MOLECULAR BIOLOGY AND THE IMMUNOPATHOGENESIS OF RHEUMATOID ARTHRITIS

Organizers: William Arend and William Koopman

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Molecular Biology and the Immunopathogenesis of Rheumatoid Arthritis

Role of Infectious Agents

O 001 INTERACTION BETWEEN GENETIC AND ENVIRONMENTAL FACTORS IN RHEUMATOID ARTHRITIS, Dennis A. Carson, Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0945. Rheumatoid arthritis is a complex disease that almost certainly involves the interaction of several different genetic and environmental factors. Genes demonstrated to predispose to rheumatoid arthritis include those encoding histocompatibility antigens, immunoglobulins, and estrogen/androgen production. However, environmental factors must also determine disease susceptibility. Despite the fact that rheumatoid arthritis is now a common illness affecting 1% of the population, it apparently did not become prevalent in Europe until the 18th century. Because the incidence of rheumatoid arthritis is relatively similar throughout the world, it seems unlikely that acute infection with an uncommon microorganism triggers the disease. Moreover, even in identical twins growing up in the same environment, the concordance of rheumatoid arthritis is only 30-40%. This suggests that a random environmental event, such as occurs during the somatic diversification of the immune system, also contributes to rheumatoid arthritis incidence and severity. It is essential to discern how the three types of factors, i.e., genetic, infectious, and somatic, combine to produce the final disease entity. The introduction of this session will summarize available data on the genetic and environmental risk factors for rheumatoid arthritis. An attempt will be made to explain how the interaction of common elements can produce a disabling chronic joint disease.

O 002 LENTIVIRUS ETIOLOGY AND PATHOGENESIS OF CAPRINE ARTHRITIS, William P. Cheevers, Donald P. Knowles and Travis C. McGuire, Department of Veterinary Microbiology and Pathology and USDA Animal Disease Research Unit, Washington State University, Pullman, WA 99164. Caprine arthritis-encephalitis (CAE) is an inflammatory disease of domestic goats characterized predominantly by chronic arthritis and mastitis in adults. A retrovirus capable of inducing lesions after experimental inoculation was isolated by in vitro explantation of carpal joint synovium of an arthritic goat. The virus was designated CAEV and classified as a lentivirus based on biological and biochemical criteria. Clinically affected joints exhibit periarticular swelling with excessive synovial fluid containing inflammatory cells. Histologic lesions are indicative of proliferative synovitis with lymphocyte, macrophage and plasma cell infiltration of hyperplastic synovial membrane and subsynovial stroma. A comprehensive understanding of the pathogenesis of CAE arthritis must explain the relationship between indefinite virus persistence in the presence of anti-viral immune responses and progressive development of immune-mediated inflammatory lesions. Our studies have established the following features of CAE arthritis pertinent to these questions: Goats challenged with CAEV after vaccination with inactivated virus experience more severe arthritis than non-vaccinated controls, and inoculation of persistently-infected goats results in exacerbation of arthritis. Recurrent virus isolates from chronically arthritic joints exhibit antigenic variation of neutralization epitopes expressed by the virion gp135 surface glycoprotein. These virus variants are antigenically cross-reactive with respect to gp135 non-neutralization epitopes, which elicit much higher antibody titers than variable neutralization epitopes. Goats respond to CAEV infection with a preferential immune response to the virion gp135, and the severity of joint inflammation in chronically infected goats correlates with the titer of synovial fluid anti-gp135 antibody. These observations indicate that (1) antigenic variation of neutralization epitopes may contribute to virus persistence and (2) joint inflammation is mediated by or involves cumulative immune responses to immunodominant gp135 domains of persistently replicating CAEV. To pursue more specific studies, we have cloned and sequenced the envelope gene encoding gp135 of two antigenic variants of CAEV and expressed these genes in recombinant vaccinia virus. These molecular clones have provided recombinant gp135 and synthetic peptide reagents to analyze the role of specific anti-gp135 immune responses in persistent virus replication and arthritis.

O 003 T CELL TOLERANCE TO SELF MHC PEPTIDES. IMPLICATIONS FOR THE ASSOCIATION OF RHEUMATOID ARTHRITIS WITH HLA DR4.

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Susceptibility to rheumatoid arthritis maps to residues QKRAA in the third hypervariable region of the β1 chain of HLA DR4/Dw4. The Epstein Barr virus glycoprotein gp110 and the E. coli heat shock protein DNA J contain the QKRAA sequence. In normal, non HLA Dw4 humans, EBV infection triggers T cells that recognize peptides from gp110 and HLA DR4/Dw4 encompassing the QKRAA sequence.

To test whether molecular mimicry between the HV3 of the β chain of MHC class II molecules and a foreign antigen can influence the immune response to the foreign antigen or trigger autoimmunity, we tested whether peptides from the HV3 of MHC class II molecules are subject to self tolerance. We worked in a mouse system, evaluating T cell proliferation to a peptide from the third hypervariable region of $IE\beta^s$ (HV3 $IE\beta^s$ peptide, PEFLEQRRAAVDTYC) after footpad immunization. We found that mice that express the $IE\beta^s$ chain on their cell surface (B10S9R), or that express it as a cytoplasmic protein (B10S), do not respond to HV3 $IE\beta^s$ peptide. Conversely, strains that express a different IEβ allele, like B10BR ($H2^k$), B10D2($H2^d$), develop T cell proliferation to HV3 $IE\beta^s$ peptide. In B10S and B10S9R, non response to HV3 $IE\beta^s$ peptide was not due to absence of binding to MHC class II molecules, since HV3 $IE\beta^s$ peptide was shown to bind to IA^s. This suggests that HV3 $IE\beta^s$ peptide is tolerated in strains that express $IE\beta^s$ or have it in their cytoplasm. To confirm that non response to HV3 $IE\beta^s$ peptide was due to self tolerance, we bred B10D2 mice (strong responders) with B10S9R mice (non responders) and found that the hybrid was tolerant to HV3 $IE\beta^s$ peptide.

Thus, in this example, a peptide from the third hypervariable region of an MHC class II molecule is subject to self tolerance. This finding suggests that peptides from foreign antigens mimicking the third hypervariable region of an MHC class II molecule may escape T cell response.

O 004 IMMUNE RESPONSES TO HEAT SHOCK PROTEINS OF BACTERIA AND PARASITES.

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Stress response proteins play an important role in the humoral and cellular immune responses to many bacterial and parasitic pathogens including the etiologic agents of tuberculosis, leprosy, Q fever, malaria, filariasis, and schistosomiasis. Immunoreactive protein antigens have been identified that have homology with members of five families of heat shock proteins: the hsp90, hsp70, hsp60, low molecular weight, and hsp10 families. The heat shock proteins of the pathogenic microbes display 50-60% amino acid sequence identity with the sequences of the homologous human proteins. The antibodies and T cells elicited during an infection are directed against nonconserved as well as conserved sequences of the heat shock proteins. In addition, certain heat shock proteins also contain regions that cross-react with other host proteins, such as MHC molecules and cartilage proteins. The immune response to such shared sequences may have important consequences for the host with respect to the recognition of self and non-self and may play an important role in the pathogenicity and autoimmune consequences of infections.

Molecular Biology and the Immunopathogenesis of Rheumatoid Arthritis

MHC and Rheumatoid Arthritis

O 005 MECHANISMS OF MHC ASSOCIATIONS WITH RA. G. T. Nepom and B. S. Nepom, Department of Immunology, Virginia Mason Research Center, Seattle, WA 98101

The association between HLA and rheumatoid arthritis is one of the most thoroughly studied and well-documented examples of genetic susceptibility to an autoimmune disease. The most striking feature of this association is the high prevalence (from 65-80% in different population studies) of HLA-DR4 in patients with RA. The association between DR4 and RA, however, is incomplete in two important respects: First, not all haplotypes which carry an HLA-DR4 specificity are associated with RA; and secondly, a substantial number of patients (from 20-35%) with RA do not carry DR4-related genes. Recent molecular and genetic studies which address these issues provide important insights into the basis for genetic susceptibility. In populations in which DR4 is prevalent, two subtypes of DR4, called Dw4 and Dw14, are each independently associated with RA; in non-DR4 populations, principle associations are with two other HLA-DR alleles termed Dw1 and Dw16. These four genes associated with RA all encode a highly homologous alpha-helical loop segment within the HLA molecule. Functional parameters of this alpha-helical segment have been tested using site-directed mutagenesis, which indicate a critical role for selected amino acid residues in the HLA structure necessary for specific immune recognition.

Role of T Lymphocytes

O 006 T CELL RECEPTOR REPERTOIRE ABNORMALITIES IN RHEUMATOID ARTHRITIS, Brian Kotzin, Xavier Paliard, Yongwon Choi, Sterling West, John Kappler, and Philippa Marrack, Departments of Pediatrics and Medicine, and Howard Hughes Medical Institute at Denver, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

Analysis of the human T cell repertoire is currently limited by the small number of monoclonal antibodies to different human T cell receptor (TCR) variable region elements. As an alternative approach, we modified the polymerase chain reaction (PCR) to quantitatively amplify specific T cell receptor β-chain (Vβ) and α-chain constant region (Cα) gene segments. With 22 different Vβ-specific oligonucleotide primers, >75% of the Vβ repertoire could be defined in a mixed T cell population. By using this method, we established the principal Vβ elements involved in the response of human T cells to different staphylococcal enterotoxins, and characterized Vβ-specific T cell repertoire alterations in patients with toxic shock syndrome.

Although the etiology of rheumatoid arthritis (RA) is unknown, previous studies have implicated T cells in the pathogenesis of this autoimmune disease. Furthermore, the association of disease with particular class II MHC alleles suggests that the disease may involve $CD4^+$ T-cells bearing αβ T-cell receptors. We used the above PCR technique to examine T cell receptor repertoires in the synovial fluid and blood of patients with RA. All patients had active inflammatory disease at the time of fluid and blood sampling and all but one expressed HLA-DR4. The T cell receptor Vβ repertoires of the T cells in the synovial fluid were variable among patients and idiosyncratic mismatches compared to blood were present. In each individual, the data indicated that the synovial fluid infiltrating T-cells were not just a passive reflection of the peripheral blood repertoire. In addition to the individual variations, in all seven patients studied, we noted an elevation of the percentage of T cells expressing Vβ14 in the synovial fluid compared to peripheral blood. Such skewing of Vβ14 expression was not observed in other types of inflammatory arthritis, including psoriatic arthritis and Reiter's syndrome. Contributing to the difference between synovial fluid and blood levels in RA was a remarkable decrease in circulating $V\beta14^+$ T-cells, not found in HLA-DR4 matched normal individuals. In four of the seven RA patients, peripheral blood Vβ14 levels were undetectable. These findings suggest the involvement of T cells bearing Vβ14 in RA and furthermore suggest that a Vβ-specific superantigen may be involved in the pathogenesis of this disease. Longitudinal and family studies are currently in progress to help determine the basis for the T cell repertoire abnormalities.

O 007 A SUPERANTIGEN MODEL FOR ARTHRITIS IN Vβ8 TCR TRANSGENIC *lpr/lpr* MICE, Tong Zhou, Hörst Bluthmann[1], Renate E. Gay, Carl K. Edwards, III[2], and John D. Mountz, Dept. of Medicine, The University of Alabama at Birmingham, Birmingham, AL 35294. [1]Hoffmann LaRoche, Basel, Switzerland, [2]Dept. of Immunology, Marion Merrell Dow, Inc., Kansas City, MO 64137. Certain bacterial products have the ability to combine with class II MHC and stimulate a wide spectrum of T cells specificities through the TCR Vβ chain. T cells expressing the Vβ8 TCR can be stimulated by staphylococcal enterotoxin B (SEB) superantigen. To determine if the SEB superantigen can lead to a chronic arthritis, Vβ8 TCR transgenic I-E$^+$ MRL-+/+ and MRL-*lpr/lpr* mice were injected intra-articular (IA) with 10-fold dilution of SEB ranging from 10 mg to 10 ng. Nearly all of the T cells in the TCR transgenic mice were Vβ8$^+$ and should be capable of interacting with SEB. Although both Vβ8 TCR transgenic MRL-+/+ and MRL-*lpr/lpr* mice developed arthritis within 3 weeks of injection of 100ug and 10 ug of SEB, the arthritis was more severe in the *lpr/lpr* mice. To determine if the severity of arthritis was related to a defect in T cell tolerance, TCR transgenic *lpr/lpr* and +/+ mice were treated with SEB from birth to 2 weeks of age. Although clonal deletion occurred equally in *lpr/lpr* and +/+ mice, the remaining Vβ8 T cells in *lpr/lpr* mice proliferated in response to Vβ8 cross linking by anti-Vβ8 antibody or SEB, whereas T cells from +/+ mice were anergic. Furthermore, neonatal tolerance induction in the T cells in *lpr/lpr* mice was completely lost 2 weeks after the last SEB injection, whereas +/+ T cells remained anergic for at least 2 months. These results suggest that the SEB superantigen can induce a chronic arthritis after a single IA in mice. Furthermore, the severity and chronicity of arthritis is related to a T cell tolerance defect in *lpr/lpr* mice. We propose that in certain predisposed individuals with a T cell tolerance defect, superantigen released during a systemic bacterial or viral infection can lead to a chronic inflammatory arthritis such as RA.

O 008 THE ROLE OF T CELLS IN RHEUMATOID ARTHRITIS (RA), Samuel Strober, Department of Medicine, Division of Immunology & Rheumatology, Stanford University School of Medicine, Stanford, CA 94305.
Evidence from several different experimental approaches suggest that T cells contribute to the immune injury in the inflamed synovial tissues in RA. Prolonged depletion of T lymphocytes, in particular CD4$^+$ cells, after total lymphoid irradiation (TLI) results in an improvement in joint inflammation in controlled studies. Analysis of autoantibodies in the blood and joints in the latter patients show no change in the levels of IgM, IgG and IgA rheumatoid factor despite improvement. Spontaneous secretion of IL-1 by synovial biopsy specimen is reduced by about 10-fold after TLI. Synovial fluid T cells from patients with RA proliferate vigorously to mycobacterial antigens, and mycobacterial 65 Kd heat shock protein reactive T cell clones can be established from synovial tissue T cells. A high proportion of such clones are γ δ T cells which are not restricted in their antigen recognition by either Class I or II MHC molecules. Mixed cultures of synovial T cells and synoviocytes stimulated initially with mycobacterial antigens and cultured in the presence of IL-2 form organized tissue outgrowths which have the microscopic appearance of pannus and can be serially propagated. The outgrowths are dependent on the presence of IL-2, and can be inhibited by anti-T cell monoclonal antibodies. Bacterial proteins such as tetanus toxoid cannot substitute for the mycobacterial proteins. These experimental findings suggest that interactions between T cells and synoviocytes from RA patients results in expansion of both populations in long-term tissue culture without further exogenous antigenic stimulation.

Autoantibodies in RA

O 009 RHEUMATOID FACTORS ISOLATED FROM PATIENTS WITH AUTOIMMUNE DISORDERS ARE DERIVED FROM GERMLINE GENES DISTINCT FROM THOSE ENCODING THE WA, PO AND BLA CROSS REACTIVE IDIOTYPES, Kimberly D. Victor, Virginia Pascual, Ingrid Randen, Keith Thompson, Oystein Forre, Jacob B. Natvig, Shu Man Fu, and J. Donald Capra, Department of Microbiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas, 75235-9048.

Studies of human rheumatoid factors isolated from patients with mixed cryoglobulinemia suggested that a limited repertoire of V_H and V_L region gene segments was expressed in these antibodies. Additionally, these studies suggested that a single germline gene could generate rheumatoid factor specificity and that affinity maturation was not required. In order to better understand the structural basis for rheumatoid factor activity, the V region gene segments of nine human monospecific IgM rheumatoid factors were analyzed. Rheumatoid factors were isolated from three patients with rheumatoid arthritis, a patient with systemic lupus erythematosis and a normal individual. The V region gene usage of these rheumatoid factors is not as restricted as previous work on mixed cryoglobulin rheumatoid factors had suggested. For example, each of the different V_K families are represented and there are two examples where a V_λ gene segment is used. Thus, the Wa and Po CRI, characteristic of mixed cryoglobulin rheumatoid factors, do not represent major idiotypes in rheumatoid factors isolated from rheumatoid arthritis patients. While there are clear examples of rheumatoid factors which are direct copies of germline genes, most of the sequence data suggests that the processes of antigenic selection and somatic mutation contribute significantly to the generation of rheumatoid factors in patients with autoimmune disease.

O 010 POSSIBLE DELETION OF A DEVELOPMENTALLY REGULATED HEAVY-CHAIN VARIABLE REGION GENE IN AUTOIMMUNE DISEASES, Pojen P. Chen*, Tsaiwei Olee, Pei-Ming Yang, Katherine A. Siminovitch+, Nancy J. Olsen#, Franklin Kozin^ and Dennis A. Carson, Departments of Medicine and *Pathology, University of California, San Diego, La Jolla, CA 92093; +Department of Medicine, University of Toronto, Canada; #Department of Medicine, Vanderbilt University, Nashville, TN 37232; and ^Department of Medicine, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Several autoantibody-associated variable region (V) genes are preferentially expressed during early ontogenic development, suggesting strongly that they are of developmental and physiological importance. Accordingly, we hypothesized that a homozygous deletion of a critical autoantibody-associated Ig variable (V) gene may alter the immune system and thus predispose the host to autoimmune disorders. We searched extensively for a probe related to a developmentally regulated V gene that has the power to differentiate among highly homologous V genes in human populations. Using such a probe (i.e. Humhv3005/P1) related to both anti-DNA and anti-IgG autoantibodies, we studied restriction fragment length polymorphisms (RFLP) in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The results showed that one of the four major hybridizing bands was missing in about 20% of patients with either RA or SLE, but only 2% of normal subjects. Subsequently, we employed the polymerase chain reaction (PCR) to amplify and analyze hv3005, 1.9III and homologous genes in individuals with characteristic RFLP genotypes. The results revealed that there are several hv3005-like genes and 1.9III-like genes in humans, and that a complete deletion of the hv3005-like genes is relatively restricted to a subset of autoimmune patients. Combined, these findings provide evidence for deletion of developmentally regulated autoreactive V genes in autoimmune diseases.

O 011 RHEUMATOID FACTOR (RF) DIVERSITY IN RHEUMATOID ARTHRITIS (RA). **William J. Koopman, Ralph E. Schrohenloher, Antonio del Puente, and Peter H. Bennett.** Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, and NIH, Phoenix, AZ 85014.

Considerable circumstantial evidence suggests that RF contributes to the pathogenesis of tissue injury in RA. However, the genetic basis of RF expression in RA remains unclear. Previous studies indicated that monoclonal antibodies 6B6.6 and 17.109 identify mutually distinct VκIII-RF-associated cross-reactive idiotopes (CRI) encoded by germline genes Humkv328 and Humkv325, respectively. While these two VκIII CRI are expressed on ~2/3 of all IgMκ RF paraproteins, they account for only 1-2% of IgM RF in established RA. The unexpectedly low proportion of VκIII CRI(+) RF in established RA may reflect somatic mutation with consequent loss of detectible CRI and/or preferential utilization of alternative Vκ genes in the RF response in RA. We investigated this issue among Pima Indians enrolled in a 19-year NIH epidemiologic study. Serial serum samples obtained from 25 RA patients prior to disease onset (pre-RA), at disease onset (RA-onset), and following onset (post-onset) were analyzed for IgM RF, 17.109(+) RF and 6B6.6(+) RF using quantitative ELISA. Levels of 6B6.6(+) and 17.109(+) RF did not differ significantly in the pre-RA, RA-onset, and post-onset samples. Moreover, the proportion of CRI(+) RF at each time point was small (17.109: 2.9%, 1.6%, and 0.7%; 6B6.6: 2.5%, 1.1%, and 1.1%). In parallel studies we have compared the $V\kappa/V_H$ idiotope expression among RF occurring in patients destined to develop RA compared to benign RF(+) controls using monoclonal antibodies 17.109, 6B6.6, and G6 (a V_H1 CRI likely encoded by germline gene 783c). Pre-onset serum samples from 24 RF(+) individuals who subsequently developed RA and samples obtained from 24 age- and sex-matched RF(+) healthy Pimas who did not develop RA (controls) were analyzed for the presence of IgM RF, 6B6.6(+) RF, 17.109(+) RF, and G6(+) RF. Levels of IgM RF did not differ ($P>0.05$) between the pre-RA and control groups. Levels of V_H/V_L CRI(+) RF and the proportion of RF expressing these CRI also did not differ significantly between the two groups.

In view of the low proportion of 6B6.6(+) and 17.109(+) RF present prior to disease onset and the minimal change in levels of these two RF CRI over time, we conclude that Humkv325 and Humkv328 likely encode only a small fraction of RF in RA. Other as yet unidentified V_L genes appear to account for most RF synthesized in RA. Furthermore, the similarity in patterns of V_H1 and VκIII CRI expression among RF in benign RF(+) adults and individuals destined to develop RA suggest that germline genes Humkv325, Humkv328, and 783c do not encode disease specific RF.

O 012 IMMUNOGLOBULINS IN ARTICULAR CARTILAGE OF PATIENTS WITH RHEUMATOID ARTHRITIS, Mart Mannik, Division of Rheumatology, RG-28, University of Washington, Seattle, WA 98195

Immunoglobulins and complement components were identified in the superficial layers of articular cartilage in most patients with rheumatoid arthritis (RA) and in a smaller percentage of patients with osteoarthritis (OA) (1). By immunofluorescence microscopy, a granular pattern of immunoglobulins and complement components was identified in RA cartilage specimens that was not encountered in OA specimens (2). A linear deposit of IgG at the surface was the most frequent pattern seen in cartilage of patients with OA. Human serum albumin (HSA) was found diffusely in the cartilage matrix close to the surface in RA and OA specimens.

In order to characterize the nature of immunoglobulins in cartilage, sequential elution steps were carried out. The amount of IgG and HSA in elutes was measured with a sensitive radioimmunoassay. Serial neutral buffer elutions were followed with 6M guanidine hydrochloride (GdnHCl). After GdnHCl was removed by dialysis, the cartilage was digested with bacterial collagenase. Alternatively, the cartilage was serially eluted with 6M GdnHCl and then degraded by collagenase. The recovery with buffer alone was incomplete for IgG and for HSA. After elution with GdnHCl, 5.18 ± 3.66% of total IgG present remained in RA cartilage, whereas 29.14 ± 14.03% of total HSA remained after GdnHCl and was removed by collagenase ($p = 0.0009$). The recovered materials were analyzed by SDS-PAGE, under non-reducing and reducing conditions, and transfer blotting, using specific antisera to IgG and to HSA. Under non-reducing conditions, some IgG and HSA were bound to other molecules, giving a diffuse staining pattern in regions of the blot where molecules heavier than the monomers would migrate. Under reducing conditions, nearly all of the HSA and gamma chains of IgG appeared as monomers on the blots. These results indicated that intermolecular disulfide bonds linked some IgG and HSA in the superficial layers of articular cartilage to matrix molecules.

The nature of the molecules to which IgG and HSA are bound by intermolecular bonds and the antibody specificities of the recovered IgG are now under investigation.

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2. Vetto and Mannik, Rheumatol Int 10:13, 1990.

O 013 **PREFERENTIAL UTILIZATION OF AUTOANTIBODY-ASSOCIATED AND DEVELOPMENTALLY RESTRICTED IMMUNOGLOBULIN GENE SEGMENTS IN RHEUMATOID SYNOVIUM.**

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Diseased synovium in severe, active rheumatoid arthritis (RA) contains lymphocyte germinal centers able to produce immunoglobulin at splenic levels. Murine models suggest that autoantibodies generated during nonspecific stimulation (e.g. LPS) are polyclonal and reflect the germline repertoire; whereas in autoimmune states, highly self-reactive antibodies are clonally related and demonstrate all the characteristics of antigen-driven immunoglobulin production. Studies of these immunoglobulins have focused on rheumatoid factor content and have shown that, in seropositive RA, 5-10% of the repertoire consists of both IgM and IgG anti-IgG Fc. There is strong circumstantial evidence that the resultant immune complexes are involved in the pathogenesis and progression of the disease. The 6B6.6 and 17.109 κ light chain cross-reactive idiotypes (CRI) recognize 35% and 26% of IgMκ RF paraproteins, respectively. However, these germline-encoded CRI (Humkv328 by 6B6.6, and 325 by 17.109) recognize less than 1% of polyclonal RF from patients with RA. We generated an oligo d(T) primed, unrestricted cDNA library from diseased synovial tissue obtained from a 62 y/o white female with an 18 year history of seropositive RA whose synovium was negative for 6B6.6 and 17.109 reactivity. We randomly isolated 15 Vκ-containing and 14 VH, Cγ-containing cDNA transcripts. 4 of 15 (27%) κ light chains were drawn from the Humkv328 and 325 germline elements suggesting that the absence of the CRI in the synovium is due to somatic mutation of the loci which encode the epitope. 9 of 14 (69%) of the VH transcripts share high homology with germline gene segments which are potentially autoreactive, and/or developmentally restricted. In particular, 29% (4 of 14) may be drawn from germline gene segments which potentially encode rheumatoid factor. 5 of 15 (33%) of the κ light chains contained N-region addition of random nucleotides at the site of the V-J join. Preferential utilization of developmentally restricted gene heavy chain segments and light chains with N-region addition suppports the hypothesis that the rheumatoid synovial antibody repertoire is derived from unusual B cell progenitors. Although none of this limited sample of synovial transcripts were clonally related, extensive somatic mutation in the codons of the classic antigen binding site in both the heavy and light chains support the hypothesis that the expansion of the synovial antibody repertoire is antigen-driven. These studies were supported in part by NIH grants AI26394, AI30879, and AR03555. HWS is an RJR Nabisco Research Scholar in Immunology. SLB is a Terri Gotthelf Lupus Research Fellow.

Cytokines - I

O 014 BIOLOGICAL PROPERTIES OF NAP-1/IL-8 AND RELATED CHEMOTACTIC CYTOKINES,

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NAP-1/IL-8 consists of a single, non-glycosylated peptide chain. It is produced by mononuclear phagocytes and a variety of tissue cells upon stimulation with TNF or IL-1 (1). It is released after cleavage of a signal peptide of about 20 aa, and the major extracellular form consists of 72 aa. Structurally, NAP-1/IL-8 belongs to a family of small proteins with four conserved Cystein residues that form two disulphide bridges. Two other neutrophil-activating peptides belong to this family, NAP-2, a cleavage product of platelet basic protein, and gro/MGSA, a peptide that was originally reported to be mitogenic for melanoma cells. NAP-1/IL-8, NAP-2 and gro/MGSA activate human neutrophils inducing shape change, chemotaxis, a rise in intracellular free calcium, exocytosis of the contents of azurophil and specific granules and secretory vesicles, upregulation of CR1 and CR3 and the respiratory burst (1). They act via related receptors and share the signal transduction machinery. All three peptides are unusually selective for neutrophils: They have only weak effects on monocytes, eosinophils and basophils. NAP-1/IL-8 was also reported to be chemotactic for lymphocytes.

In vivo, these peptides induce massive neutrophil infiltration, and are therefore thought to be involved in the recruitment of neutrophils in a variety of in inflammatory conditions. NAP-1/IL-8 is found in large quantities in psoriatic lesions, synovial fluids of arthritic joints and, in some instances, in bronchoalveolar lavage fluids. Blood monocytes and synovial mononuclear cells from RA patients spontaneously release high amounts of NAP-1/IL-8, and production can be further enhanced by stimulation with LPS, immune complexes, zymosan and IL-1.

1. Baggiolini, M., Walz, A., Kunkel, S.L. NAP-1/IL-8, a novel cytokine that activates neutrophils. J. Clin. Invest. 84: 1045-1049 (1989)

O 015 ROLE OF INTERLEUKIN-1, TUMOR NECROSIS FACTOR, INTERLEUKIN-6 AND THEIR INHIBITORS IN RHEUMATOID ARTHRITIS, Jean-Michel Dayer, Division of Immunology and Allergy, Dept. of Medicine, Hôpital cantonal universitaire, 1211 Geneva 4, Switzerland.
Tissue destruction which occurs in inflammatory osteoarticular diseases, such as rheumatoid arthritis, is in part mediated by cytokines. These are produced by immune and non-immune cells present in the synovial tissue and fluid, in cartilage and bone. The stimulation of the production of proteases and arachidonic metabolites by synovial cells is mainly due to IL-1 and to a lesser extent by TNFα produced by monocyte-macrophages. Direct cell-cell contact and cell-associated cytokines are part of a most efficient system for stimulating synovial cells and chondrocytes as well as for activating monocyte-macrophages by lymphocytes. IL-6, found at high concentrations in synovial fluid, is unable to stimulate collagenase and PGE_2 production by synovial cells; it even decreases PGE_2-induced IL-1 production and does not affect collagenase-induced IL-1 production. Whilst IL-1 stimulates synthesis of proteoglycan and hyaluronic synthesis in synovial cells it decreases it in chondrocytes. Numerous mechanisms exist that counteract the biological activities of pro-inflammatory cytokines. We reported the IL-1 receptor antagonist (IL-1ra) in the urine of patients with monocytic leukemia [1] or with juvenile rheumatoid arthritis and high fever [2]. IL-1ra is also present in synovial fluid and culture supernant of synovial fluid macrophages or of blood monocytes aged in vitro, particularly in the presence of GM-CSF [3]. IL-1ra which competes with IL-1 at the receptor level [4], blocks $^{45}Ca^{++}$ release in bone when induced by IL-1 but not by PTH [5]. Biological activities of TNFα are blocked by a different mechanism involving soluble TNFα-binding proteins (TNFα-BP I and -BP II) which are exra-membranous fragments of the two TNFα receptors [6]. These inhibitors may be present in greater amounts in chronic inflammatory diseases, representing an attempt to control the expansion of inflammation.

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O 016 GM-CSF AND CYTOKINE NETWORKS IN RHEUMATOID ARTHRITIS, Gary S. Firestein, Department of Medicine, UCSD Medical Center, San Diego, CA 92103.
Synovial lining hyperplasia and destruction of extracellular matrix in rheumatoid arthritis (RA) are, in part, a consequence of increased numbers of activated fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes (MLS). The synovial cytokine milieu in the inflamed joint is thought to be influential in these changes. For instance, IL-1 and TNF-α increase cultured synoviocyte proliferation and synthesis of collagenase. Also, cytokines like GM-CSF are likely important in determining the surface phenotype of synoviocytes, particularly the expression of class II major histocompatibility markers. Recent studies have focused on identifying cells responsible for cytokine production in the joint. For instance, in situ hybridization studies using enzymatically dispersed synovial tissue cells indicate that MLS produce IL-1 and TNF-α, while FLS are responsible for most IL-6 production. We have recently attempted to identify the cells in the joint that produce GM-CSF. Long term cultured RA FLS do not spontaneously produce GM-CSF. If FLS are stimulated with IL-1β or TNF-α (but not IFN-γ, M-CSF, TGF-β, or IL-6) they express the GM-CSF gene and secrete the protein. RNA transcripts are detected 2 h after stimulation and persist for at least 24 h. Interestingly, IFN-γ specifically inhibited TNF-α mediated GM-CSF production. IFN-γ also inhibited TNF-α mediated FLS proliferation and collagenase production. FACS purified fresh MLS spontaneously synthesize GM-CSF in vitro. After one week, additional stimulation with IL-1 or TNF-α is required for further GM-CSF production. In situ hybridization to cytospun subpopulations of fresh synovial cells demonstrate that the MLS population is enriched for GM-CSF mRNA.

These data are consistent with paracrine/autocrine models of chronic RA, since GM-CSF is produced by non-T cells in the synovium. GM-CSF (produced either by MLS or FLS) induces HLA-DR expression and cytokine production (IL-1 and TNF-α) by MLS. IL-1 and TNF-α can, in turn, induce FLS to produce IL-6, GM-CSF, collagenase, and other soluble mediators. Novel therapeutic interventions directed at cytokine loops in the synovium have great potential in RA. For instance, IFN-γ therapy, which has a modest anti-inflammatory effect in RA, might function through inhibition of TNF-α pathways. Because the cytokine network is highly redundant, combination therapy will likely be required.

Molecular Biology and the Immunopathogenesis of Rheumatoid Arthritis

Cytokines - II

O 017 GROWTH FACTOR CONTROL OF ANGIOGENESIS, Daniel B. Rifkin, Department of Cell Biology New York University Medical Center, 550 First Avenue, New York, 10016. The process of angiogenesis is a normal physiological process in which cells invade. Like other invasive processes, when endothelial cells invade during neovascularization there is an increase in the production of plasminogen activator(PA). This is controlled by the angiogenesis factor, basic fibroblast growth factor (bFGF). bFGF increases both the transcription and translation of mkNA. In addition, bFGF increases the number of cell surface PA receptors thereby focusing the increased pericellular proteolytic activity. The importance of the proteolytic balance in angiogenesis is also apparent from the observation that one class of antiangiogenic compounds- angiostatic steroids - may inhibit angiogenesis via the induction of high levels of the PA inhibitor PAI-1.

The inhibition of angiogenesis may be controlled by transforming growth factor β (TGF-β). TGF-β suppresses PA synthesis, increases PAI-1 synthesis, but has no effect on the uPA receptor in bovine endothelial cells. Interestingly, the generation of TGF-β may, itself, be mediated by PA/plasmin. TGF-β is released as a latent high molecular weight complex (LTGF-β) which is inactive. We have shown that the activation of LTGF-β is plasmin mediated. The activation appears to involve a surface assemblage of protease, substrate, and several additional binding proteins. One of these binding proteins is the cation independent mannose-6-phosphate/insulin-like growth factor II receptor.

O 018 GROWTH FACTORS IN INFLAMMATORY SYNOVITIS, Ronald L. Wilder, National Institutes of Health, Bethesda, MD 20892.

Although rheumatoid arthritis is frequently described as a cell mediated immune process, an increasing body of data suggests that the synovial disease shares many cellular and molecular characteristics with highly proliferative and invasive tumors. Indeed, a salient pathologic feature of this disease is massive hyperplasia of synovial stromal cells (fibroblast-like cells and blood vessels) and invasive destruction of periarticular bone and cartilage. This proliferative and invasive disease is associated with marked upregulated expression in vivo of a number of biochemical markers commonly associated with invasive tumors, i.e., vimentin, c-myc, c-fos, c-jun, phosphotyrosine, transin/stromelysin and collagenase (Case, J. et al., Amer. J. Pathol. 135:1055, 1989; Sano, H. et al., J. Cell Biol. 110:1417, 1990). In addition, numerous observations in vitro are consistent with the tumor-like analogy. For example, freshly explanted rheumatoid synovial fibroblast-like cells can be grown under anchorage-independent conditions, which is an in vitro correlate of the transformed phenotype (Lafyatis, R. et al. J. Clin. Invest. 83:1267, 1989). Rheumatoid synovial tissues will also form short-lived tumor-like nodules when implanted in athymic mice. These observations lead us to ask: what growth factors regulate this tumor-like phenotypic behavior? Although many factors are undoubtedly involved, recent data have implicated three major families of cytokines/growth factors--platelet-derived growth factors (PDGF), heparin-binding (or fibroblast) growth factors (HBGF), and type-beta-transforming growth factors (TGF) (Lafyatis, R. et al., J. Clin. Invest. 83:1267, 1989; Kumkumian, G. et al., J. Immunol. 143:833, 1989; Lafyatis, R. et al., J. Immunol. 143:1142, 1989; Wilder, R. et al., Ann. NY Acad. Sci. 593:197, 1990; Sano, H. et al., J. Cell Biol. 110:1417, 1990; Remmers, E.F. et al., Growth Factors 2:179, 1990; Remmers, E.F. et al., J. Rheumatol., in press). These factors are produced in abundance in rheumatoid synovial tissues in vivo and modulate the phenotypic behavior of synovial connective cells in vitro. For example, PDGFs and the HBGFs are potent stimuli of anchorage-independent growth of rheumatoid synovial fibroblast-like cells. TGF-beta 1 and 2, on the other hand, tend to induce a more mature and less invasive cellular phenotype. HBGF-1 is a potent known angiogenic factor in vivo and may play an important role in driving angiogenesis in the rheumatoid synovium. In other words, these factors, generated as part of the inflammatory process, appear to stimulate rheumatoid synovial connective tissues to behave like a locally invasive, but non-malignant, tumor.

Cytokines - III

O 019 IL-1 RECEPTOR ANTAGONIST: REGULATION OF PRODUCTION, William P. Arend, Fenneke G. Joslin, Robert W. Janson, Mark Malyak, Carl F. Bigler, Arthur Gutierrez-Hartmann and Michael F. Smith, Jr., Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262.

A 17 kD monocyte-derived specific receptor antagonist of interleukin-1 (IL-1ra) has recently been purified, sequenced, cloned and expressed. IL-1ra is produced by human monocytes after culture with LPS or on adherent IgG. As human monocytes mature into macrophages they undergo a progressive transcriptional repression of IL-1β production. However, in vitro-derived or alveolar macrophages exhibit a constitutive production of IL-1ra which is further enhanced after culture with GM-CSF. Other cells also produce IL-1ra: polymorphonuclear leukocytes (PMN) contain IL-1ra mRNA and synthesize small amounts of protein. Lastly, unstimulated human keratinocytes synthesize, but do not secrete, a variant IL-1ra molecule that lacks a leader peptide.

The possible mechanisms whereby monocytes regulate production of an agonist (IL-1β) and a receptor antagonist (IL-1ra) have been explored in recent studies. Relative steady-state IL-1β and IL-1ra mRNA levels were determined using specific cDNA probes; protein levels in cell lysates and supernatants were measured using specific ELISA's. LPS stimulated transcription and translation of both proteins in monocytes to the same degree and with similar kinetics. In contrast, monocytes cultured on adherent IgG transcribed the IL-1β gene to low and variable levels but failed to synthesize any detectable IL-1β protein. However, IgG induction led to a delayed but greater level of transcription of IL-1ra with a high level of protein production. The specific stimulatory effect of adherent IgG on IL-1ra production was due both to induction of transcription and to enhancement of IL-1ra mRNA stability.

In order to further explore the differential regulation of transcription of IL-1β and IL-1ra, 1.7 kb of DNA from the 5'-flanking region of the IL-1ra gene has been cloned, mapped and sequenced. Deletional mutants of this IL-1ra promoter region have been prepared and coupled to the reporter gene for luciferase. These constructs have been transfected into cell lines of varying types and exhibit specificity for macrophage cell lines. The results of initial studies have revealed the presence of both positive and negative cis-acting DNA elements for LPS-induced IL-1ra transcription in the promoter region. Further studies will characterize these DNA elements and analyze the presence and mechanisms of trans-acting nuclear protein factors important in the functional regulation of IL-1ra transcription.

In summary, IL-1ra production by human monocytes and macrophages is regulated at both transcriptional and post-transcriptional levels. IL-1β and IL-1ra production by these cells appears to be regulated in a differential, and possibly reciprocal, fashion.

O 020 STRUCTURAL AND BIOLOGICAL STUDIES ON IL-1 RECEPTOR ANTAGONIST, Stephen P. Eisenberg, David J. Dripps, Ron J. Evans, John D. Childs, and Robert C. Thompson; Synergen, Inc., 1885 33 Street, Boulder, CO 80301.

IL-1α and IL-1ß are two distantly related proteins that are believed to be mediators of both inflammatory and immune responses. Recently, a human monocyte derived IL-1 receptor antagonist (IL-1ra) was isolated, cloned and characterized (1,2). This protein has significant homology to IL-1ß; furthermore their hydropathy profiles are remarkably similar. These facts suggest that the two proteins are related. Characterization of genomic clones for IL-1ra support the idea that IL-1ra is a member of the IL-1 gene family. Furthermore, site directed mutagenesis studies suggest that the structures of IL-1ß and IL-1ra are similar.

IL-1ra binds with high affinity to the type 1 IL-1 receptor. The Kd for this interaction is ≈100-150 pM. It binds with lower affinity to the type 2 receptor. However, IL-1ra does not induce a response in any of the standard assays for IL-1 activity (1,3). Two early events following receptor binding that may be important for IL-1 activity are the induction of a protein kinase activity and the internalization of the IL-1·IL-1R complex. IL-1ra does not induce this kinase activity, nor is it internalized. Thus, IL-1ra appears to be a pure receptor antagonist.

Investigators at Synergen have collaborated with several groups in using IL-1ra to study the role of IL-1 in a number of inflammatory diseases. First, F. Cominelli and co-workers have shown that IL-1ra can block immune complex-induced inflammation of colon tissue in a rabbit model for ulcerative colitis (4). Second, K. Ohlsson and co-workers found that LPS-induced septic shock in rabbits can be prevented by the administration of IL-1ra (5). Finally, J. Schwab and colleagues have found that IL-1ra injected into rats can block SCW-induced joint inflammation.

1. C. H. Hannum et al. (1990) Nature **343**, 336-340.
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3. W. P. Arend et al. (1990) J. Clin. Invest. **85**, 1694-1697.
4. F. Cominelli et al. (1990) J. Clin. Invest. **86**, 972-980.
5. K. Ohlsson et al. (1990) Nature, in press.

Adhesion Molecules

O 021 MODULATION OF VLA PROTEIN ADHESIVE FUNCTIONS DURING THE IMMUNE RESPONSE, Martin E. Hemler, Bosco M.C. Chan, Joaquin Teixido, Mariano J. Elices, Hamid Band, Stefan Carrel, Justin Wong and Anjana Rao, Dana-Farber Cancer Institute, Harvard University School of Medicine, Boston, MA 02115

Stimulation of antigen specific mouse T cell clones with arsenate-derivatized ovalbumin resulted in a transient increase in T cell adhesion to the matrix proteins collagen, laminin and fibronectin. This increase in adhesion was not observed when mutant T cell clones lacking the T cell receptor were utilized. To show that the mutant T cells did not have faulty adhesion receptors, they were stimulated with phorbol esters (thus bypassing the TCR), and a transient increase in adhesion to collagen, laminin and fibronectin was observed. This adhesion was blocked almost completely by antibodies to the integrin β_1 subunit. Together these results indicate that T cell interaction with specific antigen can transmit signals through the T cell receptor (TCR), resulting in the increased adhesiveness of β_1 integrins (VLA proteins) toward matrix protein ligands.

In addition, we have demonstrated that the integrin VLA-4 can directly mediate transmission of activating signals to T cells. Using the human T cell line Jurkat, we discovered that an unusual anti-VLA-4 MAb could directly stimulate a rapid increase in intracellular calcium, and also caused interleukin-2 secretion when added together with phorbol ester. Furthermore, this antibody caused peripheral blood T cells to proliferate. It appears that direct MAb activation through VLA-4 might be TCR-dependent because no calcium flux or lymphokine secretion was observed (in response to triggering through VLA-4) when the TCR was either absent on non-functional. Notably, the ability of VLA-4 to mediate binding to its ligands fibronectin and VCAM-1 occurred in both the presence and absence of functional TCR, thus indicating that VLA-4 adhesion functions are not necessarily coupled to TCR-dependent signal transduction through VLA-4.

Together, all of these results suggest a circular mechanism, whereby integrins in the β_1 family (VLA proteins) can both facilitate T cell activation, and be functionally upregulated as a result of T cell activation.

O 022 REGULATION OF THE MIGRATION OF LYMPHOCYTES INTO INFLAMMATORY SITES, Peter E. Lipsky, Laurie S. Davis and Nancy Oppenheimer-Marks, Harold C. Simmons Arthritis Research Center, Southwestern Medical School, Dallas, TX 75235

One of the critical events in the initiation and propagation of an immunologically mediated inflammatory response is the binding of circulating lymphocytes to endothelial cells (EC) of postcapillary venules, and their subsequent migration into the extravascular tissue. Especially remarkable is the degree to which lymphocytes infiltrate into perivascular tissues that are involved in chronic inflammatory reactions, such as the rheumatoid synovial membrane. The adhesion of lymphocytes to endothelial cells is mediated by a variety of receptors whose utilization is determined by the activation and differentiation status of the T cell and the cytokine exposure of the EC. Cytokines, such as IFN-γ , IL-1 and TNF, favor the development of increased lymphocyte adhesion by increasing the expression of adhesion ligands, such as ICAM-1 and VCAM-1 by EC. These molecules, by binding to their appropriate lymphocyte receptors, LFA-1 and VLA-4, respectively, mediate interactions of EC with T cells. Expression of LFA-1 and VLA-4 by T cells is not sufficient for their utilization during T cell-EC binding. Rather this is regulated by the activation status of the T cells, with a variety of stimuli inducing the capacity to employ these receptors for adhesion to EC. The gp90 receptors, CD44 and LECAM-1 also mediate adhesive responses of lymphocytes, and like LFA-1 and VLA-4, their utilization during binding to EC is regulated by the activation and differentiation status of the T cells. Similarly, the transendothelial migration of adherent lymphocytes appears to be a receptor mediated process. The capacity of T cells to utilize these receptors as well as their intrinsic motility determines their ability to migrate into inflammatory sites. The activation status of the T cells modulates their competency to migrate. Similarly, activation of T cells, in combination with exposure of EC to IL-1 or TNFα , enhances transendothelial migration by inducing an LFA-1/ICAM-1 mediated event. CD44 also mediates part of the migration process of activated T cells, whereas VLA-4/VCAM-1 interactions play a lesser role. By contrast, resting T cells migrate through control EC in an LFA-1/ICAM-1 and CD44 independent manner, but depend in part on the activity of VLA-4/VCAM-1. These results indicate that adhesion of T cells and their subsequent transendothelial migration are distinct events that use specific panels of receptor-ligand pairs and are altered differentially by the activation status of the T cell and the cytokine exposure of the EC. Knowledge of the receptor-ligand pairs involved in these processes should permit the development of therapeutic interventions that limit entry of T cells into inflammatory sites.

New Therapeutic Approaches

O 023 ANTI-CD4 ANTIBODIES AS AN IMMUNOTHERAPEUTIC APPROACH TO TREATING AUTOIMMUNE DISEASE, C. Garrison Fathman, Susan Alters, Davida Grossman, Judy Shizuru, Division of Immunology, Stanford University Department of Medicine, Stanford, CA 94305.

The inductive events which surround immune response in experimental models are well understood. It has been clearly demonstrated that CD4+ helper inducer T-cells respond to peptide antigens presented within the putative binding cleft of major histocompatability complex class II cell surface glycoproteins. The ternary (three-part) complex formed by the CD4+ T cell receptor, the antigenic peptide and the MHC class II gene product form an inviting target for specific immunotherapy. Several strategies have recently evolved for the treatment of autoimmune disease based upon an understanding of this ternary complex in the inductive events of experimental models of autoimmunity caused by purposeful vaccination with "autoantigens". Unfortunately, rheumatoid arthritis does not have a good animal model; and the inductive events are not as well understood. Nonetheless, the same basic philosophy which has allowed insights into innovative strategies for immunotherapy of murine models of autoimmune disease can be tested in patients with rheumatoid arthritis. Experiments in my laboratory have recently begun to unravel the mechanisms involved in anti-CD4 mediated immunotherapy. We have begun to develop technologies to allow us to isolate those CD4+ inducer lymphocytes which are involved in the inductive events of autoimmune response in animal models. Extrapolation of these models to treatment of human rheumatoid arthritis is currently underway in many laboratories. The use of monoclonal anti-CD4 antibodies as an immunotherapeutic reagent will be discussed. One advantage of this particular form of immunotherapy has been the demonstrated retention of specific immunologic memory in the experimental models developed to date. Preliminary studies suggest that anti-CD4 monoclonal antibodies may have a role in immunotherapy of rheumatoid arthritis.

Infectious Agents/MHC and RA

O 100 CATIONIC BACTERIAL ANTIGENS AS INITIATORS OF INFLAMMATORY JOINT DISEASE, Stephen Batsford, Klaus Gondolf, Andreas Mertz and Enrico Curschellas, Department of Immunology Institute of Med. Microbiology, 7800 Freiburg, FRG.
A local antigen driven immune reaction can initiate joint injury and may be an early event in RA. Experimental studies (JCI 74:1850,1984) have shown that cationised antigens can bind to negatively charged joint structures, provoke an immunologic reaction and produce arthritis. Our goal was to validate this mechanism for bacterial antigens. Simple criteria established in experimental studies with model antigens and used for selecting candidate antigens were: 1) cationic (isoelectric point > 8.5), 2) size > 40kD (aggregated), 3) immunogenic (man/rodents). First efforts were directed at 2 bacterial species associated with joint complications, Borrelia burgdorferi (Bb) and Yersinia enterocolitica (Ye). For further testing intra-articular challenge was performed in pre-immunised rats, induction of arthritis was assessed by histology and ^{99m}Tc scintigraphy. A number of bacterial products were considered, with Bb we concentrated on a cationic outer surface protein complex (Osp A(31kD) + Osp B(34kD) + 22kD protein, mw ~800kD) prepared in aqueous solution by butanol extraction. This Osp complex is a major immunogen in man and induced severe arthritis in rats. In the case of Ye an intracellular cationic protein was isolated from the ribosomal pellet (putative nucleic acid binding protein - histone-like?), it is apparently restricted to Ye 0:3. This protein was arthritogenic in rats and was also immunogenic in man. These studies establish the principle that cationic bacterial antigens can be potent inducers of arthritis. Studies to examine the relevance of such antigens in human reactive arthritis are underway. It is conceivable that similar mechanisms operate in RA.

O 101 AN ANALYSIS OF HLA CLASS II GENES AND SUSCEPTIBILITY TO JUVENILE RHEUMATOID ARTHRITIS. AB Begovich, W Klitz, R Mantegazza, F Mercuriali, and HA Erlich, Department of Human Genetics, Cetus Corporation; Department of Integrative Biology, U.C. Berkeley; Department of Neurology, Stanford University; and Servizio di Immunogenatologia e Transfusionale, Instituto Ortopedico "G. Pini", Milan.
Non-radioactive sequence specific oligonucleotide probes have been used in a dot blot format to HLA-DRB1, -DQA1, -DQB1, and -DPB1 type PCR-amplified DNA from 48 Italian patients with pauciarticular juvenile rheumatoid arthritis and 36 Italian controls. Previous serological analyses had shown the DRw8 and DRw11 alleles to be increased in patients with this disease. Our molecular analyses verify the DRw8 association and allow us to pinpoint the specific allele involved. An increase in the DPB1*0201 allele, which is independent of the DRw8 association, is also noted. Comparisons will be made between these HLA alleles and others involved in susceptibility to different forms of arthritis including seropositive adult rheumatoid arthritis, seropositive juvenile rheumatoid arthritis, and lyme arthritis.

O 102 OUTGROWTH OF DOUBLE POSITIVE T CELLS FROM THE JOINTS OF ARTHRITIC RATS, Denise E. Casentini-Borocz, Hansha R. Bhayani, Somesh D. Sharma, Biospan Corporation, Redwood City, CA 94063

Adjuvant-induced arthritis in rats has been extensively used as a model for human rheumatoid arthritis. A number of studies have demonstrated that both αβ and γδ receptor bearing T cells can be cultured from human synovial tissues. We have induced adjuvant arthritis in Lewis rats in order to determine the phenotypic characteristics of the cells present in the joints of the arthritic animals. At either 6 or 12 weeks after the immunization and during the course of arthritis, cells were isolated from the joint tissues using collagenase and dispase digestion. The cell mixture was cultured in the absence or presence of an acetone precipitate of M. tuberculosis H37Ra (AP-MT) for the first 6 days and thereafter with 20 u/ml. of rIL-2. The predominant cell type obtained from rats 6 weeks after immunization and maintained for 5 months in rIL-2 containing media were >95% αβ TCR+CD8+CD4+ lymphocytes, whereas, the cells isolated from the 12 week rats after immunization were 75% αβTCR+ and >95% CD8+CD4+ after 2 weeks in culture. Only fibroblastic cells were seen in the non-antigen stimulated cultures. Normal rat lymph node cells also contained, albeit in significantly lower numbers (15%), CD8+CD4+ αβTCR+ T lymphocytes. Therefore, it appears that CD8+CD4+ T lymphocytes are present in the joints of arthritic rats, and that preferential expansion of CD8+CD4+ T cells occurs under these experimental conditions.

O 103 DOWN-REGULATION OF MHC CLASS II ANTIGEN EXPRESSION ON MACROPHAGES BY ADENOSINE AND RELATED ANALOGS, Carl K. Edwards, III*, Lynnetta M. Watts, Marsa D. Hatfield and David R. Borcherding, Department of Immunology and Chemical Research, Marion Merrell Dow, Inc., Kansas City, MO 64134

Purine nucleosides such as adenosine (ADO) have been shown to have several immunoregulatory properties. A close association between the expression of Major Histocompatibility Complex (MHC) Class II antigens (Ag) on cells of the immune system and the pathogenesis of autoimmune diseases such as Rheumatoid Arthritis (RA) exists. The effects of ADO and ADO analog compounds 2-Chloroadenosine (2Cl-ADO), ML1111 and ML1112 on Lewis female rat peritoneal macrophage (MØ) MHC Class II Ag expression *in vitro* and *in vivo* by flow cytometry are addressed. MØ ($2x10^{+6}$; > 98%α-naphtyl esterase positive) which were > 97%OX-42 positive and < 2.0%OX-19 positive, incubated for 18 hr *in vitro* with recombinant rat IFN-γ (rRaIFN-γ; 500 U/mL) resulted in significant ($p < 0.05$) enhancement of Ia^+ (MRC OX-6) Ag levels (47.5% versus 15.1% in untreated MØ). rRaIFN-γ- induced Class II Ag on MØ could be blocked with a specific anti-rat IFN-γAb (20.7%Ia^+). MØ incubated with rRaIFN-γ and either ADO, 2Cl-ADO, ML1111, or ML1112 (1.0μM) resulted in levels of Ia^+ which were reduced by nearly 50% (21.8%, 25.5%, 28.8% and 24.9% respectively). Similarly, MØ obtained from Lewis rats (N = 4) treated *i.p.* for 3d with heat killed *Bordetella pertussis* (200 OD Units) had elevated Ia^+ expression in comparison to vehicle treated rats (82.0 ±7.0% vs. 18.3±4.0% Ia^+, respectively) and these levels could be significantly ($p < 0.05$) reduced by either ADO or ML1112 treatment (20mG/kG/d) given *s.c.* for 3d after *B pertussis* injection (64.3±6.3% vs. 42.3% ±8.4% Ia^+, respectively). These data demonstrate that ADO analogs which down-regulate MHC Class II Ag on MØ have important therapeutic potential in the treatment of autoimmune diseases such as RA.

O 104 A HIGH PROLIFERATIVE RESPONSE TO A 65 kDa MYCOBACTERIAL HEATSHOCK PROTEIN AND IN VITRO EXPANSION OF $V\delta1^+$ T CELLS FROM SYNOVIAL FLUID OF RA PATIENTS.

Alvar Grönberg°, Karl Söderström*, Anders Bucht°, Lars Klareskog# and Rolf Kiessling*.°Dept. of Biomedical and Chemical Research, Kabi Pharmacia Therapeutics, Uppsala, Dept. of Immunology Karolinska Institute, Stockholm, Dept. of Clinical Immunology, University Hospital, Uppsala, Sweden.

We have analyzed the ability of mononuclear cells from synovial fluid (SFMC) and from peripheral blood (PBMC) of patients with RA to proliferate in response to a mycobacterial 65 kDa heat shock protein (hsp65), BCG and to rat collagen type II. The SFMC showed a higher response to hsp65 and to BCG as compared to PBMC from the same patient. With collagen type II, only a small proportion of the patients showed a proliferative response, although also with this antigen SFMC responded better than PBMC. A high proportion of cells in SFMC derived short term T cell lines stimulated with IL-2, BCG or hsp65 were of TcR γ/δ type, often exceeding the percentage of TcR α/β cells. The SFMC lines were predominantly $\delta TCS1^+$ ($V\delta1^+$). In contrast, lines from PBMC contained a lower percentage of γ/δ^+ cells the majority of which expressed $V\gamma9$ ($Ti\gamma A^+$) and Vδ2 ($BB3^+$). cDNA from a SFMC line containing a high percentage of $\delta TCS1^+$ cells was amplified using Vδ1 and Cδ specific primers and the polymerase chain reaction. The products were subcloned and sequenced. A preferential rearrangement to Jδ1 (11/12) over Jδ3 (1/12) and a high degree of junctional diversity was observed. These data confirm previous reports of elevated anti-hsp65 response and further demonstrate a characteristic distribution of τ/δ cells in synovial fluid as compared to peripheral blood of RA patients.

O 105 ANALYSIS OF T-CELL RECEPTOR V REGION GENE EXPRESSION IN SYNOVIAL FLUID T-CELL CLONES SPECIFIC FOR MYCOBACTERIAL STRESS PROTEIN, Judy Henwood[1], Jason A. Loveridge[2], John I. Bell[2], J.S. Hill Gaston[1], Department of Rheumatology[1], University of Birmingham, Birmingham, UK and Department of Molecular Immunology[2], Institute of Molecular Medicine, Oxford, UK.

T-cell responses to mycobacterial antigens have been implicated in the pathogenesis of inflammatory arthritis. We used synovial fluid T-cell clones isolated from patients with inflammatory arthritis, previously analysed for their MHC restriction and responsiveness to M. leprae 65 kD stress protein (SP) epitopes. The α and β chains of the T-cell receptor (TCR) were identified for each clone, using polymerase chain reaction.

All four SF T-cell clones from a patient with an acute self-limiting disease were DR3 restricted and all recognised the same epitope at the N terminal of the SP. These clones were all found to express a previously unidentified Vα 2 gene and a Vβ 5 gene.

A second set of four clones, DP restricted, were from a DR4 homozygous individual with classical rheumatoid arthritis. These clones all recognised an epitope towards the C terminal of the SP, and showed a different but similarly restricted Vα and Vβ gene usage.

This is one of the first demonstrations in humans of restricted TCR V region gene usage in defined T-cell clones.

O 106 REGULATION OF CLASS II MHC GENES BY cAMP, IFN-γ, AND TRANSCRIPTION FACTOR mXBP IN CULTURED CELLS AND TRANSGENIC MICE. Lionel B. Ivashkiv, Maryann Z. Whitley, Mark D. Fleming, Mark R. Boothby, and Laurie H. Glimcher, Department of Rheumatology/Immunology, Harvard Medical School, and Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115. David Lo, Scripps Clinic, La Jolla, CA 92037.

Aberrant and elevated expression of class II MHC genes is found in many autoimmune diseases, including rheumatoid arthritis. IFN-γ causes aberrant expression of class II in many cell types, including synoviocytes. This induction is inhibited by agents, such as prostaglandin E2, which cause elevations in intracellular cAMP. We have shown that cAMP can down-regulate baseline class II gene expression in a B cell line as well as inhibit IFN-γ induction of class II in a macrophage cell line. This effect occurs at the level of transcription, and we have mapped it to a particular DNA element shared by most class II gene promoters. Another important element present in all class II promoters is the X box. We have generated transgenic mice which overexpress murine X box binding protein in their B and T lymphocytes. We expect that these mice will express abnormal levels of class II antigens and may have an autoimmune or immunodeficient phenotype. The results of experiments characterizing the immune responses of these mice will be presented.

O 107 INDUCIBLE mRNA AND CELL SURFACE EXPRESSION OF HUMAN HEAT SHOCK PROTEIN hsp60 ON HUMAN MYELOMONOCYTIC CELL LINES AND SYNOVIAL MACROPHAGES, R. Kiessling*, M. Ferm*, K. Söderström*, S. Jindal°, R. Young°, L. Klareskog# and A. Grönberg×. Department of Biomedical and Chemical Research, ×Kabi Pharmacia Therapeutics, Uppsala,* Department of Immunology Karolinska Institutet, Stockholm, #Department of Clinical Immunology, Uppsala University Hospital, Uppsala, °Whitehead Institute for Biomedical Research, Cambridge, MA.
Heat shock proteins (hsp) are evolutionary highly conserved in their structure between procaryotes and eucaryotes and are of potential interest in relation to mechanisms of autoimmune diseases, especially rheumatoid arthritis. The ML-30 monoclonal antibody (mAb) crossreactive between the mycobacterial hsp65 and human hsp60 was used to screen 21 human tumor cell lines in Western blot analysis. All T cell and B cell lymphomas were found to have high constitutive expression of hsp60. Two human monocytic leukemia lines showed low expression under normal culture conditions, but enhanced levels of hsp60 mRNA and protein could be induced by heat-shock, retinoic acid or IFN-gamma. Highest expression was seen after the combined treatment with TNF-alpha and IFN-gamma. The anti-hsp60/65 mAb epitope was expressed on the cell surface and two proteins of 60 and 67 kDa could be immunoprecipitated from lysates of cell surface ^{125}I-iodinated cells treated with IFN-gamma/TNF-alpha. FACS analysis of mononuclear cells from rheumatoid synovial tissue revealed a population of HLA-DR+, CD14+ cells stained by the ML-30 mAb. These results demonstrate that hsp60 can be expressed at the cell surface of monocytic cell lines and inflammatory macrophages and that its synthesis can be enhaced by inflammatory cytokines.

O 108 ARTHRITIS BY PEPTIDOGLYCAN POLYSACCHARIDE COMPLEXES OF THE HUMAN ANAEROBIC INTESTINAL FLORA AND TRANSFER OF THE DISEASE BY A T CELL-LINE, Ina S. Klasen, Marie-José Melief, Jeanette Kool, Wim B. van den Berg*, Anton J. Severijnen and Maarten P. Hazenberg, Department of Immunology, Erasmus University Rotterdam and *Department of Rheumatology, University Nijmegen, The Netherlands.
A pivotal role for the normal intestinal flora in the induction of arthritis in man has been hypothesized by Bennett (1). We have supported this hypothesis by demonstrating the arthritis inducing capacity in a rat model of the cell wall peptidoglycan polysaccharide complexes (PPC) of Eubacterium aerofaciens and Bifidobacterium species, anaerobic species that are present in high numbers (i.e. $>10^9$/g) in the faecal flora. PPC's can also be isolated in soluble form from faeces or ileostoma fluid. PPC from faeces was not arthritogenic. PPC from ileostoma fluid, likely to be less degraded by intestinal enzymes, appeared to be arthritogenic when injected in incomplete Freund adjuvant in the base of the tail of Lewis rats.
The role of T cells in the peptidoglycan induced arthritis was investigated by T cell transfer studies. A T cell-line was isolated from the lymph nodes of rats with an E. aerofaciens PPC arthritis. This cell-line, B13, which has the helper phenotype, proliferated after stimulation with syngeneic or class II compatible spleen cells without additional antigen. The proliferation of B13 appeared to be significantly higher when PPC from faeces or ileostoma fluid was added. Most interesting, the B13 cell-line was arthritogenic when injected intravenously in 6Gy irradiated rats. 5 Out of 7 rats developed a joint inflammation in one or both knee joints. The arthritis was confirmed by a ^{99m}Technetium scanmethod and histological examination. F344 rats, that are insusceptible for active arthritis induction by bacterial antigens, are also insusceptible for arthritis induction by B13.
These results reinforce the hypothesis that bacterial antigens from the intestine and T cell responses directed against these antigens, can be of great importance in rheumatic disease.

1. Bennett JC. The infectious etiology of rheumatoid arthritis. Arthritis Rheum 1978;21:531-8.

O 109 T CELL LINES DERIVED FROM SYNOVIAL FLUID OF A PATIENT WITH YERSINIA-TRIGGERED REACTIVE ARTHRITIS RECOGNIZE AN ANTIGEN ON AUTOLOGOUS ANTIGEN PRESENTING CELLS, Riitta Lahesmaa, Timo Veromaa, Sirpa Jalkanen, Reijo Luukkainen and Paavo Toivanen, Departments of Medical Microbiology and Medicine, Turku University, Turku, and Satalinna Hospital, Harjavalta, Finland.
T cell lines and clones generated from Yersinia stimulated peripheral blood lymphocytes (PBL) and synovial fluid lymphocytes (SFL) of a patient with Yersinia-triggered reactive arthritis were analysed. Considerably higher frequency for growth of cell lines following stimulation with Yersinia was observed for SYL compared to PBL. Altogether 98 T cell lines from synovial fluid were screened for their specificity against a panel of antigens including Yersinia, PPD, tetanus and feeders alone. The feeders were patient's own peripheral blood mononuclear cells taken at the early stage of the disease. Interestingly enough, 41 out of the 51 lines (78%) showing specificity for the antigens used, had responses also against the feeder cells alone. These responses were comparable to those obtained against Yersinia. Out of the remaining 10, seven reacted only against Yersinia and 3 were stimulated by PPD only. Some of the lines also reacted to Mycobacterial derived 65 kD heat shock protein. Antigen specificity was class II restricted as shown by anti HLA class I and class II antibodies. More specifically, the response was DR7 restricted demonstrated by the HLA matched and mismatched antigen presenting cells. The lines obtained were predominantly TCR$\alpha\beta$ and of CD4 phenotype. Eight lines, originated from 1 cell/well, were analysed for their TCR V region usage with the panel of V_β-specific monoclonal antibodies. Three out of the eight lines were expressing $V_\beta 8$. Selected lines showing the predominant specificity pattern with reactivity to the patient's own mononuclear cells were tested further. Interestingly, the reactivity was diminished when PBL taken at the later stages of the infection were introduced to the T cell lines. This suggests for the presence of Yersinia antigens within the cells taken at the early stage of the infection, presented to the T cell lines on autologous feeder cells.

O 110 STRONG PRIMARY SELECTION FOR DR4 SUBTYPES ACCOUNTS FOR THE HLA-DQw7 ASSOCIATION WITH FELTY'S SYNDROME, Jerry Lanchbury, Emma Jaeger, Margaret Hall, David Sansom, Paul Wordsworth & Gabriel Panayi, Molecular Immunogenetics & Rheumatology Units, UMDS, London, UK, The Bath Institute for Rheumatic Diseases, Bath, UK, Institute of Molecular Medicine, Oxford Univ., UK.
Approximately 1% of rheumatoid arthritis (RA) patients develop Felty's syndrome (FS). FS was previously shown to be highly associated with HLA-DR4 and DQw7. To map the likely susceptibility locus for FS within the HLA class II region, we used DR4 and DQ specific PCR together with specific oligonucleotide probes for detection of disease associated sequences. 43 FS patients, 107 random controls and 122 DR4 controls were studied. As expected HLA-DR4 was strongly associated with FS (93% vs. 32% controls) as was DQw7. The DR4 increase is almost entirely accounted for by Dw4 (88%) with a secondary Dw14.1/.2 association (26%). Analysis of FS and control DR4-DQW7/8 combinations suggests that the DQw7 association derives from selection for Dw4. Dw10 and Dw13.1/.2 alleles are absent from FS patients. Susceptibility to RA has previously been analysed in terms of a DRB1 epitope with a distinct hierarchy of allelic association. In FS this hierarchy is greatly skewed towards Dw4 and has important implications for immunoregulation in RA.

O 111 DETECTION OF PARVOVIRUS B19-SPECIFIC DNA SEQUENCES IN BONE MARROW (BM) ASPIRATES FROM CHRONIC B19 ARTHROPATHY PATIENTS, Stanley J. Naides, Frank Foto, Laura L. Scharosch and Elizabeth J. Howard, Department of Internal Medicine, The University of Iowa College of Medicine, Iowa City, IA 52242.
Acute B19 infection may lead to chronic rheumatoid-like arthropathy. In order to determine whether B19 infection persists in chronic B19 arthropathy, acute infection in adults was documented by anti-B19 IgM serum antibodies detected by ELISA. BM aspirates were obtained from 3 patients with chronic joint symptoms at 24-29 months following acute infection. Each had symmetric polyarthralgia/polyarthritis characterized by 1-3 hours of morning stiffness and tenderness in up to 28 of 69 joints evaluated. Genomic DNA was prepared from whole BM aspirate or purified BM buffy coat cells. B19-specific DNA sequences in the viral capsid gene were amplified by polymerase chain reaction methodology and probed by B19-specific radiolabelled oligonucleotides in Southern analysis. Specificity of amplification was confirmed by product sequencing. BM from 3 of 3 chronic B19 arthropathy patients contained B19-specific DNA sequences compared to 0 of 7 anti-B19 IgM^-, IgG^- and 0 of 6 anti-B19 IgM^+, IgG^+ normal donor BM. The 3 chronic arthropathy patients had no anti-B19 IgM antibodies in late convalescent sera, but remained anti-B19 IgG^+; B19 DNA was not detected in convalescent sera. Therefore, B19-specific DNA sequences may be found in BM from chronic B19 arthropathy patients without detection in serum. Absence of B19-specific DNA sequences in BM from normal anti-B19 IgM^-, IgG^+ donors argues against nonspecific viral persistence after acute infection. These findings suggest that chronic B19 arthropathy results from persistence of either B19 infection or select B19 DNA.

O 112 T CELL ACTIVATION BY 60KD HEAT SHOCK PROTEINS IN PATIENTS WITH INFLAMMATORY SYNOVITIS, Richard M. Pope, Rosa M. Lovis and Radhey S.Gupta, Department of Medicine, Northwestern U. and the VA Lakeside Medical Center, Chicago, and Department of Biochemistry, McMaster U., Hamilton.

In an attempt to define which mycobacterial antigens were responsible for the activation of synovial fluid(SF) lymphocytes, acetone precipitated-M. tuberculosis(AP-MT) antigens were separated into 5 fractions following SDS-PAGE and added to SF mononuclear cell cultures of patients with inflammatory synovitis. Fraction 2 (50 to 70kD) and 5 (<28kD) resulted in significantly more proliferation that fractions 1, 3, and 4. The response to a purified mycobacterial 65kd heat shock protein (hsp), which migrated in fraction 2, was highly correlated ($r = 0.89$, $p < 0.001$) with the response to the crude AP-MT. Studies were performed to determine if the proliferative response to the 65kD mycobacterial hsp was due to cross-reactivity of an autoimmune response to the homologous human 60kD mitochondrial hsp. Of the 23 synovial fluids examined, 69% demonstrated positive proliferative responses (stimulation index ≥ 4.0 and $> 3,000$ counts per minute[cpm]) to the AP-MT, with comparable reactivity to the purified mycobacterial 65kD hsp. In contrast only 35% of the SFs demonstrated positive proliferative responses to the purified, homologous, recombinant human protein. The proliferation induced in response to the AP-MT was often quite intense, 52% of the fluids demonstrating > 20,000 cpm in response to the mycobacterial proteins. In contrast, only 2 (9%) of the SFs responded strongly to the human hsp (proliferation > 20,000 cpm). Although autoreactivity against the human 60kD hsp may occur in some individuals, cross-reactivity of an autoimmune response does not appear to be the principal mechanism for the induction of the majority of the SF T cell proliferative responses to mycobacterial antigens.

O 113 Dw4 MAY BE ASSOCIATED WITH PERSISTENCE BUT NOT INDUCTION OF ARTHRITIS. Mike Salmon, Paul Emery, B Paul Wordsworth, Edward J Tunn, Paul A Bacon, John I Bell. Dept of Rheumatology, Birmingham University, and Institute for Molecular Medicine, Oxford UK.

Rheumatoid arthritis (RA) is associated principally with subtypes of DR4, (Dw4, Dw14 and Dw15 but not Dw10 or Dw13) but also with DR1. The RA associated alleles share a common sequence in the third allelic hypervariable region (3AHVR) of the DR molecule; DR1 Dw14 and Dw15 are identical, Dw4 has a conservative substitution (lys for arg at position 71). It is not clear whether this class II susceptibility locus acts to induce arthritis or to facilitate its persistence. We analysed PCR amplified DNA from the DR B1 locus (encoding the first domain of the DR chain) from 55 patients who four years previously were recruited to an early synovitis clinic. All of the patients fulfilled the criteria for possible or probable RA when first seen. After 4 years 29 patients had developed definite or classical rheumatoid arthritis, while 26 had never fulfilled the criteria for this disease. We wished to find out whether those patients who failed to develop persistent disease would share the same MHC profile as those who developed RA, to determine whether Class II antigens in RA are associated with the induction of symmetrical polyarthritis or with its persistence. Oligonucleotide probing of dot-blotted amplified DNA was used to determine the presence of allele specific sequences for DR1 and DR4. DNA from all DR4 positive individuals was reamplified with DR4 specific primers before assignment of subtypes: Dw4, Dw10, Dw13, Dw14 and Dw15.

There was, as expected, a marked excess of DR4 among the RA patients compared with 100 healthy Caucasian controls ($p < 0.001$) and also with the non-rheumatoid group in this study ($p < 0.05$). This difference was largely accounted for by an excess of Dw4. 17 of the 18 DR4 positive RA patients expressed the Dw4 allele, compared with 5 of the 9 DR4 positive patients with self limiting disease ($p < 0.01$). Dw14 was slightly raised in both synovitis groups (NS) DR1 was slightly elevated in patients with RA (NS). None of the patients in this study expressed Dw10, Dw13 or Dw15.

These findings suggest that the 3AHVR characteristic of Dw4 is associated with persistence but not the induction of RA; or alternatively synovitis in these two groups, though indistinguishable on presentation, arises by totally different mechanisms.

O 114 **INCIDENCE AND EXPRESSION OF PARVOVIRUS RA-1 DNA SEQUENCES AMONG ARTHRITIS PATIENTS AND HEALTHY SUBJECTS**, Robert W. Simpson[1], Jaya P. Gaddipati[1], Carol A. Smith[2] and Moti L. Tiku[3], Rutgers University, Waksman Institute, Piscataway, NJ 08855[1], Montefiore Hospital, Bronx, NY 10467[2] and Robert Wood Johnson University Hospital, New Brunswick, NJ 08903[3]

The RA-1 parvovirus originated from the synovium of a rheumatoid arthritis (RA) patient [SCIENCE 223:1425 (1984)]. This virus can cause permanent crippling, kyphosis, alopecia and other symptoms in rodents surviving neonatal infection. The physical map of the RA-1 viral genome and the DNA sequence of its major coding regions show that this virus is a unique member of the parvovirus family and shares closest DNA homology with a bovine parvovirus (BPV). Polymerase chain reaction (PCR) assays have been used for detecting RA-1 virus-specific DNA sequences in clinical specimens from human patients with various rheumatic disorders and from healthy controls. In a screening of more than 100 subjects, most of whom were RA patients, all persons tested positive for RA-1 target DNA sequences which are amplified by specific RA-1 DNA primer sets. The specificity of selected PCR product DNA was verified by sensitivity to restriction endonucleases unique for RA-1 DNA and unable to cleave replicative form DNA of such heterologous parvoviruses as BPV. Using PCR for quantitative measurements, preliminary findings suggest that RA-1 DNA is more highly represented in humans with overt rheumatic disease compared with apparently healthy persons. This DNA appears to be ubiquitous in humans.

O 115 ANTIBODIES AND T CELLS RESPONSES TO MYCOBACTERIAL PROTEINS IN RA AND JRA. Mouldy Sioud, Jens Kjeldsen-Kragh, Dag Sørskaar, Jacob. B. Natvig and Øystein Førre. Oslo Sanitetsforenings Rheumatism Hospital and Institute of Immunology and Rheumatology, University of Oslo, Oslo, Norway.

Recently T cell reactivity to 65 kDa mycobacterial HSP has received great interest in the pathogenesis of RA. In the present study we have used immunoblotting to search for antibodies against mycobacterial antigen in the sera of RA and JRA patients. In these conditions no detectable 65 kDa HSP antibodies were found in the 40 sera of RA and 15 sera of JRA patients. In contrast, immunoblotting has demonstrated a predominance of antibodies to mycobacterial proteins 18 kDa (P18) and 30 kDa (P30) in the majority of the sera of JRA and RA patients respectively. We have obtained a significant proliferative response of mononuclear cells from only synovial fluid to P18. Clones specific for P18 were also established. The data indicate that P18 and P30 may be candidate antigens in RA and JRA.

O 116 **STAPHYLOCOCCAL SEPTIC ARTHRITIS: PROTEOGLYCAN RELEASING FACTOR IS A GLUCOSAMINE-RICH GLYCOPROTEIN,** R. Lane Smith, Ph.D., G. Kajiyama, B.A., J. Lerman, B.A., R.J. Williams, III, B.A., David J. Schurman, M.D., Orthopaedic Research, R171, Stanford University School of Medicine, Stanford CA, 94305-5326.

Staphylococcal septic arthritis remains a serious complication in chronic rheumatoid arthritis and often results in residual joint damage in spite of proper treatment. In animal models, S. aureus joint infection results in rapid loss of cartilage proteoglycan. Cartilage degradation is induced in vitro by a soluble Staph Factor that requires living chondrocytes. We report here that Staph induction of proteoglycan release is stimulated by a soluble glucosamine-rich glycoprotein. Characterization of the active material was carried out by toluidine blue staining and radioisotope labeling with ^{14}C-glucose and ^{14}C-amino acids using polyacrylamide electrophoresis. Identification of glucosamine was by thin-layer chromatography. The presence of active Staph Factor is associated with multiple forms of toluidine blue staining material with the most active regions having a molecular weight range > 90 kDA. The active Staph Factor is a potent stimulator of stromelysin and collagenase and may be a significant contributor to final outcome in management of Staphylococcal septic arthritis in chronic rheumatoid arthritis.

O 117 **A DR4/DR1 RESTRICTED IMMUNODOMINANT EPITOPE ON A 19 KD MYCOBACTERIAL PROTEIN AND RHEUMATOID ARTHRITIS.** Paul L.J.Tan, Margot Skinner, Sarah Farmiloe, Judy Young and James D Watson. Department of Molecular Medicine, University of Auckland School of Medicine, Auckland, New Zealand.

Two Major histocompatibility complex (MHC) epitopes associated with DR4 (Dw4 and Dw14) and DR1 (Dw1) are known to contribute to the genetic basis of rheumatoid arthritis. Peptides which are DR4 or DR1 restricted may thus be used to analyse T cell responses dependent on the disease-associated MHC epitopes.

The 19kD protein from Mycobacterium tuberculosis is known to contain a DR4/DR1 restricted epitope. Peripheral blood lymphocytes (PBL) , synovial fluid (SF) and synovial tissue (ST) lymphocytes from rheumatoid patients were stimulated with eleven 20-residue, overlapping, synthetic peptides spanning the 159 amino-acid 19kD protein. After 48 hr culture, responses were assessed by the uptake of 3H-thymidine.

Results were consistent with a DR4/DR1 restricted site at the N-terminal (1-20) end of the protein. This site was also an immunodominant epitope as responses to other peptides were not detected in this assay. There was no difference in the responses of PBL from rheumatoid patients compared with healthy individuals. Likewise SF and ST lymphocytes were responsive only to the N-terminal peptide. Derivative peptides with critical amino-acid substitutions may serve as competitive inhibitors of antigens presented by an arthritis-associated MHC.

O 118 THE HLA CLASS II ASSOCIATION OF RHEUMATOID ARTHRITIS, Paul Wordsworth, Judith Stedeford, Jerry Lanchbury, William Rosenberg, John Bell. Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.
Conserved epitopes on certain HLA-DR molecules may be responsible for susceptibility to RA while a role for DQ is controversial. We have used a PCR-based oligonucleotide typing system to assign DR, DQ and DP genotypes to 249 patients with classic/definite RA and a large number of ethnically-matched controls. Significant associations were found with DR4 ($p < 10^{-6}$), DR1 ($p<0.001$) and DRw10 ($p=0.02$) and 89% of patients carried at least one of these antigens. In the remaining 11% there was no preponderance of other class II alleles (in particular no increase in DP2.1 or 3). Comparisons of 178 DR4-positive patients and 185 DR4-positive controls showed a disproportionate increase in Dw4 ($p=0.02$) and Dw14 ($p=0.05$) while there was a reduction in Dw10 ($p<0.01$) and Dw13 ($p=0.01$). Alleles of Dw14 with either glycine or valine at position 86 of the DRβ chain were associated with RA. In DR4-positive RA there was no association with particular DQ alleles (DQw7 was 50% in patients and controls). Furthermore in a small group with Felty's syndrome Dw4 was more strongly associated (12/12) than DQw7 (8/12). These data strongly support the importance of the Dw4-related epitope (DRβ67-74) a the main factor influencing both susceptibility to and severity of RA.

O 119 A SUPERANTIGEN MODEL FOR ARTHRITIS IN Vβ8 TCR TRANSGENIC *lpr/lpr* MICE, Tong Zhou, Hörst Bluthmann[1], Renate E. Gay, Carl K. Edwards, III[2], and John D. Mountz, Dept. of Medicine, The University of Alabama at Birmingham, Birmingham, AL 35294. [1]Hoffmann LaRoche, Basel, Switzerland, [2]Dept. of Immunology, Marion Merrell Dow, Inc., Kansas City, MO 64137. Certain bacterial products have the ability to combine with class II MHC and stimulate a wide spectrum of T cells specificities through the TCR Vβ chain. T cells expressing the Vβ8 TCR can be stimulated by staphylococcal enterotoxin B (SEB) superantigen. To determine if the SEB superantigen can lead to a chronic arthritis, Vβ8 TCR transgenic I-E$^+$ MRL-+/+ and MRL-*lpr/lpr* mice were injected intra-articular (IA) with 10-fold dilution of SEB ranging from 10 mg to 10 ng. Nearly all of the T cells in the TCR transgenic mice were Vβ8$^+$ and should be capable of interacting with SEB. Although both Vβ8 TCR transgenic MRL-+/+ and MRL-*lpr/lpr* mice developed arthritis within 3 weeks of injection of 100ug and 10 ug of SEB, the arthritis was more severe in the *lpr/lpr* mice. To determine if the severity of arthritis was related to a defect in T cell tolerance, TCR transgenic *lpr/lpr* and +/+ mice were treated with SEB from birth to 2 weeks of age. Although clonal deletion occurred equally in *lpr/lpr* and +/+ mice, the remaining Vβ8 T cells in *lpr/lpr* mice proliferated in response to Vβ8 cross linking by anti-Vβ8 antibody or SEB, whereas T cells from +/+ mice were anergic. Furthermore, neonatal tolerance induction in the T cells in *lpr/lpr* mice was completely lost 2 weeks after the last SEB injection, whereas +/+ T cells remained anergic for at least 2 months. These results suggest that the SEB superantigen can induce a chronic arthritis after a single IA in mice. Furthermore, the severity and chronicity of arthritis is related to a T cell tolerance defect in *lpr/lpr* mice. We propose that in certain predisposed individuals with a T cell tolerance defect, superantigen released during a systemic bacterial or viral infection can lead to a chronic inflammatory arthritis such as RA.

T Lymphocytes/Autoantibodies in RA

O 200 RESISTANCE TO COLLAGEN-INDUCED ARTHRITIS IN RHESUS MONKEYS: A ROLE OF CD8+ CELLS AND IL2 IN T CELL LOW-RESPONSIVENESS, Nicolaas P.M. Bakker, Monique van Erck, Margreet Jonker, Bert A. 't Hart, Institute for Applied Radiobiology and Immunology TNO, P.O.Box 5815, 2280 HV Rijswijk, the Netherlands.

The induction of experimental arthritis in rhesus monkeys was studied by intradermal immunization of bovine type II collagen (B-CII) and with cell membranes derived from mycobacterium tuberculosis, Streptococcus pyogenes and Eubacterium aerofaciens. The tested bacterial antigens proved to be not arthritogenic (1).

Ten out of fourteen rhesus monkeys developed arthritis after a single immunization with bovine type II collagen (B-CII), while four animals were fully resistant to the disease. In contrast to resistant monkeys, arthritic animals showed during the induction phase of arthritis a B-CII specific T cell proliferation and anti-B-CII antibodies, which were predominantly of the IgM isotype (2). Four arthritic animals were euthanized for ethical reasons. The surviving arthritic animals showed a full remission of the disease. Two monkeys acquired complete resistance to arthritis after one period of disease, but in three animals a booster immunization with B-CII induced a slight flare-up. It has been demonstrated that rhesus monkeys have the capacity to develop or restore resistance to arthritis and that resistance is reflected at the level of T cell low-responsiveness to B-CII in vitro. In the 'flare-up' animals the cellular non-responsiveness can be restored by depletion of CD8+ cells or by adding exogenous IL2. These findings suggest a role for both in the T cell low-responsiveness to B-CII.

1) Bakker NPM, van Erck MGM, Zurcher C, Faaber, P, Lemmens A, Hazenberg M, Bontrop RE, Jonker M (1990); Rheumatol.Int. 10,21
2) Bakker NPM, van Erck MGM, Botman MGM, Jonker M, 't Hart LA (1990); Arthritis Rheum., accepted for publication

O 201 PRE-B CELLS IN THE PERIPHERAL BLOOD OF RHEUMATOID ARTHRITIS (RA) PATIENTS. Marie C. Béné, Francis Guillemin, Jacques Pourel, Alain Gaucher and Gilbert C.Faure. Lab Immunology and Clinique Rhumatologique B, CHU & University of Nancy, 54500 Vandoeuvre les Nancy, France.

One of the early maturation stages of B-cells in the bone marrow is characterized by the presence of intracytoplasmic mu chains (c-mu+), without light chains and surface immunoglobulins (s-mu-). We investigated the presence of such cells among peripheral blood lymphocytes (PBL) in 92 RA patients and 23 controls. Cytospins of PBL were fixed in cold ethanol, rehydrated, and stained with monospecific fluorescent antibodies to mu, kappa or lambda chains. Nine of the controls had small numbers of c-mu+ cells (mean 1.6% of PBL). The mean value for the whole group of RA patients was 4.8% of PBL, with 58% of positive patients (p=.0002). Light chains were not observed in these cells, suggesting that they were truly pre-B. The absence of surface immunoglobulins was further confirmed by double immunofluorescence for sIg and c-mu. Magnetic beads were used to enrich or deplete PBL in CD19+ cells. This method confirmed that the c-mu+ cells observed belonged to the B-lineage and expressed this membrane antigen which disappears on later stages of B-cell maturation. Investigation of the patients' clinical features further indicated that the presence of pre-B cells in the PBL of RA patients appears as a new immunologic feature of RA of early onset.

O 202 CHARACTERIZATION OF T CELL RECEPTOR GENES OF A COLLAGEN TYPE II REACTIVE HUMAN T LYMPHOCYTE CLONE. Wolf M. Bertling, Thomas Ritter, Tan Yan, Barbara Bröker, Klaus von der Mark, Frank Emmrich, Clin. Res. Units, Rheumatology, Max-Planck Soc. at the University of Erlangen, Schwabachanlage 10, 8520 Erlangen, Germany

There is evidence, that T cells are involved in the pathogenesis of chronic joint inflammation in animal models as well as in human reactive and rheumatoid arthitis (RA). However, no specific antigen is known in RA. Cartilage components are considered as source of putative autoantigens. One of the major structural elements of joint cartilage, collagen type II (CII), might be responsible for such an autoimmune reaction.

Recently we have cloned and characterized a HLA-DR restricted T-cell specific for human collagen type II (see also Poster Burkhardt et al.). A cDNA clone representing the TCR β-chain of this clone could be isolated by PCR and was sequenced. An antibody directed against the subfamily Vβ6 reacted positive. The analysis of the sequence revealed that the V-region of the active receptor is a member of the subclass 6.7, and that the C1-isotype and a not yet described D-element are used.

More Collagen II specific T-cells will have to be screened to answer the question if CII specific TCRs are restricted to one or a few V-, D, or J-families.

O 203 TOLERANCE SUSCEPTIBILITY OF CD5+ VERSUS CD5- B CELLS, K. H. Brooks, C. Banotai and L. Lindesmith, Department of Microbiology, Michigan State University, East Lansing, MI 48824

The CD5+ B cell has an, as yet, unknown role in systemic autoimmune diseases such as rheumatoid arthritis (RA). The size and activation status of this minor B cell subset have recently been correlated with disease activity in RA. In the context of determining the signals regulating activation and Ig secretion by this subset, we have compared the ability of anti-immunoglobulin (Ig) to inhibit growth of CD5+ and CD5- neoplastic B cell clones. Anti-Ig-mediated growth inhibition has been used for several years as a model for the mechanistic dissection of tolerance induction. However, to date, all of the cell lines used have been found to be CD5+ and thus may not be representative of the entire B cell spectrum. We found that two murine CD5- B cell clones derived from the same spontaneous tumor were significantly less sensitive to anti-Ig-mediated inhibition than the BCL_1-3B3 cells, a CD5+ neoplastic, murine B cell clone. The growth of the BCL_1-3B3 cells was 90-95% inhibited by 25 μg/ml goat anti-mouse Ig whereas ^{3}H-thymidine incorporation by the CD5- clones 225-11 and 225-2 was only inhibited approximately 50%. This difference was not due to any differences in the kinetics of growth inhibition. The possible significance of enhanced susceptibility to antigen-induced growth inhibition in the CD5+ B cell is discussed.

O 204 AN AUTOANTIBODY TO COLLAGEN TYPE II INTERFERES WITH PROCESSING OF THE ANTIGEN THUS GENERATING AN INHIBITORY PEPTIDE FOR AUTOREACTIVE HUMAN T CELLS,

Harald Burkhardt, Tan Yan, Annette Beck-Sickinger, Rikard Holmdahl, Klaus von der Mark, Frank Emmrich, Max-Planck-Gesellschaft, Klinische Arbeitsgruppen für Rheumatologie am Institut für Klinische Immunologie, Medizinische Klinik III, der Universität Erlangen-Nürnberg, D-8520; University of Uppsala (R.H.); Universität Tübingen (A.B.-S.)

Cartilage components are considered as source of antigens that could continuously fuel an autoimmune arthritis. One of the most interesting candidate autoantigens whose occurrence is restricted to joint cartilage is collagen type II (CII). In general, it is very difficult to establish and to maintain human CII-reactive T cell clones for detailed analyses.

Here we describe a human CD4+ T cell clone that uses HLA-DR7 as restriction element and recognizes an epitope within the CNBr-fragment 11 of human CII. Fragment 11 is the only CNBr-fragment proven to be arthritogenic in rodent arthritis models. By investigating two potentially disease-related anti-CII monoclonal antibodes (mAb), we made an unexpected observation. One of the mAb (C1) inhibited the proliferative T cell response to CII very effectively while the other (D3) did not. The antibody C1 recognizes a conformational epitope within the CNBr-fragment 11. Inhibition was not observed by using denatured instead of native CII together with the C1 antibody - a finding that argues against direct inhibition of T cell funtion or monocyte function by the antibody. Compatible with all experimental findings is the idea that the C1 antibody would protect a collagen sequence sensitive to regular lyosomal degradation thereby giving rise to a novel peptide which is presented by HLA-molecules and competes with the stimulatory peptide. Ideed, two homologous CII-sequences were identified which may comprise the proposed HLA-binding site shared by both peptides. The putative inhibitory peptide was synthesized and is in fact able to inhibit a CII-mediated proliferative T cell response as predicted by the concept. Put in a more general way, the conclusion would be that autoantibodies to collagen type II may be able to block autoreactivity of T cells to the very same molecule.

O 205 SHARED DOMINANT REARRANGEMENTS AMONG DIFFERENT CLONES DERIVED FROM RHEUMATOID SYNOVIAL TISSUES. May Chatila and James Kurnick, Arthritis and Pathology Research Units. Massachusetts Gen. Hosp., Boston, MA. 02114. Rheumatoid arthris (RA) synovial tissue infiltrating T cell clones were evaluated for T cell receptor β (TCRβ) gene rearrangement. In one RA patient 2 of 21 clones matched the TCR-β gene rearrangements of the corresponding bulk culture (using 5 different restriction endonucleases). More importantly, up to 40% of the clones from this and additional RA patients shared one of the dominant bands with the bulk culture. These findings reaffirm previous studies indicating dominant TCRβ gene rearrangements among syovial infiltrating cells, but imply that multiple clones with shared rearrangements are likely to contribute to the non-germline restriction fragments. The original suggestion of oligoclonality is probably more appropriately referred to as TCRβ restriction fragment sharing, suggesting selective TCR gene segment usage among the infiltrating cells. PCR amplification with Vα and Vβ primers followed by DNA sequencing are being used to further validate these observations and to determine the extent of sharing among TCR gene elements.

O 206 A MONOCLONAL ANTIIDIOTYPE DEFINES A SHARED DETERMINANT ON RHEUMATOID FACTORS FROM PATIENTS WITH RHEUMATOID ARTHRITIS. Anne Davidson, Dept. of Medicine, Albert Einstein College of Medicine, Bronx, New York, 10461.

Genetic analysis of human rheumatoid factors (RF) have focused on the monoclonal RF produced by malignant B cells. However, crossreactive idiotypes shared by these monoclonal proteins are present on only 1-2% of polyclonal RF found in patients with rheumatoid arthritis (RA). By immunizing mice with purified polyclonal IgM RF from a patient with classic RA, we have generated a new antiidiotype to human RF (4C9). Using an ELISA in which equivalent amounts of IgM are bound to the plate, 4C9 reactivity is high (more than 4 SD over normal) in the serum of 31/35 seropositive RA patients and is correlated with RF titer. 4C9 reactivity is absent in 12/13 seronegative RA patients and present in low titer in 1/13. 4C9 reactivity is found in the serum of 8/16 RF positive SLE patients but is found, in low titer, in only 1/22 sera from patients with malignant RF. 4C9 activity is found on purified IgM RF but not on non-RF IgM from the original patient and 7 other patients tested. Semi-quantitative assays demonstrate that 4C9 is expressed on more than 20% of RF in the serum of the original patient. Thus 4C9 defines a shared determinant on RF in RA patients different from those previously described for monoclonal RF. We have now generated a RF-positive 4C9-positive EBV transformed B cell line from a patient with RA. Genetic analysis of this line reveals that the light chain is encoded by a VkIII gene most homologous to Vk3g whereas the heavy chain is encoded by a member of the VH4 gene family. Thus, molecular analysis of this 4C9-reactive RF demonstrates that it is encoded by different genes than those encoding malignant RF.

O 207 RHEUMATOID ARTHRITIS SYNOVIAL FLUID LYMPHOCYTE RESPONSES TO COLLAGEN: REQUIREMENT FOR EXOGENOUS IL-2. Robert O. Endres, Department of Medicine and Department of Microbiology and Immunology, University of Tennessee, Memphis, TN 38163.

Mononuclear cells from rheumatoid arthritis (RA) synovial fluids were tested for their ability to proliferate in response to six types of collagen that are known to be present in either synovial membrane or cartilage (types I, II, III, IV, V, IX, and XI). In the absence of exogenous IL-2, no responses were observed to any of the collagens used. In the presence of IL-2 alone, proliferation increased due to the existence of IL-2 receptors on a portion of the synovial T cells. When collagen and IL-2 were added together, synergistic effects on proliferation were seen. Synovial cells from most patients exhibited IL-2-dependent responses to more than one type of collagen, but the patterns varied between individuals. All six types of collagen stimulated IL-2-dependent responses in cells from at least some patients. Peripheral blood mononuclear cells from normal individuals did not exhibit synergistic responses with any type of collagen and IL-2. Our results suggest that cell mediated immune responses to collagen are occurring in the synovium of RA patients, but that these responses (at least in vitro) are characterized by insufficient IL-2 production by the predominantly CD29+ T cells. Sequence homologies between different types of collagen show the potential for shared T cell epitopes among multiple types as well as unique epitopes. Our data indicate that individual patients exhibit different patterns of collagen epitope recognition, which has implications for the search for common T cell receptor usage in this disease. This work is supported by Specialized Center of Research grant AR-39166 from the National Institutes of Health.

O 208 GENETIC CONTROL OF ARTHRITIS IN lpr CONGENIC MICE, Gilkeson, G.S., Ruiz, P., Kurlander, R., Pritchard, A.J., Pisetsky, D.S., Medical Research Service, Durham VA Hospital and Duke University Medical Center, Durham, NC 27705

MRL-lpr/lpr mice spontaneously develop a complex autoimmune disease characterized by immune complex glomerulonephritis, vasculitis, and inflammatory arthritis. These mice also produce anti-DNA antibodies as well as IgM and IgG rheumatoid factors. Previous reports from our laboratory and others have demonstrated that both the lpr gene and another gene(s) in the MRL background are necessary for the development of clinical manifestations of autoimmune disease. In contrast, the lpr gene alone appears sufficient to induce autoantibody production. To determine if the same genetic features determine the expression of both arthritis and renal disease in lpr mice, F_1 mice obtained by crossing B6-lpr/lpr mice with MRL-lpr/lpr mice were studied. The number of mice in each group with pathologic involvement of the synovium and glomeruli at 6-7 months of age was determined by a pathologist blinded as to the group of origin.

	synovial hypertrophy	glomerular pathology
MRL-lpr/lpr	13/17	17/17
B6-lpr/lpr	0/14	0/14
MB-lpr/lpr	14/28	7/21
BM-lpr/lpr	9/22	5/22

Synovial involvement in the F_1 mice was similar in severity to that of MRL-lpr/lpr mice. In contrast, renal involvement in the F_1 mice consisted of minimal focal glomerular hypercellularity as opposed to crescentic glomerulonephritis in the majority of the MRL-lpr/lpr mice. Only one F_1 mouse had both renal and synovial abnormalities. Furthermore, the presence or titer of autoantibodies did not correlate with either arthritis or renal disease.These results indicate that renal disease and arthritis develop independently in these mice possibly on a genetic basis and that the incidence and degree of arthritis in F_1 mice suggests a role for a dominant gene.

O 209 ANTIGENIC SPECIFICITY OF γδ T CELLS FROM RHEUMATOID ARTHRITIS SYNOVIAL FLUID, Joseph Holoshitz, Dawn R. McKinley and Nancy K. Bayne, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109-0531

The antigenic specificity of γδ T cells and the role of MHC molecules in antigen presentation to these cells are still elusive. We have previously reported a panel of $CD4^-CD8^-$ γδ T cell clones isolated from the synovial fluid of a patient with rheumatoid arthritis. These cells proliferated in response to a mycobacterial antigen without MHC restriction. Further studies have shown that a clonal cell population, whose monoclonality was confirmed by T cells receptor Southern blot analysis, displayed two distinct proliferative responses: an MHC-unrestricted proliferative response to a non-mitogenic mycobacterial antigen, and a DR-restricted proliferative response to another antigenic peptide. Both reactivities were mediated by the T cell receptor and could be effectively blocked by anti-LFA-1monoclonal antibodies. In addition to their dual proliferative reactivity, the γδ T cell clones displayed MHC unrestricted cytotoxic activity against various tumor cell lines including NK targets.
These results indicate that a clonal population of γδ T cells can display multiple reactivities. These results further indicate that similar to T cells bearing the αβ receptor, γδ T cells recognize antigenic peptides presented by class II MHC. In addition to their reactivity to a nominal anitigen, some γδ T cells respond to a mycobacterial antigen which resembles a superantigen. The potential relevance of these findings to the pathogenesis of rheumatoid arthritis will be discussed.

O 210 INTERACTION OF TYPE II COLLAGEN ANTIBODIES WITH ARTICULAR CARTILAGE SURFACES, Hugo E. Jasin and Joel D. Taurog, Rheumatic Diseases Division, University of Texas Southwestern Medical Center, Dallas, TX 75235
These studies deal with the availability of cartilage collagen epitopes for interaction with antibodies at the surface of articular cartilage. Intact rabbit patellas or bovine cartilage slices were incubated with rat antibodies to collagen types II, V, VI, and IX. After washing, surface-bound antibodies were quantitated with ^{125}I-goat anti-rat Ig. Results are expressed in pg anti-Ig bound/mg tissue. Surprisingly, more anti-type V antibodies were bound to intact articular cartilage than anti-collagen type II. The latter antibodies bound no more than control rat sera (anti-type V: 16.3±1.6; anti-type II: 6.9±1.1; control: 9.4±1.8). Anti-type II antibodies binding increased significantly in articular cartilage obtained from rabbits with acute antigen-induced arthritis (anti-type V: 17.7±1.7; anti-type II: 17.9±3.0; control: 6.3±1.1). In vitro incubation of cartilage with as little as 100 PMN/mm^3 for 1 hr resulted in a significant increase in antibody binding, which increased linearly with increasing concentrations of cells. In vitro incubation with human PMN lysates resulted in 564% increase in antibody binding over control. Collagen anti-type II antibody binding was not significantly increased by incubation with hypochlorous acid or hyaluronidase, or by cartilage matrix degradation mediated by cytokine-induced chondrocyte activation in organ culture. Antibody binding increase mediated by PMN lysates was almost completely inhibited by serine-esterase inhibitors and by a specific neutrophil esterase inhibitor (peptidyl-chloromethylketone). Finally, a 5-fold increase in antibody binding was obtained by incubation of cartilage with purified human neutrophil elastase. These results indicate that a serine-esterase-susceptible protein/s present at the surface of intact articular cartilage prevent interaction of collagen type II with antibodies. Disruption of this protein layer early in inflammatory arthritis may lead to increased permeability to macromolecules and further tissue damage.

O 211 DEVELOPMENT OF AN ANTIGEN-SPECIFIC MICROCULTURE T-CELL COLONY ASSAY, Edward C. Keystone, Lorraine Poplonski, Krista M. Snow and Chris J. Paige, The Wellesley Hospital and Ontario Cancer Institute, Toronto, Canada M4Y 1J3
Evidence has accumulated implicating T-cell hypersensitivity in the pathogenesis of many rheumatic diseases. Therefore, we have developed a limiting dilution microculture T-cell cloning technique to generate antigen-specific T-cell clones. Purified peripheral blood (PB) T-cells obtained by E-rosetting were seeded in limiting dilution numbers in round-bottomed microwells containing 10^5 irradiated PB mononuclear cell feeders in Aim V media supplemented with 5% AB serum. Cultures were further supplemented with recombinant IL-2 (Cetus) at 48 hours and 7 days. Additional feeders (10^4) were added at 7 days. Colonies were enumerated microscopically at 14 days. Under these conditions no colonies were detected in the absence of PHA or antigen. The results of limiting dilution studies revealed that the data fit the limiting dilution model of Porter and Berry (Brit. J. Cancer 17:583, 1963). Studies of cloning efficiency using PHA activated T-cells in 5 experiments revealed the mean frequency of colony-forming cells to be one cell in 1.98 with a range between 1/1.07 and 1/3.35. The mean precursor frequency of tetanus toxoid (TT) reactive T-cells was 1/2528 in 8 healthy subjects. The average colony size determined from 19 randomly selected colonies ranged from 1.5 to 5.4 x 10^5 cells per colony.
Restimulation of 14 days T-cell colonies revealed marked proliferation to PHA and TT but only background responses to PPD.
These studies demonstrate the development of a rapid antigen-specific liquid culture T-cell cloning system which is likely to detect T-cell clones that are representative of the original T-cell population. As well, we have generated clones of sufficient size to be useful in studies of antigen cross-reactivity in rheumatic diseases.

O 212 FIBROBLASTS MEDIATE RETENTION OF T CELL VIABILITY: A PROPOSED MECHANISM FOR IMMUNOLOGICAL MEMORY. James T. Kurnick, Sumi Scott, Franco Pandolfi, Dept. of Pathology, Massachusetts General Hospital, Boston, MA 02114
Synovial fibroblasts and the conditioned medium from these and other stromal cell are responsible for a salvage pathway whereby activated T lymphocytes revert to non-proliferating cells in the absence of antigen or mitogenic signals. In the absence of mitogenic cytokines, T lymphocytes cease dividing and rapidly begin to die. However, the addition of synovial fibroblasts results in both enhanced responsiveness to IL-2, and to the prolonged survival (over 8 months) of the previously activated T lymphocytes in the absence of proliferation. Survival is enhanced on both CD4+ and CD8+ subsets and clones of T cells from different sources and different specificities. Preservation for up to 7 weeks is also achieved by the culture of IL-2 propagated lymphocytes in conditioned medium from the fibroblasts. The surviving cells retain specificity and function. The prolonged survival of the activated T cells is not accounted for by IL-1 to 7 or PGE_2. The studies suggest a stromal cell-mediated, antigen non-specific mechanism for survival of memory T lymphocytes in a non-proliferating state at sites of inflammation and in lymphoid tissues.

O 213 ANTIGEN SPECIFIC CYTOTOXIC T LYMPHOCYTES IN INFLAMMATORY SYNOVIAL TISSUES AND SYNOVIAL FLUIDS OF RHEUMATOID ARTHRITIS PATIENTS. Shu Guang Li, Yamin Shen, Alison Quayle, Jens Kjeldsen-Kragh, Øystein Førre and Jacob B. Natvig, Institute of Immunology and Rheumatology, University of Oslo, Fr. Qvamsgt. 1, N-0172 Oslo 1, Norway

BCG specific T cell lines and clones raised from the inflammatory synovial tissues (ST) or synovial fluids (SF) of RA patients were tested for their cytotoxic capacity. Autologous adherent monocytes from the ST/SF or peripheral blood (PB) were used as the targets. BCG lines from all patients tested so far showed specific lysis of the adherent autologous ST or PB monocytes pulsed with BCG, but not control antigens such as tetanus toxoid. Interestingly, from one RA patient, BCG specific T cell lines driven from both ST and PB did not lyse BCG pulsed adherent PB monocytes, in contrast, they did lyse BCG pulsed adherent ST monocytes. These long-term-cultured, IL-2 dependent BCG cell lines did not have NK activity or LAK activity, since they did not lyse K562 cell line. The majority of these BCG specific cytotoxic T lymphocytes (CTL) from the synovial compartment are $CD4^+$ cells. The data suggest that antigen specific CTL may play a role in the pathology of RA.

O 214 SURFACE PHENOTYPE AND T CELL RECEPTOR ANALYSIS OF RHEUMATOID SYNOVIAL TISSUE LYMPHOCYTES. Peter E Lipsky, Allan D Duby, Ellis Lightfoot, John J Cush. Univ. of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235
Lymphocytes eluted from the synovial tissue of rheumatoid arthritis (RA) patients were analyzed for surface phenotype and T cell receptor rearrangement patterns. Ten patients with a mean disease duration of 14 yrs. and active, erosive arthritis were studied. 6 of 9 patients were HLA-DR4(+). Peripheral blood samples from 7 healthy volunteers and from 8 of the patients were analyzed for comparison. Synovial tissue was digested with collagenase to obtain mononuclear cells and these were enriched for synovial T cells by passage over nylon wool columns. Nearly 50% of the freshly eluted synovial cells were CD3+ T cells, of which the majority were CD4+. The percentage of CD4+/CD29+ memory T cells was markedly increased and the percentage of CD4+/CD45RA+ naive T cells was markedly reduced when compared to autologous and control PBL. No enrichment for CD8+/CD57+ T cells or CD5+/CD20+ B cells was observed in the synovium. In addition, synovial lymphocytes were enriched in HLA-DR+, LFA-1 (CD11a+/CD18+) bright cells and cells expressing VLA-1. To examine whether T cells infiltrating the rheumatoid synovium were oligoclonal, T cells were cloned at 0.3 cells/well with PHA, irradiated feeder cells and IL-2. Using this technique, a mean cloning efficiency of 48.3% was achieved (range from 10-86.5%). 144 synovial tissue and 132 peripheral blood T cell clones were generated from 8 of these RA patients. All of the synovial clones were CD3+, 61% were CD4+, and 24% were CD8+. Genomic DNA was extracted from each T cell clones and analyzed for T cell receptor gene rearrangement patterns using $C_{\beta 1}$ and $J_{\beta 2}$ probes after restriction enzyme digestion with EcoRI. Three pairs of the synovial T cell clones demonstrated identical rearrangement patterns, although each pair was unique. A similar degree of *oligoclonality* was manifest by peripheral blood T cell clones. Thus, although the predominant cell in the rheumatoid synovium is the CD3+, CD4+, CD29+, HLA-DR+ activated, memory T cell that expresses VLA-1, no oligoclonality was observed. Therefore, the majority of cells in the rheumatoid synovium are unlikely to be the progeny of individual antigen-reactive T cells.

O 215 THE IDENTIFICATION OF T CELL EPITOPES OF TYPE II COLLAGEN IMPORTANT IN MURINE COLLAGEN-INDUCED ARTHRITIS, Linda K. Myers, Jerome M. Seyer, James M. Stuart and Andrew H. Kang, Departments of Pediatrics and Medicine, University of Tennessee, Memphis, Memphis, TN 38163
Collagen-induced arthritis is an autoimmune disease induced by immunizing susceptible animals with type II collagen (CII). In order to identify epitopes important for disease, we cleaved type II collagen with CNBr and purified the resulting peptides. When peptides were then used to immunize animals to test for the ability to induce disease, only α1(II)-CB11 was able to induce arthritis in DBA/1 mice. In order to determine immunogenic epitopes recognizable by T cells, polypeptides 22-26 amino acid residues in length were generated which correlated with the sequence of α1(II)-CB11. Pooled splenocytes and lymph node cells from immunized DBA/1 mice were then utilized in proliferation assays and lymphokine assays to determine immunogenicity of the peptides. Four separate T cell epitopes of α1(II)-CB11 were then identified. In order to determine which epitopes play a role in arthritis, these same polypeptides were then given to neonatal mice as toleragens before the mice were subsequently immunized with type II collagen. We have used this system previously to identify α1(II)-CB11 122-147 as a major immunogenic epitope. By using polypeptides representing the entire length of α1(II)-CB11 as toleragens, the same four critical regions were again identified as having the ability to suppress arthritis. We are in the process of fully characterizing the critical residues of these epitopes using overlapping peptides and peptides with substitutions in critical regions.

O 216 PREVALENCE AND POLYMORPHISM OF HUMAN FETALLY EXPRESSED HUMAN GERMLINE GENES, Eric H. Sasso, Ko Willems van Dijk, Andrew Bull, and Eric C.B. Milner, Virginia Mason Research Center, and the University of Washington, Seattle, WA 98101

Variations in the germline V_H repertoire may underlie individual differences in predisposition to autoimmune disease. We have thus compared the germline representation of the fetally expressed germline elements 56p1 (V_H3 family) and 51p1 (V_H1) with those of the highly homologous germline elements hv3005 and 1.9III (V_H3) and hv1263 (V_H1), respectively. These elements have been implicated in both RF and anti-DNA Ab.

Employing sequence-specific oligonucleotide probes (ONP), 56p1 and hv3005 were localized to comigrating 5.0 kb TaqI fragments. 1.9III was found on a 3.5 kb TaqI fragment. The prevalences of 56p1, hv3005, and 1.9III among 52 unrelated individuals were 62%, 35%, and 92%, respectively. The distribution of 56p1, hv3005, 1.9III indicated they cannot be alleles of a single locus, and 56p1 and 1.9III are probably not alleles of each other. These 3 elements were cloned and sequenced from a single individual, and by family segregation assigned to [56p1,1.9III], and [hv3005] haplotypes. Thus, while V_H3 elements are commonly polymorphic, single V_H3 loci appear to be dominated by 2-4 alleles.

ONP analysis of the 51p1 and hv1263 sequences in genomic DNA of 48 individuals found several coding region varients occuring on 6 different restriction fragments (RF). Two of the prevalent 51p1-related RF were in linkage disequilibrium, and likely represent a gene duplication. The distribution of all elements detected by the 51p1- and hv1263-derived probes was non-random, and strongly suggested that 51p1, hv1263, and the related elements are alleles of either a single locus, or a neighborhood of closely linked loci.

O 217 OCCURRENCE OF IgG GALACTOSYLATION DEFICIENCY PRIOR TO THE ONSET OF RHEUMATOID ARTHRITIS (RA), Ralph E. Schrohenloher, Milan Tomana, William J. Koopman, Antonio del Puente and Peter H. Bennett, The University of Alabama at Birmingham, Birmingham, AL 35294 and NIH, Phoenix, AZ 85014
Previous work has shown that deficient galactosylation of serum IgG occurs in RA and other autoimmune disorders. Subsequent studies of families characterized by high frequencies of autoimmune diseases revealed that deficient galactosylation of IgG can also occur in the absence of clinical disease among family members with autoantibodies as well as those with normal serologies and raised the possibility that defects in IgG galactosylation may be a risk factor for development of autoimmune disease. In order to further investigate the relationship of IgG galactosylation to disease status, the glycosylation of serum IgG was examined by gas chromatography in Pima Indians enrolled in an NIH epidemiological study. The mean galactose content of the serum IgG from 11 RA patients collected prior to disease onset was significantly lower than that of serum IgG from seronegative controls without RA from the same population ($P=0.05$). This difference became more significant in the RA-onset ($P=0.02$) and post-onset ($P=0.002$) sera; however, differences in the mean IgG galactose content obtained prior to, at, or post RA-onset were not significant. In addition, the mean serum IgG galactose contents of the RA patients did not differ from those of seropositive Pima controls who did not develop RA over the course of the study. These results indicate that deficient galactosylation of IgG can occur prior to the onset of RA and support the contention that IgG galactose deficiency is a risk factor for development of RA.

O 218 ANTI-RA33 – A NEW DIAGNOSTIC MARKER FOR RHEUMATOID ARTHRITIS: PURIFICATION OF THE ANTIGEN AND FIRST FUNCTIONAL STUDIES, Günter Steiner, Wolfgang Hassfeld, Aleksandra Sinski, Karl Skriner and Josef Smolen, 2nd Department of Medicine, Lainz Hospital, Vienna, Austria

Recently we have described autoantibodies directed against a nuclear protein of molecular weight 33 kD (RA33) which were present in about 35% of sera from rheumatoid arthritis (RA) patients but in less than 1% of various controls. Further studies revealed that anti-RA33 was frequently associated with the occurrence of antibodies directed against three additional nuclear antigens in the molecular weight range of 31–37 kD. We have purified these proteins to approximately 80% homogeneity by a combination of chromatographic procedures including anion exchange chromatography, affinity chromatography on Heparin–Sepharose, preparative gel electrophoresis and immuno–affinity chromatography. There was no demonstrable association of these antigens with nuclear RNA or with the core proteins (snRNPs) of the nuclear pre–mRNA splicing machinery. Affinity purified anti-RA33 crossreacted with the other antigens demonstrating a strong immunological relationship between the proteins. In sucrose gradient density centrifugation experiments performed with DNase/RNase treated nuclear extracts the RA specific antigens were found both in the top layer and in the pellet. Based on these findings we hypothesize that RA33 and the related antigens may be associated with a large nuclear structure. Their chromatographic properties indicate that they are probably basic proteins which might be associated with DNA. It is not yet clear whether these antigens are transcribed from individual genes, or generated by an alternate splicing mechanism, or whether they are degradation products derived from a larger precursor. It is also unknown which role the autoantibodies against these proteins play in the pathogenesis of RA. Currently, work is in progress to purify the proteins to homogeneity in order to obtain sequencing data and information about their structure and function. Future experiments using the purified antigens should help us to find answers to the many open questions mentioned above.

O 219 NEUTROPHIL OXYRADICAL PRODUCTION MIGHT FORM THE CONNECTION BETWEEN (AUTO)IMMUNITY AND ARTHRITIS IN HETEROLOGOUS TYPE II COLLAGEN-IMMUNIZED RATS, Bert A. 't Hart and Nicolaas P. M. Bakker, Department of Chronic and Infectious Diseases, Institute for Applied Radiobiology and Immunology TNO, P.O. Box 5815, 2280 HV Rijswijk, the Netherlands.

In a high proportion of patients with rheumatoid arthritis immunity to type II collagen (CII) has been found. The causal relation between this immunity and the disease, however, is still uncertain. Collagen-induced arthritis (CIA) is a suitable experimental animal model to dissect which factors from the CII-specific immunity are involved in the pathogenesis of arthritis. Recent results from our group demonstrate that on the one hand CII-specific IgM antibodies together with T cells (1) and on the other neutrophil-derived oxyradicals (2) are involved in the triggering of CIA. A short treatment of CII-immunized rats (between day 9 and 23 after immunization) with apocynin, a selective inhibitor of the neutrophil oxidative burst (3), dramatically reduced the severity and incidence of CIA (2). Interestingly, no flare-up of the disease was observed after termination of the treatment, although cellular and humoral (auto)immunity to CII was not affected by apocynin. On the basis of these data we conclude that neutrophil oxyradical production early in the disease induces processes which have a profound effect on the course of the disease. One possible mechanism is the enhanced exposure of self CII by the cartilage destructive capacity of oxyradicals. Furthermore, we provide evidence that oxyradicals induce the expression of the 65 kD heat-shock protein, which is considered as a target antigen of the immune system in the chronic phase of the arthritis. Thus, oxyradical production by neutrophils which have chemotactically migrated towards the joint-cartilage as a consequence of (auto)immune processes adjacent to the cartilage, might incite the arthritis.

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O 220 ABERRANT SURFACE IgM-MODULATION IN HUMAN RHEUMATOID FACTOR-EXPRESSING B CELL LINES. Doris B. Tse, Ivan Robbins, Glenn Krasinski and Nicholas Chiorazzi. Division of Rheumatology, Allergy-Clinical Immunology, Department of Medicine, North Shore University Hospital-Cornell University Medical College, Manhasset, NY 11030.

An EBV-transformed B cell line (Q-34) obtained from the spleen of a patient with hypergammaglobulinemic purpura was identified as rheumatoid factor-positive by reactivity of secreted Ig with rabbit gamma globulin ($R_\gamma G$) in solid phase enzyme immunoassay. FACS analyses of these cells modulated at 37° with goat anti-human Ig, IgM, IgD or IgM and IgD ($G_\alpha HIg$, $G_\alpha H_\mu$, $G_\alpha H_\delta$, $G_\alpha H_{\mu+d}$) showed increased surface binding of GIgG over 2h compared to the same cells treated at 0°. Modulated cells restained indirectly with anti-human IgM mAb showed up-regulation of sIgM by 2.0±0.5-fold with $G_\alpha HIg$ (N=11), 1.8±0.5-fold with $G_\alpha H_\mu$ (N=8), 2.3±0.4-fold with $G_\alpha H_{\mu+\delta}$ (N=2) and 1.1±0.3-fold with $G_\alpha H_\delta$ as control (N=5). Since Q-34 treated with biotin-conjugated non-immune GIgG (B-NGIgG) at 37° increased sIgM expression by 2.1±0.4-fold, secreted Ig was analyzed and found to react with B-NGIgG, NGIgG and heat-aggregated $G_\gamma G$ and $R_\gamma G$; but only B-NGIgG was effective in up-regulating sIgM. Subsequent analyses of other EBV-transformed B cell lines from normal donors and patients with autoimmune disorders showed that aberrant sIgM down-regulation was frequently associated with RF^+ (92%, N=12) but not RF^- cells (0%, N=3). Activation of cord blood mononuclear cells with PMA-ionomycin over 3 days showed that sIgM on $CD20^+$ cells became progressively insensitive to $G_\alpha H_\mu$-induced down-regulation. This suggests that the aberrant sIgM responses of RF^+ B lymphoblastoid cells may be related to maturation-specific differences in their signaling pathways.

O 221 ANALYSIS OF T-CELL RECEPTOR ALPHA AND BETA CHAIN SEQUENCES EXPRESSED IN THE SYNOVIAL FLUID OF A PATIENT WITH RHEUMATOID ARTHRITIS , Yasushi Uematsu, Helmut Wege#, Jerry S.S. Lanchbury*, Gabriel S. Panayi* and Michael Steinmetz, Central Research Units, F. Hoffmann-La Roche, Basle, Switzerland, #Institut for Virology and Immunovirology, University of Würzburg, Würzburg, Germany and *Rheumatology Unit, Division of Medicine, Guy's and St. Thomas's Hospitals, University of London, London, U.K.

One of the major questions in the pathology of rheumatoid arthritis is whether there are few or many distinct disease-inducing T cells. We have developed a method based on PCR which allows us to amplify T-cell receptor (TCR) cDNA sequences directly from synovial exudating lymphocytes without cellular expansion and cloning. We have analyzed one HLA-DR4 positive patient with erosive joint disease but no extraarticular manifestations. Data from 69 and 50 productively rearranged TCR alpha and beta genes, respectively, showed neither oligoclonality nor preferential usage of any particular variable or joining gene segment in the synovial fluid of this patient. Analysis of activated T cells from synovial membranes is in progress.

O 222 ANALYSIS OF T-CELL RECEPTOR Vα AND Vß GENE REGION USAGE IN RHEUMATOID ARTHRITIS. Peter van den Elsen, James Kurnick, Gail Hawes, Jorge Oksenberg*, Lawrence Steinman*, Rene de Vries, Ferry Breedveld# and Linda Struyk. Departments of Immunohematology/Bloodbank and #Rheumatology, University Hospital Leiden, 2300 RC Leiden, The Netherlands. *Department of Neurology, Stanford University, Stanford, CA 94305.

To define T-cell Receptor (TcR) determinants that are associated with rheumatoid arthritis (RA), we have analysed the frequency of TCR V-region usage at the transcriptional level of the αßTcR in T-lymphocytes present in the PBL and Synovial Fluid (SF) of ten RA patients. For these studies we have employed a semi-quantitative PCR-technique, using Vα and Vß family specific amplimers. Several conclusions can be drawn from these analyses: 1) Modulations in the relative frequency of TCR Vα and Vß gene usage can be observed as measured by the level of their respective transcripts in paired samples of PBL and SF T-lymphocytes. 2) The relative frequencies of TCR Vα and Vß gene usage are variable between different patients both in PBL and SF. 3) No preferential increase or decrease in the frequency of usage of a given TCR Vα and/or Vß gene segment can be observed that correlates with RA both in the PBL and SF samples tested.

O 223 THE RHEUMATOID FACTOR REACTIVITY OF A HUMAN IgG MONOCLONAL AUTOANTIBODY IS ENCODED BY A NEWLY IDENTIFIED GERMLINE Vk LIGHT CHAIN GENE. R.H. Weisbart, A.L. Wong, D.T. Noritake, A. Kacena, G. Chan, C. Ruland, E. Chin, I.S.Y. Chen, and J.D. Rosenblatt, Department of Medicine, Veterans Administration Medical Center, Sepulveda, CA, 91343 and The UCLA Center for the Health Sciences, Los Angeles, CA 90024

In order to determine the genetic and molecular basis for rheumatoid factor (RF) autoantibody reactivity in patients with rheumatoid arthritis (RA), we established a human lymphoblastoid cell line (hRF-1) from a patient with RA that produced an IgG RF monoclonal autoantibody, mAb hRF-1. Studies of isolated heavy and light chains showed that RF reactivity was conferred by mAb hRF-1 light chains. The light chain gene was cloned from a cDNA library prepared from hRF-1 cells. The nucleotide sequence was similar to known V_{kII} light chains except for a two nucleotide change corresponding to a change of two amino acids in an invariable region of FR3. Either or both of these amino acid changes may contribute to the RF reactivity, since an antibody with the same V_{kII} light chain except for these two amino acid changes in FR3 did not have RF reactivity. The RF reactivity of isolated light chains from mAb hRF-1 was confirmed by transfecting COS cells with an expression vector encoding the hRF-1 kappa chain gene and showing that the secreted kappa chains had RF reactivity. In addition, the variant coding sequences of mAb hRF-1 V_k were shown by polymerase chain reaction (PCR) to be germline in genomic DNA, including K562 proerythroblast cells that do not rearrange immunoglobulin genes. Expression of this newly identified germline V_k light chain gene may form the basis for RF autoantibody reactivity in some patients.

O 224 T CELL RECEPTOR HETEROGENEITY IN THE CLONAL RECOGNITION OF HLA Dw14.

Katsuo Yamanaka, Jocyndra Wright, William W. Kwok, Eric Mickelson, Gerald T. Nepom, and Patrick Concannon. Department of Immunology, Virginia Mason Research Center, Seattle, WA 98101

Rheumatoid arthritis (RA) is associated with HLA-DR4 subtypes Dw14 and Dw4, which differ from Dw10 at three amino acid positions in the third hypervariable region of the DRβ1 molecule. In order to identify structural features of T cell receptors (TCR) which can recognize Dw14 and discriminate between Dw14 and the closely related Dw10, TCR Vβ nucleotide sequences from T cell clones which recognize Dw14 were analyzed. EM025 and EM036 are alloreactive T cell clones specific for Dw14-associated residues on DR4-positive molecules. EM025 recognizes the Dw14.1, Dw14.2, and Dw15 subtypes and EM036 reacts with only Dw14.1. TCRβ cDNA was amplified first using an anchored polymerase chain reaction technique and subsequently with Vβ and Cβ specific gene primers for asymmetric PCR and direct sequencing. Both clones EM025 and EM036 express the same TCR Vβ gene family; however, they have several different residues at the Vβ-Dβ-Jβ junctional region. These junctional differences may be important in contributing to the different fine specificity between the EM025 and EM036 reactivity profiles.

Cytokines, Collagen and Mediators of Inflammation

O 300 A C1q PEPTIDE AND A MODIFIED FORM OF C-REACTIVE PROTEIN (MCRP) THAT BIND IMMUNE COMPLEXES, **Byron Anderson, Michael Baumann and Lawrence Potempa, Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, IL 60611 and Immtech International, Inc., Evanston, IL 60201**

Circulating and tissue bound ICs are characteristic of rheumatoid arthritis (RA) and other autoimmune diseases and are bound by certain proteins including the C1q component of complement. A particular peptide sequence of the globular region of the B chain of the C1q molecule was synthesized, purified and shown to exhibit inhibitory activity of the binding of both Protein A and C1q to IgG. The peptide covalently coupled to a solid phase matrix also bound ICs of peroxidase - anti-peroxidase and heat aggregated human IgG (HAG) from buffer solution and from diluted sera indicating that the peptide sequence may be the portion of C1q that interacts with ICs. CRP is an acute phase reactant with a pentameric structure and is markedly elevated in sera of RA. When the subunits of CRP are dissociated, a modified form of CRP (MCRP) results with different physical-chemical properties and which exhibits a number of biological activities. We have shown that MCRP adsorbed to ELISA plate wells and covalently bound to various solid phase matraces will bind ICs of different antibody-antigen natures and of HAG. The mCRP also potentiates the binding of ICs by C1q. The studies suggest that one of the functions of CRP, after conversion to a modified form is in the clearance of ICs.

O 301 INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN (IRAP) mRNA REGULATION IN U937 CELLS, Ann E. Berger, Donald B. Carter, Susan O. Hankey and Robert N. McEwan, Cell Biology & Molecular Biology Units, The Upjohn Company, Kalamazoo, MI 49001

A naturally occurring receptor-level antagonist of Interleukin-1 (IRAP or IL-1ra) has recently been cloned. (Nature 344:633). To determine what stimuli might regulate this inhibitor, cytokines were tested for their effects on the steady-state level of IRAP mRNA in phorbol ester-differentiated U937 cells. The cytokines tested fell into one of three groups: 1) Inducers (GM-CSF, IL-4), 2) Weak inducers (<2 fold stimulation) (IL-1α, IL-1β, IL-2, TGF-β and PDGF) and 3) Cytokines with no effect (αFGF, βFGF, EGF, GCSF, IL-3, IL-5, IL-6, IFNγ, MCSF and TNFα). 100 U/ml of either GM-CSF or IL-4 was the dose inducing peak IRAP mRNA expression; that peak expression occurred 12 hr after addition of cytokine. GM-CSF induced a 34 ± 15 fold (n = 5) increase in IRAP mRNA, and IL-4 induced a 15 ± 6 fold (n = 4) increase. In the same RNA samples, GM-CSF increased IL-1β mRNA 5.9 ± 1.7 fold (n = 5), but IL-4 decreased IL-1β mRNA to half that of control levels (0.45 ± 0.17, n = 5). Thus, a single stimulus (IL-4) decreased the expression of an agonist (IL-1) while it increased the expression of an antagonist (IRAP). When U937 cells were treated with both IL-4 and GM-CSF, the level of IRAP mRNA induced was additive, suggesting that the cytokines acted differently to increase IRAP mRNA levels. The level of IL-1 mRNA in cells treated with both IL-4 and GM-CSF was intermediate. Dexamethasone inhibited all mRNA increases and did not reverse IL-4-induced decreases in IL-1 mRNA. Cycloheximide slightly superinduced IL-4- and GM-CSF-stimulated IRAP mRNA levels, and completely reversed IL-4-induced IL-1 mRNA decreases.

O 302 INHIBITION OF PHOSPHOLIPASE A2 ACTIVATION AND PROSTAGLANDIN RELEASE BY PHOSPHOLIPASE A2 ACTIVATING PROTEIN (PLAP) ANTISENSE OLIGODEOXYNUCLEOTIDES. John S. Bomalaski and Mike A. Clark. V.A. Medical Center, Medical College of Pennyslvania and University of Pennsylvania, Philadelphia, PA 19104 and Schering Corp., Bloomfield, NJ 07003.

High levels of phospholipase A2 (PLA2) activity have been observed in synovial fluid from patients with rheumatoid arthritis. PLA2 is thought to be the rate limiting step in the generation of potent lipid mediators, including prostaglandins (PG). We have recently isolated and characterized a mammalian protein which activates PLA2, termed PLAP. High levels of PLAP are also found in rheumatoid synovial fluid, suggesting a role for PLAP in PLA2 regulation as well as the generation and perpetuation of the rheumatoid lesion. To further characterize this protein and to determine its role in the generation of PG, we have cloned PLAP and have used antisense DNA to the PLAP mRNA to attenuate its synthesis. Sequence analysis predicted a region in PLAP having a high degree of homology with melittin, a PLA2 activating protein in bee venom. Tumor necrosis factor and leukotriene D4, known receptor-ligand stimulators of PLA2 and PG synthesis, caused endothelial and smooth muscle cells to rapidly increase the production of PLAP mRNA as well as PLAP protein synthesis. Pre-treatment of these cells with antisense complementary to a 20 bp region near the 5' end of the open reading frame not only inhibited the ability of these ligands to increase PLA2 activity, but also blocked PG release. These experiments suggest a role for PLAP and PLA2 in PG synthesis.

O 303 CHARACTERIZATION OF A NOVEL FAMILY OF TRANSCRIPTIONAL FACTORS REQUIRED FOR IL-1β EXPRESSION. Jon A. Buras, Brian G. Monks, Maria A. Monroy, Gary W. Hunninghake, and Matthew J. Fenton, Departments of Pathology and Medicine, Boston University Medical School, Boston MA 02118, and University of Iowa Hospital and Clinic, Iowa City IA 52242.

Interleukin 1 (IL-1) α and β are pluripotent proinflammatory cytokines that play a critical role in the pathogenesis of rheumatoid arthritis. We have identified a novel nuclear protein, termed NFIL-1βA, that binds to a highly conserved sequence upstream of the TATA box of the human and murine IL-1β promoters. NFIL-1βA is present in virtually all cells which are capable of expressing IL-1. Some cell types express multiple forms of NFIL-1βA, and it is possible that these isoforms represent either distinct DNA-binding proteins, heterodimers that share a common DNA-binding moiety, or post-translationally modified forms of the same protein. Distinct isoforms can be distinguished by their elution profile from DEAE columns and sensitivity to brief heat treatment. UV-crosslinking studies have been performed to determine the molecular sizes of these isoforms. Studies of the functional role of NFIL-1βA suggest that this factor is required for basal promoter function, as well as for the ability of the promoter to be trans-activated by CMV immediate early gene products. These findings are consistent with the role of NFIL-1βA as a "bridging" factor that may receive or channel stimulatory signals from inducible upstream enhancer elements.

O 304 IL-1 EXPRESSION IN HUMAN MONOCYTES IS TRANSCRIPTIONALLY AND POSTTRANSCRIPTIONALLY REGULATED BY IL-4, Raymond P. Donnelly, Matthew J. Fenton*, Joshua D. Kaufman and Theresa L. Gerrard, Division of Cytokine Biology, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892; and *Department of Medicine, Boston University School of Medicine, Boston, MA 02118

The T cell-derived lymphokine, IL-4, inhibits production of IL-1β by normal human monocytes. In order to determine whether IL-4 suppresses IL-1 expression by a transcriptional and/or posttranscriptional mechanism(s), we evaluated the half-life of LPS-induced IL-1β message and transcriptional rate of the pro-IL-1β gene in human monocytes following treatment with IL-4. Although the initial steady-state IL-1 mRNA levels in control and IL-4-treated monocytes were comparable during the first 2 hr following stimulation with LPS, the IL-1 message levels subsequently decreased at a significantly greater rate in the IL-4 treated cells. Thus, IL-4 did not prevent the initial expression of IL-1 message but accelerated down-regulation of IL-1 mRNA in these cells. The initial 2 hr lag period may be necessary for production of a protein(s) which mediates this inhibitory effect because the protein synthesis inhibitor, cycloheximide, abrogated the marked reduction of IL-1 message levels induced by IL-4. Nuclear run-on analyses demonstrated that IL-4 decreases IL-1 mRNA levels, in part, by reducing IL-1 gene transcription. Furthermore, mRNA half-life studies showed that IL-4 also significantly increases the rate of IL-1 message turnover in these cells. These findings demonstrate that IL-4 inhibits IL-1 production in human monocytes by acting at both the transcriptional and posttranscriptional levels. In addition, the kinetics of inhibition and the fact that cycloheximide blocks this process suggest that IL-4 induces or enhances synthesis of a secondary protein(s) which mediates these effects.

O 305 **BIOCHEMICAL PHARMACOLOGY OF CI-986, A NOVEL INHIBITOR OF ARACHIDONIC ACID (AA) METABOLISM.** R. D. Dyer, J. Kennedy, D. Bornemeier, A. M. Egloff, M. Adamchak F.-Z. Chung, M. Mullican, D. Connor, and D. J. Schrier, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Michigan, 48105.

Compounds which inhibit both cyclooxygenase- (CO) and 5-lipoxygenase- (5-LO) catalyzed conversions of AA are expected to be potent antiinflammatory agents with novel pharmacologic profiles. CI-986 (5-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,3,4-thiadiazole-2(3H)-thione, choline salt) inhibits CO and 5-LO end product ($PGF_{2\alpha}$ and LTB_4) formation by Ca^{++}-ionophore- (A-23187) stimulated RBL cells with respective IC_{50}'s of 0.47 and 3.2 μM. In this assay the reference compound indomethacin inhibited $PGF_{2\alpha}$ production (IC_{50} = 0.5 μM) but did not reduce LTB_4 production at concentrations up to 100 μM. CI-986 was active in a biological matrix, inhibiting TxB_2 and LTB_4 production in A-23187-stimulated whole human blood. 5-LO inhibition in the 20,000xg supernatant from sonicated RBL cells (IC_{50} = 1.4 μM) was associated with both a delay in enzyme activation and a decrease in the ratio of the initial reaction velocity to the catalysis-related inactivation constant. CI-986 also inhibited the conversion of [^{14}C]AA to [^{14}C]5-HETE by recombinant human 5-LO (IC_{50} = 0.35 μM). CI-986 only weakly inhibited isolated CO's prepared from bovine seminal vesicles and RBL cells ($IC_{50} \geq 29$ μM) whereas indomethacin potently inhibited ($IC_{50} \approx 1.6$ μM) both preparations. Including exogenous AA (5 μg/ml) in A-23187-stimulated cellular incubations caused no change in the CI-986 inhibition of LTB_4 production but reduced its potency for inhibition of $PGF_{2\alpha}$ production 20-fold. Furthermore, CI-986 inhibited A-23187-stimulated release of radioactivity from RBL cells prelabeled with [^{3}H]AA. CI-986's interference with cellular AA metabolism at several points contributes to its potency and desirable inhibitory profile.

O 306 **IDENTIFICATION OF RESIDUES OF IL-1 RECEPTOR ANTAGONIST IMPORTANT FOR RECEPTOR BINDING,** Ron J. Evans, John D. Childs, Robert C. Thompson and Stephen P. Eisenberg, Synergen Inc., Boulder, CO 80301
Interleukin-1 receptor antagonist (IL-1ra) is a protein that binds to the type 1 IL-1 receptor with an affinity equal to that of IL-1α and IL-1ß, but does not induce a response in any of the standard assays for IL-1 activity. IL-1ra has homology to IL-1ß and their hydropathy profiles are remarkably similar, indicating that the two proteins are related. Here, we examine the function of specific residues of IL-1ra using site directed mutagenesis. The two mutagenized residues in IL-1ra are trp16 and tyr34. These residues correspond (based on sequence alignment) to residues in IL-1ß previously reported to be important for either receptor binding or biological activity. Changing either trp16 or tyr34 (corresponding to arg11 and his30 of IL-1ß respectively) to a glycine results in a 75 to 80% reduction in affinity to the type 1 IL-1 receptor on human and mouse cells. Chromatography elution profiles suggest that there is no difference in tertiary structure between these muteins and wild-type IL-1ra. These results suggest that trp16 and tyr34 are important residues for binding IL-1ra to the receptor. Bioassay data are consistent with the observation that these muteins bind with decreased affinity to the IL-1 receptor. In contrast, other changes such as changing tyr34 to a histidine have no significant effect on receptor binding or biological activity.

O 307 NON-IL-1-DRIVEN ACTIVATION OF T-LYMPHOCYTES IS NOT INHIBITED BY AN IL-1 RECEPTOR ANTAGONIST, William E. Fleming, Gregory E. Winterrowd and Martin E. Sanders* Hypersensitivity Diseases Research, The Upjohn Company, Kalamazoo, MI, 49007 USA and *Clinical Immunology Research, Centocor, Maevern, PA, 19355 USA

A predominant feature early in the pathogenesis of rheumatoid arthritis is the infiltration of lymphocytes (mainly of the T-lineage) into the synovial tissue. This infiltrate of T-lymphocytes as well as their activation initiates a complex sequence of immune reactions which if unchecked can lead to irreparable damage to the integrity of the synovium.

The recent cloning and expression of an IL-1 receptor antagonist protein (IRAP) has allowed examination of the role of IL-1 in T-lymphocyte activation by diverse stimuli. We show that IRAP inhibits the IL-1-driven proliferation of T-lymphocytes, however, neither IRAP nor specific antibodies to IL-1α or to IL-1β inhibit the proliferation of human peripheral blood mononuclear cells (PBMCs) stimulated with mitogens, specific antigen or alloantigen. Additionally, IRAP does not block the synthesis of IL-1β from PBMCs stimulated with lipopolysacharide. Although IRAP binds to the IL-1 receptor and blocks IL-1-mediated signalling, the activation of T-lymphocytes stimulated with any of the aforementioned agents proceeds unabated.

O 308 REGULATION OF EXPRESSION OF MURINE AMYLOID P COMPONENT. Antony J. Hansen, Jacqueline K. Anderson and John E. Mole. Department of Biochemistry, University of Massachusetts Medical Center, Worcester, MA, 01655.
Amyloid P component (AP) is the major acute phase reactant in mice. A substantial variation in AP inducibility between mouse strains exists, however the underlying mechanism for this difference is unknown. In a low-responder strain (DBA/2J), lipopolysaccharide (LPS) injection results in an 8-fold increase in AP mRNA levels. By contrast, mRNA levels increase 30 to 40-fold in C57Bl/6J (high responder) mice. In at least the high responder strain the observed increases in mRNA levels are due, in part, to increased transcription of the AP gene. Approximately 2.4 kb of DNA upstream of the AP gene in both the high and low responder strains has been sequenced. Sequence comparison revealed 97% similarity between these regions. Computer analysis of this region of both AP genes also revealed several putative binding sites, including an HNF-1 binding site and consensus heat shock sequences. Since it is unlikely that strain-dependent expression of AP is due to any differences in nucleotide sequence so far obtained, control of AP may also be exerted post-transcriptionally. This is currently under investigation.

O 309 **IL-1β AND IL-1ra PRODUCTION IN HUMAN ALVEOLAR MACROPHAGES,** **Robert W. Janson, Talmadge E. King, Jr., Kenneth R. Hance, and William P. Arend, University of Colorado Health Sciences Center, Denver, CO 80262.**

Alveolar macrophages (AM) play a key role in local immunoregulation. The objective of these studies was to compare the production of IL-1β and IL-1ra, a specific receptor antagonist of IL-1, in AM from patients with interstitial lung disease (ILD) and from normal non-smoking volunteers. cDNA probes for IL-1β and IL-1ra were utilized to measure steady-state mRNA levels. IL-1β and IL-1ra protein levels in lysates and supernatants were determined by ELISA's. No detectable IL-1β protein production in AM from normals and ILD patients was observed when these cells were cultured in medium for 20 h. AM from normals and patients with ILD produced IL-1β mRNA and protein when stimulated with high doses of LPS but not with GM-CSF in concentrations up to 2000 U/ml. In contrast, AM from patients with ILD produced IL-1ra protein when cultured in medium alone for 20 h. Stimulation of these cells with LPS did not lead to a further increase in IL-1ra protein levels. Culturing these cells in GM-CSF further augmented IL-1ra mRNA levels and protein production. Preliminary data showed that normal AM produced substantially less IL-1ra protein when cultured in medium in comparison to AM from ILD patients. IL-1ra protein production by normal AM was again enhanced in the presence of GM-CSF but not LPS. These preliminary results suggest that AM from ILD patients have an enhanced capacity to produce IL-1ra in comparison to AM from normals. In addition, enhanced production of IL-1ra is noted in AM from normals or ILD patients after GM-CSF but not LPS stimulation.

O 310 **DIFFERENTIAL SYNTHESIS OF 5-LIPOXYGENASE, ONCOSTATIN M AND THROMBOSPONDIN IN NEUTROPHILS FROM RHEUMATOID ARTHRITIS.**

Christian Jobin, Josée Gauthier, Jocelyne Letarte and Christophe Kreis. Centre de recherche, Hôpital Saint-François d'Assise, Québec (Canada) G1L 3L5.

A hallmark of rheumatoid arthritis (RA) is the dysregulation which occurs at the cellular and molecular levels. Abnormal expression of certain proinflammatory molecules (i.e. transient appearance and disappearance of their mRNAs and proteins) may be implicated in the amplification phase of this disease. We are studying the expression of three genes in RA which play important roles in normal cellular physiology: 5-lipoxygenase (5-LO), oncostatin M (OCS) and thrombospondin (TSP). The studies were performed on matched peripheral blood (PB) and synovial fluid (SF) neutrophils from RA patients. RNA was isolated from cells *ex vivo* and protein synthesis studies were performed on cells cultured *in vitro*. We found that 5-LO mRNA and protein were present at high levels in PB neutrophils but at low levels in matched SF cells (J. Immunol., in press). We present preliminary evidence on the regulation of 5-LO which may in part be due to alternative splicing and to the presence of a consensus AU-rich sequence in the 3'-untranslated region of the RNA. A hypothesis is presented to show how leukotrienes may regulate the levels of 5-LO via an autoregulatory loop. OCS RNA is present at equal levels in PB and SF neutrophils but the protein is detected only in SF cells. Since OCS mRNA contains AU-rich motifs in the 3'-UTR, regulation of expression appears to be controlled posttranscriptionally. TSP protein is expressed only in SF neutrophils. The TSP mRNA is either present in small amounts or more likely is unstable due to the presence of extensive AU-rich motifs in the 3'-UTR region. In all three cases, the features of the 3'-UTR of the mRNAs provide a potential explanation for the regulation of these messages and proteins in RA.

O 311 CONVERSION OF THE IL-1 RECEPTOR ANTAGONIST INTO AN AGONIST BY A SINGLE AMINO ACID SUBSTITUTION,

G. Ju*, C.A. Campen#, W.R. Benjamin#, E. Labriola-Tompkins*, P. Kilian#, S.P. Eisenberg+ and R.J. Evans+, Depts. of *Molecular Genetics and #Immunopharmacology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110, and +Synergen, Inc., Boulder, CO 80301.

A naturally occurring protein, the interleukin-1 receptor antagonist (IL-1ra), is a pure antagonist for the T cell IL-1 receptor. The IL-1ra protein has no detectable agonist activity in a number of bioassays. By site-specific mutagenesis, we have created an analog of IL-1ra containing a substitution of a single amino acid near the COOH-terminus. This analog exhibited significant bioactivity in the D10 proliferation assay. The newly acquired agonist activity could not be neutralized by antisera to IL-1α or IL-1ß, but it could be blocked by a monoclonal antibody to the T cell IL-1 receptor. The analog also showed agonist activity in an IL-1-dependent PGE_2 release assay. These results have implications for the function of the COOH-terminus of IL-1 in signal transduction and triggering of the biological response.

O 312 CYTOKINE REGULATION OF COLONY-STIMULATING FACTOR PRODUCTION IN CULTURED HUMAN SYNOVIOCYTES. T. Leizer*, J. Cebon**, J.E. Layton**, and J.A. Hamilton#. *DNAX Institute, Palo Alto, CA; **Ludwig Institute of Cancer Research, Melbourne, Australia and #University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Melbourne, Australia Intra-articular production of colony-stimulating factors (CSFs) may be an important event in the initiation and perpetuation of chronic inflammation as seen in rheumatoid arthritis. To determine the effects of potential cytokine mediators of CSF production by human synovial fibroblasts, specific bioassays and immunoassays for granulocyte-macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF) were used. Recombinant interleukin-1 (IL-1α and IL-1β) and tumor necrosis factor (TNF) induced production of both CSFs in a dose-dependent manner with significant secreted levels first detected within 6-12 hrs and maximal levels 24-48 hrs after stimulation. IL-1 was 5-10x more potent in inducing CSF than TNFα, and 1000x more potent than TNFβ. Secreted levels of G-CSF were 10-20x higher than GM-CSF. CSF stimulation by IL-1 and TNF was not due to endogenous cross-induction of synovial cell TNF or IL-1 production. Furthermore, monokine-stimulated CSF secretion was a measure of de novo synthesis as neither CSF was detected in synovial cell lysates, and actinomycin and cycloheximide both abolished production. IL-1 and TNFα together synergized for both GM-CSF and G-CSF secretion. Interleukins 2, 3 and 6, interferons α, β and γ, macrophage CSF (CSF-1), platelet-derived growth factor, epidermal growth factor, and transforming growth factors α and β had no effect on synovial cell CSF production with or without IL-1.

O 313 THERAPEUTIC DRUG MONITORING OF CI-986, A NOVEL INHIBITOR OF ARACHIDONIC ACID METABOLISM, DURING PHARMACOLOGICAL SCREENING IN RAT INFLAMMATION MODELS,
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CI-986 is a potent antiinflammatory agent which inhibits cellular leukotriene and prostaglandin biosynthesis. CI-986 also inhibits the enzyme 5-lipoxygenase and inhibits arachidonic acid release from cell membranes. Plasma drug concentration monitoring during pharmacological screening was utilized to assist in identification of the optimal salt formulation for oral administration of CI-986 and to correlate in vivo pharmacological activity with plasma concentrations of CI-986. The Mycobacterium footpad edema (MFE) model of inflammation was utilized during salt selection studies and, along with the adjuvant-induced polyarthritis (AIP) model, in therapeutic drug monitoring studies. Based on physical stability data and activity in the MFE study, CI-986 was developed as the choline salt. In therapeutic drug monitoring studies, plasma CI-986 concentrations of 0.07 and 0.14 μg/mL were associated with a 57% and 56% inhibition of swelling in the MFE and AIP screens, respectively. In the MFE screen, a plasma CI-986 AUC (1-4 hours postdose) of 0.2 μg·h/mL was correlated with a 50% inhibition in joint swelling. Therapeutic drug monitoring during pharmacological screening served as a powerful tool for optimizing drug delivery and for correlation of efficacy with plasma drug concentrations.

O 314 CELLULAR ENERGY AND GLUCOSE TRANSPORTER IN IL-1ß PRODUCTION IN LPS-ACTIVATED MONOCYTES, Urszula Orlinska and Robert C. Newton, Inflammatory Diseases, DuPont Co., Glenolden, PA 19036.
Interleukin-1ß (IL-1ß) is produced in large quantity in monocytes upon their activation with lipopolysaccaride (LPS). We investigated the cellular energy requirement for this process and the direct involvement of glucose transporter in export of IL-1ß. Both DNP and CCCP inhibited extracellular and intracellular IL-1ß. These processes were accompanied by the reduction of intracellular pools of nucleotides as compared to LPS-activated cells only. 2-deoxyglucose inhibited extracellular and intracellular IL-1ß with IC_{50}=0.45 mM and 2 mM respectively. The stimulated by LPS (^{3}H)-2-deoxyglucose uptake was inhibited by cytochalasin B with IC_{50}=10 nM. Accordingly, extracellular IL-1ß declined and intracellular IL-1ß accumulated in dose response to cytochalasin B. Phloridzin caused accumulation of intracellular IL-1ß in comparison to LPS treatment only. PGE_2 production paralleled the changes in intracellular IL-1ß. The kinetic studies showed inhibition of extra- and intracellular IL-1ß in time response to applied agents. All these data suggest the cellular energy requirement for IL-1ß production and a link between glucose transporter and Il-1ß export.

O 315 IDENTIFICATION OF A CHEMOTACTIC DOMAIN IN THE PROPEPTIDE PORTION OF HUMAN IL-1β,

Arnold E. Postlethwaite and Jerome M. Seyer, University of Tennessee, Memphis and the Veterans Administration Medical Center, Memphis, TN 38163.

Human IL-1β is synthesized as a precursor or "pro" molecule which is cleaved between Asp 116 and Ala 117 sometime during its cellular processing. Although the mature IL-1β molecule has been extensively characterized, little is known of the function or fate of the cleaved pro IL-1β peptide. In the present study we have synthesized pro IL-1β peptide as oligopeptide fragments of various lengths and have tested them in in vitro assays for IL-1-related biologic activities. Each oligopeptide was tested for its ability to stimulate T cell, dermal and synovial fibroblast proliferation, synthesis of collagen, hyaluronic acid and collagenase, and chemotaxis of neutrophils and monocytes. All of the peptides except an oligopeptide containing residues 88-116 lacked biologic activity in these assays. Peptide 88-116 consistently induced chemotaxis of human neutrophils and monocytes at concentrations ranging from 10^{-5} to 10^{-6}M but was not active in the other bioassays.

These data suggest that the pro IL-1β peptide 1-116 contains a chemotactic domain that could function upon release from the cell of origin to attract neutrophils and monocytes to participate in inflammatory and immune reactions.

O 316 PRESENCE OF AN AP-1-LIKE SEQUENCE MOTIF IN THE FIRST INTRON OF HUMAN PROα1(I) GENE: EXPLORATION OF ITS ROLE IN CONSTITUTIVE AND PMA-MEDIATED TRANSCRIPTIONAL REGULATION, Rajendra Raghow, Jane. D. Stephenson, Hitoshi Katai, Carl P. Simkevich, and James P. Thompson, Departments of Medicine and Pharmacology, University of Tennessee, Memphis and VA Medical Center, Memphis, TN 38104

The first intron of the human Proα1(I) collagen gene interacts with the promoter-proximal sequences in an orientation-dependent manner; the intronic DNA consists of both positive and negative *cis*-acting elements. Using a Sau 3A fragment (+494 to +854; S360) and DNase footprinting, we identified an Ap-1-like motif (+590 to +615) and discovered that nuclear extracts prepared from mesenchymal and nonmesenchymal cells contain *trans*-acting factors which bind to this element. Deletion of the Ap-1 binding site (+598 to +604) from pCOL-KT (1) caused increased expression of the reporter gene. Functional consequences of site-directed mutations in the Ap-1 element were also tested by transient expression assays. One point mutation, which resulted in the loss of protein binding to S360, led to increased CAT activity while another mutant, which retained binding of the Ap-1-like *trans*-acting factor(s), showed decreased CAT expression. Thus, the Ap-1-like *cis*-regulatory element plays a critical role in the constitutive transcription of the human Proα1(I) gene. Potential role of this Ap-1 site in down regulation of Proα1(I) gene expression in response to phorbol esters will be discussed.

1. Thompson, J., Simkevich, C., Holness, M., Kang, A., and Raghow, R. *JBC* (in press).

O 317 **The antiinflammatory effects of CI-986, 5-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,3,4-thiadiazole-2(3H)-thione, choline salt, a novel inhibitor of arachidonic acid metabolism.** **D.J. Schrier, V.M. Baragi, D.T. Connor, R.D. Dyer, J.H. Jordan, M.E. Lesch, M.D. Mullican, G.C.N. Okonkwo, M.C. Conroy, Warner Lambert Pharmaceutical Research, Ann Arbor, MI, USA.**

CI-986 is a potent dual inhibitor of cellular leukotriene and prostaglandin biosynthesis which also inhibits the enzyme 5-lipoxygenase and interferes with arachidonic acid release from cell membranes. Based on these biochemical findings the compound was tested in several models of inflammation in rats including carrageenan footpad edema (CFE), Mycobacterium footpad edema (MFE) and adjuvant arthritis (AIP). CFE and MFE are acute models taking one and three days, respectively, for maximal swelling to occur. AIP is a more chronic model requiring 21 days for full expression of the inflammatory response. CI-986 was administered daily (1 hour before carrageenan injection in CFE) and found to be active in each of the models, with ID_{40} values of 1.0, 7.7 and 7.2 mg/kg, respectively. The potency of the compounds in the three models was roughly equivalent to naproxen (ID_{40} = 0.7, 6.3 and 3.8 mg/kg). Because NSAID's frequently produce G.I. side-effects, CI-986 was tested in a gastric irritation model. In contrast with results obtained with standard NSAIDs, CI-986 caused no gastro-intestinal damage at doses up to 200 mg/kg. In other GI studies the compound inhibited the release of PGE_2 and LTC_4 by gastric mucosa and prevented mucosal and vascular (as assessed by Evans blue extravasation) damage to the gut after ethanol administration. These results indicate that CI-986 is a potent nonulcerogenic antiinflammatory agent with a novel pharmacologic profile.

O 318 DETERMINANTS OF TRANSCRIPTIONAL TISSUE SPECIFICITY OF THE HUMAN PROα1(I) GENE: EVIDENCE FOR INTERACTION BETWEEN PROMOTER-PROXIMAL NEGATIVE *CIS*-REGULATORY ELEMENTS AND THE FIRST INTRON, Carl P. Simkevich, James P. Thompson, Helen Poppleton, and Rajendra Raghow, Departments of Medicine and Pharmacology, University of Tennessee, Memphis and VA Medical Center, Memphis, TN 38104

Expression of pCOL-KT, in which human Proα1(I) upstream sequences to -804 and the first intron (+474 to +1440) drive transcription of a reporter gene (1), was tested in several cell types; pCOL-KT was readily expressed in HFL-1, SL-29, and A204 (mesenchymal) cells, but failed to be expressed in K562 and PC12 (nonmesenchymal) cells indicating that pCOL-KT contained regulatory elements required for tissue-specific expression. To delineate the *cis*-acting sequences which determine tissue specificity of Proα1(I) collagen gene expression, functional consequences of deletions in the promoter and first intron of pCOL-KT were tested. *Cis*-elements in the promoter-proximal and intronic sequences displayed either positive or negative influence depending on the cell type except the *Xba I** deletion (-804 to -609) which resulted in very high expression in all cells regardless of their collagen phenotype. The *Xba I*(-Sst II)* construct which contained the intronic *Sst II* fragment (+670 to +1440) in the reverse orientation was not expressed in either mesenchymal or nonmesenchymal cells. Based on these results, we conclude that stereo-specific interactions between negatively-acting 5'-upstream sequences and the first intron dictate optimal tissue-specific expression of the human Proα1(I) gene.

1. Thompson, J., Simkevich, C., Holness, M., Kang, A. and Raghow, R. *JBC* (in press).

O 319 **ISOLATION AND CHARACTERIZATION OF THE IL-1RA PROMOTER**, M.F. Smith, Jr.*, W.P. Arend*, and A. Gutierrez-Hartman#, Departments of *Rheumatology and #Endocrinology, Univ. of Colorado HSC, Denver, CO 80262.

We have cloned and sequenced approximately 1700bp of 5'-flanking DNA of the human IL-1ra gene. This region was cloned proximal to the luciferase reporter gene in order to study its promoter activity. This construct (pRA-1700.Luc) was transfected into a variety of cell lines in order to assess the cell type specificity of this promoter. When transfected into the murine macrophage cell line RAW 264.7, this region demonstrated strong promoter activity, as determined by the induction of luciferase expression. However, this promoter was inactive in all other non-macrophage cell lines examined. A series of 5'-truncated promoter constructs, all having identical 3' ends were prepared in order to locate regions of the promoter necessary for its maximal activity. Removal of the region between -1700 and -1022 led to a four-fold decrease in promoter activity. A further deletion to -843 led to a partial restoration of promoter function while deleting sequences to -506 resulted in a two-fold loss of promoter activity. Thus, we have identified three regions which appear to be operative in controlling IL-1ra promoter activity; a positive element between -1700 and -1021, a strong negative element between -1021 and -843, and another positive element between -843 and -506.

O 320 PLATELET-DERIVED GROWTH FACTOR POTENTIATES CELLULAR RESPONSES IN ARTICULAR CHONDROCYTES TO INTERLEUKIN-1, Robert J. Smith, James J. Justen, Laurel M. Sam, Norman A. Rohloff, Patricia L. Ruppel and Jia En Chin, Hypersensitivity Diseases Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

We have examined the interactions between recombinant human interleukin-1 alpha (IL-1α) and recombinant human platelet-derived growth factor (PDGF), a synovial cell mitogen which, like IL-1, is found in rheumatoid synovial fluid. IL-1α (0.01-10 ng/ml) stimulated cartilage and bone matrix-degrading metalloproteinase (MMP: collagenase, gelatinase) production by rabbit articular chondrocytes (RAC), whereas PDGF (2-200 ng/ml) was inactive. Simultaneous exposure of cells to PDGF and IL-1α, however, under serum-free circumstances, resulted in a time- and concentration-dependent potentiation by PDGF of MMP production in IL-1α activated RAC. The highest concentration of PDGF (200 ng/ml) caused a 430% increase in IL-1α -induced MMP release. Although PDGF (2-200 ng/ml) did not stimulate prostanoid production by RAC, it caused a concentration-dependent potentiation of prostaglandin E_2 release elicited by IL-1α (0.1 ng/ml) alone. Pretreatment of RAC with PDGF induced a significant increase (50-100%) in the number, but not affinity, of IL-1 receptors using iodinated IL-1α as the ligand. Thus, the PDGF-mediated enhancement of chondrocyte responsiveness to IL-1 may constitute a component of the pathogenesis of inflammatory joint disease.

O 321 PURIFICATION OF A CYTOSOLIC PLA2 FROM RAW 264.7 CELLS, Lisa A. Sultzman, Nina S. Milona James D. Clark, Jasbir Seehra, Chakkodabylu S. Ramesha* and John L. Knopf, Drug Discovery, Genetics Institute, Inc. Cambridge, MA 02140, *Department of Inflammation Biology, Syntex Research, Palo Alto, CA 94303. Although there is accumulating evidence that PLA2 activity is regulated by G-proteins and / or kinases, little is known about the cytosolic PLA2 which interacts with these signal transduction elements. We have purified the major dithiothreitol-insensitive PLA2 from the murine macrophage cell line RAW 264.7 by 30,000-fold using sequential chromatography on phenyl-5PW, Mono Q, HPHT, and G3000-SW columns. This novel 105 kDa PLA2 selectively hydrolyzes fatty acids at the sn-2 position and preferentially releases arachidonic acid when incubated with neutrophil membranes. Interestingly, a greater than 5-fold increase in PLA2 activity is noted as the calcium concentration increases from the level found in resting cells to that observed in activated cells. The identification of PLA2 as a 105 kDa protein is consistent with our purification of a 110 kDa PLA2 from the human cell line, U937(Clark et al. PNAS 87 7708, 1990). Recently, Leslie et al. (BBA 963 476,1988) and Diez and Mong (JBC 265 14654,1990) have attributed PLA2 activity to a 60 kDa protein purified from RAW 264.7 and U937 cell lines, respectively. The final specific activities reported by these two groups however were 10 and 200 times lower, respectively, than the specific activities we have observed. Interestingly, in both of our purifications, a 60 kDa contaminant was one of the last proteins to be separated from the PLA2 activity. This 60 kDa contaminant has been shown by amino terminal sequencing to have 100% sequence identity with the murine 60 kDa enzyme, protein disulfide isomerase, thus eliminating the possibility that the 60 kDa polypeptide which we have observed is a proteolytic fragment of our 110 kDa PLA2.

O 322 IRAP BLOCKS MURINE TYPE 2 IL-1 RECEPTORS AND INHIBITS IL-1 INDUCED NEUTROPHILIA AT HIGH CONCENTRATIONS, Daniel E. Tracey, Marilyn M. Hardee, John A. Shelly, John G. Chosay, Stephen F. Fidler, Alice L. Laborde and Colin J. Dunn, Departments of Hypersensitivity Diseases Research and Chemical and Biological Screening, The Upjohn Company, Kalamazoo, MI 49007.

We previously reported (Nature 344:633, 1990) that the recombinant human IL-1 receptor antagonist protein, IRAP (= IL-1ra), was a potent competitor (IC_{50} 0.8 nM) for IL-1 on YT cell type 1 IL-1 receptors and was a potent inhibitor (ID_{50} 0.5 μg) of IL-1-induced serum corticosterone in mice, but failed to compete for IL-1 binding to murine bone marrow cell type 2 IL-1 receptors and failed to inhibit IL-1 (10^4 U)-induced blood neutrophilia in mice. Further studies have shown, however, that high concentrations of IRAP do inhibit IL-1 binding to bone marrow cells and IL-1-induced neutrophilia. In competition binding experiments IRAP inhibited the binding of 250 pM ^{125}I-rhIL-1α to C3H/HeJ murine bone marrow cells with an IC_{50} of 600 nM. Irrelevant proteins, such as IL-3 or human serum albumin, did not compete for ^{125}I-rhIL-1α binding at concentrations at which IRAP gave complete inhibition. Blood neutrophilia in C3H/HeJ mice, measured as total neutrophil counts or as a percentage of blood leukocytes three hours after s.c. injection of 10^3 U rhIL-1α or β, was substantially inhibited by coinjection of 300-1000 μg IRAP. These results demonstrate that IRAP binds with very low affinity to type 2 IL-1 receptors on mouse bone marrow cells and inhibits IL-1-induced blood neutrophilia in mice with very low potency compared with the effects of IRAP on type 1 receptor dependent activities.

Metalloproteinases, Adhesion Molecules and Therapy

O 400 ENHANCEMENT OF T CELL ACTIVATION THROUGH CD2 BY THE FIBRONECTIN/VLA-4 INTERACTION, Marie-Luise Blue, Patricia Conrad, and Gary Davis, Institute for Arthritis and Autoimmunity, Miles Research Center, West Haven, CT 06516

The interaction of lymphoid cells with fibronectin potentially plays an important role in wound healing and lymphocyte homing. The VLA-4 integrin molecule, which is highly expressed on most lympoid cells and only sparsely on some non-lymphoid cells, binds to a unique site on the alternatively spliced V region of fibronectin. We found that VLA-4 by its interaction with fibronectin can enhance the proliferation of resting T lymphocytes stimulated via CD2, an antigen independent activation pathway. Suboptimal concentrations of CD2 (anti-T11$_2$ and -T11$_3$) and an activating antibody (D6γ) directed against VLA-4 were able to induce T cell proliferation when incubated with immobilized fibronectin. The proliferation due to D6γ was abrogated by the GPEILDVPST peptide, which represents the binding site on V fibronectin for VLA-4 . These results indicate that the VLA-4 molecule can be activated by fibronectin and suggest that the VLA-4/fibronectin interaction may play a role in the activation and expansion of T lymphocytes at tissue sites containing alternatively spliced V fibronectin.

O 401 Non-integrin Laminin Receptors on Lymphocytes

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We have discovered a novel multigene family defined by homology to a previously described metastasis associated laminin receptor (not an integrin). Messenger RNA from this family is expressed at high levels in resting T and B lymphocytes and is found at even higher levels in activated and transformed T cells.

Multiple members of this gene family have been retained throughout mammalian evolution, possibly because they have differentiable functions, and/or patterns of expression. We are testing the hypothesis that these laminin receptor (LR) gene family members play a roles in lymphocytes similar to that previously demonstrated in nonlymphoid cell tumor metastasis by examining their roles in: a) the recirculation of normal T lymphocytes, b) the acquisition of invasiveness by activated lymphocytes, and c) the invasiveness of T cell leukemia lines.

O 402 CLINICAL AND IMMUNOLOGIC STUDIES OF RHEUMATOID ARTHRITIS PATIENTS TREATED WITH AN ANTI-CD5 IMMUNOCONJUGATE. John J Cush, Lisa A Nichols, Peter E Lipsky. Univ. of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235

Nine patients with long standing, seropositive rheumatoid arthritis (RA), refractory to numerous DMARDs were treated with a 5 day infusion of an anti-CD5 ricin A chain immunoconjugate (CD5 Plus) and maintained on their nonsteroidal antiinflammatory agents with or without low dose prednisone (<10mg./d). Two patients each continued to receive oral azathioprine or methotrexate. Collectively, these patients failed an average of 4.3 DMARDs, 55% had evidence of extra-articular disease, and 6 patients were ACR functional class II and 3 were functional class III prior to infusion. A significant clinical response was based on >20% improvement in four of the following 6 measures: AM stiffness, tender joint score, swollen joint score, modified HAQ, physician and patient estimates of disease activity. 4 of 9 patients (44%) were responders 8 and 60 days post-infusion. Four of these patients continue to do well 2 months after treatment, with the longest response lasting for greater than 7 months. Four patients have been retreated. One of the 2 patients who had an earlier response benefitted from retreatment, while both of the initial nonresponders continued to do poorly. Immunofluorescent analysis of circulating lymphocytes revealed that the number of circulating CD3+CD5+ lymphocytes was rapidly depleted in all patients. The majority of responders demonstrated sustained reductions in the number of CD3+CD5+ T cells. No significant differences in memory vs. naive CD4+ cells were noted. The number of CD8+CD57+ was also reduced in most patients following infusion and tended to remain low in the responders. T lymphocytes were tested for in vitro responsiveness to mitogens, anti-CD3 and PHA. Although, both responders and nonresponders manifested significantly decreased T cell proliferative responses to OKT3 post-infusion, this effect was transient. These studies demonstrate that T cell depletion with an anti-CD5 immunotoxin leads to clinical improvement in patients with refractory RA without permanent immunosuppression.

O 403 **IN VITRO EFFICACY OF A MOUSE/HUMAN CHIMERIC CD4 ANTIBODY: FUNCTIONAL CONTRIBUTIONS OF ISOTYPE AND Fc.** M. Dalesandro[1], K.Y. Pak[1], S. Tam[1], E. Wilson[1], C. Reiter[2], D. Knight[1], J. Looney[1], J. Ghrayeb[1], E.P. Rieber[2], G. Riethmuller[2], P. Daddona[1], [1]Centocor, Inc., Malvern, PA, [2]University of Munich, Munich, FRG.

Murine monoclonal CD4 antibodies have been tested clinically for the treatment of allograft rejection and rheumatoid arthritis. To decrease immunogenicity, increase circulating half-life and recruitment of human effector functions, mouse/human chimeric CD4 Igγ1 and Igγ4 antibodies were constructed with murine M-T412 variable and human Fc constant regions. $F(ab')_2$ and Fab fragments of the murine and chimeric γ1 (cM-T412) Mabs were generated by enzymatic digestion. The cM-T412γ1 and its fragments retained the affinity ($5x10^9$ M^{-1}) and specificity of the parental murine M-T412γ2a Mab.

The effect of intact CD4 antibody or antibody fragments on $CD4^+$ T cell activity was evaluated in in vitro assays of Ig production by pokeweed mitogen-stimulated cells, soluble IL-2 receptor production by phytohemagglutinin-stimulated PBMC, and cell proliferation in response to: tetanus toxoid, anti-CD3 Mab, and mixed lymphocyte culture. Representative findings were seen with tetanus toxoid where the cM-T412γ1 Mab inhibited PBMC proliferation by 90% at 0.1 μg/ml. The cM-T412γ4 and cM-T412γ1 $F(ab')_2$ achieved a maximum of 65% inhibition at 10 μg/ml while the cM-T412γ1 Fab required 100 μg/ml for similar inhibition. These data show that cM-T412γ1 exhibits superior down regulation of T cell function with a strong contribution by the γ1 Fc region. Based on these results, cM-T412γ1 is being actively evaluated in human clinical trials.

O 404 **A SIGNIFICANT REDUCTION IN THE INCIDENCE OF COLLAGEN INDUCED ARTHRITIS IN MICE TREATED WITH ANTI-TCR V_β ANTIBODIES,** Chella S. David, Kevin G. Moder, Gary D. Anderson, and Harvinder S. Luthra, Departments of Immunology and Rheumatology, Mayo Clinic, Rochester, MN 55905.

Collagen induced arthritis (CIA) is an animal model of inflammatory polyarthritis. Previous studies have shown that T cells bearing specific receptors (TCR) are necessary for the development of CIA. The incidence of CIA in B10.RIII mice injected with porcine type II collagen was significantly reduced in those treated with a single injection of the monoclonal antibody F23.1 which deleted $V_\beta 8$ bearing T cells (33% vs. 74% in controls, $n=63$, $p<.05$). Arthritis score and antibody level to type II collagen were also reduced in the treatment group. No deleterious effects were noted in the treated animals. Use of the monoclonal antibody 466B5 to delete $V_\beta 6$ in combination with F23.1 was no more effective than F23.1 alone and the incidence of CIA in animals treated with 466B5 alone was not significantly different from controls. While F23.1 is a mouse antibody, 466B5 is a rat IgM antibody and is neutralized rapidly in mice in vivo. Thus, $V_\beta 8$ family of TCR's may be expressed on self reactive T cells in CIA. Deletion of only the specific subset of T cells involved in a disease is a promising therapy for autoreactive disorders because it will prevent the development of disease yet leave the host immunocompetent.

O 405 ANTI-INFLAMMATORY AND CHONDROPROTECTIVE ACTIVITIES OF A POTENT METALLOPROTEINASE INHIBITOR, M.J. DiMartino, C.E. Wolff, W. High, M.J. Crimmin and W.A. Galloway, SmithKline Beecham, King of Prussia, PA 19406, U.S.A. and British Bio-Technology Ltd., Oxford, OX4 5LY, U.K.

Metalloproteinases (e.g., collagenase and stromelysin) are believed to contribute to connective tissue damage occurring in rheumatoid arthritis. The purpose of this report is to describe the anti-inflammatory and chondroprotective activities of BB16 (2S, 3R-N-[3(N-hydroxycarboxyamido)-2-(2-methylpropyl)-butanoyl]-0-methyl-L-tyrosine-N-methylamide), a potent inhibitor of collagenase (IC_{50}=5nM) and stromelysin (IC_{50}=40nM), in the adjuvant arthritic (AA) rat model. The administration of BB16 to AA rats at 0.6 to 50 mg/kg, i.p., bid, significantly inhibited hindpaw swelling. In contrast, BB15, a stereoisomer of BB16 with weak enzyme inhibitory activity (IC_{50}>1μM), suppressed hindpaw swelling only at doses $\geq$ 50 mg/kg, i.p. bid. The arthritic lesions were also evaluated radiographically and histologically. BB16 produced moderate decreases in soft tissue swelling, the extent of the inflammatory process, and the severity of bone and cartilage loss. In contrast, indomethacin caused moderate to profound decreases in soft tissue edema and/or the inflammatory process but equivalent decreases in cartilage loss were not observed. These results suggest that metalloproteinase inhibitors may offer a new disease modifying therapy for rheumatoid arthritis.

O 406 AN ANTI-CD4 TREATMENT TRIAL IN RHEUMATOID ARTHRITIS.

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Monoclonal antibodies (mAb) to CD4 inhibit the function of $CD4^+$ T cells in vitro and have been used for treatment of autoimmune diseases in several animal models. We have prepared an anti-CD4 mAb (MAX.16H5) that binds with high affinity to human CD4. It is of the IgG1 isotype and does not mediate cell lysis. The antibody was given daily at a dose of 0.3 mg/kg body weight for 7 days in order to treat 10 patients with severe rheumatoid arthritis resistant to conventional therapy. 14 treatments were performed. More than half of the patients that have been fully evaluated so far showed a reduction of ESR, CRP, rheumatoid factor and total immunoglobulin. Reduction of Ritchie index, increase of grip strength, reduction of morning stiffness and reduced numbers of swollen joints demonstrate the clinical benefit of the therapy which persisted for 6-8 weeks. MAX.16H5 accumulates at the site of inflamed joints, thus permitting imaging with 99m-Tc-labelled antibody. Affected digital joints can be detected earlier in this way than by conventional bone scans. The antibody elicits a marginal anti-mouse-immunoglobulin response including anti-idiotypic antibodies. However, significant improvement of the clinical situation as well as of the laboratory parameters was seen in spite of the presence of anti-mouse-immunoglobulin antibodies at the second treatment trial. The antibody depletes $CD4^+$ cells from circulation and modulates the CD4 molecule very effectively. However, both parameters did not correlate with therapeutical efficacy. Instead, evidence of direct negative signalling by MAX.16H5 was found. The antibody was able to inhibit significantly the T cell receptor-mediated increase of intracellular Ca^{++} in human helper T cells.

O 407 DEFECTIVE EXPRESSION OF LFA1 ON PERIPHERAL LYMPHOCYTES FROM RHEUMATOID ARTHRITIS (RA) PATIENTS.

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The leukocyte function adhesion 1 molecule (LFA1) is a non polymorphic heterodimer involved in cellular interactions and T-cell activation in immune responses, physiologically expressed on all lymphocytes, monocytes and polymorphonuclears. Total and partial deficiencies have been reported, respectively associated with recurrent infections, viral infection and auto-immune diseases. We report a study performed on peripheral blood lymphocytes (PBL) from a cohort of 150 RA patients. The percentage of PBL expressing LFA1 was significantly lowered, with a mean value of 70.5% (SD 21.9, range 6-98). No significant correlation was found with classical clinical parameters of RA activity. The PBL subsets involved in this defect was further analyzed using magnetic beads to enrich PBL in CD4+ or CD8+ cells, or to deplete them from LFA1+ cells. These studies showed that LFA1 deficiency was significantly associated with the CD4+ subset. Flow cytometry analysis of LFA1 expression, allowing to approach the amount of molecules expressed showed that even when positive, CD4+ cells expressed significantly lower levels of LFA1. These data suggest the participation of LFA1 in the pathogenesis of RA. This defect could be linked with viral infection of the CD4+ subset, or might indicate a tentative regulation of auto-immune interactions in this disease.

O 408 SYNTHETIC INHIBITORS OF COLLAGENASE: DESIGN OF POTENT INHIBITORS WITH ANTI-ARTHRITIC PROPERTIES.

W.A.Galloway[1], M.J.Crimmin[1] and M.J.DiMartino[2]. [1]British Bio-technology Limited, Oxford, UK and [2]SmithKline Beecham, King of Prussia, PA 19406.

For some time, metalloproteinases have been implicated as the enzymes responsible for the degradation of extracellular matrix in chronic inflammatory diseases such as rheumatoid arthritis. To investigate the pathological role of these enzymes, we have synthesised a series of potent hydroxamic acid-containing pseudopeptide inhibitors using diasterioselective routes. Structure activity relationships for these inhibitors have been derived by measuring their activities against human fibroblast collagenase. A series of inhibitors, specific for matrix metalloproteinases, with IC_{50} values between 10^{-8} and 10^{-9} M have been designed. In an adjuvant arthritis model in the rat, therapeutic administration of these compounds at 1 to 50 mg/kg, i.p., u.i.d., significantly inhibited disease progression. These results indicate that, in adjuvant arthritis in the rat, collagenase is a primary mediator of connective tissue destruction. Thus, collagenase inhibitors may offer novel disease-modifying approaches to rheumatoid arthritis therapy.

O 409 REDUCED THYMIDINE UPTAKE AND AUGMENTED DGTP ACCUMULATION IN MOLT-4 T CELLS AND STIMULATED NORMAL HUMAN LYMPHOCYTES TREATED WITH DEOXYGUANOSINE AND CI-972, A NEW INHIBITOR OF PURINE NUCLEOSIDE PHOSPHORYLASE (PNP), R. B. Gilbertsen, M. K. Dong, J. C. Sircar, L. M. Kossarek, M. K. Shaw, D. J. Wilburn, C. R. Kostlan, and M. C. Conroy, Parke-Davis Pharm. Res., Warner-Lambert Co., Ann Arbor, MI 48105

CI-972 (2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4H-pyrrolo[3,2-d]-pyrimidin-4-one) is a novel, competitive inhibitor of PNP (K_i= 0.83 μM). We evaluated CI-972 for its ability to block proliferation (^{3}H-TdR uptake) of human MOLT-4 and MGL-8 (B cell) lymphoblasts, and for effects on intracellular nucleotide pools. CI-972 (50 μM, highest dose tested) or 2'-deoxyguanosine (GdR, 10 μM) alone failed to inhibit ^{3}H-TdR uptake by MOLT-4 or MGL-8 cells, but in the presence of 10 μM GdR, the IC_{50} for CI-972 decreased to 3.0 μM for MOLT-4 but remained at >50 μM for MGL-8. The decrease in ^{3}H-TdR uptake was associated with an increase in dGTP that was dependent both on GdR and on CI-972 concentration, and was prevented by 10 μM 2'-deoxycytidine. No alterations in GTP pools were noted in MOLT-4, and neither GTP nor dGTP was altered in MGL-8. Inhibition of ^{3}H-TdR uptake by human MLR cultures was dependent on CI-972 concentration, but minimally dependent on GdR concentration. However, significant dGTP accumulated in the MLR, and this was dependent both on CI-972 and on GdR concentration. Further, dGTP pools increased only in highly inhibited MLRs. GTP pools were unaffected in the MLR. These results suggest that the mechanism whereby CI-972 inhibits T cell lymphoblasts and MLR cultures is via blockade of PNP, resulting in dGTP accumulation and cessation of DNA synthesis. CI-972 is a novel compound which could be used to test the concept of T cell-selective immunoregulation effected through inhibition of PNP.

O 410 LOCALIZATION OF COLLAGENASE AND STROMELYSIN mRNA PRODUCTION IN RHEUMATOID SYNOVIUM, Ellen M. Gravallese, Jama M. Darling, Amy L. Ladd and Laurie H. Glimcher, Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115

Destruction of cartilage and connective tissue in rheumatoid arthritis is mediated largely by the neutral proteinases, collagenase and stromelysin. The production of both proteinases has been demonstrated in cultured synovial cells, and several lines of evidence support the theory that the collagenase and stromelysin genes are coordinately regulated. The production of collagenase mRNA has been previously localized to cells of the hyperplastic synovial lining layer, but the localization of stromelysin mRNA production has not been elucidated. In this study, frozen sections of synovial tissue from eight patients with rheumatoid arthritis and two patients with osteoarthritis were examined for collagenase and stromelysin mRNA production using the technique of *in situ* hybridization. All patients were female with classical or definite RA. The mean age was 56.6 years with a mean disease duration of 19.5 years. Disease modifying therapy included plaquenil (1 patient), IM gold (4), methotrexate (1); four patients were receiving prednisone. Production of stromelysin mRNA localized almost exclusively to the hyperplastic synovial lining layer cells with grain counts ranging from 90 counts/cell (background grains 4.6) to 7.3 counts/cell (background grains 1). Endothelial cells, lymphocytic aggregates and synovial fibroblasts were negative, except for an occasional positive stromal cell. Serial 6 micron sections localized collagenase mRNA production to the same areas producing stromelysin mRNA. In addition, grain counts revealed a direct correlation between the level of production of stromelysin and collagenase mRNA. One OA synovium was weakly positive for stromelysin and collagenase mRNA production, whereas the second was negative. These data support the identity of the synovial lining cells as the effector cells in RA and provide further evidence for the coordinate production of collagenase and stromelysin.

O 411 CHARACTERIZATION OF TYPE V COLLAGENASE (GELATINASE) IN SYNOVIAL FLUID FROM PATIENTS WITH INFLAMMATORY ARTHRITIS, Tomohiko Hirose, Robert A. Reife, Gerald N. Smith, Jr., Randall M.Stevens, Carlo L. Mainardi and Karen A. Hasty, Departments of Anatomy and Neurobiology and Medicine, University of Tennessee, Memphis, TN 38163

Cartilage degradation in inflammatory arthritis is thought to be mediated by a family of matrix metalloproteinases. A subfamily of these enzymes consists of two distinct gene products which are similar in their substrate specificity degrading native types IV and V collagens and gelatin. The 92 kDa form of this enzyme is expressed by macrophages and tumor cells and is contained in specific granules of neutrophils whereas the 70kDa enzyme is expressed constitutively by fibroblasts and synovial lining cells. These enzymes can be identified by the technique of zymogram analysis from which one can qualitatively and semi-quantitatively estimate the presence of each of these enzymes. Thus, in order to characterize the metallogelatinases involved in joint degradation in inflammatory arthritis, we analyzed 19 synovial fluid samples from 14 patients including 6 RA, 3 Reiter disease,1 gout, 4 pseudogout. Three samples from OA patients were also analyzed. By zymogram analysis some samples showed a neutrophil pattern with predominant 92 kD gelatinase activity, while others showed predominantly 70kD. 92kD gelatinolytic activity was directly proportional to the number of neutrophils in the synovial fluid. Furthermore, we purified type V collagenase from synovial fluid using gelatin-sepharose affinity column. The purified 92kD enzyme was then analyzed by immunoblotting with polyclonal antibodies prepared to human neutrophil gelatinase. Staining on the blot was evident at the correct molecular weight for 92 kD gelatinase. In addition, the purified gelatinase was capable of degrading type XI collagen, which is a component of articular cartilage and is resistant to the action of fibroblast or neutrophil collagenase. These data suggest that both the 70 kDa and 92 kDa gelatinases contribute to the joint destruction seen in RA but the prevalence of free 92 kDa enzyme in most samples would implicate the neutrophil enzyme in articular destruction associated with active, intense inflammation.

O 412 DEGRADATION OF CARTILAGE PROTEOGLYCAN IN VITRO BY INFLAMMATORY CELLS AND MACROPHAGE AND FIBROBLAST CELL LINES, Michael J. Janusz, Andrea D. Ackermann and Michelle Hare, Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215

We have been investigating models of connective tissue matrix degradation that involve proteolytic attack at the sites of cell-substrate contact. Human neutrophils stimulated with opsonized zymosan degraded cartilage matrix proteoglycan in a dose-dependent manner in the presence of serum anti-proteases. Supernatants from stimulated neutrophils cultured in the presence of serum did not degrade cartilage matrix, indicating that neutrophil mediated degradation in the presence of serum was confined to the protected subjacent region between the inflammatory cell and substratum. A combination of elastase and cathepsin G inhibitors each at 500 μM or each at 100 μM blocked subjacent cartilage matrix degradation by stimulated human neutrophils by 91%±3 and 54%±8(mean±SEM, n=5) respectively, whereas either inhibitor alone was much less effective. These data suggest that protease inhibitors may be useful in inhibiting cell-mediated tissue degradation. To more closely simulate the cartilage degradation that occurs beneath the synovial pannus; macrophages, fibroblasts or combinations of macrophage and fibroblast cell lines were allowed to adhere and grow on the surface of ^{35}S labeled heat-killed cartilage disks in 96 well microtiter plates for 7 days in the presence of 10% FCS. Incubation of cartilage discs with the transformed mouse macrophage cell line J774A.1 resulted in the release of 41%±7(n=6) of the radiolabeled cartilage matrix proteoglycan. The transformed mouse fibroblast cell line 10 MEHDA.5R.1 alone did not degrade cartilage discs (3.1%±.1, n=3). However, incubation of J774A.1 macrophages with 10 MEHDA.5R.1 fibroblasts resulted in 73%±5 (n=6) release of cartilage proteoglycan suggesting an interaction between the macrophage and fibroblast cell lines. These experimental systems provide useful methods for the study of cell-mediated proteolytic tissue degradation.

O 413 ANTI-CD4 TREATMENT WITH CHIMERIC MONOCLONAL ANTIBODY RESULTS IN PROLONGED CD4+ CELL DEPRESSION, M. Jonker[1], W. Slingerland[1], G. Treacy[4], K.Y. Pak[4], E. Wilson[4], S. Tam[4], A.F. LoBuglio[2], M. Khazaeli[2], T. Liu[2], G. Riethmuller[3], E.P. Rieber[3], P.E. Daddona[4] and J.D. Iuliucci[4]. [1]TNO Primate Institute, HV Rijswijk, The Netherlands, [2]University of Alabama, Birmingham, AL, [3]Universitat Munchen, Germany, [4]Centocor, Inc. Malvern, PA.

Murine anti-CD4 monoclonal antibodies have shown therapeutic effectiveness in several animal models of autoimmune disease and have been tested in limited clinical studies. To prolong serum half-life and reduce immunogenic potential, an anti-CD4 murine/human chimeric IgG1 monoclonal antibody (cM-T412) was developed. To assess the safety, pharmacokinetics and CD4 depressant effects of cM-T412, 4 chimpanzees received 5 mg/kg/day, i.v., for 7 days. Clinical toxicity/pathology and lymphocyte subsets were monitored for up to 4 months.

cM-T412 was well tolerated. Circulating CD4+ cell numbers were markedly decreased from the first dose through 2-3 wks after the last dose (10-500 cells/μl). CD4+ cells increased 3-4 wks post dose but remained depressed for 3-4 months (<1,000 cells/μl). Saline treated controls had a mean CD4 cell number of 1,500/μl. The first CD4+ cells to reappear after treatment were CD3 negative mononuclear cells. Selective loss of naive or memory CD4 cells was not observed. Mean cM-T412 serum concentrations were 89 ± 3 and 42 ± 5 μg/ml 30 min and 24 hr post initial infusion and 267 ± 13, 212 ± 15 and 36 ± 10 μg/ml at 30 min, 24 hr and 7 days post 7th infusion. The data fit a 2 compartment model with an α t1/2 of 12 hr and a β t1/2 of 98 hr (R=0.87). No antichimeric antibody response was detected. Thus, cM-T412 was shown to induce prolonged depletion of circulating CD4+ cells in chimpanzees with no incidence of adverse events or immunogenicity. This supports a potential clinical application of cM-T412 for the treatment of autoimmune disease.

O 414 **INHIBITION OF INTERLEUKIN-1 CHONDOCYTE ACTIVITY BY CORTICOSTEROID AND AN NSAID**, Nancy Lane, Riley J, Williams, David J. Schurman, R. Lane Smith, Department of Medicine, University of California, San Francisco, and Department of Orthopaedics, Stanford University School of Medicine, CA 94303

Common treatment of inflammatory arthritis includes administration of NSAID and or intra-articular corticosteroid injections. The aim of this study was to determine the effects of methylprednisolone (MP) and naproxen (NAP), two commonly used agents, alone and in combination on IL-1 induced chondrocyte proteolytic activity. These experiments were carried out in serum-free medium. Chondrocytes were treated with IL-1 (200 units/ml) and with either MP, range 0.005-8.0ug/ml, or NAP, range 7-120 ug/ml. In combination, MP concentrations were tested with NAP at 30 ug/ml. Proteolytic activities in cell culture medium samples were determine using radiolabeled collagen and casein, following APMA activation. PGE2 was determined by RIA. MP reduced IL-1 induced caseinolytic activity by 25% at 0.05 ug/ml and 50% at concentrations of 0.5, 2.0, and 8.0 ug/ml (p=.01). MP inhibited collagenase activity 10-17% at concentrations from 0.5 to 8.0 ug/ml (p=.02). NAP alone had no effect on either enzyme. MP, in combination with NAP at 30 ug/ml, inhinited caseinolytic activity from 37-62% (range 0.05-8 ug/ml) p=0.001. NAP and MP, alone and in combination, significantly decreased IL-1 induced PGE2 production. Thus, MP and NAP significantly increased the effectiveness of MP in inhibiting IL-1 induced caseinolytic activity, but this was not as apparent with collagenolytic activity. These data suggest that this combination therapy may be useful in the treatment of inflammatory arthritis and osteoarthritis.

O 415 CYTOKINE-ENHANCED EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) BY CULTURED RHEUMATOID SYNOVIOCYTES, HB Lindsley, DD Smith, LS Davis, PE Lipsky, Dept Med, Univ KS Med Ctr, Kansas City, and UTSMCD, Dallas. ICAM-1 (CD54), a ligand for LFA-1, is thought to play a major role in mediating the entry of lymphocytes into inflammatory sites. Expression of ICAM-1 is increased in the rheumatoid synovium, but which cytokines are involved in its induction is not clear. To examine the capacity of cytokines to alter expression of ICAM-1, adherent synoviocytes were stimulated with recombinant cytokines: interferon-Γ (IFN), interleukin-1-ß (IL-1), tumor necrosis factor-α (TNF), interleukin-6 (IL-6), and granulocyte-macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), and transforming growth factor-ß1 and ß2 (TGFß1, TGFß2) for 24-96 hours. ICAM-1 was measured using monoclonal Ab RR1/1 or 84H10. By flow cytometry, ICAM-1 was detected on 5-40% of synoviocytes but at low density. Both the percentage of cells expressing ICAM-1 and the density of expression were increased variably by the different cytokines. By cell ELISA, ICAM-1 density was enhanced (50-150%) most consistently by IFN (1-100 U/ml), TNF (0.5-50 U/ml) or IL-1 (0.1-10 U/ml); whereas it was unchanged by GM-CSF (0.01-100 U/ml), PDGF (0.1-10 ng/ml), TGFß1 or TGFß2 (0.01-30 ng/ml). IL-6 stimulation (30-300 U/ml) resulted in variable enhancement of ICAM-1 (0-30%). Enhancement of expression of cell adhesion molecules may facilitate entry (or retention) of lymphocytes into the synovium, thereby enhancing rheumatoid inflammation.

O 416 METALLOPROTEINASE AND METALLOPROTEINASE INHIBITOR EXPRESSION IN HUMAN ARTHRITIC SYNOVIUM, S. Spence McCachren, GRECC, VAMC and Department of Medicine, Duke University Medical Center, Durham NC 27710

The neutral metalloproteinases are implicated in the tissue destruction accompanying rheumatoid arthritis. Expression of interstitial collagenase and stromelysin and of the tissue inhibitor of metalloproteinases (TIMP) were evaluated in rheumatoid and osteoarthritic synovia by combined in situ hybridization and immunohistochemistry. Interstitial collagenase and stromelysin were expressed mainly by synovial lining cells in active rheumatoid arthritis. Expression was noted both in LeuM3 positive and negative cells, consistent with both monocyte/macrophage and fibroblast sources of the mRNA. TIMP expression was also predominately along the synovial lining cells but subintimal cells also expressed significant levels. TIMP expression was detectable in most cases of RA and OA, and its level of expression was similar in most synovia. Protease expression correlated with inflammation score in RA. Protease expression was generally low in OA synovium, perhaps reflecting the generally lower inflammation scores.

O 417 **TREATMENT OF REFRACTORY RHEUMATOID ARTHRITIS (RA) WITH A CHIMERIC ANTI-CD4 MONOCLONAL ANTIBODY. L. W. Moreland, R. P. Bucy, P. W. Pratt, M. B. Khazaeli, A. F. LoBuglio, J. Ghrayeb, P. Daddona, M. E. Sanders, C. Kilgarriff, G. Riethmüller, and W. J. Koopman.** University of Alabama at Birmingham, Birmingham, AL 35294; University of Munich, Federal Republic of Germany; and Centocor, Inc., Malvern, PA 19355.

CD4 T-cells have been implicated in the pathogenesis of RA. In a pilot study to examine whether treatment with a monoclonal antibody against CD4 would be safe and effective in the treatment of RA, we treated 15 patients with active disease by ACR criteria with single doses of IV human/murine chimeric CD4 mab cMT412. All patients had failed at least one remittive drug and were taking methotrexate at the time of study. Groups of 3 patients were treated with 10, 50, 100, 150, or 200 mg of cMT412. Immediate dramatic drops in CD4 counts were observed (from a mean baseline of 1070/mm^3 to 196/mm^3 at one hour post dose) which gradually returned toward baseline (618/mm^3 at day 14 and 667/mm^3 at day 35 post dose). Non-blinded assessment of tender joint counts suggested clinical response (50% or greater reduction in 8/15 at day 35) with some patients showing sustained improvement up to 90 days. Rheumatoid factor, CRP, and complement did not change significantly. Flu-like symptoms lasting 24 hours after dosing were common, but no serious adverse effects were noted. We conclude that chimeric CD4 mab cMT412 is well tolerated, induces a sustained depression of circulating CD4 T-cells, and may prove efficacious in the treatment of RA.

O 418 INCREASED EXPRESSION OF THE HYALURONATE RECEPTOR (CD44) ON SYNOVIAL CELLS AND IN SYNOVIAL FLUID IN RHEUMATOID ARTHRITIS (RA) COMPARED TO OSTEOARTHRITIS (OA), Karen L. Patton, Laura P. Hale, Margaret E. Martin, Rex M. McCallum, and Barton F. Haynes, Duke Medical Center, Durham, NC 27710
The receptor for hyaluronate (CD44) is an 85kd molecule whose N-terminal amino acids are homologous to cartilage link proteins. Ligand binding to CD44 synergizes with CD2 or CD3/TCR ligands to activate T cells and induce T cell IL2 production, and induces monocyte IL1 production. We have measured CD44 in RA and OA using indirect immunofluorescence (IF) and Western blot analysis. Tissue sections of 7 RA synovial tissues (mean inflammation index [II] 13±2.1) and 9 OA synovia with a (mean II 6.3±1.2) were graded in indirect IF assays on a 1 to 4 scale for the number of CD44+ cells present. RA tissue had a CD44 score of 3.5±.2 whereas OA tissue had a CD44 score of 1.78±.22 ($p<.001$). Representative synovial tissues were detergent-extracted and analyzed by Western blot for the relative amount of CD44 present. For example, RA synovium 154 (II=21) had 9 times more CD44 present than OA synovium 198 (II=3). Western blot analysis of 15 RA synovial fluids (SF) (mean WBC=11,279/mm^3) and 5 OA SF (mean WBC=1539/mm^3) demonstrated a mean of 54% more CD44 in RA SF compared with OA SF ($p<.001$). These data demonstrate marked upregulation of CD44 expression in RA synovial tissue and fluid and suggest a role for CD44 in promoting ongoing inflammation in inflammatory synovitis.

O 419 SOLUBLE CD2 INHIBITS ADHESION AND SUPPRESSES T CELL ACTIVATION. E. M. Rabin, K. Gordon, E. Neidhardt, M. Knoppers, M. Recny, and D. Dwyer, Procept, Inc, 840 Memorial Dr., Cambridge, MA 02139.
The CD2 (T11) receptor, found on the surfaces of human T lymphocytes, NK cells, and thymocytes, functions as an adhesion molecule and binds the surface glycoprotein LFA-3. The adhesion of T cells with accessory cells through the binding of CD2/LFA-3 plays an important role in augmenting the interaction of the T cell receptor (TcR) with the MHC/Antigen complex during T cell activation. The present studies examined whether soluble CD2 (sCD2) could interfere with T cell activation in a similar fashion to Mabs against CD2. sCD2 lacking the membrane-anchoring portion of the receptor was prepared in the baculovirus system and in CHO cells. Rosette formation between T lymphocytes and sheep red blood cells (SRBC) was completely inhibited by the addition of 10 uM (200 ug/ml) sCD2; 1.0 uM inhibited 80%. Proliferation of T cells specific for Tetanus toxoid and the viral antigens Herpes Simplex Virus - 1 and Rubella was inhibited 60-80% by the addition of 10 uM sCD2. T cells responding to alloantigens were also inhibited by addition of sCD2 to the cultures, but the inhibitory effect was less striking, 35% inhibition was observed. Time course studies showed that sCD2 had to be added early during the culture period to be most effective. The involvement of CD2 in T cell stimulation makes it an attractive molecule to target with immunotherapeutic reagents for abrogating the T cell activation that occurs in the course of autoimmune diseases .

O 420 AN IN VIVO MODEL OF HUMAN INFLAMMATORY SYNOVITIS: GROWTH OF HUMAN SYNOVIAL TISSUE IN IMMUNE DEFICIENT (SCID) MICE, Karen E. Rendt, Todd E. Barry, Conrad E. Richter, Dawn M. Jones, S. Spence McCachren, and Barton F. Haynes, Duke University Medical Center, Durham, NC 27710
The study of human inflammatory synovitis is hampered by the lack of in vivo models of many forms of human synovial disease. In this study, we have engrafted RA synovium under the renal capsule of immune-deficient C.B-17 scid/scid (SCID) mice to determine synovial xenograft morphology, growth potential and cytokine production. At 1 to 2 months post-engraftment, we found xenograft morphologies similar to the original synovial tissue including the presence of synovial macrophages, T cells, multinucleated giant cells and bone deposition. Of 8 synovial grafts placed into non-immunosuppressed SCID mice, all tissues engrafted at 1-2 months. Of 9 synovial grafts placed into SCID mice explants with anti-asialo GM-1 to eliminate SCID NK cells, 8 grafts grew. Human synovial fibroblast and vessels engrafted well and routinely invaded SCID renal parenchyma. While synovial lining cells prior to engraftment contained collagenase, stromelysin, tissue inhibitor of metalloprotease (TIMP) and gelatinase, only TIMP and gelatinase-expressing synovial cells could be indentified in synovial xenografts. These data suggest human synovial tissue engrafted into SCID mice can provide a new in vivo model of human inflammatory synovitis.

O 421 TREATMENT OF RHEUMATOID ARTHRITIS WITH MURINE AND CHIMERIC HUMAN/MOUSE MONOCLONAL CD4 ANTIBODIES, E. Peter Rieber[1], Christian Reiter[1], Bahram Kakavand[1], Manfred Schattenkirchner[2], Klaus Krüger[2], Peter Daddona[3] and Gert Riethmüller[1], [1]Institute for Immunology and [2]Dept. of Rheumatology, Med. Poliklinik, University of Munich, Munich, FRG, [3]Centocor, Inc., Malvern, PA.

Monoclonal CD4 antibodies (CD4-mAb) efficiently inhibit antigenic activation of T cells. Since $CD4^+$ T cells seem to play a crucial role in the pathogenesis of rheumatoid arthritis a therapeutic trial was initiated where patients with chronic active disease received daily infusions of 20 mg or 50 mg of murine CD4-mAb M-T151 for 7 days. Treatment was well tolerated. Ten out of 18 patients showed a significant clinical response lasting for several weeks up to 6 months, the improvement was only of short duration in 5 patients and 3 patients were unresponsive. In 4 patients treatment was successfully repeated after 6 months. The clinical improvement was paralleled by a decrease of the CRP values. The serum concentration of the CD4-mAb reached peak values of 2 μg/ml within 1 h after infusion of 20 mg and 20 μg/ml after infusion of 50 mg antibody. It was undetectable already after 6 h. A close up immunological monitoring revealed a selective and transient depletion of $CD4^+$ T cells after each infusion, while on residual circulating $CD4^+$ cells complement components and CD4-mAb were detected. A transient appearance of soluble CD4 was observed in the serum with a peak value of 100 ng/ml after 3 h. The proliferative response of blood T cells to PPD was still diminished 4 weeks after cessation of mAb-treatment.

A therapeutic trial following the same schedule was initiated with the recombinant human/mouse chimeric CD4-mAb cM-T412. Results of this study will also be presented and discussed.

O 422 T CELL ACTIVATION VIA CD3 IS INHIBITED BY A MONOCLONAL ANTIBODY TO CD44
Barbara L. Rothman, Marie-Luise Blue, Keith A. Kelley, David Wunderlich, Diane V. Mierz, and Thomas M. Aune, Institute for Arthritis and Autoimmunity, Miles Research Center, West Haven, CT 06516

The CD44 molecule, also known as Hermes-1 lymphocyte homing receptor, human Pgp-1, and extracellular matrix receptor III, has been shown to play a role in T cell adhesion and activation. Specifically, anti-CD44 mAb block binding of lymphocytes to high endothelial venules, inhibit T cell-erythrocyte rosetting, and augment T cell proliferation induced by the CD2 or CD3-T cell receptor pathways. We have characterized a new anti-CD44 mAb (212.3) which immunoprecipitates an 80-90 kDa protein, and is specific for CD44 as shown by peptide mapping and antibody competition studies. Interestingly, our studies with 212.3 have demonstrated that this CD44-specific mAb completely inhibits CD3-receptor mediated PBMC proliferation. Further investigations into the mechanisms involved in this inhibition have revealed that this effect is not a result of cell death, but is associated with 1) inhibition of IL-2 production, 2) inhibition of IL-2R expression, and 3) inhibition of CD3-receptor mediated increase in intracellular Ca^{2+} levels. In addition, 212.3 does not induce PBMC proliferation alone, has no effect on PBMC proliferation induced by mitogens (PHA or PWM) or an MLR, and enhances PBMC proliferation when induced by a pair of CD2 mAb. Thus, these studies describe a novel CD44-specific mAb (212.3) that inhibits CD3-receptor mediated T cell activation by blocking early signal transduction. Furthermore, these studies suggest that 'receptor cross-talk' between CD2 and CD44 or between the CD3-T cell receptor complex and CD44 may regulate T cell regulation.

O 423 EXPRESSION OF CELL ADHESION MOLECULES IN SJOGREN'S SYNDROME (SS),
E. William St.Clair and Kay H. Singer, Department of Medicine, Duke University Medical Center, Durham, NC 27710.

Often accompanying rheumatoid arthritis, SS is characterized by mononuclear cell (MN) infiltration of lacrimal and salivary glands (SGL) leading to ocular and oral dryness. To study cellular interactions in this autoimmune glandular disorder, frozen tissue sections of labial SGL from 5 patients with SS were stained with monoclonal antibodies to determine expression of cell adhesion molecules. By indirect immunofluorescence, the infiltrating MN cells were positive for LFA-1, ICAM-1 (the ligand for LFA-1), LFA-3, CD2 (the ligand for LFA-3), and CD44. In patients as well as non-autoimmune controls, the SGL acinar and ductal epithelium expressed LFA-3 and CD44. Although ICAM-1 is present on keratinocytes in inflamed skin and on synovial cells in the rheumatoid joint, it was not expressed on epithelial cells in any of the SS biopsy specimens. Analysis by flow cytometry demonstrated that SGL epithelial cells were nonetheless capable of expressing ICAM-1 insomuch as 35 % SGL epithelial cells grown in vitro were positive for this cell surface molecule. Moreover, ICAM-1 expression was upregulated by treatment of cultured cells with interferon-γ. These results show that cell adhesion molecules are widely distributed in the SGL microenvironment of SS and suggest that interactions between MN cells and epithelial cells may occur and perpetuate the immune response in this disease.

O 424 INTERFERON-GAMMA INHIBITS INTERLEUKIN-1β-INDUCED COLLAGENOLYTIC ACTIVITY VIA A DECREASE IN STROMELYSIN EXPRESSION, Elaine N. Unemori, Matthew J. Bair, Edward P. Amento, Department of Developmental Biology, Genentech, Inc., S. San Francisco, CA., 94080.

The expression of collagenolytic activity by cells represents the rate-limiting step in the turnover of collagen during remodeling. The metalloproteinase collagenase is the only enzyme known to initiate cleavage of the interstitial collagens at conditions of neutral pH and salt. Interleukin-1β (IL-1β), a product of activated macrophages, is a potent stimulator of procollagenase expression in fibroblasts. The addition of interferon-gamma (IFN-γ), which is secreted by activated lymphocytes, inhibits IL-1β-induced collagenolytic activcity by up to 70%. The elevation in procollagenase protein induced by IL-1β was not altered by IFN-γ, as assessed by Western blot analysis, gelatin zymography, and Northern analysis. The expression of tissue inhibitor of metalloproteinases (TIMP) was stimulated by IL-1β treatment, while the addition of IFN-γ decreased IL- 1β-induced TIMP levels. Instead, the ability of procollagenase to be activated, either by trypsin or organomercurials, is decreased in a significant way in conditioned media from IFN-γ treated cultures due to the down-regulation in expression of stromelysin, a metalloproteinase activator of procollagenase. These data indicate the pivotal role of stromelysin in the activation of procollagenase and suggest that independent regulation of stromelysin and collagenase expression represents another level at which collagenolysis can be regulated by cytokine mediators of connective tissue turnover, such as IFN-γ.

O 425 CHIMAERIC (MOUSE-HUMAN) AND CDR-GRAFTED ANTIBODIES TO HUMAN IL2 RECEPTOR, Ulrich H. Weidle, Eberhard Russmann, Helmut Lenz and Brigitte Kaluza, Boehringer Mannheim GmbH, Nonnenwald 2, D-8122 Penzberg, FRG.

Murine antibodies to human IL2 receptor α-chain and ß-chain were made chimeric by transferring V-regions to human constant γ1- and -regions. In addition, the CDR-regions of a murine antibody to human IL2 receptor α chain were grafted onto the framework of a human antibody ("humanized antibody").

Both chimeric and humanized antibodies were expressed in transfectoma clones and tested for their biological activity. Upon administering a combination of anti-α- and anti-ß-chain antibodies, a synergistic inhibitory effect on T lymphocyte proliferation was observed.

O 426 EXTRAVASCULAR FIBRIN FORMATION AND DISSOLUTION IN SYNOVIA FROM HUMANS WITH OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS: THE ROLE OF MACROPHAGES. J. B. Weinberg, A. M. Pippen, T. S. Wortham, C. S. Greenberg. VA & Duke Med. Ctrs., Durham, NC 27705.

Fibrin deposition is prominent in the synovium of patients with rheumatoid arthritis (RA). Macrophages are found in increased numbers in RA syn; these cells are known to produce various procoagulant and anticoagulant molecules. The purpose of this study was to determine the content and distribution of several important components of the coagulation system in the syn of humans with RA, osteoarthritis (OA), or traumatic joint abnormalities requiring surgery. Samples from nine patients (three from each category) were examined in detail. Results demonstrate that RA syn (as compared to that of patients with OA or traumatic joint abnormalities) has increased numbers of mac, and increased expression/content of fibrinogen, tissue factor, factor XIII, tissue transglutaminase, cross-linked fibrin (fibrin D-dimer), urokinase type plasminogen activator, and α2-plasmin inhibitor. Macrophage content in syn from RA patients was increased in both the lining cell areas and the interstitial cell areas. Fibrinogen was distributed throughout the entire tissue of all the samples, and was greater in those of RA patients. In syn of trauma and OA patients, tissue factor was seen only associated with the vessels (endothelial cells), but in RA syn, it was markedly increased throughout the tissues. While fibrin D-dimer was seen in small amounts in syn lining cell areas of trauma and OA patients, it was increased in the lining cell and interstitial cell areas of RA syn. Factor XIII and tissue transglutaminase were present in scant amounts in syn of trauma and OA patients, but there were increased amounts of both (especially tissue transglutaminase) in RA syn in the vessel, lining cell, and interstitial cell areas. Urokinase and α2-plasmin inhibitor were also markedly increased in the tissue from RA patients. These results suggest that in the inflammatory syn, there is ongoing extravascular tissue fibrin formation and dissolution that correlates with the degree of inflammation and macrophage content. Extravascular coagulation/fibrinolysis in RA represents a target for therapeutic intervention.

O 427 SUPPRESSION OF IN VITRO AND IN VIVO T CELL FUNCTION BY AN INHIBITOR OF S-ADENOSYL HOMOCYSTEINE HYDROLASE, Jeffrey A. Wolos, Ann L. Akeson and Kathleen A. Frondorf, Marion Merrell Dow Research Institute, Cincinnati, OH 45215

The enzyme s-adenosyl homocycteine (AdoHcy) hydrolase catalyzes the cleavage of AdoHcy to adenosine and L-homocysteine. AdoHcy hydrolase indirectly regulates biological methylation reactions by controlling intracellular levels of AdoHcy, which is a potent inhibitor of s-adenosylmethionine-dependent transmethylation reactions. Lymphocytes appear to have a greater requirement for transmethylation for their activation than other cell types. Therefore, inhibition of AdoHcy hydrolase might be immunosuppressive. We have examined the effects of a novel enzyme-activated irreversible inhibitor of AdoHcy hydrolase, 4',5' unsaturated 5'vinylfluoroadenosine (VFA) on T cell, B cell, and macrophage function. In vitro, the proliferative response of rat or mouse spleen cells to Concanvalin A, a T cell mitogen, was inhibited by VFA. VFA did not affect the stimulation of rat or mouse B cells by lipopolysaccharide (LPS), a B cell mitogen. VFA did not inhibit Interleukin 1 production by a macrophage cell line. In vivo, VFA given i.p. or p.o. at 5 or 10 mg/kg/day inhibited the production of IgG antibodies in mice immunized with ovalbumin, a T-dependent antigen. T cells from the lymph nodes of treated animals demonstrated a decreased responsiveness to ovalbumin in vitro compared to controls. VFA given at 5 mg/kg/day to mice immunized with LPS, a T-independent stimulus, had no effect on the production of IgM antibodies to single-stranded DNA. VFA given i.p. at 0.3 mg/kg/day inhibited the development of adjuvant arthritis in rats, a T cell mediated autoimmune disease. The data indicate that VFA is a potent immunosuppressive agent, with selectivity for T cells.