cells.

Prevention and treatment of experimental autoimmune encephalomyelitis using non-cytotoxic anti-class II MHC antibodies
R M Smith, D C Wraith
Cambridge University Department of Pathology, Cambridge CB2 1QP, United Kingdom

Despite the proven immunosuppressive efficacy of anti-class II major histocompatibility complex (MHC) antibodies, the demonstration that they may cause marked and longlasting depletion of antigen presenting cells, not only provided a trivial explanation for their action, but also questioned their safety in human disease. The aim of this work was to demonstrate whether the cytotoxicity is a prerequisite for the immunosuppressive action of such antibodies and to investigate alternative mechanisms of action. We have demonstrated thatOX6 is poorly cytotoxic in the Biozzi AB/H mouse, coats class II positive cells only briefly, modulates class II MHC from the surface of cells incompletely and transiently, but is still effective in prevention and treatment of experimental autoimmune encephalitis. Histology, flow cytomtery and proliferation assays confirmed that OX6 is not preventing priming of T cells in the draining lymph node. These findings are in keeping with the previous demonstration of active suppression of delayed type hypersensitivity responses following anti-class II antibody administration. The recent description of functionally antagonistic T cell subsets suggests an identity for these suppressor cells. However, coadministration of 1B11 did not abrogate the therapeutic effect of OX6, arguing against induction of Th2 cells in this model. Alternatively, effector mechanisms may be inhibited directly. We found both in vivo antibody production in vitro macrophage tumour necrosis factor α production to be unaffected by anti-class II antibody administration. Further work on these mechanisms is in progress.

Effects of antibodies to the CD40 ligand (gp39) on cell and humoral mediated immunity
*Department of Microbiology, Dartmouth Medical School, Lebanon, NH, USA; Departments of Medicine and Pediatrics, University of Massachusetts Medical School, Worcester, MA; †Department of Internal Medicine, University of Texas, Southernmost Medical Center at Dallas, Dallas, TX, USA; ‡TNO Biological, The Netherlands; §Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle WA, USA

Accumulating evidence in animal models indicates that administration of anti-gp39 eradicates a variety of autoimmune phenom mon and interferes with allograft rejection. Anti-gp39, administered before the induction of disease, blocked the occurrence of both collagen induced arthritis and peptide induced experimental allergic encephalo myelitis. In addition to these autoimmune disorders, anti-gp39 blocked the acute and chronic forms of graft versus host disease by inducing T cell tolerance to host allo antigens. The basis for the induction of T cell tolerance by anti-gp39 is believed to lie in via prevention of the upregulation of co stimulatory molecule expression on the antigen presenting cell. This hypothesis was supported by the observation that anti-gp39 administered with allogenic B cells, induced T cell tolerance to alloantigens—in both the CD4 and CD8 compartments. Proof that this process of T cell transplantation tolerance was supported by the observation that mice rendered tolerant accepted the long-term engraftment of allogeneic, pancreatic islet cells. These results provide compelling evidence that antibodies to human (h) gp39 may have significant therapeutic value in the management of human autoimmune disease and in the transplantation of allogenic tissue and organs in humans. To gain greater insights into the functional impact of anti hgp39 monoclonal antibodies (MAbs) on the human immune system, a panel of anti hgp39 MAbs were generated, tested extensively for functional inactivation of gp39 in vitro, then tested in huSCID mice. This system was used to evaluate the immunosuppressive effects of in vivo administration of anti-gp39 on the immune response of human T and B cells.

Interference with the function of CD28
D Wofsy
University of California, San Francisco, USA

On interaction with antigen, B cells and other antigen presenting cells (APC) are stimulated to produce certain surface molecules that are not present on resting APC. Among these, the B7 related molecules (B7-1, B7-2, etc) appear to have a pivotal role in determining T cell responses. Specifically, the interaction of B7 related molecules on APC with CD28 on T cells provides an important co stimulatory (‘second’) signal for T cell activation. Selective inhibition of the B7-CD28 interaction induces antigen specific T cell unresponsiveness in vivo. Attempts to extend this observation to in vivo systems have focused on a new strategy that takes advantage of the homology between CD28 on (all T cells) and another T cell surface molecule, designated CTLA4. CTLA4 is expressed on activated T cells, and it binds the B7 related molecules with considerably greater avidity than does CD28. Therefore, a fusion protein encoded by genetic fusion of CTLA4 to an immunoglogulin Cy chain (CTLA4ig) binds B7 molecules, blocks the B7/CD28 interaction and inhibits T cell activation. As B7 related molecules are preferentially expressed by activated APC, it has been postulated that this addition may be limited to T cells that recognise particular antigens (for example, autoantigens in people with autoimmune diseases; alloantigens in transplant recipients). We have used a mouse CTLA4ig that blocked autoantibody production and retarded autoimmune nephritis in a mouse model for systemic lupus erythematosus. This observation suggests a potential role for CTLA4ig in the treatment of autoimmune diseases in humans.

Interference with the function of CD4
A M Solinger
IDEC Pharmaceuticals Corporation, San Diego, California 92121, USA

Recent investigations with monoclonal anti-T cell antibodies have resulted in significant, prolonged CD4 suppression without evidence of immunomodulation. A Primatized anti-CD4 was developed that contains a macaque variable and human constant region in order to reduce adverse experiences and increase immunomodulatory effects. Five patients with rheumatoid arthritis were treated with IDEC-CE8.1 in an open label, single dose, dose escalation study
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(0.03-4.0 mg/kg intravenous infusion) and a trial of 40 patients receiving a repeat dose regimen in early rheumatoid arthritis is in progress. The initial suppression of CD4 counts lasted no more than seven days after infusion in both cases. Cell surface markers were studied (CD3, CD4, CD5, CD8, CD16, CD19, CD20, CD25, 45RO, 45RA, and polymorphic DR), and significant changes were noted in CD4 and CD8 cell populations. A marked, early, subjective response was seen in the initial trial and has been confirmed in the repeat dose trial. With intravenous doses up to 4 mg/kg, no important adverse experiences were noted in any patient. Drug related adverse experiences were classified as mild and were rarely considered as related to the drug study. Fever, headache, rigors, or chills were not observed. Laboratory safety evaluations revealed no significant abnormalities. In vitro proliferation studies in the single dose trial demonstrated suppression of response to mitogen (concanavalin A, phytohemagglutinin, and pokeweed mitogen) for up to one month in the higher dose groups. Similar suppression was seen in response to soluble recall antigens.

No quantifiable anti-IDEC-CE9.1 levels were observed at any time during the single dose trial. IDEC-CD9.1 antibody appears to be well tolerated, causes depletion and quick recovery of CD4 levels, does not produce anti-IDEC-CE9.1 responses, and shows some early evidence of clinical activity.

**RELEVANT PUBLICATIONS**


**CELL SIGNALLING**

**Signalling by TCR/CD3, CD28 and CTLA-4**

C E Rudd

Division of Tumour Immunology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115, USA

T cell activation is regulated by at least two steps, one from the T cell receptor (TCR)/CD3-CD28 complex and another from CD28 and its binding to B7-1/B7.2 on presenting cells. TCR/CD3-CD4 generate signals via the tyrosine kinases p56lck, p59fyn, and ZAP-70, leading to the phosphorylation of multiple substrates. Receptor aggregation stimulates kinase activity, leading to the phosphorylation or recruitment of downstream targets such as TR66, CD5, and ZAP-70. Tyrosine phosphorylation independent recruitment of phosphatidylinositol-3 kinase (PI-3 kinase) regulates the signaling of multiple pathways. The PI-3 kinase is a lipid kinase that is central to signalling growth factor receptors, generating the lipids PI 3-PI, PI 3,4-P2, and PI 3,4,5-P3.

Despite the intracellular TCR/CD3-CD4 signalling, optimal stimulation requires costimulation mediated by CD28 and other receptors. CD28 has been implicated in anergy, and in models of autoimmunity and autoimmunity. CD28 possesses a pYNNM motif that binds to the SHD2 domains of PI-3 kinase and GRB-2/SOS. CTLA-4 was also found to bind to PI-3 kinase. In the case of CD28, GRB-2-SOS converts p21 to a GTP-bound state. Both PI-3 kinase and GRB-2 are recruited upon CD28 ligand. CD28 is thus capable of generating multiple ‘costimulatory signals’ in T cells.

**Setting thresholds for B lymphocyte activation**

D T Fearon

**Welcome Trust Immunology Unit, University of Cambridge School of Clinical Medicine, Cambridge CB2 2SP, United Kingdom**

The response of the B lymphocyte to the binding of antigen to its membrane immunoglobulin (mIg) receptor is not ‘hard-wired’, as is that of most growth factor receptors, but differs when, for example, the antigen is self rather than foreign. We are studying how the B lymphocyte can change the intensity of signals emanating from mIg. CD19 and CD22 are two B lymphocyte surface immunoglobulins that, in addition to the IgG1/2 heterodimer of the mIg complex, are tyrosine phosphorylated after the ligation of mIg. Both proteins interact with cytosolic effectors having SH2 domains, and can alter the threshold for activation of B lymphocytes by the mIg complex. Phosphorylated CD19 binds and activates phosphatidylinositol 3-kinase, and its coligation to mIg decreases the thresholds for activation of both the mIg complex and also lowers by two orders of magnitude the threshold for activation of B lymphocytes through mIg. As the ligands for CD22 are glycoconjugates containing sialylated linked glycosphingolipids, the affinity of the mIg complex also lowers by a lower order of magnitude the threshold for activation of B lymphocytes through mIg. Both the mIg complex and B lymphocytes may promote host defense when challenged by foreign antigen, but may contribute to autoimmune responses when involving self antigen.

**Protein kinase C inhibitors**


Research Centre, Roche Products, Welwyn Garden City, Herts AL7 3AY, United Kingdom

The protein kinase C (PKC) isoenzyme family is believed to mediate a wide variety of immunological events in T and B lymphocytes. Much of the evidence for this comes from studies with phorbol esters, which are potent direct PKC activators, but may not mimic physiological triggering of the enzyme, or from the use of non-selective protein kinase inhibitors such as H2 and staurosporine. Studies with selective PKC inhibitors suggest that the role of PKC in cellular processes may have been overstated and that these agents may actually be toxic. A new generation of bisindolylmaleimides, derived from the lead provided by staurosporine, show a high degree of selectivity for PKC over closely related protein kinases: for example, Ro31-8422 exhibits 350-fold selectivity for PKC over cyclic AMP dependent protein kinase, and 160-fold selectivity for PKC over phosphatidylinositol 3-kinase. Bisindolylmaleide PKC inhibitors such as Ro31-8425 inhibit antigen, but not IL2 driven T cell proliferation. This might be mediated through inhibition of the binding of the transcriptional control protein NF-kB to its specific DNA sequence and a critical role in T cell activation necessary for cytokine production and clonal expansion.
One of these bisindolylmaleimides, Ro32-0432, shows oral anti-inflammatory activity in a phorbol ester induced model of hind paw inflammation. This agent was ineffective in models of acute inflammation, but selectively inhibited the systemic immune response associated with the second phase of adjuvant arthritis. Ro32-0432 also inhibited the development of paralysis in an experimental acute encephalomyelitis model. These effects are consistent with an immunosuppressive action of Ro32-0432 and suggest that selective FKC inhibitors may have therapeutic potential in organ transplantation and in the treatment of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.

**Immunophilin binding drugs**

M H Schreier

Sandoz Pharma Ltd, Basel, Switzerland

For most immunosuppressive drugs, the understanding of the molecular mode of action lagged far behind their beneficial clinical application. This is true particularly for cyclosporin (Sandimmune®) which, together with a number of macrolides (FK506, FK906) and a ClB non-immunosuppressive analogues, proved to be invaluable tools to dissect and understand the different components of the signalling pathways within lymphoid cells. Antigen recognition by the T cell receptor (TCR)/CD3 complex initiates a signalling cascade flowing from the cell surface to the regulatory elements of a set of lymphokine genes and leading to their coordinated transcription. Remarkable progress has been made in elucidating the molecular interactions which lead to a blockade of this signal transduction pathway. A detailed understanding of the complex formation between different cyclophilins and different cyclophilins and of various macrolides with their binding proteins (FKBPs) has emerged. Immunochemical and enzymatic studies followed by nuclear magnetic resonance and molecular modelling have provided much needed detail information about the structural features of these binary immunophilin-ligand complexes. A big gap in our understanding of signal transduction in the cytoplasm was filled when it was unambiguously established that both the cyclosporin-cyclophilin and the FK506-FKBP complex (but not the FKBP-rapamycin complex) interact with high affinity with calcineurin and thereby inhibit its serine threonine phosphatase activity. This phosphatase controls the nuclear transport of cytosolic components of the transcription machinery that regulates lymphokine gene activation.

Calcineurin, the common target of the cyclophilin A-cyclosporin A and the FKBP12-FK506 complex, is a heterodimer consisting of a large calmodulin binding subunit (61 kDa) and a ClB binding subunit (19 kDa). The topology of the cyclosporin-cyclophilin-calmodulin complex has been studied by chemical cross linking and photofinity labelling. By using recombinant partial stretches of the catalytic subunit A, the minimal sequence necessary for immunophilin-ligand-calcineurin interaction could be defined. Functional expression of calcineurin and the limited downstream element in the TCR/CD3 mediated signalling, changes in the stoichiometry of the structural elements involved in this complex formation greatly affects the susceptibility of T cells to immunosuppression. There is a good correlation between the relative immunosuppressive potency of cyclosporins and their calcineurin modulating effect which can be readily determined by measuring release of phosphate from suitably phosphorylated substrates. These insights into the molecular mode of action of immunophilin binding drugs provide a basis for a rational insight into the interactions of synergistically acting drugs according to their mode of action.

**Phase II clinical trials with leflunomide in rheumatoid arthritis**


†Leflunomide Study Group Croatica, Slovenia and Yugoslavia. *Synergis; ‡Hochot AG, 65926 Frankfurt, Germany

Leflunomide is an isoxazole with immunosuppressive characteristics which has been acutely tested in patients with active rheumatoid arthritis (RA). Phase II included a double blind randomised placebo controlled trial in 402 patients, comparing daily doses of 5, 10 or 25 mg for six months. Significant improvement of primary and secondary outcome measures, and responder analyses, occurred in the 10 and 25 mg dose groups compared with placebo. Twenty one patients in the active treatment group died during the study because of adverse events. Gastrointestinal complaints, weight loss, skin rash, allergic reactions, and reversible alopecia were more common in the 10 and 25 mg dose groups. The incidence of infections was similar between treatment and placebo groups, and did not include opportunistic infections. Transient increases in liver function test values occurred in 6% (placebo) and 13–14% (10 and 25 mg dose groups). Three hundred of the 402 patients subsequently participated in an open label extension study. Improvement occurred in patients previously receiving placebo and they began receiving active drug treatment, and in those already taking low doses of leflunomide. Leflunomide is effective in daily doses of 10 and 25 mg in patients with active RA without major organ system toxicity. Phase III randomised controlled trials are currently in progress in the United States and in Europe.

**MECHANISMS OF SYNNEVTIS**

Mesenchymal reaction

S Gay, U Muller-Ladner, J Kriefgmann, R E Gay

Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, Alabama 35294-0006, USA

Invasive and destructive proliferation of synovial lining cells characterizes joint destruction in rheumatoid arthritis. The transformed appearing phenotype of synovial lining cells is accompanied by the upregulation and expression of oncoenges fos, myc and ras, and the early growth response zinc finger protein encoding gene (c-myc), in addition to the production of matrix degrading cathepsins (B, L, D), and collagenase. To study the interaction of synovial cells with cartilage in detail, we developed a novel model by engraving rheumatoid synovial cells and normal human cartilage into severe combined immunodeficiency disease (SCID) mice. Vascular cell adhesion molecule-1 is expressed largely in fibroblast like cells prone to attach and invade the cartilage. On the basis of the concept that the regulation between cell proliferation and apoptosis appears initiated by an up-regulation of c-myc and then by an additional signal, we examined the gene expression of apoptosis regulating molecules in rheumatoid arthritis (RA) synovial tissue. Expression of the 'cell death suppressor' gene bcl-2 was decreased in the lining layer of rheumatoid synovial T cells that bcl-2 may prevent certain cells from undergoing apoptosis and thereby maintain protease producing invasive synovial lining cells. The transformed appearing phenotype is sustained in the arthritis synovial T cells as observed not only in the SCID mouse model, but also in a sad experience of nature. Most recently, we observed progressive joint destruction by proliferating RA synovial lacking T cells as a result of HIV infection, and demonstrated for the first time progression of joint destruction associated with the production of cathepsins by fibroblast like cells at the site of invasion in the absence of inflammation.


RELEVANT PUBLICATIONS


Matrix metalloproteinases and cartilage degradation


Pharmaceutical Research Centre, Roche Products Ltd, PO Box 8, Welwyn Garden City, Hertfordshire AL7 9AY, United Kingdom

The sequential destruction of aggrecan, followed by collagen, in articulating cartilage is a feature of both rheumatoid arthritis and osteoarthritis. These two main extra-cellular components of cartilage (aggrecan and type II collagen) give the tissue its shape and physical properties: the collagen fibrils provide structure and form, while the aggrecan molecules, entrapped by the collagen fibrils, produce a large swelling pressure which allows the cartilage to resist compressive forces. Recent reports have implicated the putative enzyme "aggrecanase" in the metabolism of aggrecan in cartilage in arthritis. We have studied using a range of synthetic inhibitors of stromelysin (a member of the matrix metalloproteinase family) in an in vitro cartilage explant assay, we have demonstrated that inhibition of aggrecan breakdown results in the effective inhibition of the compound against stromelysin. This suggests that stromelysin (or a closely related metalloproteinase) has a role in the breakdown of cartilage aggrecan. We propose that stromelysin is involved in upstream processing of aggrecan.

Additional studies with synthetic inhibitors of the matrix metalloproteinase collagenase have demonstrated that this enzyme is responsible for the pathological destruction of cartilage collagen in models of cartilage destruction. Furthermore, R031-9790 (a potent orally active inhibitor of collagenase; K_i 1-1 nmol/L) will prevent collagen destruction in vivo both in mechanistic models of collagenase induced cartilage destruction, and in the Propionibacterium acnes mono-arthritis model of rheumatoid arthritis. These results confirm the hypothesis that drugs blocking aggrecan degradation using inhibitors of collagenase will provide a treatment for the underlying pathological destruction of extracellular tissue observed in rheumatoid arthritis.

Metalloproteinases, cytokines and angiogenesis

A H Drummond

British Biotech Pharmaceuticals Ltd, Oxford OX4 5LY, United Kingdom

The role of metalloproteinases, particularly matrix metalloproteinases, in the progression of the arthritides is receiving increasing attention. A variety of different approaches (immunohistochemical, in situ hybridisation, etc) have led to a consensus that the enzymes are involved in the breakdown of cartilage in arthritic patients at levels in excess of those seen in unaffected individuals. The ability of members of the matrix metalloprotease family such as collagenase and stromelysin to break down the constituents of bone and cartilage is well established. Recent data suggest two further mechanisms by which the enzymes contribute to the arthritic process: by affecting angiogenesis and by their role in the production of soluble tumour necrosis factor (TNFα). Our work has focused on the discovery of potent, specific, and orally active inhibitors of matrix metalloproteinases. A number of these inhibitors (for example BB-94 (batimastat) and BB-2516) are now in preclinical and clinical development for the treatment of arthritis.

The compounds are effective inhibitors of both paw oedema and bone and cartilage destruction in animal models of arthritis. Moreover, batimastat has been investigated in a number of models of angiogenesis with positive results. The effects of certain matrix metalloproteinase inhibitors (so called TMI drugs: TNF and Metalloproteinase Inhibitors) that have the additional ability to block the production of TNFα have been investigated.

Regulation of cellular adhesion in rheumatoid arthritis by corticosteroids

C Pitzalis

Rheumatology Unit, Guy's Hospital, London SE1 9RT, United Kingdom

Having been out of favour for many years, the usage and mechanisms of action of corticosteroids in rheumatoid arthritis (RA) are now enjoying a renewed interest. This is due to several factors, including their clear effectiveness when given during acute flares of disease and their ability to accelerate disease improvement when used together with second line drugs. More interesting still is the recent evidence that corticosteroids themselves might have disease modifying activity. Despite this renewed clinical interest and the large number of studies, the mechanisms of action are not yet fully understood, though they are known to be multi-factorial. Some of the anti-inflammatory effects of corticosteroids have been attributed to the synthesis of lipocortins, whereas the immunosuppressive effects are thought to be mediated through the inhibition of several immune functions such as chemotaxis, phagocytosis, cytotoxicity, and the down-regulation of cytokine gene expression, including that of interleukins (IL)-1, -2 and IL-6, interferon gamma and tumour necrosis factor alpha. Another important mechanism of action of corticosteroids may be related to their ability to interfere with the phenomena of adhesion and migration of inflammatory cells responsible for the initiation and perpetuation of rheumatoid synovitis. This hypothesis is based on both in vivo and in vitro evidence. In vivo, it has been known for a long time that treatment with corticosteroids causes polymorphonuclear (PMN) cell leucocytosis, thought to be related to decreased adhesion of the 'marginated pool' to vascular endothelial cells. More importantly, it has been shown that intra-vavenous corticosteroid treatment induces a decrease in synovial PMN numbers, suggesting that corticosteroids can interfere with the process of migration itself. The mechanism of regulation of leukocyte migration by corticosteroids is at a cellular level, corticosteroids, by binding to the glucocorticoid response elements, can inhibit binding of transcription factors, such as the AT and AP-1 nuclear proteins, to promoter sequences. Finally, corticosteroids can also act at post-transcriptional level by altering mRNA stability, with subsequent decreased translation. We are currently characterising whether these cellular mechanisms predominate in controlling adhesion molecules gene expression.
In summary, corticosteroids are potent inhibitors of adhesion related mechanisms as demonstrated both in vitro and in vivo. Targeting these mechanisms specifically may have important implications for treatment, in terms of developing more powerful and less toxic steroid regimens.

Regulation of gene expression by glucocorticoids
D T Boumpas
National Institutes of Health, Bethesda, MD 20892, USA

The ordered regulation of gene expression during the inflammatory immune response relies on sequence specific DNA-protein interactions during the process of nuclear transcription. Glucocorticoids may up- or downregulate the transcription of several of these genes. One major mechanism by which they inhibit gene transcription is through non-covalent interaction of the activated glucocorticoid-receptor complex with the c-Jun-Fos heterodimer (nuclear factor AP-1). AP-1 binds to the AP-1 site of metalloproteasine (such as the collagenase) and cytokine (such as the interleukin (IL)-2) genes and activates their transcription. In the presence of glucocorticoids, binding to the AP-1 site is inhibited. Glucocorticoids also inhibit the binding of nuclear factor of activated T cells (NFAT) on the IL-2 promoter through a mechanism which involves both interference with the binding of its AP-1 component, and downregulation of calcineurin dependent pathways for its activation. In the case of IL-8, a leucocyte chemoattractant cytokine, glucocorticoids modulate its nuclear transcription by interfering with the binding of nuclear NF-κB to its corresponding site on the IL-8 promoter. Glucocorticoids are also effective repressors of major histocompatibility complex (MHC) class II gene expression by inhibiting the binding of the X box DNA binding protein to the X box of the MHC class II gene IAB promoter. In addition to their downregulatory effects on gene transcription, glucocorticoids, under certain conditions, upregulate the transcription of cytokine genes such as the IL-4 gene. The mechanism for this upregulation is not known at present.

Angiostasis and vascular regression in chronic granulomatous inflammation induced by diconfene with hyaluronan induced targeting technology (HYAL CT-1101)
C A S Alam, M P Seed, S S Ascula, D A Willoughby

Department of Experimental Pathology, St Bartholomew’s Hospital Medical College, London EC1M 6QX, United Kingdom

Angiostasis during inflammation is induced by diconfene and with hyaluronan induced targeting technology (HYAL CT-1101). Diconfene modulates angiogenesis, and non-steroidal anti-inflammatory drugs (NSAIDs) reduce inflammation angiogenesis. A topical diconfene formulation in hyaluronan (HYAL CT-1101: 6 mg/kg diconfene in 0.1 ml 2.5% hyaluronan) was tested for its ability to modulate neovascular development and regression in allergic inflammation in the chinchilla. Air pouches were dosed topically or into the pouch with HYAL CT-1101 prophylactically for five days or therapeutically on the established 7 day neovascularization for a further seven or 14 days. Vascular casts containing carmine were dissolved and assayed spectro-photometrically. HYAL CT-1101 injected into the air pouch reduced the vascular density by p < 0.01; n = 16), whilst diconfene in saline, and hyaluronan alone were without effect. Topical HYAL CT-1101 induced a 50% inhibition (p < 0.01) of vascularity, whilst diconfene in aqueous cream, and hyaluronan alone were ineffective. Topical treatment for seven and 14 days with HYAL CT-1101 induced a 20-5% (p < 0.05) and 22.5% (p < 0.02) reduction of the established 7 day neovascularization accompanied by a reduction in tissue mass (seven days: 46-1%; p < 0.0001; 14 days: 50-8%; p < 0.001) and vascular content (seven days: 60-0%; p < 0.001; 14 days: 60-0%). Topical HYAL CT-1101 is not only antiangiostatic, but induces neo-vascular regression, resulting in reduced granulomatous disease. HYAL CT-1101 hyaluronan induced targeting (HIT) technology is therefore effective as a drug delivery system for diconfene, as it is for other drugs.

RELEVANT PUBLICATIONS

CYTOKINES

Cytokines and cytokine inhibitors: basic principles and clinical perspective J-M Dayer, D Burger

Division of Immunology and Allergy, Department of Medicine, University Hospital, 1211 Geneva 14, Switzerland

It has been evident during recent years that interleukin-1 (IL-1) and tumour necrosis factor α (TNFα), mainly produced by monocyts and macrophages, are the principal mediators of tissue destruction in many immune inflammatory diseases such as rheumatoid arthritis (RA). In synergy, the two cytokines induce the production to high levels of matrix metalloproteinases (MMP) by fibroblasts, synovial cells and chondrocytes. The biological activity of MMP is controlled by tissue inhibitor of MMP (TIMP) which also displays the presence of cytokines in the microenvironment.

One of the principal stimuli of the production of IL-1 and TNFα is the direct contact between the membranes of activated lymphocytes and monocyte-macrophages. Several glycoproteins expressed on the surface of activated lymphocytes (CD11, CD69) are implicated in this activation process and can be partially blocked by their respective antibodies. These prompt the decrease of cytokines and proteases in the lymphocyte-monocyte interaction. In the past few years, two pathways for inhibiting the activation of macrophages, fibroblasts, and synovial cells have been elucidated. One of them is via the action of anti-inflammatory cytokines such as IL-4 and IL-10 which considerably decrease production of IL-1, TNFα, and metallo-proteases. (In contrast to IL-4, IL-10 is also capable of stimulating the production of TIMP). The other, more specific, inhibitory mechanism involves molecules that act as true receptor antagonists, such as the IL-1 receptor antagonist (IL-1ra) or protein (CT-1101). This promotes the decrease of soluble inhibitory fragments derived from the extramembranous domain of the two receptors for TNF (sTNFRIp55 and sTNFRIp75), or the two receptors for IL-1 (sIL-1RI and sIL-1RII). Both IL-1ra and sTNF, initially observed in our laboratory in their natural form, have been cloned by different investigators and their efficacy is being tested in a number of rodent models for various pathologies, in articular sepsis, allergic diseases, and rheumatoid arthritis.

Inhibition of biological activities and production of IL-1 and TNFα may provide a pharmacological approach which will help to prevent the destruction of connective tissue and that will not almost exclusively consist of anti-inflammatory and painkilling effects.

Cytokine profile dependent on the type of arthritis: dominant role of interleukin-1 in cartilage destruction
M van der Bent, P Josten, M A Helsen, P L van Lent, F A J van Leeuwen

Rheumatology, University Hospital Nijmegen, The Netherlands

Cytokines are believed to have an important role in rheumatoid arthritis. Initial experiments with humanised tumour necrosis factor (TNF) antibodies in patients with rheumatoid arthritis (RA) have been encouraging, and the focus of treatment with TNF is based on the observed cascade of TNF-interleukin (IL-1) in the rheumatoid synovium. We have investigated the role of interleukin-1 (IL-1) in various models of arthritis, including antigen induced arthritis (AIA), immune complex arthritis, collagen induced arthritis (CIA), and streptococcal cell wall (SCW) arthritis. We used neutralising antibodies and IL-1 receptors (IL-1ra) (Synergex, Boulder) in vivo to investigate the involvement of TNF and IL-1. Joint inflammation and cartilage destruction were assessed in joint sections and chondrocyte function was measured with 3S-proteoglycan labelling in
Transgenic analyses of the role of tumour necrosis factor and interleukin-1 in arthritis

G Kollias, L Alexopoulou, S Georgopoulos, D Plows, L Probert

Department of Molecular Genetics, Hellenic Pasteur Institute, Athens, Greece

We have reported previously that transgenic mice expressing a 3-modified human tumour necrosis factor (TNF) transgene develop typical chronic inflammatory polyarthritis with 100% phenotypic penetrance and timedisease onset. However, no information about the contribution in these pathologies of the secreted or the membrane associated forms of TNF was obtained. We have therefore introduced a transgenic line of SCW mice with a TNF transgene for non-secretable membrane bound TNF. Interestingly, typical chronic inflammatory polyarthritis similar to the arthritis triggered by wild type TNF transgenes was induced. These results have important implications for a specific involvement of membrane associated TNF in the development of arthritis and provide an in vivo model system in which systemic or contact dependant modes of TNF action and their contribution to the pathogenesis of autoimmunity may be further investigated.

TNF and interleukin-1 (IL-1) share many biological activities and are known to mediate the key events in the pathogenesis of chronic arthritic disease, but it is still unclear whether these molecules act independently or in concert to induce development and progression of disease. The type I mouse IL-1 receptor was blocked with antibodies (kindly provided by Immunex) in the arthritic TNF transgenic line Tg197 and this treatment was found to prevent completely the development of arthritis. This study thus indicates that the IL-1 receptor accepts the whole pathogenic load of TNF, acting as a potent downstream mediator in the pathogenesis of chronic arthritis.

Anti-tumour necrosis factor treatment in arthritis in mice and in humans

M Feldmann, F M Brennan, M Elliot, R Williams, R N Maini

Kennedy Institute of Rheumatology, London, United Kingdom

We have established that tumour necrosis factor (TNF) is of major importance in rheumatoid arthritis by using three different systems: an in vitro analysis of cytokine regulation using cultures of dissociated rheumatoid synovium cells, a DBA/1 collagen induced model of arthritis, and clinical trials in patients with long standing active rheumatoid arthritis. The results from the last of these have triggered other groups to enter the field and encouraging results are also being reported with other antibodies and with IgG-TNF receptor fusion proteins, confirming our earlier conclusions.

Our current interests are to learn how to improve on the clinical benefit of anti-TNF, and for this purpose we have focused on the animal model. Most encouraging is the very clear synergy between anti-CD4 and anti-TNF antibodies, and the mechanism of this synergy is being explored. TNF has a multitude of effects on various cell types, and it is of importance to ascertain which may be likely to produce clinical benefit.

The advantage of TNF antibody treatment using the cA2 antibody is the marked efficacy in almost all patients, depending on dose, and improvement in many clinical variables. The speed of onset of benefit is also notable. It is too early to know what the disadvantages may be. One identified so far is the induction of antibody against double stranded DNA in two individual patients who ended subside after cessation of treatment, without symptoms. The major potential problem is increased susceptibility to infection, but to date this has not emerged in the placebo controlled study. There was no difference between placebo, low, and high dose cA2 in terms of infection, but with longer term treatments there remains a possibility of increased susceptibility to infection.

Treatment of rheumatoid arthritis with engineered human antibody to tumour necrosis factor α: CDPS71

E H S Choy, E C C Rankin†, D Kassimos, G H Kingsley, S Stephens*, M Sopwith*, D A Isenberg†, G S Panayi

Rheumatology Unit, United Medical and Dental Schools, Guy’s Hospital, London, United Kingdom; †Bloombury Rheumatology Unit, Middlesex Hospital, London, United Kingdom; *Celitech Therapeutic Limited, Slough, United Kingdom

Tumour necrosis factor α (TNFα) is a potent proinflammatory cytokine which is thought to have a major role to play in the pathogenesis of rheumatoid arthritis (RA). A placebo controlled trial using a chimeric TNFα antibody showed that it produced significant disease improvement in RA, but because engineered human antibodies are thought to be less antigenic than chimeric antibodies, we tested the efficacy of a humanised TNFα antibody, CDPS71 (Celitech) in a two phase study.

The initial study was a double blind, placebo controlled, dose escalation study using three doses of CDPS71: 0.1, 1, and 10 mg/kg. The second phase was an open study in which patients were treated with either 1 or 10 mg/kg of CDPS71. During the first phase, 36 patients were recruited: 12 received placebo and eight received each dose of CDPS71. Disease activity was measured by the European League Against Rheumatism core data set for disease assessment in RA and performed before and at one, two, four, and eight weeks after treatment. A disease activity score (DAS) was used to summarise results.

Treatment was well tolerated. Statistically significant reductions were seen in the visual analogue scale of pain, tender joint count, erythrocyte sedimentation rate (p = 0.005), and C reactive protein (p = 0.057) in the 10 mg/kg group. The median reduction in the DAS was 0.45 (p < 0.05). After a single dose of CDPS71, no significant anti-streptolysin response was seen. Serum levels of CDPS71 showed a clear dose kinetic with a serum half life of one to two weeks. Twenty patients completed the second phase of this study. Repeated treatment was again effective in supporting disease improvements.

We conclude that TNFα is an effective treatment target in RA. A 10 mg/kg dose of CDPS71 produced significant disease improvement lasting four to eight weeks. The long term efficacy of CDPS71 in RA remains to be determined.

Treatment of rheumatoid arthritis with recombinant soluble tumour necrosis factor receptor p80 fusion protein L W Moreland

The University of Alabama at Birmingham, Birmingham, Alabama 35294, USA

Tumour necrosis factor α (TNFα) has a central role in the pathogenesis of rheumatoid arthritis (RA). Evidence supporting such a role includes the presence of TNFα at the cartilage-pannus junction in RA patients, and increased levels of TNFα in RA synovial fluid. Furthermore, TNFα production has been found to increase by the synovial cells of patients with active RA, but not in synovial cells from patients with inactive RA. Several proinflammatory actions of TNFα may contribute to its role in the pathogenesis of RA. In addition to stimulating the release of other proinflammatory cytokines, including interleukins (IL) IL-6, IL-8, IL-1β, and leukasemia inhibitory factor, TNFα also induces the release of proteases from neutrophils, fibroblasts, and chondrocytes. These enzymes, including collagenase and other neutral metalloproteinases, are likely to be responsible for joint destruction in RA. TNFα also induces the expression of adhesion molecules (e.g. intercellular adhesion molecule-1 and E-selectin), leading to rapid transmigration of leucocytes to extravascular sites. In addition, transgenic mice expressing human TNFα spontaneously develop destructive arthritis. Other animal models of arthritis have also provided further evidence of the involvement of TNFα in arthritis. TNFα gives intra-articularly in collagen induced arthritis model led to a more severe and accelerated course of arthritis. Monoclonal TNF antibody decreases the severity of the joint destruction in these animal models. Thus TNFα may be ‘pathogenic’ cytokine in regard to the development of other inflammatory mediators in RA. This view has led to therapeutic interest in developing strategies to modulate TNFα activity in patients with RA. Biological activities of TNFα require binding to specific membrane bound TNF receptors; two such receptors are known for TNFα, and are expressed by numerous cell types, including polymorphonuclear leucocytes, vascular endothelial cells, and fibroblasts. TNF binding to its receptors...
mediates a wide variety of actions, including its proinflammatory effects. Soluble TNF receptors (sTNFR) are the extracellular portions of the specific cellular receptors for TNFα that have been detached from the cell surface. Two sTNFR have been identified: type I (p55 or 60) and type II (p75 or p80). sTNFR levels have been shown to be increased in serum and synovial fluid of patients with RA.

A recombinant human sTNFR fusion protein (rhu sTNFR) has been formed by linking the two monomeric forms of the soluble p80 receptor to an Fc portion of a human IgG molecule. The fusion protein has a substantially greater affinity for TNFα than does the monomeric soluble receptor. In addition, the fusion protein has a longer half life in vivo compared with the monomer. This rhu sTNFR:Fc significantly reduced both the incidence and severity of collagen induced arthritis in preventive and therapeutic programmes. Safety studies in normal human volunteers demonstrated no adverse events after intravenous administration.

A phase I study using rhu sTNFR:Fc in patients with RA was recently completed at The University of Alabama at Birmingham. Sixteen patients with severe, refractory RA were treated for four weeks and observed for an additional one month. Criteria for entry were that patients had failed to respond to two or more DMARDs or that they had a disease duration of at least one year, but had severe and uncontrolled failure of treatment with at least one disease modifying antirheumatic drug, active disease (>5 swollen joints and >9 painful joints), and functional class I, II, or III. Concomitant treatment was with a non-steroidal anti-inflammatory drug and stable dose of prednisolone (≤10 mg/day for >1 month) were allowed. Patients were enrolled in groups of four: three in each group received active drug and one received placebo in a double blind fashion. Groups of patients received rhu sTNFR:Fc as an intravenous (IV) load followed by four weeks of twice weekly subcutaneous administration (maintenance dose).

There were no serious adverse effects and all patients completed four weeks of treatment. There was no clear dose response among the treatment groups, therefore all treated patients were grouped together. At day 31, there was a 44% mean improvement in total pain and total joint scores in patients receiving active drug (n = 12), compared with a 22% improvement in those receiving placebo (n = 4). Average morning stiffness improved by 55% in treated patients. Compared with baseline, there was a significant (p < 0.05) decrease in Westergren erythrocyte sedimentation rate (32%). Levels of C reactive protein (CRP) also decreased (27%) significantly in the treated patients compared with those who received placebo (13%); this was most pronounced in the highest dose group of 10 mg/kg RA and 10 mg/kg sTNFR:Fc in CRP at 31 days in the three patients in group IV). These data indicate that rhu sTNFR:Fc is well tolerated. Efficacy of rhu sTNFR:Fc in RA is being further evaluated in a multicentre phase II randomised controlled clinical trial that is in progress.

Ribozymes: possible use in rheumatic disorders
O Forre
Institute of Immunology and Rheumatology and Oslo Sanitetsforenings Rheumatism Hospital, University of Oslo, 0172 Oslo, Norway

Ribozymes are RNA molecules that cleave other RNA molecules. They thus offer a new way of turning expression of specific genes for which the nucleotide sequences are known. It has been shown that the HIV-1 gene expression in ACH-2 cells could be blocked by endogenously expressed ribozymes. Large quantities of tumour necrosis factor α (TNFα) can produce destructive changes such as those seen in rheumatoid arthritis and in transgenic mice. As a first step to interfere with the TNFα gene expression and to see whether a preformed ribozyme could be delivered to living target cells, we used cationic liposome mediated transfection to deliver a TNFα specific ribozyme into human promyelocytic leukaemia cells (HL60) and human peripheral blood mononuclear cells. Delivering a ribozyme in this manner reduced TNFα mRNA and protein by 90% and 85%, respectively. A modified ribozyme with a bacteriophage T7 transcription terminator at its 3' end was more stable in vivo than one lacking this sequence. Our data demonstrate that a presynthesised ribozyme could be delivered to the target cells, and in addition could be useful for evaluating modification of ribozyme structure to improve in vivo catalytic activity. Intracellular stability to ribozyme is an important factor for ribozyme efficacy. We previously showed that hammerhead ribozyme directed against mRNA encoding TNFα is acutely capable of resistance to degradation in cultured human cells. In order to explain this resistance, studies have been made of endogenous cellular protein(s) that bind TNFα ribozyme in solution to form stable complexes during native gel electrophoresis, and it is planned to use ribozymes specific for TNFα locally in the diseased joint, to treat patients with rheumatic disease.

Inhibition of transfer of collagen induced arthritis into SCID mice by ex vivo infection of spleen cells with retroviruses expressing soluble tumour necrosis factor receptor
Y Chernajovsky, A Adamś, O I Poznanski, P D Robbins, M Feldman†
†Kennedy Institute of Rheumatology, London, United Kingdom; †Instituto de Investigaciones Bioquímicas and Departamento de Genética y Bioquímica, Universidad de Buenos Aires, Argentina; †Department of Genetics and Biochemistry, University of Pittsburgh, Pennsylvania, USA

The feasibility was assessed of using cells of the immune system, capable of transferring arthritis from DBA/1 mice to severe combined immunodeficiency disease (SCID) mice, as local carriers of therapeutic agents introduced by ex vivo gene transfer.

Splenocytes from diseased DBA/1 mouse were incubated in vitro for 48 hours with bovine collagen type II and in vivo during the last four hours were infected with retroviruses expressing high levels of a monoclonal form of the human soluble extracellular domain of the p75 TNF receptor (TNFRp75) or expressing a low amount of a tumour necrosis factor inhibitor in polyclonometric mRNA with the herpes simplex virus thymidine kinase (HSV-tk) coding sequence. As a control we used a retrovirus expressing solely the HSV-tk gene.

Splenocytes from DBA/1 mice infected with a retrovirus expressing monomeric TNFRp75 were inhibited from transferring arthritis to SCID animals (p < 0.014). The inhibition correlated with a decrease in collagen antibody levels (p = 0.006). The levels of TNFRp75 in serum did not correlate with the collagen antibody titres or with outcome of treatment. The virus expressing only the HSV-tk gene had no effect in this arthritis transfer with or without ganciclovir.

However, the polyclonometric virus expressing HSV-tr and TNFRp75 was therapeutically more effective than ganciclovir and arthritis could be abrogated by targeting the animals with ganciclovir.

In conclusion, ex vivo gene transfer of splenocytes with a soluble monomeric TNFRp75 was successful, and this system is a good model of arthritis transfer. Therefore the cells of the immune system are a good target for ex vivo transfer with therapeutic cytokine inhibitors and, probably, inhibitory cytokines.

Immunomodulatory properties of tenidap
A M M Mittenburg, F C Breedveld
University Hospital, Department of Rheumatology, Leiden, the Netherlands

To investigate the effect of tenidap on T cell functions in vitro, we measured proliferation and cytokine production of CD4 T cell clones. Tenidap dose dependently (5–25 μg/ml) inhibited the proliferation of anti-CD3 or anti-TNFα (TNFα) activated T cell clones. Tenidap also inhibited the proliferative response of the cytokine indicator cell lines D10 (IL-1), B9 (IL-6), and CTL122 (IL-2), which suggests that signal transduction leading to cell proliferation driven by multiple cytokines may be influenced by this drug. In another set of experiments we did not obtain evidence for synergy of the effect of tenidap with that of cyclosporin A with respect to T cell proliferation. Tenidap also inhibited interferon gamma (IFNγ) production of anti-CD3 stimulated T cells in a dose dependent manner. Tenidap 25 μg/ml inhibited the replication of HIV, but did not inhibit IFNγ that occurs 24 hours after stimulation with peripheral blood mononuclear cells (PBMC). Furthermore, tumour necrosis factor α (TNFα) lipopolysaccharide or phorbol ester (PMA) stimulated DNA synthesis of PBMC as six hours was significantly inhibited by the presence of 2.5 μg/ml of tenidap. These effects of the drug were specific, as the housekeeping gene β-actin was not influenced under these conditions. Different non-steroidal anti-inflammatory drugs in different concentrations did not have the effects described above. These data suggest that part of the clinical spectrum of tenidap may be the result of its effect on T cell function.
Interleukin-1 (IL-1) has an important role in the pathogenesis of rheumatoid arthritis (RA). It induces the release of metalloproteinases from RA cells, including matrix metalloproteinase-1 and collagenase, and stimulates bone resorption, T cell activation, and the expression of adhesion molecules. There are increased levels of IL-1α and IL-1β in the synovial fluids of RA patients. Increased serum levels of IL-1β have also been reported and correlated with disease activity and radiographic progression, and immunohistochemical staining has been used to localize the cytokine in synovial membrane vessels at the cartilage-panus junction. Inhibition of IL-1 activity is thus a logical strategy in an attempt to downregulate the inflammatory process in RA.

Cell surface receptors specific for mediating cellular events for IL-1α and IL-1β are found on several different cell types. Soluble IL-1 receptors (sIL-1R) are the extracellular portion of specific cellular receptors for IL-1 that have been detached from the cell surface. They are of two types: type I and type II. The role of the sIL-1Rs has not been clearly established, but type I sIL-1R has been found on most cell types and is important for transducing the action of IL-1. Type II sIL-1R has been detected primarily on B lymphocytes, monocytes, neutrophils, and bone marrow cells, and increased levels have been reported in serum and synovial fluids of RA patients. Arend and colleagues have reported differential binding of IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) to soluble forms of types I and II sIL-1Rs. IL-1β bound more avidly to sIL-1R (type I) than did IL-1α or IL-1Ra. In addition, sIL-1R (type I and II) inhibited the detection of IL-1α and IL-1β, respectively, by enzyme linked immunosorbent assay (ELISA). These properties of these two soluble receptors may have significant importance with regards to the measurement of IL-1β and IL-1Ra in body fluids when measured by ELISA and may, in addition, prove important in dictating which type (I or II) of sIL-1R will be most useful clinically.

sIL-1R (type I) has been produced by recombinant techniques. Recombinant human sIL-1R (type I) has been shown to suppress inflammation in various animal models including antigen induced arthritis and experimental autoimmune encephalomyelitis. Administration of the recombinant sIL-1R to mice with cardiac allografts increased survival and markedly reduced lymph node swelling. Recombinant sIL-1R has been safely administered to humans with allergies, and inhibited the late phase response in these subjects. The recombinant sIL-1R has been tested in a double blind, randomised trial in 16 RA patients given intra-articular administration of sIL-1R (type I) was well tolerated and some beneficial effects were noted. A phase I study using recombinant human sIL-1R subcutaneously to treat patients with active rheumatoid arthritis has been completed. Twenty three patients with active RA were entered into a double blind, placebo controlled dose escalation, two centre trial (Northwestern University, University of Alabama at Birmingham). For 28 days, patients received daily subcutaneous doses of recombinant sIL-1R (type I) 125, 250, 500 or 1000 mg/m². One patient in each dose group received placebo. There were no serious significant adverse events. In addition, a phase II multicentre, double blind, placebo controlled trial using recombinant human sIL-1R (type I) has been completed. In this phase II trial, 70 patients with active RA were randomised to receive a single intravenous loading dose (sIL-1R or placebo) followed by daily subcutaneous low dose sIL-1R (type I) for 34 consecutive days. All patients completing the first cycle received a larger intravenous loading dose or placebo followed by 34 days of subcutaneous administration of a larger dose of sIL-1R (type I) or placebo.

Gene therapy for arthritis

C H Evans
University of Pittsburgh Medical Centre, Pittsburgh, USA

Many novel compounds with promising anti-arthritic potential are proteins and thus difficult to administer as drugs, especially in chronic disease. Here, delivery of a gene (or cDNA) encoding the protein of interest holds promise as a means of obviating this limitation. We are developing methods for the appropriate delivery of potentially antiarthritogenic genes.

In general, genes may be delivered 'locally' to individual diseased joints or 'systemically' to the whole body. The appropriate delivery of potentially antiarthritogenic genes should be transduced into the synovial lining of the diseased joint in situ and express the therapeutic protein 'locally' to individual diseased joints or 'systemically' to the whole body.

RELEVANT PUBLICATIONS


ASSESSMENT OF THERAPEUTIC BENEFIT

Clinical and radiological outcome measures

D L Scott
Department of Rheumatology, King's College Hospital, London SE5 9RS, United Kingdom

Outcome measures should be based on the core data set accepted by the World
Functional mended for European Leagues

Health Organisation, the International and European Leagues Against Rheumatism, and other groups. These measures include tender joint counts, swollen joint counts, patient's assessment of pain, global assessments of disease activity, and laboratory evaluation of an ESR, together with the radiography as a functional measure. Radiology is recommended for long term analysis. There are a number of articular indices. Their validity and reliability do not differ substantially. Taking simplicity into account, the 28 joint index, not graded and not weighted, is preferred. In clinical trials it is common to apply a number of endpoint measures. The number of measures could be reduced by using few indices which are generally considered important, sensitive to change, and able to differentiate between drugs. An example is to combine a joint count, assessment of pain, and measurement of erythrocyte sedimentation rate. An alternative is to look at changes in the core data set for rheumatoid arthritis disease activity. For example, changes in >20% in five of the seven criteria may indicate a satisfactory response. There are several approaches to joint scoring, including plain radiology, magnetic resonance imaging (MRI), bone scans, iso-tape labelling methods and dual energy x ray absorptiometry (DEXA) scans for peri-articular bone loss. Conventionally, radiographs are best for looking at drug efficacy; current radiograph scoring methods are predominantly those of Sharp et al and Larsen et al. MRI seems better for defining synovitis.

Biochemical measurement of disease activity

M H van Rijsijk
University Hospital Groningen, The Netherlands

In rheumatoid arthritis (RA), measurement of the acute phase response has proven to be a suitable parameter for long term monitoring of disease activity. Macrophages and synoviocytes in the articular membrane have been shown to be producers of cytokines such as interleukins (IL) 1 and 6, and tumour necrosis factor α (TNFα). The proinflammatory cytokines IL-1 and TNFα are thought to play a major role in chronic inflammation and joint destruction in RA; IL-6 is not a tissue damaging cytokine, but rather appears to be the major inducer of the systemic acute phase response. In clinical practice the acute phase response can be measured indirectly by erythrocyte sedimentation rate (ESR) or directly by C reactive protein (CRP) or serum amyloid A (SAA). The low basal levels, rapid increase and short half life of CRP compared with fibrinogen (the main determinant of ESR) indicate that CRP is a more appropriate marker of disease activity. Longitudinal studies in patients with early RA have shown that CRP and ESR were correlated mainly with radiological damage and with joint swelling, but not with joint pain. In general, CRP levels in RA patients with active arthritis do not reach values greater than 100 mg/l. Higher concentrations, in particular >150 mg/l, are highly suggestive of a complicating bacterial infection. In a chronic inflammatory disorder like RA the nature of the acute phase activity and its relationship to outcome is important for the prognosis and for the assessment of the long term effects of therapeutic interventions. Serial measurements of acute phase proteins, concentrations, reflecting current disease activity, should ideally be transformed into time integrated values (area under the curve) to allow comparison with outcome measures which are not changeable in nature, such as radiographic damage.

Although, theoretically, IL-6 could be a good indicator of synovitis activity, CRP and ESR show a better correlation with radiological progression, and can be more easily measured, and therefore appear to be more suitable for clinical use.

Prognosis features and outcome of early rheumatoid arthritis

P Emery
Department of Rheumatology, Birmingham University, Birmingham B15 2TT; United Kingdom

The treatment of rheumatoid arthritis has changed considerably in recent years. This has resulted from a self fulfilling prophecy of the poor outcome (including increased mortality) of conventionally treated patients and the realisation that, once established, the disease is permanent. Furthermore, the contrasting effect of treatment on the inflammatory and erosive activity of the disease function has been instructive. The former results in a delay in the progression of disease by modifying the rate, while the latter stops the progression. This theme is reflected in the continued emphasis on the treatment of rheumatoid arthritis which is a chronic, progressive disease and which needs to be chronically treated. For this and other reasons, early treatment has major advantages. However, there are also several practical problems which need to be appreciated if a more widespread application of these principles is to be achieved.

Quantitative measures such as the number of swollen joints, the level of acute phase response, and the amount of disability have been recognised as predictors of outcome but, with the exception of rheumatoid factor (RF), most are factors which evolve with disease progression; their development is therefore part of a self fulfilling prophecy. RF is more suitable for severe disease. To alter the disease fundamentally it is necessary, from both a clinical and a theoretical standpoint, to treat patients before the development of the signs indicating poor prognosis. Therefore prognostic features must be independent of the disease stage. There is now evidence that genetic markers in combination with RF can provide this information. The obstacles which, in the past, have prevented a disease remission approach have been removed. Such disease remission programmes are currently being undertaken and should provide the answer to the question whether rheumatoid arthritis is truly treatable.

RELEVANT PUBLICATIONS

To verify the hypothesis that IVIg may be effective also by solubilising immunocomplexes from the tissues, we examined biopsy specimens of non-lesional, sun-exposed skin of the dorsal forearm from six patients with SLE before and after treatment with high doses of IVIg. All patients were women (ages 15–49 years); disease duration was one to five years. Three cycles of monthly infusion of IVIg 400 mg/kg/day for five consecutive days were administered. Before treatment, all subjects had large amounts of IgM and C1q cutaneous deposits, and the pattern of immunofluorescence was always filamentous or granular, involving the dermo-epidermal junction. After the third cycle of IVIg infusion, the amount of IgM and C1q deposits decreased dramatically and the pattern of immunofluorescence also changed, shifting from diffuse D-E junction staining to papillary (focal). The linear D-E junction staining correlated with severe forms of SLE.

Our results support the hypothesis that high dose IVIg therapy may be effective by solubilising immunocomplex deposits from the tissues.

IL-1 and IL-1ra production by different macrophage subsets in rheumatoid arthritis synovial membranes

A Cauli, G Yanni, G Panayi

Rheumatology Unit, Guy’s Hospital, United Medical and Dental Schools, London, United Kingdom

Rheumatoid arthritis (RA) is a disease in which an unknown antigen is presumed to drive T cell responses leading to macrophage activation, production of monokines, and tissue destruction. As the antigen remains unknown, new treatments for RA have targeted T cells (cyclosporin A, CD4 monoclonal antibody (MABs)) or monokines (tumour necrosis factor α (TNFα) antibodies; interleukin-1 receptor antagonist (IL-1ra)).

Macrophage maturation and the proinflammatory cytokines IL-1α, IL-1β, IL-6, IL-1ra are studied in an attempt to define proinflammatory and anti-inflammatory subsets of macrophages.

Using double labelling immunofluorescence and immunoperoxidase techniques, synovial membranes from 10 RA patients were stained to define early (2E70, CD14), mature (25F9), and anti-inflammatory macrophages (RM3/1), and monokines (IL-1α, IL-1β, IL-1ra). Cells double labelled for monokines and macrophages were expressed as a percentage of the total number of positively staining macrophages.

IL-1α and IL-1β were found in the sublining layer, while mature macrophages were more abundant in the lining layer. IL-1α and IL-1β were co-expressed in the macrophages examined. In the sublining layer, fewer early macrophages were IL-1α positive (33.2 ± 17.1%) and IL-1ra positive (31.7 ± 29.5%) compared with mature macrophages (IL-1α 89.2 ± 9.7%; IL-1β 91.2 ± 4.6%; IL-1ra 90.2 ± 4.6%). Anti-inflammatory macrophages gave results similar to mature ones. CD14 macrophage positivity for the two cytokines was intermediate between 2E10 and 25F9 macrophages, showing that a subset of staining was noted in the lining layer. The majority of IL-1α positive cells were also IL-1ra positive (79.8 ± 12.3% sublining layer; 98.3 ± 2.2% lining layer).

We conclude that, after monocytes have migrated into the RA joint, they undergo phenotypical and functional changes from an early profile (2E10 and CD14 positive, low percentage of IL-1α and IL-1ra positive cells) to a mature profile (CD14 positive and negative, 25F9 and RM3/1 positive, high percentage of IL-1α positive and IL-1ra positive cells).

Prospective sonographic and arthroscopic evaluation of proliferative knee joints in RA (Join, T 0–2 months)

F Chicco Bianchi, C Rigon, A De Candia*, G Gallo, L Rubaltelli*, U Fiocco, S Todesco

Division of Rheumatology and *Institute of Radiology, University of Padua, Italy

Monitoring of treatment by ultrasound is a promising field, with the advantages of non-invasiveness and objectivity. Quantitative estimates of synovial effusion and proliferation can be useful for objective assessment of the severity and changes of local disease activity and for early therapeutic decisions in the treatment of both rheumatoid (RA) and psoriatic (PsA) arthritis. The objective of our study was to establish the accuracy of ultrasonography in assessing the topography, morphology, and extent of synovial proliferation in knee joint synovitis of RA and PsA using arthroscopic visualisation as a ‘gold standard’ reference and anatomical guideline for measuring synovial thickness at several sites in the knee joint. Sonographic examination was carried out in 12 RA patients (13 knees) and in 13 PsA patients (14 knees) with knee joint synovitis, followed within one week by arthroscopy to compare the topography (intra-articular localisation) and the morphometry (ultrasonographic pattern) of synovial proliferation. In 15 knees undergoing arthroscopic synovectomy, preoperative sonographic measurements of synovial thickness, the suprapatellar, medial, and lateral parapatellar recesses was compared with arthroscopic visualisation of synovial proliferation. Control measurements were taken by ultrasonography on the same knees two months after arthroscopic synovectomy in 13 knees. Three distinct sonographic patterns of synovial proliferation were confirmed by arthroscopic examination: villonodular (12 knees), uniform thickening (eight knees), and over-lapping layers (seven knees). More than 50% of knees showed more than one sonographic pattern, with no differences in distribution between RA and PsA. A significant correlation was found between ultrasonographic and arthroscopic evaluation of synovial thickness in the suprapatellar (p < 0.02) and medial parapatellar recesses (p < 0.02)—the sites of maximal synovial proliferation in all our patients. The number of arthroscopic synovectomy, ultrasonographic measure of synovial thickness showed a significant reduction in all the three joint recesses explored (supratellar: p < 0.005; medial parapatellar: p < 0.005; lateral parapatellar: p < 0.02). The significant correlation of ultrasonographic findings with clinical findings both before and after synovectomy suggests that sonography should be an objective method in monitoring the response to treatment of inflammatory knee joint disease.

Various monoclonal antibodies (MABs) have been used in the treatment of rheumatoid arthritis (RA). The use of mouse MAB showed that almost all patients developed an antiglobulin response with increased risk of anaphylaxis and reduced efficacy on retreatment. The use of chimeric MAB may reduce the antigenicity of MAB. We treated 19 RA patients with the chimeric CD4 MAB, cm-T412, and assessed their chimeric antibody response. They were treated with four different dosing regimens: a single 50 mg dose (group I); 50 mg weekly for four weeks (group II); 50 mg daily for five days followed by weekly 50 mg for five weeks (group III); 50 mg daily for five days followed by a repeated course after five weeks (group IV).

The table shows the number of patients and the incidence of a significant human chimeric antibody (HCA) response (titre >1/20).

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<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tr>
<td>Total No of patients</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HCA positive</td>
<td>(25) (90)</td>
<td>(2) (67)</td>
<td>(4) (67)</td>
<td>(5) (87)</td>
</tr>
<tr>
<td>(No (%))</td>
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Group III patients became HCA positive at week 2 or 4 with a peak response at weeks 8 and 10. This may have reflected the weekly nature of treatment from week 2 to week 6. Conversely, group IV patients showed a positive response from week 2 which subsequently decreased, but showed a sudden transient increase after retreatment at week 6. All HCA titres declined after cessation of treatment. The HCA titres observed were in general five to 10-fold lower than responses to similar murine antibodies. One patient in group III had an urticarial rash, but no serious anaphylactic response was seen.

In conclusion, many patients treated with chimeric CD4 MAB developed a human chimeric antibody response, but this was less than that seen after treatment with murine MAB. This may be the advantage of using chimeric MAB. As HCA titres declined after cessation of treatment, repeated treatments may be possible.

Long term follow up of patients with active rheumatoid arthritis injected with murine idiotype class II monoclonal antibodies

L Cozzi, U Fiocco, E Cozzi, S Todesco

Division of Rheumatology, Department of Internal Medicine, University of Padua, Italy

Rheumatoid arthritis (RA) is a progressive disease associated with severe joint damage and functional decline. Although the pathogenesis remains unclear, elucidation of the
molecular events underlying the inflammatory reaction of rheumatoid synovitis has provided specific targets for selective immunotherapy. Several studies in animal models have shown the effectiveness of monoclonal antibodies in the resolution of inflammatory abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis and clinical outcome after immunotherapy with the anti-Id MAb, F3-C25, elicited with the syngeneic anti-HLA DR, DP, MAB CR 11.462, were determined in a phase I clinical trial with active RA and controls. The subsequent five years follow up. Each patient received four or five injections of 500 μg of MAB F3-C25.

In all the patients complete physical examination, further the immunohistological studies and blood samples for laboratory investigations were collected weekly, monthly, and quarterly during a 2.5 year periodic.

During the follow up, clinical improvement (50% reduction of Ritchie’s index, morning stiffness, and daily drug intake for more than two months) was seen in one patient one year after the second injection. In three other patients a clinical improvement was observed six months after the second injection (two patients) and third injection (one patient).

No allergic or anaphylactic reactions or infections were observed during the observation period.

We conclude that the active immunotherapy of RA with MAB in the doses studied is safe and well tolerated long term. On the basis of these preliminary data further investigations are necessary to validate the effectiveness of this immunotherapeutic approach.

**Immunohistological analysis of synovial membrane in ankylosing spondylitis: implications for immunotherapy**

G Cunnane, O FitzGerald, B Bresnihan

Department of Rheumatology, University College Dublin, St Vincent’s Hospital, Dublin, Ireland

Ankylosing spondylitis (AS) is an inflammatory arthropathy with distinguishing clinical features. Although recent immunohistological studies in AS have highlighted an immunological pathogenesis, there has been little systematic investigation of immune based treatment. This study was undertaken to characterise further the immunohistological features of synovitis in patients with severe AS and peripheral arthritis. A large panel of monoclonal antibodies was used to document the cell populations accumulating in both the lining and sublining layers. Five patients underwent synovial biopsy of inflamed knees. In all specimens, features observed included: a thin layer with predominant macrophage (CD14 and CD68) accumulation; prominent macrophage infiltration of the sublining layer; and very sparse T lymphocytes (CD3, CD4, CD8) infiltration. These histological characteristics differ from those usually described in rheumatoid arthritis (RA). A surprising feature in two patients was the occurrence of B cell (CD19 and CD22) infiltration, with small follicles forming in the sublining layer. The predominance of macrophages in the lining and sublining layers in all samples, and the accumulation of B cells in some, have implications for immunotherapy in AS which may differ from those in RA.

**Endocrine disorders in rheumatoid arthritis**

A Eijssbouts, F van den Hoogen, R Laan, A Hermus, C Sweep, L van de Putte

Departments of Rheumatology and Endocrinology, University Hospital, Nijmegen, The Netherlands

It has become clear that there is communication between the endocrine system and the immune system. Various studies have shown that cytokines activate the hypothalamic-pituitary-adrenal (HPA) axis after immune stimulation.

In animal studies cytokines, especially interleukin-1 (IL-1) and tumour necrosis factor α (TNFα) were found to have an important role in arthritis. Furthermore, cytokines such as IL-1, IL-6, and TNFα have been detected in rheumatoid synovium and serum concentrations of several cytokines in patients with RA were increased compared with normal controls.

By activating the HPA axis, cytokines elicit a cortisol response which has an anti-inflammatory effect. A defective cortisol response after stimulation by cytokines could lead to a higher susceptibility for developing (chronic) arthritis. Supporting evidence for this hypothesis has been found in animal studies and in humans; in the latter studies patients with rheumatoid arthritis were found to have greater cytokine concentrations, but smaller ACTH and cortisol concentrations after stress, compared with control groups (patients with osteoarthritis and chronic osteomyelitis) and greater levels of prolacin, which has been shown to have pro-inflammatory effects.

These findings suggest that patients with rheumatoid arthritis may have a hypothalamic dysfunction leading to a pro-inflammatory imbalance in their hormonal status, which might play a part in the pathogenesis of rheumatoid arthritis.

On the basis of this assumption, we set up a trial to study the hormonal and cytokine reactions to stress in patients with recent onset rheumatoid arthritis before treatment, after two to four weeks of treatment with non-steroidal anti-inflammatory drugs and after six months treatment with modifying antirheumatic drug. Subjects were compared with normal controls, patients with RA of five years duration, either active or in remission, and patients with other chronic inflammatory disease and chronic pain syndrome. The study aimed to confirm the assumed endocrine disorder, and examine whether it is influenced by treatment and is specific for RA.

**Radiographic assessment of damage in rheumatoid arthritis: a contribution to a standardised approach in multicentre studies**

G Pasero, F Prinoli, A Ceresa, L Bacarin, M Cammissa, R Ferrari, O Della Casa-Alberighi, E Marubini, G F Ferraccioli

Department of Rheumatology, University of Pisa, Pisa, Italy; Institute of Radiology, Rome, Italy; *Institute of Radiology, Treviso, Italy; *Institute of Radiology, S Giovanni Rotondo, Italy; †Medical Department, Sandoz PF, Milan, Italy; ‡Institute of Biometrics, Milan, Italy; §BDU Department of Internal Medicine, Udine, Italy

Radiographic scoring of anatomical damage remains the best approach to evaluate the severity of rheumatoid arthritis (RA) and the effects of disease modifying antirheumatic drug (DMARD) therapy on the aggressiveness of the disease. In order to standardise the scoring for a multicentre prospective, randomised trial with a radiological endpoint in early active RA treated with DMARDs, we undertook a methodological evaluation of the most reliable assessment and scoring of joint damage.

Radiographs were taken (posteroanterior view) of the hands, wrists and feet of 284 RA patients, at entry and 12 months later. All were of the same quality and granularity. After being collected centrally, they were all jointly scored by an independent central committee of three radiologists, blind as to the patients’ clinical and laboratory findings, and to treatments. The radiographs were evaluated under identical scoring conditions, according to three different types of reading: as single radiographs, randomly ordered in terms of sequence and patient; as paired radiographs for each patient, without knowledge of their chronological sequence as ordered radiographs for each patient (with knowledge of their chronological sequence). The ability of each type of reading to detect differences within patient and between methods was measured. The radiographs were scored according to the Larsen-Dal effect method, the 12 months t baseline progression of erosion score (PES) and progression of damage score (PDS) being the main variables analysed. The table shows the results.

**Results of progression of erosion scoring (PES) and progression of damage scoring (PDS) of radiographs evaluated singly, in pairs, or in chronological sequence, for RA patients**

<table>
<thead>
<tr>
<th>Variable and reading</th>
<th>Score (mean (SD))</th>
<th>Quartile Comparison of readings (t)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td><strong>PES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>1.98 (3.92)</td>
<td>0</td>
</tr>
<tr>
<td>Paired</td>
<td>1.87 (3.07)</td>
<td>0</td>
</tr>
<tr>
<td>Ordered</td>
<td>2.42 (3.53)</td>
<td>0</td>
</tr>
<tr>
<td><strong>PDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>6.24 (15.47)</td>
<td>0</td>
</tr>
<tr>
<td>Paired</td>
<td>5.27 (9.10)</td>
<td>0</td>
</tr>
<tr>
<td>Ordered</td>
<td>7.00 (9.74)</td>
<td>0</td>
</tr>
</tbody>
</table>

Test for parallelism of regression line with the diagonal line r = 1: ***p < 0.001.
The differences between the means of the three methods represented a measure of the possible overestimation of one reading over the others (single > ordered > paired). Single reading data were always much more dispersed than those of the other two methods; PDS data were more dispersed than those for PES. The paired and ordered readings performed in a similar way because the rater was able to make the correct comparison. However, knowledge of the sequence may inflate the mean difference between the entry and final values of the scores, as bias towards the expected progression of the disease may occur. Paired reading therefore seem to offer a more conservative rating, and for these reasons appear to be the method of choice for inferential purposes.

T cell reactivity to human type II collagen

G U Gimsa

Deutsches RheumaForschungsZentrum, Berlin, Germany

T cell reactivity to type II collagen is of interest for many reasons. Although it is unlikely (but not impossible) that this protein plays a part in the pathogenesis of rheumatoid arthritis (RA), it may do so in other more rare rheumatological conditions. Autoimmunity to type II collagen, however, provides one of the best animal models of RA. The current interest in oral tolerance has excited renewed interest in reactivity to this protein. Finally (and this is what excites us most), T cells reactive with type II collagen may provide the ideal carriers of T cell cytokine genes for treatment of RA. We have examined various aspects of this problem.

First, we have been able to demonstrate, for the first time, that lymphocytes of mice that have been immunised with foreign (bovine) type II collagen acquire the ability to leach self collagen out of their homologous.

Second, we have discovered that a mouse class II allele (H-2Aα) which is known to have a suppressive effect on several other immune responses can also suppress collagen-induced arthritides. Our working hypothesis is that these widespread effects reflect promoter polymorphism: for instance an allele or locus which is better expressed in B cells than in macrophages is likely to favour a Th2 response. To test this hypothesis we have sequenced all four class II promoters (Aa, Ab, Ea, Eb) from four mouse haplotypes. Interestingly, H-2Aα is the only one to have entirely unique features in the promoter region, namely an A substitution for G. Furthermore, the H-2Aβ promoters are the closest in sequence to the homologous HLA-DQ promoters, as might be expected from the similarity of the functions which they are hypothesised to perform.

Third, we have been attempting to clone collagen II reactive T cells out of RA patients. To date, this task has frustrated us, as it has others before. Nevertheless, we will draw on the accumulated wisdom of years of experience with Lyme disease to apply other methods in pursuit of this aim. We can draw on information concerning the T epitopes of type II collagen in the mouse.

Evaluation of immunological and disease parameters for type II collagen induced arthritis in disease susceptible and resistant rhesus monkeys

(Macaca mulatta)

B A Hammerschmidt, M E Koppelet, M Jenker, R E Bontrop

Biomedical Primate Research Centre-TNO, PO Box 5815, 2280 HV Rijswijk, The Netherlands; TNO Prevention and Health, Leiden, The Netherlands

After immunisation with bovine type II collagen (bCII), about 70% of the rhesus monkeys studied developed severe polyarthritis. All animals in the disease resistant group share one particular major histocompatibility complex class I allele, designated Mamu-A26. Animals lacking this allele are disease susceptible. We have compared the course of type II collagen induced arthritis (CIA) in two groups of Mamu-A26 positive and negative animals, and investigated different parameters associated with autoimmune arthritis.

All Mamu-A26 negative monkeys produced IgM and IgG antibodies to bovine and rhesus CII, and a proliferative T cell response to rCII could be demonstrated. Mamu-A26 negative monkeys produced bCII antibodies of the IgG, but not the IgM, isotype and bCII induced T cell proliferation was absent. In all Mamu-A26 negative monkeys, increased values of serum C reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) were found during clinically active arthritis, whereas no changes were observed in Mamu-A26 positive monkeys.

Enhanced articular cartilage degradation products were found in the urine of Mamu-A26 negative, but not of Mamu-A26 positive monkeys. In particular, the major collagen cross link in articular cartilage, hydroxylysino pyridinoline (HP), showed a strong increase (nine- to 15-fold). Excretion rates of lysino pyridinoline, which is considered as a marker of bone specific degradation, increased only four- to sixfold.

The capacity to generate IgM antibodies and a T cell response to bCII is associated with susceptibility to CIA. Candidate markers for the course of the disease are ESR and CRP for inflammation, and HP for inflammatory cartilage destruction.

Induction of autoantibodies against a self protein

I Dalum, P Hindersson, M R Jensen, H Einers, S Mouritsen

M/E&E Lersoe Parkalle 40 DK 2100 Copenhagen East, Denmark

To demonstrate the abrogation of tolerance towards ubiquitin in mice, four variants of ubiquitin were constructed, each containing a foreign immunogenic T cell epitope implanted into the sequence.

Synthetic oligonucleotides encoding the T cell epitopes HEL50.61 or OVA325-336 were implanted into a gene encoding ubiquitin. These epitopes bind specifically to Aα and Aβ major histocompatibility complex (MHC) class II, respectively. They replaced ubiquitin segments and were inserted in a way which disturbed the overall structure of the ubiquitin protein as little as possible. The ubiquitin variants were expressed in Escherichia coli and purified. BALB/c and BALB/k mice were immunised with ubiquitin-OVA (containing OVA325-336) and ubiquitin-HEL (containing HEL50-61).

Within two weeks, BALB/k mice (H-2k) immunised with ubiquitin-HEL or ubiquitin-OVA responded to the unique epitope of ubiquitin-OVA elicited autoantibodies in this strain. Native ubiquitin was non-immunogenic in both mouse strains. Serological studies showed that the antibodies reacted against soluble native ubiquitin epitopes, but this response to ubiquitin did not follow the known MHC restriction of the induced T cell epitopes. The immunological basis of the observed antibody response was studied by competitive MHC class II binding experiments using peptides representing the entire ubiquitin protein. The specificity of the antibody response against individual overlapping ubiquitin peptides varied for different constructs and was not identical to the antibody response against a carrier conjugated ubiquitin protein.

These results show that insertion of a foreign immunogenic T cell epitope into a self antigen is sufficient to abrogate tolerance. This observation supports the concept that lack of T cell help is the major mechanism responsible for immunological B cell responses against autoantigens. We therefore hypothesised that implantation of epitopes in a cytokine self antigen can interfere with an animal model of autoimmune arthritis.

Thalidomide and pentoxifylline in rheumatoid arthritis: preliminary results of an open study

T W J Huizinga, B A C Dijkman, L van Rensen, C L Verweij, F C Breedveld

Academisch Ziekenhuis Leiden, Department of Rheumatology, Leiden, The Netherlands

Use of monoclonal antibodies against tumour necrosis factor α (TNFα) is an effective treatment of rheumatoid arthritis (RA), with a convincing relation between the dose of anti-TNFα antibodies and the clinical response. Pentoxifylline inhibits TNFα gene transcription and thalidomide decreases the half-life of mRNA encoding TNFα; therefore both drugs could be effective in the treatment of RA. In an open study we treated nine patients with active RA with 1200 mg pentoxifylline and 100 mg thalidomide daily for 12 weeks. Preliminary data from the first eight weeks of treatment showed that TNFα production in a whole blood system significantly decreased in six of eight patients in stimulation with lipopolysaccharide 100 ng/ml blood and in seven of eight patients on stimulation with lipopolysaccharide 1000 ng/ml blood (p < 0.04, Wilcoxon paired pair test). The amount of TNFα produced on stimulation with lipopolysaccharide at week 0 was 7.4 ± 2.4 ng, reducing to 3.6 ± 1.6 ng at week 8. According to the Paulus 20% criteria, three of nine patients were responders; this low number might be related to the relatively weak inhibition of TNFα production by these drugs. In the analysis of the responders versus non-responders, the plasma TNF level in the responders was 45, 40, and 0.2 ng/ml, compared with 2, 5, 0, 0, and 0 ng/ml in the non-responders (differences p = 0.06, Mann-Whitney U test). We suggest that a high plasma TNF level in the non-responders of TNFα might be a predictor for response to treatment with pentoxifylline and thalidomide.
Does rheumatoid arthritis improve after HIV-1 infection? A case report

G Lapadula, F Iannone, C Zuccaro, M Covelli, R Bucci*, V Pipitone

Department of Rheumatology, University of Bari, Bari, Italy; *Ospedali Riuniti-Foggia, Foggia, Italy

A 59 year old woman presented 12 years ago with arthritis of both shoulders, wrists, second and third metacarpophalangeal and proximal interphalangeal (PIP) joints and with early morning stiffness >60 minutes. Laboratory findings were: erythrocyte sedimentation rate (ESR) 108 mm/1st h, C reactive protein (CRP) 36 mg/l, haemoglobin 107 mg/l, Waaler Rose test 1/128. Radiographic assessment of the hands revealed symmetrical bone erosions of the third PIP joint and joint space narrowing. Treatment with oral gold salts and prednisone at low dose was started. At the age of 56 years, the patient was accidentally discovered to be HIV-1 positive, after which her rheumatic disease ameliorated and she suffered just arthralgies without synovitis. Recently she had only swelling of tendon sheaths of the hands; ESR was 120 mm/1st h (maybe because of Treponema gondii and Candida albicans infections), CRP 5 mg/l; RF was negative and, surprisingly, the bone erosions disappeared.

The T cell phenotype and peripheral blood mononuclear cell reactivity to bound phase CD3 and phytohaemagglutinin were compared with those of a patient with rheumatoid arthritis (RA), a normal control, and a patient with longstanding AIDS (table): HIV-1 infection induces a profound perturbation of the immune system: depletion of T' cells, mainly CD4; T cell anergy, most probably as a result of impaired interleukin-2 and interferon-gamma synthesis, with a concomitant increase in interleukin-10; and a defective antigen presenting cell function. This immunosuppression would account for the regression of RA seen in our patient. This case reported stresses once more the importance of CD4 T cells in the pathogenesis of RA and the relevance of immunomodulation in the treatment of RA.

Biological effects of a human IgG4 monoclonal antibody in vivo

J D Isaacs, J Greenwood, M Wing*, P Rebello†, G Hale, H Waldmann

Cambridge University Departments of Pathology, *Neurology and †Surgery, Cambridge, United Kingdom

The biological effects of chimaeric humanised monoclonal antibodies (MABs) of IgG4 and IgG1 were compared in vitro and in vivo. MAbs with specificity for the CAMPATH antigen (CD52) of either IgG4 or IgG1 isotype were administered to patients with rheumatoid arthritis (RA). Peripheral blood lymphocytes (PBL) counts and serum concentrations of tumour necrosis factor α (TNFα) were monitored before and after MAB administration, and related to 'first dose' symptoms. Lymphocyte proliferation and antibody dependent cell mediated cytotoxicity assays were compared with in vivo effects.

IgG1-CAMPATH-1H resulted in profound and lasting lymphocyte depletion. In contrast, there was an average 66% depletion of PBL 48 hours after a single infusion of IgG4-CAMPATH-1H, with an apparent polymorphism in depletion ability between patients. First dose symptoms were less with IgG4-CAMPATH-1H. The study design did not permit a direct contrast between isotypes with regard to TNFα release, but comparison with historical data suggested that this was less than with IgG1-CAMPATH-1H. Although patients were polymorphic in the in vitro analyses, this did not correlate with in vivo depletion data.

Contrary to most predictions from in vitro data, MAbs of human IgG4 isotype do possess significant effector function in vivo, although this is less than that seen with IgG1 MAbs. It is not currently possible to correlate in vivo and in vitro effector functions for human IgG4 MAbs.

Pharmacokinetic profile of a new formulation of cyclosporin in patients with rheumatoid arthritis after conversion from the standard formulation

A Marchesoni, G Paserot, S Zeni, N Battafarano, G Riufo, A Padula†, E Taglione, L Leon*, F Fantini, O D Casa-Albergih

Department of Rheumatology and *Laboratory of Analysis, University of Milan, Milan, Italy; †Department of Rheumatology, University of Pisa, Pisa, Italy; ‡Medical Department, Sandos, Milan, Italy

A new oral microemulsion formulation of cyclosporin (CyA-NOF) was assessed in patients with rheumatoid arthritis (RA) to establish if it had better bioavailability than the standard formulation commonly used (cyclosporin soft gel capsules: CyA-SGC). Fifteen patients (11 women and four men, mean age 50.3 ± 10.9 years) with longstanding RA (mean disease duration 7 ± 7 years) receiving stabilised treatment with CyA-SGC had their medication changed to CyA-NOF during a multicentre, open-switch over six week trial. After three weeks receiving CyA-SGC in a stabilised dose, the patients received CyA-NOF in the same dose for an additional three weeks. Pharmacokinetic profiles were obtained at the end of both three weeks by evaluating the CyA concentration in whole blood at time 0 (predose), and 1, 1.5, 2, 3, 5, 7, and 12 hours after drug administration. A thorough clinical evaluation was also made.

The mean dose of cyclosporine was 3.0 ± 0.7 mg/kg/day. The table shows the main pharmacokinetic variables.

Interpatient variability was lower with CyA-NOF. Despite the greater cyclosporin bioavailability, CyA-NOF was not more toxic than CyA-SGC, and the two formulations were comparable with regard to clinical efficacy.

In patients with RA, the new oral microemulsion formulation of cyclosporin seems to give more consistent pharmacokinetic profiles than the standard formulation, without being more toxic. The greater and more predictable drug bioavailability associated with CyA-NOF should allow better management of those RA patients who require treatment with cyclosporin.

Biologically relevant and evolutionarily conserved proteins are major target antigens for the cellular immune system in yersinia triggered reactive arthritis

A Merts, M Grolms, J Braun, J Sieper

Klinikum Benjamin Franklin, Free University of Berlin, Berlin, Germany

Yersinia triggered reactive arthritis is a T cell mediated disease that is initiated and driven by persisting bacterial antigens. Although the outcome is usually favourable, long lasting and relapsing forms exist. Moreover, associated extra-articular phenomena such as uveitis and enthesitis suggest that there is autoimmunity, although there is no direct evidence (no autoantibodies) for it. The identification of immunodominant antigens and peptides is of great importance both for the understanding of the disease mechanisms in general, and for the development of new forms of immunotherapy, by means of altered peptide ligands for T cell receptors (TCR). The yersinia genome codes for many hundred proteins, all of which represent potential antigens for T cells. In a recent study we prepared different antigenic fractions from whole bacteria by biochemical methods and found two proteins to be immunodominant for T cells in bulk culture proliferation assays of synovial fluid mononuclear cells of 20 yersinia arthritis patients: a 19 kDa protein and a 13 kDa protein. The former was subsequently identified as the β subunit of the urease by molecular cloning and sequencing, and the 13 kDa protein was identified as the evolutionarily conserved ribosomal L23 protein. The physiological
role of the yersinial urease remains unclear, but striking homology with the corre-
sponding protein of helicobacter suggests a central role as a facilitating factor for bacterial
survival in the host’s acidic intracellular milieu. The RA associated at the clonal level using native and
recombinant 19 kDa protein, and the fine
specificity of the synovial T cells with over-
lapping 20-mer synthetic peptides deter-
mixed. Identification of the T cell stimulating
immunodominant epitope might sub-
sequently open a new treatment option via
construction of appropriate TCR peptide
antagonists.

Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association
Z A Nagy, J Hammer, F Sinigaglia†
Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc, Nutley, NJ, USA; †Roche Milano Richerche, Milano, Italy

We have investigated whether sequence 67 to 74 shared by β chains of rheumatoid arthritis (RA) associated HLA-DR molecules
imparts a specific pattern of peptide binding. The peptide binding specificities of the RA associated molecules, DRB1*0401, DRB1*0404, and the closely related, RA non-associated DRB1*0402 were deter-
mined using designer peptide libraries. The effect of single key residues of β chain was tested with site directed mutants of DRB1*0401. The results have demonstrated important differences between RA linked and
unlinked DR allotypes in selecting the portion of peptides that interacts with the 67-74 area. The most striking difference was associated with a single amino acid exchange at position 71 of the DR β chain. The RA linked molecules with Lys or Arg at this position bound peptides with negatively charged residues in their central portion up to 500-fold better than the non-linked molecule with Glu at position 71. Con-
versely, positive charges in the peptide were accepted up to 1000-fold better by the RA non-linked molecule than the RA associated allotypes. Thus the 67-74 region, in par-
cular position 71, induces a dramatic difference in peptide binding specificity that correlates with the genetic linkage of RA susceptibility.

Nimesulide, a sulphonanilide non-
steroidal anti-inflammatory drug, inhibits mediator release from human synovial mast cells
A de Paulis, D de Crescenzo, A Ciccarelli, I Marino, G Marone
Division of Clinical Immunology and Allergy, University of Naples Federico, II School of Medicine, 80131 Naples, Italy

Mast cells are found in normal and diseased synovial tissue; they comprise approximately 3% of the cells in normal synovium. An increased density of mast cells has been identified in synovial tissues and fluids in diverse arthritides. Increased levels of mast cell products (histamine, tryptase, and prostaglandin D$_2$ (PGD$_2$)) are found in synovial fluid in several rheumatic diseases, suggesting that these cells and
their chemical mediators have an important role in several joint disorders. Nimesulide is a sulphonanilide non-steroidal anti-
flammatory drug (NSAID) used in the treatment of various inflammatory diseases and chemically related to other acidic
NSAIDs such as acetilsaliclyc acid and indomethacin. We investigated the effects of nimesulide and of its in vivo metabolite, 4-hydroxy-nimesulide (OH-nimesulide), on the release of histamine (hsp60) and de
ovo synthesised mediators (sulphoteteprotein leukotriene C4 (LTC4) and PGD$_2$) from human synovial mast cells isolated by enzymatic digestion of synovium obtained from 35 patients suffering from inflammatory arthritis and osteoarthritis. Preincubation of nimesulide and its metabolite, OH-
nimesulide 10$^{-6}$–10$^{-8}$ mol/l caused concentration dependent inhibition (16-86% and 18–90%, respectively) of histamine release from human synovial mast cells challenged with rabbit antihuman IgE antibody. Nimesulide also markedly inhibited the de
ovo synthesis of PGD$_2$ and LTC4 from the mast cells. The hydroxylation of the hydroxyl groups of acetilsaliclyc acid 10$^{-6}$–10$^{-8}$ mol/l did not inhibit the release of histamine. Our findings are at variance with the concept that NSAID are pharmacologically inactive on mast cell mediator release, and suggest a new mechanism of action of nimesulide that can explain, at least in part, its in vivo pharmacological properties.

Treatment with antibodies to CD23 markedly ameliorates an established arthritis in DBA/1 mice
C Plater-Zyberk, J-Y Bonnefoy
Glaxo IMB, Immunology Department, CH-1228, Geneva, Switzerland

It has been observed that soluble CD23 (sCD23) regulates the production of pro-
flammatory mediators, including tumour necrosis factor α (TNFα) and interleukin-1β (IL-1β) by human monocytes in vitro, supporting the hypothesis that CD23 is involved in inflammatory processes. Pro-
flammatory cytokines appear particularly important in rheumatoid arthritis (RA), and a central role for TNFαs and IL-1β in the destruction of the synovium has been postulated. In view of the increased expression of CD23 in RA and the role played by sCD23 in the synthesis of pro-
flammatory cytokines, we have studied the effect of neutralising CD23 in type II collagen induced arthritis (CIA) in DBA/1 mice—a model for RA. DBA/1 mice were immunised intradermally with 100 μg bovine type II collagen. At the first sign of clinical arthritis, groups of mice were injected intraperitoneally with different doses of CD23 antibodies (25–100 μg monoclonal antibody B3B4; 50–400 μg rabbit polyclonal anti-CD23). A dose related disease amelioration was achieved with either polyclonal or monoclonal antibodies to mouse CD23 (therapeutic doses ≥50 μg). The significantly reduced clinical scores and number of affected paws was accompanied by a marked decrease in cellular infiltration of the synovial sublining layer, and limited destruction of cartilage and bone (0% and 94% of joints with severe arthritis in B3B4 vs controls). These findings demonstrate the involve-
ment of CD23 in a model of human RA.

Prostanoid modulation of synovial CD4 T cell cytotoxic function in rheumatoid arthritis
L T Ratcliffe, P T Lukey, O L Meyers, S R Ress
Clinical Immunology Laboratory of the Rheumatic Diseases Unit, Department of Medicine, University of Cape Town, Cape Town, South Africa

Cytolytic mediators within rheumatoid synovial CD4 T cells have recently been demonstrated. The aim of this study was to investigate the in vitro function and regulation of class II restricted cytotoxic
T cells from the site of pathology (synovial fluid mononuclear cells (SFMCN) and circu-
ation (peripheral blood mononuclear cells (PBMCN)) in rheumatoid arthritis (RA). The mechanism involved in the anti-
gen specific cytotoxicity of SFMCN and PBMCN from 20 patients with seropositive
RA were measured using \(^3\)H-thymidine incorporation and chromium-51 release assays, and correlated with clinical data. Regulatory factors including prostaglandin E\(_2\) (PGE\(_2\)), interferon gamma and interleukin-4 were measured in cell supernatants.

A positive linear relationship between proliferation and cytotoxicity was confirmed in PBMC (r = 0.8, p < 0.001) and SFMCN (r = 0.75, p < 0.001). However, diverse cytokines in SFMCN responses permitted classification of the patients to three groups: (A) low SFMCN proliferation and cytotoxicity (proliferation < 20 000 Δcpm; cytotoxicity 4.5–21%; effect ratio target: 10:1) associated with longer disease duration (16 ± 0.1 ± 1.8 years) (p < 0.01), (B) high levels of both proliferation (Δcpm > 40 000) and cytotoxicity (41–6 ± 6.2% associated with monotherapy (NSAID) therapy in earlier disease (2.5 ± 0.9 years) (n = 7); (C) intact proliferation (Δcpm > 40 000), but reduced cytotoxicity (19 ± 2 ± 3.2%) was a feature of SFMCN from five of six patients studying disease modifying anti-rheumatic drugs and supplementary NSAIDs. Reduced cytotoxicity in group C was compartmentalised (paired PBMC: cytotoxicity: 41–7 ± 7.3%, P < 0.05) and associated with greater CD44 synovocyte PGE\(_2\) production than group B (P < 0.01). Potential mechanisms for these findings were shown to be syndial prostanoid inhibition of CD4 T cell cytotoxicity.

SFMCN mediated inhibition of CD4 T cell cytotoxicity is potentially relevant in the pathogenesis of RA. Cyclooxygenase inhibition as monotherapy in early RA may reduce the potentially beneficial effects of synovial prostanoids on T cell cytotoxicity.

**Eicosanoid regulation of human synovial CD4 T cell effector functions**

L T Racilciffe, P T Lukey, O L Meyer, S R Reis

Clinical Immunology Laboratory of the Rheumatic Diseases Unit, Department of Medicine, University of Cape Town, Cape Town, South Africa

The rheumatoid synovial compartment is characterised by an accumulation of CD4 T cells and expression of significant levels of eicosanoids. Elucidation of the effects of eicosanoids on CD4 T cell functions may have pathogenetic and therapeutic relevance.

In vitro effects of prostaglandin E\(_2\) (PGE\(_2\)), misoprostol (a synthetic PGE\(_2\) analogue), and leukotrienes B\(_4\) and C\(_4\) (LTB\(_4\) and LTC\(_4\)) on effector functions of polyclonal and monoclonal human CD4 T cell populations were investigated, and the underlying mechanisms addressed. Antigen specific and lectin dependent cytotoxicity were determined in chromium-51 release assays, and the cytokines interferon gamma (IFN\(_\gamma\)) and interleukin-4 (IL-4) measured in cell supernatants.

PGE\(_2\), 10\(^-8\) mol/l inhibited cytotoxic effector function (mean inhibition 38 ± 8 ± 4.5%; control cytotoxicity in the range 35–52%, effect ratio target: 10:1; n = 6) and cytokine production of CD4 T cell clones (inhibition of IFN\(_\gamma\) (Th1 and Th0 clones) 85–95%; inhibition of IL-4 (Th0) 65–100%; control IFN\(_\gamma\) 90–110 IU/ml; control IL-4 10–70 pg/ml; n = 6). In contrast, LTB\(_4\) 10\(^-10\) mol/l and LTC\(_4\) 10\(^-10\) mol/l caused moderate but significant increases in cytotoxicity and IFN\(_\gamma\) production (increase in cytotoxicity: LTB\(_4\) 14 ± 5 ± 3.6%; LTC\(_4\) 16 ± 1 ± 2.7%; control cytotoxicity in the range 30–50%, effect ratio target: 10:1; increase in IFN\(_\gamma\) production in the range 30–100%). Modulation of CD4 T cell cytotoxicity was studied in the result of effect of calcium dependent pathways of cytotoxicity. PGE\(_2\) suppression of cytotoxicity was a result of modulation of binding and postbinding events.

The regulatory effects of eicosanoids defined in this study may be relevant at the situs of pathology in rheumatoid arthritis, and indicate a potential target for novel therapeutic modulation of CD4 T cell effector functions at chronic inflammatory sites.

**Photodynamic treatment of antigen induced arthritis in rabbits**

L G Ratkay, R K Chowdhary, A Iamaroon, A M Richter, H Neyndorff, J D Waterfield, J G Levy

Department of Microbiology and Immunology, University of British Columbia, Vancouver V6T 1Z3, Canada

The efficacy of local transcutaneous photodynamic treatment in antigen induced arthritis was studied using a rabbit model. Arthritis was induced in sensitised New Zealand White rabbits by injecting the sensitising agent, ovalbumin, intra-articularly in both knees. Treatment, delivered on day 7 or day 14 of the arthritis, comprised intra-venous injection of benzoporphyrin derivative monoacid ring A (BPD) (Vertepronin), a three hour incubation period in the dark, and a subsequent transcutaneous light exposure of the drug in the knee joint by light at a 690 nm wavelength. At day 28 the (histo)pathology of the joints was assessed.

Dose I (1 mg/kg BPD, 50 J/cm\(^2\) light) resulted in a 25–28% lower total pathological score compared with control knees (p < 0.05) at both the early (n = 11) and later (n = 15) stages of the disease. This included a 75–85% decrease in pannus formation (p < 0.05) and an almost complete absence of bone and cartilage destruction. Dose II (0.75 mg/kg BPD, 100 J/cm\(^2\) light) had a similar but not statistically significant effect (n = 10, paired t test). BPD had a similar effect on animals receiving 0.2 mg/kg of the anti-inflammatory drug indomethacin, indicating that there were no antagonistic drug interactions. Light without drug treatment resulted in a 10% reduction of total score.

These results indicate that photodynamic therapy using BPD and transdermal light was capable of modulating arthritis in our animal model without adverse effects. As photodynamic therapy is both specific and non-invasive, these findings suggest that the treatment regimen could be used for treating human arthritis joints.

**Increased C reactive protein is a good predictor of functional outcome in early rheumatoid arthritis**


Department of Rheumatology, Faculty of Medicine and Dentistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

Distinction is drawn between laboratory measures of disease activity in rheumatoid arthritis (RA) (for example C reactive protein (CRP)) and measures of functional outcome (for example the health assessment questionnaire (HAQ)). Outcome measures are slow to alter, but measures of RA activity are capable of rapid changes. The primary aim of therapeutic intervention in RA is prevention of functional loss, but the validity of CRP as a marker of outcome has been questioned. Correlation was sought of change in CRP with HAQ score (<0.05, 0.05–0.2, >0.2) and CRP and HAQ in RA patients. In 31 patients CRP correlated with HAQ (r = 0.05). CRP on admission has been shown to be a predictor of survival. In 11 patients CRP and HAQ were measured weekly for 12 months in 37 of the 109 patients (34%), HAQ after one year of follow up was unchanged or greater compared with the score at presentation. Of these 37 patients, 29 (78%) had persistently increased CRP throughout the year of follow up. It is concluded that normalisation of a CRP value that was increased at presentation of early RA, by means of immunotherapy, is desirable and not associated with functional deterioration. In contrast a persistently increased CRP is a good predictor of functional deterioration.

**Cyclosporin A compared with methotrexate in psoriatic arthritis**

A Spadaro, V Riccieri, F Sensi, A S Scavalli, E Taccari, A Zoppini

Institute of Rheumatology, University: `La Sapienza`, Rome, Italy

The efficacy, safety, and cumulative probono, efficiency of CyA (5–10 mg/kg/day) or methotrexate 7.5 mg/week were evaluated in the treatment of psoriatic arthritis with peripheral involvement.

Fifty four patients with psoriatic arthritis (26 women) were recruited with CyA (CyA) (n = 17) or methotrexate (MTX). Non-parametric methods were used to evaluate the main clinical and laboratory parameters at baseline and after six months of therapy. The cumulative probability of continuing to take the drug was presented by Kaplan-Meier curves and the difference was tested by log-rank statistic.

The follow up time (mean SD) was 11.5 (6.2) months for the CyA group, 21.6 (20.2) months for the MTX group. After six months, 14 patients still receiving CyA were compared with 28 still receiving MTX. In the CyA group, Ritchie index (p = 0.005), number of painful/swollen joints (p < 0.005), pain analogue scale (p < 0.005), morning stiffness (p < 0.02), C reactive protein (CRP) (p = 0.02), platelets (p = 0.02) and leucocyte count (p = 0.05) were significantly reduced. In the MTX group, Ritchie index (p = 0.001), number of painful/swollen joints (p < 0.0005), pain analogue scale (p < 0.001), morning stiffness (p < 0.001), erythrocyte sedimentation rate (ESR) (p < 0.005) and CRP (p < 0.001) were significantly reduced, while albumin (p < 0.02),
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aspartate aminotransferase (p < 0.01) and alanine aminotransferase (ALT) (p < 0.005) were increased. Changes from baseline of all the variables examined were not significantly different between the CyA and MTX groups, except for the change in ALT (p < 0.05). The overall probabilities of still taking CyA and MTX were, respectively, 56% and 67% at one year, and 30% and 50% at two years, but the probability curves were not significantly different.

CyA and MTX were both effective in the treatment of psoriatic arthritis. ESR does not seem a useful means of assessing the response to CyA. During our follow up, the cumulative probability of remaining on CyA or MTX was 77% after one year, and 30% and 50% at two years.


gastrointestinal disorders, and cartilage destruction were significantly attenuated (GAG: p = 0.05 weeks 1–3; p = 0.01 weeks 4; collagen: p < 0.05 weeks 2; p < 0.01 week 3; p < 0.01 week 4). The granulomatous tissue was significantly more vascularised, and the vascular index increased by 50% (p < 0.01) at week 1.

Gastrointestinal treatment produced opposite effects. Inflammatory tissue development was retarded by over 50% (p < 0.01 weeks 1 and 2; p < 0.01 week 3). Collagen deposition (p < 0.01 weeks 1–3) and monocyte recruitment (p < 0.01 weeks 1 and 2; p < 0.01 for the change) were also retarded. Cartilage GAG (p < 0.01 weeks 1–4) and collagen loss (p < 0.01 weeks 2–4) were similarly reduced. Blood vessels were sparse in comparison with controls and inflammatory cell invasion was retarded. The vascular index was greatly reduced (p < 0.01 week 1; p < 0.01 week 2). Cortisone and heparin alone had no significant effect.

Angiogenesis is important for the development of chronic inflammation, controlling its development, cellular influx, extracellular matrix deposition, and cartilage matrix destruction. Drugs with angiostatic properties may therefore have potential in the therapy of rheumatoid arthritis.

Regression of granulomatous tissue neovascularization with angiostatic steroid therapy

M P Seed, C A S Alam, P R Colville-Nash, D A Willoughby

Department of Experimental Pathology, St Bartholomew's Hospital Medical College, London EC1M 6BQ, United Kingdom

A study was performed to establish whether the manipulation of angiogenesis by angio-static and angiogenic treatments can be achieved in chronic granulomatous inflammation, and vascular development in a dose relevant to existing neovascularization in angiostasia or vascular regression. Chronic granulomatous tissue samples were treated with cine (0.1 ml Freud's complete adjuvant in 0.1% cotton oil injected into 24 hour air pouches), and the animals treated daily with heparin 500–5000 U by mouth (angiostatic treatment) with or without cortisone 1 mg/kg (angiostatic treatment), tetrahydrocortisol, or tetrahydrocortisone. Vascularity was assessed by a spectrophotometric analysis of gelatin vascular casts incorporating carmine, and the data expressed as granuloma mass (mg), vascular volume (µg carmine), or vascular index (VI) (µg carmine/mg tissue).

Angiogenic treatment accelerated the development of the vasculature from 3.36 ± 0.53 to 8.53 ± 0.11 µg/ml VI (p < 0.01) with 5000 U heparin), in a dose related fashion, whilst angiostatic treatment reduced it (from 8.31 ± 1.46 to 6 ± 0.12 µg/ml VI with 1 mg/kg cortisone and 1000 U heparin). Tetrahydrocortisol reduced the vascular index somewhat (from 11.56 ± 0.93 to 4.956 ± 0.271 VI µg/ml (p < 0.001) at 3 mg/kg in the absence of heparin), but tetrahydrocortisone was without effect. Angiostatic treatment given to mice bearing seven day CGP prevented further vascular development, and induced a regression of the vasculature (from 10 ± 1.07 at day 7 to 6.339 ± 0.188 µg/ml VI at day 21 (p < 0.01)).

The vasculature of granulomatous inflammation will respond to classical angiogenic modulatory treatment. Tetrahydrocortisol, which is devoid of anti-inflammatory activity, is effective, whilst the 11-keto analogue, tetrahydrocortisone, is devoid of activity. More importantly, neovascular regression can be induced, making this a promising target for the identification of novel anti-rheumatic drugs.

Central role of cytokines in the pathogenesis of autoimmune diseases and the immunotherapy of different autoimmune diseases, especially rheumatoid arthritis

S Skurkovich, B Skurkovich†

Advanced Biotherapy Concepts Labs, Rockville, MD, USA; †Brown University Medical School, Providence, RI, USA

Normal functioning of the interferon (IFN) system is critical for the normal functioning of the immune system. A disturbance of IFNα synthesis lies at the core of most autoimmune diseases, leading to IFNα hyper-production and its appearance in the blood (highest in systemic lupus erythematosus) or cells. This may result from a genetic predisposition or the influence of certain viruses or bacteria. Persistent presence of IFNα in the blood is a marker of autoimmune diseases. It induces production of other cytokines including tumour necrosis factor α (TNFα) and possibly IFNβ, which then participates in the generation of autoimmune T cells. We believe that, in the immune response, every autoantigen triggers its own specific IFNα, which then participates (alone or together with TNFα) in the expression of class II antigen. Administration of IFNα or IFNγ can lead to autoimmune disease in genetically predisposed patients and injection (especially of IFNα) can exacerbate existing autoimmune disease. To treat various autoimmune diseases, at least one of these three cytokines (IFNα, IFNγ or TNFα) must be must be neutralised (neutralised). In 1975, we performed antitumour immune responses in 16 patients with very severe RA by daily injection of IFNα antibody for five days. Within three days, a marked reduction in pain, swelling, and oedema was observed, followed later by a significant improvement in relevant laboratory parameters. We have proposed treating different autoimmune diseases, including AIDS (also in part an autoimmune disease), by removing (neutralising) IFNα, IFNγ and TNFα. The most effective approach could be to neutralise (or remove) these three cytokines (the main pathological substances in autoimmune disease) simultaneously using humanised monoclonal antibodies or other antibody fragments or extracorporeal means. In future, transplantation of genes responsible for normal IFN production could be used in the treatment of autoimmune diseases.

Interleukin-10 treatment inhibits disease progression of mouse collagen induced arthritis

P D Katsikis, P D Katsikis, E Abney, S Parry, R Williams, R N Maini, M Feldmann

Kennedy Institute of Rheumatology, Hammersmith, London W6 8LW, United Kingdom

Interleukin-10 (IL-10) is a potent inhibitor of the proinflammatory cytokines tumour necrosis factor α (TNFα), IL-1β and granulocyte macrophage colony stimulating factor. All three are produced by the synovial membrane in rheumatoid arthritis (RA), and are considered to be important in the pathogenesis of the disease. IL-10 has been detected in RA synovial membrane cultures and exogenous IL-10 inhibits IL-1β and TNFα production by such cultures. The aim of the study was to determine whether IL-10 can inhibit arthritis in vivo in the mouse collagen induced arthritis (CIA) model of RA. DBA/1 mice were immunised with bovine type II collagen in adjuvant and treated daily from the onset of clinical arthritis with recombinant murine IL-10, or saline as a control. Mice were monitored over a 10 day period and the following indices recorded: number of affected joints, clinical score, and paw swelling. IL-10 treatment of CIA after onset of disease significantly inhibited paw swelling.
(p < 0.05), and progression of disease as defined by the overall clinical score (p < 0.05) and the number of affected joints (p < 0.01) compared with saline treated controls.

These results demonstrate that IL-10 is capable of suppressing the progression of CIA, possibly by inhibiting proinflammatory cytokine production, antigen presentation or immune cell recruitment. The findings in this study, taken together with those from other reports, indicate a potential therapeutic role for IL-10 in chronic inflammatory diseases such as RA.

Expression of Fcγ receptors on synovial fluid neutrophils and activation by soluble immune complexes

F Watson, J A Quayle, R C Bucknall†, S W Edwards

Department of Biochemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX, United Kingdom; † Rheumatoid Diseases Unit, Royal Liverpool University Hospital, Prescott Road, Liverpool L69 3BX, United Kingdom

Soluble immune complexes are known to be present within the synovial fluid of patients with rheumatoid arthritis (RA). The mechanism(s) by which they may activate the secretion of reactive oxygen metabolites from primed neutrophils were investigated, and the expression of Fcγ receptors (FcγRI) on the surface of synovial fluid neutrophils was measured.

Neutrophils were isolated from the blood and synovial fluid of patients with RA and from the blood of healthy controls. Cell surface expression of FcγRI was determined by fluorescence activated cell sorter analysis and mRNA levels were measured by northern blotting. Intracellular Ca²⁺ was measured by Fluo-3 fluorescence, reactive oxidant production was determined by chemiluminescence, and phospholipase D (PLD) activity was measured by thin layer chromatography.

Synovial fluid neutrophils expressed FcγRI, II, and III on their cell surface, and also possessed high levels of mRNA for these receptors. Expression of FcγRI on neutrophils has previously been reported only after treatment with interferon gamma in vitro for 18–24 hours or after granulocyte colony stimulating factor treatment, and requires activated gene expression. These observations indicate that synovial fluid neutrophils have responded to a similar agent in vivo and expression of FcγRI may alter their responsiveness to IgG containing immune complexes. Soluble immune complexes (present at high concentration in rheumatoid synovial fluid) bind to the surface of blood neutrophils and generate a transient increase in intracellular Ca²⁺, but this binding does not activate reactive oxidant production. However, if the neutrophils are primed, receptor occupancy leads to activation of PLD and subsequently the release of large quantities of reactive oxygen metabolites. Activation of PLD requires phosphorylation of tyrosine residues.

We conclude that synovial fluid neutrophils are primed and activated, and they possess cell surface receptors that are not present on blood cells. This may alter their ability to respond to immune complexes within the diseased joint. Upon priming, the neutrophils can generate novel intracellular signals that regulate the release of damaging reactive oxygen metabolites.