MOLECULAR MECHANISMS IN RHEUMATOID ARTHRITIS AND RELATED DISEASES

Organizers: William P. Arend and William J. Koopman

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Role of Major Histocompatibility Complex

EZ 001 MECHANISM OF MHC-MEDIATED ANTIGEN RECOGNITION BY T CELLS, Paul Bowness, Sarah Rowland-Jones, John Bell and Andrew McMichael, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX91AE, UK.

Cytotoxic T lymphocytes (CTL) recognise peptide fragments of virus or other cytoplamic proteins that are bound to class I molecules of the major histocompatibility complex (MHC). Cytoplasmic proteins are degraded within the cell and peptides are transported into the edoplasmic reticulum where they associate with newly synthesised class I molecules. Here they stabilize the latter which are expressed on the cell surface. The polymorphic groove on the membrane-distal face of the MHC molecule plays a critical role in the selection of the peptides. The latter are usually nonamers, octamers or decamers. For each class I molecule, there is a pattern of amino acid side-chains that fits into pockets within the groove. Thus for HLA B2705 the motif is arginine at position 2 of the peptide (p2), and aromatic residue at p3 and arginine or lysine at p9. It is clear that the arginine side chain fits into the B pocket, strong functional evidence coming from experiments (with Dr. R. Colbert and J. Frelinger and University of North Carolina at Chapel Hill) where the B pocket was replaced by that from HLA A2 and the required side chain changed from arginine to leucine. This and other experiments orientate the peptide with the amino terminus in the A pocket and the C terminus in the F pocket. The peptides presented by related members of the B27 family, B2705 and B2702 were identical in some but not all cases.
In addition to the selective effect of MHC polymorphism of epitope peptide selection there is also evidence that polymorphism in processing also contributes, affecting the nature of the peptides available for binding in the endoplasmic reticulum. Thus not all HLA class I molecules with the same sequence present the same peptide.
Peptides presented at the cell surface are recognised by the T cell receptors on the CTL. We have studied the receptors used by CTL clones specific for HLA B27 plus influenza nucleoprotein peptide 383-391. There was restricted use of receptor V genes in clones derived from three different individuals. In the alpha chains, Va 12, 14 and 22 predominated. Clones with Va12 showed a partiular fine-specificity patttern when tested on peptides with residues changed at p1. For the beta chain, Vß7 predominated and in the third hypervariable region of the ß chain *there was conservation with a dominant acidic residue at position 9.* Restricted T cell receptor usage has been observed before in CTL responding to influenza matrix peptide 58-66 but was not seen in allo-reactive B27 specific CTL.
Thes data are compatible with the arthritogenic peptide hypothesis for HLA B27 associated arthritis. Bacterial infection can initiate CTL responses. HLA B27 subtypes can present the same peptides, with the possible exception of B2703. Of the nonamer epitope peptides presented by B27, only three side-chains clearly interact with the TCR, making cross-reactions readily possible. Oligoclonal CTL responses might be expected.

Retroviruses in Autoimmune Diseases

EZ 002 EXPRESSION OF ONCOGENES IN RHEUMATOID ARTHRITIS, **Steffen Gay, Division of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham, U.S.A.**

Rheumatoid arthritis (RA) is a chronic systemic disorder that is dominated by the debilitating sequelae associated with progressive destruction of joints. Our laboratory characterized the early destructive changes in the MRL-*lpr/lpr* mouse arthropathy as being mediated by the proliferation of synovial lining cells. Since the proliferating synovial cells resemble the transformed-appearing synovial cells associated with cartilage and bone destruction in RA, we studied the expression of proto-oncogenes associated with cell transformation and cell proliferation. ras and myc oncoproteins could be detected in about 70% of RA cases and appeared largely restricted to the synovial cells attached to sites of cartilage and bone destruction. Moreover, fos and the early growth response gene-1 (egr-1), *an inducible gene encoding a zinc finger protein containing two* AP-1 binding sites, have been identified in collagenase-producing rheumatoid synovial lining cells.
Based on the observation that synovial hyperplasia in RA is associated with the proliferation of transformed-appearing synovial cells, an overexpression of proto-oncogenes and the presence of retroviral-like particles in the synovial fluid, we explore the possibility that a hitherto unknown HTLV related retrovirus is involved in the pathogenesis of RA.

EZ 003 RETROVIRUS-INDUCED IMMUNODEFICIENCY IN THE MOUSE, Herbert C. Morse III[1], Ambros Hügin[1], Richard Flavell[2], Janet Hartley[1], Sisir K. Chattopadhyay[1], [1]Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD, [2]Yale University School of Medicine, New Haven, CN.

A replication defective murine leukemia virus genome, designated BM5def, has been shown to be required for development of a syndrome (MAIDS) characterized by increasing lymphoproliferation and severe immunodeficiency. BM5 def encodes a single protein, a $Pr60^{gag}$, that appears to effect disease by stimulating a high proportion of T cells when presented in the context of MHC class II molecules on the surface of B cells. T cell proliferative responses in vitro and in vivo are dominated by $CD4^+$ cells expressing Vβ5 and to a lesser extent, Vβ11 and -12 suggesting that BM5def may encode a superantigen. The proliferation of specific responding cells is associated early on with secretion of Th0 cytokines followed by a predominance of Th2 products and a generalized immune paralysis. T and B cell activation that occur secondary to cytokine secretion are associated with appearance of autoantibodies and circulating immune complexes but no immune complex disease. Additional analyses of interactions of BM5def and the immune system utilizing transgenic mice and transfected cells lines expressing the gag product will be discussed.

Other Environmental Factors in Autoimmune Diseases

EZ 004 GENETIC SUSCEPTIBILITY AND ENVIRONMENTAL TRIGGERS IN RHEUMATOID ARTHRITIS, Dennis A. Carson, Salvatore Albani, Jean Roudier, Helen Tighe, Thomas J. Kipps, and Pojen Chen, University of California, San Diego, La Jolla, CA 92093-0663.

Family and population studies indicate that both genetic and environmental factors can increase rheumatoid arthritis (RA) susceptibility. Genetic risk factors include female sex, and the QKRAA amino acid sequence in the HLA-DRβ1 molecule. A homozygous deletion of the hv3005 Ig heavy chain gene may also predispose to RA. Because only a few genetically susceptible individuals develop disease, environmental factors must also contribute to RA pathogenesis. During immune development, some T and B lymphocytes are positively selected for autoreactivity toward abundant self-antigens, although high affinity autoreactive lymphocytes are deleted. We have collected evidence suggesting that resting B cells with low affinity rheumatoid factor (RF) autoantibody activity are positively selected, and function as a major source of antigen presenting cells. These cells cannot be induced to produce autoantibody under normal conditions. In an effort to identify antigens that might trigger RA, we have searched for environmental agents that are prevalent in a normal population, produce chronic infection with high titers of antibodies, mimic the self-antigens important for positive selection, and have the ability to stimulate lymphocytes non-specifically. We have identified two candidate antigens from organisms with these properties, the DNAj heat shock protein of *E. coli* and the gp110 protein of Epstein-Barr virus. Recent experiments indicate that humoral and cellular immune activity to the DNAj protein is abnormal in patients with juvenile RA, and probably in adult RA as well. Immune complexes containing these antigens may have the ability to trigger the expansion of self-reactive, low affinity T and B lymphocytes, with resultant B cell somatic mutation. Analyses of RF secreting hybridomas from RA synovia have revealed multiple somatic mutations, leading to high affinity self-reactivity. Once this occurs, autoimmune responses may be self-sustaining in the absence of a specific exogenous antigen. The implications of these findings for RA diagnosis and therapy will be discussed.

EZ 005 EPIDEMIOLOGY OF RHEUMATOID ARTHRITIS: IMPLICATIONS FOR AN INFECTIOUS ETIOLOGY
Alan J Silman, Arthritis and Rheumatism Council Epidemiology Research Unit, Manchester University, UK, M13 9PT

The etiology of rheumatoid arthritis (RA), despite considerable study, is essentially unknown. A genetic susceptibility, in part conferred by specific HLA class II epitope, can only explain a small proportion of disease susceptibility given that the concordance rate in monozygotic twins is only around 15%. There is thus likely to be a substantial environmental component. The epidemiological evidence is inconsistent in determining whether the most likely environmental exposure is an infectious agent.

RA is geographically ubiquitous with remarkably little variation in occurrence between most developed populations in both Northern and Southern hemispheres - variation that might have been expected if infection with a single organism or a group of related organisms was of relevance. Further, within multiple case families, as well as in disease concordance twins, there is no similarity in the timing of disease onset suggesting that shared environmental triggers do not explain the familial clustering. Reports of geographical clustering are also rare in RA. This is in contrast to clusters reported in other connective tissue diseases. By contrast other data strongly support an infectious origin. There are some populations with a very low prevalence of RA, in particular those in rural African and rural Chinese populations whereas urban African groups have the "standard" Western occurrence, suggesting that some aspect of urbanisation influences disease. There are also considerable trends in the occurrence of RA both in terms of year of birth (birth cohort effect) and year of onset (period effect). Although such data are difficult to gather, the existence of such time trends would be difficult to explain in the absence of a change in the epidemiology of a putative risk exposure. A number of case control type studies, based on sero-epidemiological investigation have implicated association of the disease with a number of infectious agents including Epstein-Barr Virus, mycobacteria and Proteus. The relative risks are small and data frequently inconsistent but these might be expected in the face of etiological heterogeneity. Any infectious etiology would also need to explain the marked female excess and the relative rarity of the disease before middle life.

EZ 006 MOLECULAR MIMICRY IN HLA-B27-RELATED ARTHRITIS, David Yu, Toru Fukazawa, Herbert Kellner, Jun Wang, and Juan Wen, Rheumatology Division, Department of Medicine, U.C.L.A., Los Angeles.

Molecular mimicry between bacteria and host has been postulated to be important in HLA-B27-related arthritis. Three experimental approaches have been available to identify such bacterial proteins. One is to search for those with linear sequences similar to the unique domain of HLA-B27. Three bacterial proteins have been described, the most striking similarity being with the Klebsiella Kp2, in which a sequence of 6 amino acids is completely identical to one in HLA-B27. The second approach utilizes monoclonal antibodies to search for bacterial proteins cross-reactive with HLA-B27. Several bacterial proteins reactive with anti-HLA-B27 antibodies have been cloned and sequenced. With one protein, the OmpA, the molecular basis of the cross-reactivity has also been analyzed using synthetic peptides of overlapping sequences, substituted residues as well as site-directed mutagenesis of the HLA-B27 gene itself. With both experimental approaches, relevance of mimicry to the patients have been tested by searching for serum antibodies directed at the mimicking epitopes. Results are unencouraging.
With the very recent revelation of the physiology and structure of the HLA-B27, the modern approach is to identify bacterial proteins which potentially provide peptides mimicking self peptides in the context of HLA-B27. Several experimental strategies again are available. One utilizes direct infection of cells or mice with the arthritis-causing bacteria, and then testing for CTL activity. These experiments demonstrate that HLA-B27-related peptides can indeed be generated during bacterial infections. A second strategy, which we have adopted, is to introduce bacterial proteins into the HLA class I pathway by transfecting into cells bacterial genes carried in eukaryotic expression vectors. We then test for patient serum antibodies as well as CTL which might recognize the complexes between the bacterial peptides and HLA-B27. Lastly, we have attempted to identify the bacterial peptides which are reactive by synthesizing all the peptides in the bacterial protein sequences which carry a motif potentially similar to those self peptides which have been reported in HLA-B27.
Identification of such bacterial peptides will potentially allow us to test whether molecular mimicry is important in HLA-B27-related arthritis.

T Cell Repertoire in Autoimmune Diseases

EZ 007 AN IN VIVO MODEL OF HUMAN INFLAMMATORY SYNOVITIS: ENGRAFTMENT OF HUMAN SYNOVIUM INTO INTO SEVERE COMBINED IMMUNE DEFICIENT (SCID) MICE AND MIGRATION OF PERIPHERAL BLOOD (PB) T CELLS TO ENGRAFTED HUMAN SYNOVIUM, Barton F. Haynes, Karen Rendt, Dawn Jones, Conrad Richter, Spence McCachren, and Todd Barry, Duke University Medical Center, Durham, NC 27710

The study of human inflammatory synovitis is hampered by the lack of *in vivo* animal models for many forms of human synovial disease. In this study, we engrafted normal and inflammatory (RA, JRA, B27+ Spondyloarthropathy) synovium under the renal capsule of immunodeficient C.B-17 *scid/scid* (SCID) mice. Both normal and inflammatory synovium grew well (95% engraftment rate), increasing in size 2 to 3 fold, after 5 to 9 weeks of engraftment. Histologic and phenotypic components of the preimplantation synovium (normal and inflammatory) including the presence of TE7+ fibroblasts, V2+ human vessels, CD11b+ macrophages, multinucleated giant cells, clusters of infiltrating TCRα/β+ T cells, cellular adhesion and activation markers (ICAM-1, LFA-3, CD44, CD29) and *in situ* mRNA expression of gelatinase and tissue inhibitor of metalloprotease (TIMP) were maintained in the synovial xenografts. However, the T cell component of inflammatory synovial xenografts diminished over time. To take advantage of the lymphodepleted state of inflammatory synovial grafts, 50 x 10^6 autologous or allogeneic PB leukocytes were injected intraperitoneally into synovium-engrafted animals. After 7 days, specific migration of PB T cells to the implanted human synovium and to abdominal murine lymph nodes was demonstrated. Whereas T cells that migrated to murine LN were >90% CD4+, T cells that migrated to synovial grafts were 60% CD8+ and 40% CD4+. TCR Vβ analysis of HLA-DR4+ RA PB T cells that had migrated to autologous RA synovial grafts demonstrated the full repertoire of TCR Vβ types. No circulating CD45+ human cells were detected and no migration of human cells to other mouse organs including kidney, spleen, liver, thymus, or appendix was observed. Thus, the migration of human T cells to human synovium in SCID mice may mimic selective *in vivo* homing of T cells to inflammatory human synovium.

EZ 008 SPECIFICITY AND FUNCTION OF RHEUMATOID ARTHRITIS-DERIVED γδ T CELLS. Joseph Holoshitz, Department of Internal Medicine, University of Michigan, Ann Arbor MI, 48105-0531.

Molecular studies have identified a disease-associated motif at the third hypervariable region of the DRB1 gene in patients with rheumatoid arthritis (RA). It is estimated that over 90% of individuals with RA express this disease susceptibility sequence. In view of the role played by this region of class II MHC molecules in antigen presentation, it has been suggested that recognition of a putative arthritogenic antigen by clonal $CD4^+$ αβ T cell populations is likely to be involved in the pathogenesis of RA. Conclusive evidence for a clonal αβ T cell population in established RA, however, is conspicuously absent. The lack of αβ clonality, and the recent observation of preferential accumulation of γδ T cells with relatively limited TCR diversity in the inflamed rheumatoid joint give a rationale for examining the role of this subset in triggering RA. To address this supposition, T cell clones expressing the Vγ9/Vδ2 TCR have been isolated from the synovial fluid of a patient with early RA. These clones displayed dual antigenic recognition: a nonclonal, MHC-unrestricted recognition of mycobacteria, and a clonal recognition of a short tetanus toxin peptide presented by HLA-DRw53, a DRB4-encoded non-polymorphic class II MHC molecule, previously found to be associated with RA susceptibility. T cell reactivity to this peptide were found in the peripheral blood and the synovial fluid of patients with early RA, but not in patients with late RA. These results suggest that recognition of this peptide by γδ T cells may be involved in the early stages of RA. Based on these results, and in accord with current knowledge of the biology of γδ T cells and the immunogenetics of RA, a postulated pathogenetic mechanism in RA will be discussed.

EZ 009 GENETIC ANALYSIS OF MRL-*lpr* MICE: RELALTIONSHIP OF THE Fas APOPTOSIS GENE TO DISEASE MANIFESTATIONS AND RENAL DISEASE MODIFYING LOCI. Mark L. Watson[1,2], Jaya K. Rao[1], Gary S. Gilkeson[1,3], Philip Ruiz[4], Eva M. Eicher[5], David S. Pisetsky[1,2,3], Akio Matsuzawa[6], Julie M. Rochelle[1,2] and Michael F. Seldin[1,2]; [1]Autoimmune Genetics Center, Department of Medicine, [2]Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC, [3]Medical Service, Durham Veterans Administration Hospital, Durham, NC, [4]Department of Pathology, University of Miami, FL, [5]The Jackson Laboratory, Laboratory Animal Research Center, Bar Harbor, ME, and [6]The Institute of Medical Sciences, University of Tokyo, Tokyo 108, Japan.

In MRL mice, the mostly recessive *lpr* mutation results in both the accumulation of $CD4^-$, $CD8^-$, $CD3^+$ T cells in lymphoid tissue and many features of generalized autoimmune disease including immune complex glomerulonephritis. To positionally clone the *lpr* mutation and analyze the effects of background genes, backcross offspring were examined from the cross (MRL/MpJ-*lpr* x CAST/Ei)F1 x MRL/MpJ-*lpr*. The *lpr* gene was found to be closely linked to a mouse Chromosome 19 marker defined by a variation of a *Fas* gene restriction fragment. Our results identified differences in RNA expression and differences in the genomic organization of the *Fas* gene between normal and *lpr* mice, and confirm the recent report that a mutation in the *Fas* apoptosis gene is the *lpr* mutation. However, our results also indicate that the *Fas* gene is expressed in spleen cells from normal mice, and spleen and lymph node cells from mice with a second mutation at the *lpr* locus (lpr^{cg}). Together these results suggest that altered *Fas* transcription results in the failure of lymphocytes to undergo programmed cell death and may lead to an altered immune cell repertoire. This mechanism may explain certain central and peripheral defects in tolerance that are present in autoimmune disease. The current study also demonstrates the profound effect of background genes on the degree of nephritis, lymphadenopathy, and anti-DNA antibody production. Of major note, our studies suggest the identification of chromosomal positions for genes that modify nephritis. Analysis of the backcross mice for markers covering most of the mouse genome suggests that over 50 % of the variance in renal disease is attributable to quantitative trait loci on mouse Chromosomes 7 and 12. Moreover, this study provides a model for dissecting the complex genetic interactions that result in manifestations of autoimmune disease.

Adhesion Molecules in Cell Migration

EZ 010 THE DISTINCTIVE FUNCTIONS OF SELECTINS, INTEGRINS, AND IG FAMILY MOLECULES IN REGULATION OF LEUKOCYTE INTERACTION WITH ENDOTHELIUM, Timothy A. Springer, The Center for Blood Research, 200 Longwood Avenue, Boston, MA 02115.

Adhesion molecules together with cytokines and chemoattractants regulate leukocyte interaction with endothelium and subsequent events such as migration through the endothelium and within tissues, and cell-cell interactions that are important in inflammatory responses including those of granulocytes and monocytes with host cells and foreign pathogens, and antigen-specific responses of lymphocytes. Adhesion receptors are also used by cells to sense information about the environment that can stimulate signalling pathways. Three families of adhesion receptors have distinct functions in cell interactions. Ig family molecule density on the surface regulates cell interactions; some molecules are inducible by cytokines such as ICAM-1 and VCAM-1 and others are constitutively expressed such as ICAM-2. Integrin adhesive activity can be regulated independently of surface expression by intracellular signals acting on integrin cytoplasmic domains that appear to affect the conformation of the extracellular ligand binding domain. Selectins mediate attachment of leukocytes ot the vessel wall under flow conditions at which integrin and Ig family members are ineffective. The initial steps in leukocyte accumulation have been reconstituted in on vitro system. Neutrophils in a parallel plate flow chamber roll on artificial phospholipid bilayers containing the selectins CD62 or ELAM-1. The results are the same on bilayers containing CD62 and ICAM-1, expect when a chemoattractant is infused, activation of integrins causes the rolling neutrophils to arrest and then spread. The accompanying adhesion developed through the integrin-ICAM-1 interaction is more than 100-fold stronger than through selectins.

EZ 011 MOLECULAR MECHANISMS OF HEMATOPOIETIC CELL ADHESION TO FIBRONECTIN, E. A. Wayner[1], A. Garcia-Pardo[2],, J. E. Schwarzbauer[3], J. B. McCarthy[1]. [1]Department of Laboratory Medicine and Pathology, University of Minnesota, [2]Centro de Investigaciones Biologicas, Madrid, [3]Department of Biology, Princeton University.

Integrin $\alpha4\beta1$ is the lymphocyte receptor for a vascular cell adhesion molecule, VCAM-1, which is up-regulated on endothelium and other cell populations during a variety of chronic inflammatory conditions. $\alpha4\beta1$ therefore plays a major role in the recruitment of lymphocytes from the vasculature to sites of inflammation. $\alpha4\beta1$ integrin is also the receptor for the carboxy terminal cell binding domain (CTCBD) of fibronectin which comprises adhesion sites in Hep II and a high affinity site, CS-1, in the type III connecting segment or V (for variable) region. Leu-asp-val or LDV is the minimal peptide in CS-1 capable of supporting stable hematopoietic cell adhesion. However, only those cells which express an active form of the $\alpha4\beta1$ complex are capable of the adhesive recognition of LDV outside the context of intact CS-1 or fragments of fibronectin which contain the CTCBD (38 kDa). The ability of the $\alpha4\beta1$ complex to bind LDV can be altered with a monoclonal antibody to $\beta1$ which specifically activates $\beta1$ dependent function. In contrast, however, the adhesive recognition of fibronectin fragments containing the entire CTCBD (38 kDa) does not require activation of $\beta1$. In the present studies we identify another peptide ligand for $\alpha4\beta1$ present in the Hep II domain of fibronectin. This is the peptide I (YEKPGSPPREVVPRPRPGV) we have previously described (McCarthy et al., Biochemistry, 27: 1380-1388, 1988). This conclusion is based on the following observations: 1) Monoclonal antibodies were made against the 38 kDa fragment and screened for their ability to inhibit T cell adhesion to intact fibronectin. This screening protocol yielded one antibody, P4H10 which reacted strongly with fibronectin, fragments of fibronectin containing Hep II but not with an 80 kDa fragment containing the CCBD or with CS-1. 2) Using overlapping recombinant fusion proteins derived from the rat fibronectin sequence (deminectins) P4H10 mapped to an amino acid sequence located in the COOH-terminal portion of the type III_{14} repeat. 3) This portion of type III_{14} contains the two adhesive peptides (I and II) described by McCarthy (2) and by ELISA, P4H10 reacted with peptide I. Since adhesion of hematopoietic cells to fragments of fibronectin which contain both CS-1 and peptide I does not require activation, it is concluded that it is the cooperative interaction of peptide I and CS-1 (when present) that together promote the high affinity interaction of $\alpha4\beta1$ with this domain. Furthermore, our data strongly suggest that it is the interaction of peptide I with the $\alpha4\beta1$ complex that activates subsequent binding to the LDV sequence in V_{120} isoforms of fibronectin. Functionally defined Mabs to CS-1 even if they can be shown to react with intact FN and the 38 kDa fragment of fibronectin (P1F11) do not inhibit adhesion of hematopoietic cells to FN or the CTCBD. Mab P4H10 has a profound effect on adhesion to fibronectin (60% inhibition), completely inhibits adhesion to Hep II (100%) and almost completely (85-90%) inhibits adhesion to the intact CTCBD strongly suggesting that $\alpha4\beta1$ interaction with peptide I is the initial step in promoting cell adhesion to this domain. Finally, soluble peptide I up-regulates binding of hematopoietic cells to LDV-containing peptides. Therefore, we conclude that hematopoietic cell adhesion to intact fibronectin involves two receptors and at least three peptide ligands: peptide I, LDV and RGD. The significance of $\alpha4\beta1$ interaction with its ligands (fibronectin and VCAM) in relationship to T cell recruitment and localization in inflammatory tissue will be discussed.

Adhesion Molecules in Cell Activation

EZ 012 SIGNAL TRANSDUCTION FROM MYELOID INTEGRINS, Eric J. Brown[1], Frederik Lindberg[1], Robert Senior[1], Irene Graham[1], Ming-jie Zhou[1], and Hattie D. Gresham[2], [1]Washington University School of Medicine, St. Louis MO 63110, and [2]University of Missori School of Medicine, Columbia, MO.

Interaction of leukocytes with extracellular matrix proteins is a potent mechanism for regulating the inflammatory functions of leukocytes. We have investigated the molecular mechanism by which extracellular matrix proteins regulate the functions of professional phagocytes, including chemotaxis, adherence, and respiratory burst. Fibronectin, laminin, fibrinogen, vitronectrin, and von Willibrand's factor all stimulate IgG-mediated phagocytosis by human PMN. Phagocytosis stimulated by all these ligands except laminin is inhibited by the monoclonal antibody 7G2, which recognizes the integrin $\beta3$ chain. By a variety of criteria, the integrin receptor involved in stimulation by these diverse ligands is not either of the two known b3 integrins, aIIbb3 or avb3. Therefore, we have called this novel $\beta3$-related integrin Leukocyte Response Integrin (LRI). Of note, unlike $\alpha IIb\beta3$ or $\alpha v\beta3$, LRI recognizes the peptide sequence Lys-Gly-Ala-Gly-Asp-Val (KGAGDV). Monovalent KGAGDV is the most potent peptide antagonist of LRI function (apparent Kd~ 3 μM), and multivalent KGAGDV is an LRI agonist. KGAGDV-coated microspheres bind only to PMN and mononuclear phagocytes. We recently have found a monoclonal antibody (B6H12) which inhibits LRI function. B6H12 does not recognize the integrin, however, but a 50 kD highly glycosylated integral membrane protein. Predicted amino acid sequence of the cDNA for this protein suggests that it spans the membrane multiple times. Interestingly, this protein has a much wider distribution than LRI, and is present at some level on all cell types so far tested. We have evidence that this protein can be physically associated with specific integrins, and B6H12 can inhibit some $\alpha v\beta3$-dependent functions as well as LRI functions. Based on the close physical and functional association of the 50 kD protein with integrins, we have called this protein Integrin-Associated Protein (IAP). Based on its close physical association with some integrins and the ability of anti-IAP to inhibit the binding of multivalent ligands to LRI and $\alpha v\beta3$, we propose that IAP is involved in an early step in signal transduction from $\beta3$ integrins.

EZ 013 REGULATION OF INTEGRIN FUNCTION AND CELLULAR ADHESION, Timothy E. O'Toole and Mark H. Ginsberg, Scripps Research Institute, La Jolla, Ca. 92109.

The adhesion of lymphocytes to other cells or extracellular matrices is mediated in part by members of the integrin family of cell adhesion receptors. Several integrins respond to extracellular signals or soluble agonists by modulating their ligand binding affinity. Disruption of these highly regulated processes can adversely affect lymphocyte function, critical in the pathogenesis of rheumatic disease. As a model to study the regulation of an integrins' affinity or activation state and role in cell adhesion, we have expressed wild type and variant forms of the platelet integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) in CHO cells. Although wild type $\alpha_{IIb}\beta_3$ assumes a low affinity state when expressed in heterologous cells, specific, high affinity ^{125}I-fibrinogen or PacI binding can be attained if α_{IIb} cytoplasmic sequences are deleted or exchanged with those from α_5. In contrast, if β_3 cytoplasmic sequences are deleted or exchanged with those from β_1, the low affinity state is retained. Constitutive, high affinity binding conferred by the chimeric α subunit is energy dependent and requires β cytoplasmic sequences. Inhibitors of glycolysis and oxidative phosphorylation, truncation of β_3 cytoplasmic sequences, or a single Ser to Pro amino acid substitution all abolish the high affinity state. In addition, β_3 cytoplasmic sequences are necessary for focal adhesion formation following ligand binding. These studies demonstrate the importance of both α and β cytoplasmic domains in integrin function and suggest the possibility of integrin-specific regulatory mechanisms.

Inhibition of IL-1 Action (Joint)

EZ 014 IL-1 RECEPTOR ANTAGONIST: PRODUCTION AND ROLE IN BIOLOGY, William P. Arend, Robert W. Janson, Jr., Mark Malyak, Charlotte Kutsch, John K. Jenkins, Lorise C. Gahring, and Michael F. Smith, Jr., University of Colorado School of Medicine, Denver, CO 80207.

L-1ra is the first described naturally-occurring human molecule that functions as a specific receptor antagonist of a cytokine. Two forms of IL-1ra have been described: secretory or sIL-1ra is produced by monocytes and macrophages whereas intracellular or icIL-1ra is a major product of keratinocytes and other epithelial cells. IL-1ra and IL-1β production by human monocytes appear to be regulated differently, and possibly in a reciprocal fashion. LPS induces both proteins in this cell whereas adherent IgG induces only IL-1ra. Other cytokines also influence IL-1ra production by monocytes. GM-CSF modestly induces IL-1ra production by fresh monocytes but is more important as a differentiating agent to enhance IL-1ra production by macrophages. LPS-induced monocytes exposed to IL-4 exhibit an inhibition in IL-1β production and an enhancement in IL-1ra *production. IL-10 is also a potent inhibitor of LPS-induced IL-1β production by* monocytes but only weakly enhances IL-1ra production. Two other cytokines, IL-1α and IL-3, are weak inducers of IL-1ra production by monocytes but do not change the effects of LPS. Thus, different cytokines may influence IL-1ra and IL-1β production by monocytes through different mechanisms.

Monocyte differentiation into macrophages leads to the acquisition of new patterns of induction of IL-1β and IL-1ra. Both *in vitro*-derived and alveolar macrophages spontaneously produce IL-1ra in culture; this production is not dependent upon adherence or the presence of serum. GM-CSF enhances IL-1ra production by macrophages but in contrast to monocytes, neither LPS nor adherent IgG have a positive effect. Another important cell as a source of IL-1ra is the *human neutrophil. Both freshly-isolated peripheral blood and synovial fluid* neutrophils contain a small amount of IL-1ra protein (but not IL-1β) without any detectable mRNA. This observation suggests that these cells were stimulated earlier in their life span to produce IL-1ra. Cultured neutrophils exhibit a modest induction of IL-1ra transcription and translation in the presence of LPS, IL-4, TNFα or GM-CSF. Although this IL-1ra is the glycosylated secretory variant, stimulated neutrophils secret little IL-1ra. These cells produce less than 1% the amount of IL-1ra per cell as do monocytes or macrophages. However, dying neutrophils present in large numbers in inflammatory exudates may be an important source of extracellular IL-1ra.

Human keratinocytes produce large amounts of icIL-1ra spontaneously in culture. This structural variant lacks a leader sequence and remains intracellular. Production of icIL-1ra by keratinocytes is enhanced by cell differentiation or by exposure to TNFα; other cytokines have no effect. The ratio of IL-1ra to IL-1 in normal human skin is $\approx$100:1, suggesting that IL-1ra may exert potent anti-*inflammatory effects in the skin when released from dying keratinocytes in the* stratum corneum. Synovial fibroblasts and alveolar macrophages can simultaneously produce both forms of IL-1ra during *in vitro* culture. The icIL-1ra to sIL-1ra ratio in cultured synovial fibroblasts is $\approx$20:1. Small amounts of icIL-1ra mRNA are produced by alveolar macrophages in response to culture with CMV.

Studies on the transcriptional regulation of sIL-1ra indicate the presence of cis-acting DNA elements in the most proximal 300-bp of the promoter that are important in both constitutive and LPS-induced IL-1ra production by macrophage cell lines. The icIL-1ra molecule is encoded for by a different first exon acting through an alternate splice receptor site in the mRNA. The icIL-1ra first exon and its promoter are located at least 5-kb upstream from the sIL-1ra genomic DNA. *The two forms of IL-1ra appear to be under different transcriptional control.*

Extracellular IL-1ra may be important as a competitive inhibitor of IL-1 in the cell microenvironment. However, intracellular IL-1ra may play additional roles in regulation of cell function that have yet to be defined.

EZ 015 CLINICAL USE OF HUMAN RECOMBINANT IL-1 RECEPTOR ANTAGONIST, Michael A. Catalano, Synergen, Inc., Boulder, CO 80301-2546.

Interleukin-1 (IL-1) is a pro-inflammatory cytokine implicated in a wide range of human diseases. There exists a naturally-occurring inhibitor of IL-1 which antagonizes its actions at the receptor level. A recombinant form of the IL-1 receptor antagonist (IL-1ra) has been effective in animal models of rheumatoid arthritis, sepsis, inflammatory bowel disease, graft-versus-host disease, and asthma. It is now being studied in clinical trials of these diseases. Some of the results will be described.

EZ 016 MODIFICATION OF ALLERGIC LATE-PHASE RESPONSE BY SOLUBLE HUMAN IL-1 RECEPTOR (RHU IL-1R).
Michael F.Mullarkey[1], Abbe Sue Rubin[2], Eileen R. Roux[2], Roberta K. Hanna[2], Cindy A. Jacobs[2], [1]Swedish Hospital Medical Center, Seattle, [2]Immunex Corporation, Seattle.

Allergic respiratory disease affects an estimated 10% of the American population. When allergens are injected under the skin of atopic patients a characteristic wheal-and-erythema reaction is noted within 20 minutes. Two to 8 hours after this immediate reaction patients experience a second reaction without additional exposure to antigen. Late-phase reactions result in sustained inflammation in the skin, nose and lungs of allergic patients. The time course, pathology and etiology of late-phase allergic responses are distinct from classic delayed hypersensitivity reactions. An initial clinical trial in humans was designed to investigate whether rhu IL-1R might inhibit late-phase response to allergen in patients with allergic rhinitis. Fifteen volunteers were skin tested with clinically relevant allergens at concentrations necessary to elicit late-phase responses. Patients were then injected S.C. with rhu IL-1R and sera collected 14 days later to determine antibody status. All patients were antibody negative. Skin tests were then repeated in duplicate on each forearm with 2x the concentration required for late-phase responses and additional control sites were placed on patients' backs. Antigen injection on the forearms was followed by the immediate S.C. injection of rhu IL-1R or placebo at allergen sites. Cohorts of three patients received 1, 10, 25, 50, and 100 ug/site. Sites were monitored for erythema, induration and itching or discomfort. Drug and placebo were administered according to a prospective, double-blind design. Induration in the tested arm versus the control arm was reduced at 1 ug/site during multiple observations obtained between 2 and 8 hours ($p<0.005$). At 10 ug/site induration was reduced in the experimental arm between 2 and 8 hours ($p<0.05$), and systemic effects were seen causing a reduction of induration at control sites on the back. At higher doses, late-phase was suppressed at both control and experimental sites in a dose-dependent fashion. All patients remained antibody negative when tested 14 days after the second exposure to rhu IL-1R. No toxicities were noted throughout the study. Rhu IL-1R is a remarkably potent inhibitor of allergen induced late-phase inflammation in the skin, with a high safety profile. It may be of value as a therapeutic agent in allergic diseases.

Inhibition of TNF Action (Joint)

EZ 017 NATURALLY-OCCURRING ANTIBODIES TO IL-1, IL-6 AND OTHER CYTOKINES.
Klaus Bendtzen, Morten Svenson, Morten B. Hansen, and Marcus Diamant. Laboratory of Medical Immunology TTA 7544, Rigshospitalet University Hospital, DK-2200 Copenhagen N, Denmark.

A very specific means of modifying the function of cytokines has recently been demonstrated by the presence in sera of specific, high-affinity autoantibodies (aAb) to IL-1α and IL-6. These aAb are found in at least 15 - 75% of normal individuals and, at variable concentrations, in patients with immunoinflammatory disorders. The Fab fragments of the respective aAb bind in a saturable manner to IL-1α and IL-6 with exquisite specificity and with remarkably high affinities (Table). The aAb to IL-1α and IL-6 are the single most important binding proteins of these cytokines in normal human sera.

'Autoantibodies' to other cytokines, e.g. IL-2, TNFα, and the interferons, have been reported in normal individuals. However, naturally occurring antibodies to cytokines, apart from those against IL-1α and IL-6, not always bind the cytokines in a specific manner or, indeed, with any appreciable affinity. Western blotting techniques, for example, may show some degree of specificity even though the binding between antibody and ligand is weak and topographically unassociated with the specific binding sites of the antibodies. Even though Western blotting, RIA and ELISA techniques may be used for screening purposes, demonstration of ligand binding to the Fab fragments of the immunoglobulins, combined with saturation binding analysis, is necessary to confirm the presence of specific aAb to a given cytokine.

The biological role of aAb to IL-1α and IL-6 is not yet understood, although their binding forces suggest that they interfere with immune and inflammatory processes, for example by blocking or destroying antigen-presenting cells carrying membrane-bound cytokine and/or by scavenging bioactive IL-1α and IL-6 released by cells at inflammatory sites. Indeed, the aAb inhibit receptor binding and thus the bioactivities of the cytokines. They are polyclonally derived, because they are recognized by antibodies to both immunoglobulin light chains. They are almost exclusively of the IgG class, IgG_4 constituting about 50% of the aAb to IL-1α (IgG_4 normally amounts to 1% of total IgG). IgG_4 does not activate complement, and lattice formation is limited. Hence, precipitation of IL-1α/anti-IL-1α IgG_4 is unlikely to occur to any significant degree *in vivo*. IgG_4 aAb to IL-1α may therefore function as specific carriers, and thus systemic regulators, of circulating IL-1α. The clinical significance of these aAb is obvious, because:

1: aAb in serum interfere with biochemical and biological assays for IL-1α and IL-6,
2: formation of aAb to cytokines in general is important when considering therapeutic use of cytokines as bioresponse modifiers,
3: inappropriate production/function of aAb to inflammatory cytokines may be pathogenetically involved in immunoinflammatory diseases, including systemic complications to infections and trauma,
4: the therapeutic use of naturally occurring aAb to cytokines is of potential interest. Thus, the presence of blocking aAb to IL-1α and IL-6 in pharmaceutically prepared normal human IgG might perhaps explain why high-dose IgG therapy is beneficial in a number of pathogenetically obscure immunoinflammatory conditions.

Table. Autoantibodies to IL-1α and IL-6 in healthy adults.

	anti-IL-1α Ab	anti-IL-6 Ab
Frequency	30-75%	15%
Increased with age and in males	yes	no
Block CK rec.-binding and bioactivity	yes	yes
Recognize native cytokines	yes	yes
Immunoglobulin classes	$IgG_{4,2,1}$	IgG_1
In pharmaceutical IgG preparations	yes	yes
Ligand binding:		
K_d	$< 10^{-11}$ M	$< 10^{-10}$ M
maximum binding	30 ng/ml serum	300 ng/ml serum
bind with Fab fragments	yes	yes

References: Svenson M et al. Scand J Immunol *1989*; **29**: 489-492.
Bendtzen K et al. Immunol Today *1990*; **11**: 167-169.
Hansen MB et al. Scand J Immunol *1991*; **33**: 777-781.

EZ 018 TNF-RECEPTORS KINETICS IN MICE,
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TNF-Receptors are considered to be more than cell signalling proteins. They are claimed to be responsible for inactivation of TNF spilling into the circulation during inflammation. The kinetics of TNF-R is studied by ELISA in mice treated with LPS. Antibodies to TNF are used to investigate the mechanism responsible for TNF-R plasma level kinetics. Furthermore the role of the kidney in the clearance of TNF-R and TNF-R-TNF complex was studied in nephrectomized and normal mice. The data indicate that TNF appears very early after TNF induction by LPS and preceding the increase in plasma TNF-levels. TNF plays a role in the regulation of TNF-R receptor levels, since anti-TNF antibodies reduced the increase in TNF-R levels. The kidney is found to be responsible for clearance of both TNF-R and TNF-R-TNF complexes.

In conclusion LPS leads to increased systemic TNF levels that are preceded by an increase in circulating TNF-R levels, a process in which TNF is a responsible factor. Both TNF-R and the complexes are primarily cleared by the kidney.

EZ 019 COUNTERACTING THE EFFECTS OF TNF WITH SOLUBLE TNF RECEPTORS, Kendall M. Mohler, Dauphine S. Torrance, Craig A. Smith, Raymond G. Goodwin and Michael B. Widmer. Immunex Corporation, Seattle, WA, 98101.

Two forms of the extracellular, ligand-binding portion of the human p80 cell surface receptor for tumor necrosis factor, a monomer (sTNFR) or a dimer linked to the Fc portion of human IgG1 (sTNFR:Fc), were used to antagonize the biological activities of TNF. As predicted from the multivalent interaction of TNF with its cell surface receptor(s), the divalent sTNFR:Fc was a more potent inhibitor of TNF binding (50-100X) and TNF bioactivity (500-1000X) *in vitro* than the monomeric sTNFR. In addition, monomeric and dimeric forms of the sTNFR were also utilized *in vivo* in two mouse models of inflammation; LPS-induced septic shock and hypersensitivity pneumonitis induced with *M. faeni*. In LPS-induced septic shock, administration of dimeric sTNFR:Fc to mice at doses ranging from 10-100 ug saved them from an otherwise lethal injection of LPS, whereas sTNFR at doses up to 260 ug did not. Surprisingly, the dimeric sTNFR:Fc construct demonstrated a beneficial effect even when administered 3h after a lethal LPS injection (i.e., after serum TNF levels had peaked and receded). To study the mechanism by which the soluble TNF receptor functions, serum TNF levels were examined in mice given LPS in the presence or absence of soluble receptor. Administration of a mortality-reducing dose of sTNFR:Fc ablated the rise in serum TNF bioactivity that normally occurs in response to LPS, as measured by an *in vitro* L929 cytolysis assay. However, TNF bioactivity was revealed in these "TNF-negative" samples when a monoclonal antibody which blocks the binding of murine TNF to the human soluble TNF receptor was included in the L929 bioassay. These results indicate that (1) the absence of direct cytolytic activity in the L929 assay was due to neutralization of TNF, rather than to an absence of TNF in the serum and (2) TNF readily dissociates from the sTNFR:Fc *in vitro*. Utilizing the L929 bioassay in conjunction with the blocking mAb to the sTNFR:Fc molecule, we have demonstrated that the sTNFR:Fc molecule can prolong the presence of serum TNF *in vivo* following administration of LPS. Moreover, administration of either monomeric sTNFR or low doses of dimeric sTNFR:Fc actually resulted in increased serum TNF levels compared to mice given LPS but no soluble receptor. However, these "agonistic" doses of receptor did not lead to increased mortality when an LD60 dose of LPS was given. In an *in vivo* model of local inflammation in the lung (hypersensitivity pneumonitis), intranasal (i.n.) installation of *M. faeni* antigen alone induced a marked influx of granulocytes and monocytes into the lung on d4. Simultaneous instillation (i.n.) of sTNFR:Fc (100 ug), but not human IgG1, decreased the inflammatory response. Instillation of monomeric sTNFR at doses up to 40 ug did not reduce the lung inflammatory response. Collectively, these results demonstrate that (1) recombinant soluble TNFR is a potent *in vitro* and *in vivo* antagonist of TNF bioactivity, especially in dimeric form and (2) soluble TNF receptors may prevent toxic effects of TNF *in vivo* by serving as biological buffers for TNF. Under some circumstances the soluble receptors function simultaneously as both TNF "carriers" and antagonists of TNF biological activity.

Abnormalities in Autoantibody Production

EZ 020 THE PATHOGENIC T CELLS OF LUPUS: PRIMARY STRUCTURE AND ANTIGENIC SPECIFICITY OF THEIR AUTOIMMUNE T CELL RECEPTORS. Syamal K. Datta, Chandra Mohan, Sharlene Adams, Ami Desai, and Changchuin Mao. *Department of Medicine, New England Medical Center, and Immunology Programme, Tufts University School of Medicine, Boston, MA 02111.*

The cause and cure of systemic lupus erythematosus (SLE) remains elusive because the primary autoantigen that drives the pathogenic autoimmune response in this disease is unknown. However, we have cloned the T helper (Th) cells that play a central role in the nephritogenic autoimmune response. Only a fraction (12-15%) of a large number (•800) of "autoreactive" T cell clones derived from patients as well as mice with lupus nephritis can selectively induce syngeneic B cells to produce pathogenic anti-DNA autoantibodies. These IgG anti-DNA autoantibodies cause lupus nephritis when injected into normal mice and they have stretches of cationic residues in their V regions that are generated by somatic mutations. Representative Th clones that selectively augment production of these pathogenic autoantibodies in vitro can also induce lupus nephritis *rapidly* when transferred into young preautoimmune mice, but conventional autoreactive T cells fail to do so. And unlike other types of autoreactive T cells, these special autoimmune Th cells are absent in young preautoimmune mice. These pathogenic Th cells of lupus interact with the pathogenic autoantibody-producing B cells in a cognate, MHC class II restricted manner indicating that they are specific for some endogenous autoantigen/s presented by the autologous B cells. The T cell receptor (TCR) V genes expressed by these pathogenic autoantibody-inducing Th clones are heterogeneous. However, the V-D-J junctional regions or CDR3 loops of TCR beta-chains expressed by the pathogenic Th clones bear a recurrent motif of one or more anionic residues. These negatively charged, junctional residues are generated in most cases by N-nucleotide additions, suggesting that the pathogenic Th clones are selected by some autoantigen/s with corresponding cationic residues. Since these Th clones preferentially interact with and help the select population of B cells that produce cationic anti-DNA autoantibodies, it is likely that those B cells in turn present the autoantigen/s with cationic residues to the pathogenic Th clones. Cationic proteins, such as histones - complexed with DNA could be taken up, processed and presented by such anti-DNA B cells efficiently. Alternatively, since the cationic anti-DNA autoantibodies produced by these B cells themselves carry streches of cationic residues in their V regions, those cationic peptides (idiopeptides) derived from their endogenous immunoglobulins could be presented by the anti-DNA B cells to the pathogenic Th clones. Indeed, we find for the first time that nucleohistones or nucleosomes stimulate about 50% of the pathogenic autoantibody-inducing Th clones in an antigen-specific manner. Identification of the critical peptide epitopes in this major autoantigen for the pathogenic T cells of lupus is in progress.

EZ 021 CELLULAR AND GENETIC MECHANISMS OF AUTOANTIBODY PRODUCTION IN SLE MICE, Robert Eisenberg[1], Deb Bloom[2], David Bradley[1], Stephen Clarke[1], Elizabeth Creech[1], Jean-Luc Davignon[3], Connie Fisher[4], Melissa Halpern[1], Takuya Katagiri[5], Richelle McDaniel[1], Danièle Nakul-Aquaronne[1], Elizabeth Reap[1], Marc Retter[1], Eric Sobel[1], Ed Treadwell[6], and Philip Cohen[1], [1]University of North Carolina, Chapel Hill, NC 27599-7280, [2]Stanford University, Stanford, [3]INSERM, Toulouse,[4]National Cancer Institute, Frederick,[5]University of Tokyo, Tokyo,[6]East Carolina University, Greenville

The lpr and gld genes induce in mice similar phenotypes of lymphoproliferation and autoantibody production. Through the use of a panel of congenic strains bearing these genes, we have tested their roles and those of other loci in this syndrome.

1. **lpr, gld**: In lethally irradiated lpr mice reconstituted with a combination of lpr and normal (+/+) bone marrows, polyclonal B cell activation and autoantibody production came entirely from the lpr donor. Similarly, the characteristic abnormal $CD4^-$,$CD8^-$ T cells that hyperproliferate were solely derived from the lpr marrow. In additional lpr-+/+ mixed chimeras, the ablation of lpr T cells with allele specific anti-Thy 1 mAbs suppressed autoantibody formation. Initial parallel experiments with gld-+/+ mixed chimeras showed an apparent correction of the gld autoimmune defect by the normal marrow. These results indicate that the lpr gene must be intrinsically expressed in those T cells and B cells that hyperproliferate and participate in autoantibody formation. Conversely, the gld gene is expressed extrinsically, at least in autoimmune B cells.

2. **MHC**: We have quantitated autoantibody levels in C57BL/6-lpr/lpr mice congenic at the major histocompatibility locus. $H\text{-}2^b$ and $H\text{-}2^{bm12}$ mice had high levels of all specificities tested (anti-chromatin, anti-DNA, rheumatoid factors), while $H\text{-}2^d$ mice had low levels. The low responder phenotype was dominant in F1 crosses. These results indicate a global rather than an antigen specific immunoregulatory effect of MHC control of autoimmunity.

3. **Igh**: In $Igh^{a/b}$ or $Igh^{j/b}$ immunoglobulin heavy chain heterozygous lpr mice, IgG2a anti-chromatin and anti-Sm antibodies were preferentially of the b allotype. In backcross analysis, anti-Sm segregated with the b allotype locus. MRL/lpr-Igh^b mice had a much higher incidence of anti-Sm antibodies than MRL/lpr-Igh^j mice. Igh recombinant lpr mice (V^bC^a) showed that this allotype effect was not due to the variable regions.

4. V_κ,V_h: Sequencing of a panel of anti-Sm hybridomas demonstrated that a variety of variable regions genes and combinations of genes could be used for this response. Certain genes were used recurrently, and some of these overlapped with those used in the anti-DNA response.

5. **Others**: Backcrossing of (C57BL/6-lpr/lpr x MRL/Mp-lpr/lpr) to MRL/Mp-lpr/lpr showed that the anti-Sm and anti-Su autoantibody responses were controlled by one or two genes in addition to those described above. The identity of these additional genes is unknown.

These studies reflect the complexity of genetic control of autoimmunity.

New Therapeutic Directions

EZ 022 T-CELL RECEPTOR PEPTIDE VACCINES AS IMMUNOTHERAPEUTICS FOR RHEUMATOID ARTHRITIS. S.W. Brostoff, D.G. Spinella, J. Diveley, K. Lundeen, and D.J. Carlo, The Immune Response Corporation, 5935 Darwin Court, Carlsbad, CA 92008

Studies in animal models of autoimmune disease such as EAE have demonstrated that the T-cells which mediate these diseases often employ a restricted repertoire of T-cell receptor (TCR) variable region genes. In such cases, it has been possible to treat or prevent the disease by vaccination with peptides derived from unique regions of the TCR V-genes known to be predominantly expressed by the autoimmune T-cells. Such a therapeutic approach may, therefore, be useful in the treatment of human-T-cell mediated autoimmune diseases in which restricted TCR repertoires can be implicated in the disease pathogenesis. In this regard, we and others have noted that activated (IL-2 receptor positive) T-cells that infiltrate the rheumatoid synovium express a limited number of TCR Vß elements suggesting that RA might be responsive to treatments based on the TCR peptide vaccine approach. We have, therefore, begun human phase I/II safety and dose-ranging studies using peptides derived from the CDR2 region of TCR Vß genes which we have found to be highly over-represented in the activated synovial T-cell pool of RA patients.

EZ 023 NEW APPROACHES TO THERAPY FOR RHEUMATIC DISEASES. William J. Koopman and Larry W. Moreland, Division of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham and Birmingham Veterans Administration Hospital, Birmingham, Alabama 35294.

Considerable evidence implicates CD4⁺ T cells in the pathogenesis of models of autoimmune disease and their human counterparts. In the case of rheumatoid arthritis (RA), several lines of evidence support this view: predominance of Ia^+ $CD4^+$ T cells in synovial mononuclear cell infiltrates; evidence that certain MHC class II alleles are associated with susceptibility to RA; improvement of RA following thoracic duct drainage; and improvement of RA in patients developing AIDS. The apparent role of $CD4^+$ T cells in the pathogenesis of RA has prompted the development of strategies intended to interrupt T cell function. Approaches currently being tested include biologicals directed against lineage-specific cell surface antigens (CDw52, CD5), accessory molecules (CD4), activation markers (IL-2R), and cell adhesion molecules (CD54).

We have utilized a chimeric monoclonal anti-CD4 (cM-T412, Centocor, Inc.) to treat patients with refractory RA in both phase I (completed) and phase II (nearing completion) trials. In the phase I study, 25 patients with active refractory RA were treated with incremental doses (10 to 700 mg) of cM-T412 in an open-label, escalating dose design. Infusion of cM-T412 was followed by an immediate rapid decline in $CD4^+$ T cells. Levels of $CD4^+$ T cells generally remained depressed at 18 months post-infusion, although in 8 of 23 evaluable patients, substantial repopulation of $CD4^+$ T cells occurred. Both CD45 RO^+ (memory) and CD45 RA^+ (naive) T cells contributed to repopulation. Lymphocyte proliferation in response to mitogens and recall antigens was depressed post-infusion with mitogen tending to recover more rapidly than antigen responses. Adverse events included fever which was generally associated with myalgia, malaise, and asymptomatic hypotension. These symptoms were self-limited and appeared to correlate with transient elevations of IL-6. One patient with multiple medical complications died 12 months after receiving a single dose of cM-T412 (pneumonia, cerebral hemorrhage). This death was felt not likely to be related to the mAb therapy. Only two patients exhibited antibody responses to the cM-T412 variable region; these were transient and low level. Significant clinical improvement, defined as ≥50% decrease in tender joint count compared to baseline, was noted in 43% of patients at 5 weeks, 33% at 6 months, and 43% at 18 months.

The data indicate that cM-T412 treatment in refractory RA appears safe and is associated with prolonged decreases in CD4 T cells and depressed *in vitro* T cell responses. Moreover, the sustained improvement observed in several patients is consistent with the view that $CD4^+$ T cells contribute to the clinical expression of RA. Further studies with cM-T412 (and other anti-T cell agents) are indicated to determine the efficacy of strategies directed toward amelioration of T cell function in RA.

EZ 024 REGULATION OF AUTOIMMUNITY IN EAE AND MS Arthur A. Vandenbark[1,2], Yuan K. Chou[1,2], George A. Hashim[3], Ruth Whitham[1,2], Dennis N. Bourdette[1,2], and Halina Offner[1,2], [1]Neuroimmunology Research 151D, V.A. Medical Center, Portland, OR, 97201, [2]Oregon Health Sciences University, Portland, [3]St. Luke's Roosevelt Hospital Center, New York.

Biased V gene use by rodent encephalitogenic T cells allowed the use of a synthetic peptide corresponding to residues 39-59 of rat Vβ8.2 to induce T cells and antibodies that could protect against EAE (1). When injected after onset of EAE, Vβ8.2-39-59 or Vβ8-44-54 prevented disease progression and speeded recovery. This rapid treatment effect was due to a boosting of a natural anti-TCR peptide response that was induced as a consequence of TCR over-expression related to the EAE disease process (2). Our working hypothesis is that the regulatory T cells and antibodies recognize naturally processed TCR epitopes that are expressed in association with MHC class I or II molecules on the encephalitogenic T cell surface. Prevention or treatment of EAE with Vβ8-44-54 reduced the frequency of BP-72-89 specific T cells in the periphery and the CNS as well as CNS inflammation but not the percentage of Vβ8.2+ T cells. These findings support the hypothesis that anti-TCR immunity regulates the function but does not delete T cells bearing Vβ8.2.

The human disease multiple sclerosis (MS) may also involve encephalitogenic T cells that might be regulated with TCR peptides. In support of this possibility, we found an increased frequency of BP-specific T cells in the blood, and of BP and PLP-139-151 specific T cells in the CSF of MS patients that approximated the frequencies observed in rats with EAE (3). The peripheral BP response was episodic and was characterized by the over-expression of Vβ5.2 and to a lesser extent, Vβ6.1 (4). The finding by others (Oksenberg et al.) that message from MS CNS plaque tissue for these same Vβ genes in association with a CDR3 motif found in BP-specific T cells lends independent support to the importance of Vβ5.2+ BP-reactive T cells in the MS target tissue. Two TCR peptides, Vβ5.2-39-59 and Vβ6.1-39-59, were injected into 11 MS patients with progressive disease to evaluate antigenicity and toxicity. In six patients, the frequency of Vβ5.2-39-59 or Vβ6.1-39-59 specific T cells increased significantly after boosting injections of 100-300 μg of the respective peptide, often with accompanying delayed type hypersensitivity reactions. Responses could be maintained for an average of 16 and 27 weeks respectively with the Vβ5.2-39-59 and Vβ6.1-39-59 peptides. At doses ≥600 μg, responses decreased. During responsive periods, peptide specific T cell clones were isolated and found to be predominantly activated CD4+ cells restricted by either MHC I or II. TCR peptide responses in the remaining patients could not be boosted with doses as high as 3,000 μg. Antibodies to Vβ6.1-39-59 were detected in only one responder. Adverse responses to the peptides were minimal. All three recipients known to have biased expression of Vβ5.2 and/or Vβ6.1 by BP-specific T cells responded to both TCR peptides, and remained clinically stable over the test period. These results indicate that TCR peptides are immunogenic and relatively safe for use in humans, although it is still not clear to what extent immunity to TCR peptides can regulate BP responses. Successful regulation of disease-associated TCR V genes might allow a critical test of the hypothesis that myelin reactive T cells participate in the pathogenesis of MS.

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Late Abstracts

MYCOPLASMA SUPERANTIGENS AND AUTOIMMUNE ARTHRITIS, Barry C. Cole, Curt L. Atkin, Marie M. Griffiths University of Utah Medical Center, Salt Lake City, Utah.

Superantigens are a newly recognized group of microbial proteins which activate T and B lymphocytes by a unique pathway. Superantigens bind directly to MHC molecules on AC surfaces without processing and are recognized by T cells via the V_β chain segments of the α/ß TCR. Each superantigen has its own characteristic receptor site(s) on the MHC molecule(s) and a characteristic pattern of V_β TCRs that it engages. Superantigens are encoded by endogenous or exogenous mouse tumor viruses are secreted by various staphylococci, streptococci, pseudomonas, and may be a part of bacterial cell walls. A superantigen is also produced by Mycoplasma arthritidis which is the agent of a chronic proliferative arthritis of rodents.

It has been proposed that a superantigen might trigger autoimmune disease by activating preexisting autoreactive T cell clones or by forming a superantigen bridge which cross links T cells with B cells resulting in polyclonal B cell activation and autoantibody production. In a number of experimental models of autoimmunity T cells bearing specific V_β TCRs are involved. However, antibody response to the autoantigen is also required for full disease expression. In collagen-induced arthritis of mice (CIA), there is evidence that disease is mediated by T cells bearing $V_\beta 6$, $V_\beta 7$ and $V_\beta 8$. MAM which also engages these V_βs was therefore used to investigate the role of superantigens in autoimmune disease.

First, we demonstrated that MAM induced a specific clonal expansion of $V_\beta 8$ and $V_\beta 6$ T cells in vivo. There was also an associated increase in immunoglobulin production and antibody responses to foreign antigens. Although IL-2 production was decreased, there was a enhanced increase in IL-4 and IL-6. The data suggest a change from T_H1 to T_H2-like activities. Inbred and hybrid mice H-2^r were immunized with porcine collagen (PII) and when disease had subsided or begun to subside, the animals were challenged iv with MAM. In other experiments, mice were immunized with PII and MAM was given at onset of CIA. The data demonstrated that the superantigen MAM could cause a flare in CIA disease activity, could increase disease severity and could trigger disease activity in mice previously subimmunized with PII. The data suggest that infections of humans with microbial agents producing superantigens similarly cause flares or even trigger autoimmune disease activity.

HUMAN T CELL RESPONSE TO RETROVIRALLY ENCODED SUPERANTIGENS, Brigitte T. Huber[1], Meena Subramanyam[1], Brian McLellan[1], Nathalie Labrecque[2], and Rafick-P. Sekaly[2], [1]Tufts University School of Medicine, Boston and [2]Institut de Reserches Cliniques de Montreal, Montreal.

Superantigens (SAGs) presented in the context of major histocompatibility complex (MHC) class II proteins stimulate a potent proliferative response in T cells expressing particular T cell receptor (TCR) Vβ genes. Although this T cell recognition is not MHC restricted, a strong hierarchy is observed in the ability of various class II molecules to present SAGs. Mls-1, encoded by the Murine Mammary Tumor Virus (MMTV) *Mtv-7 sag* gene, is the prototype of endogenous SAGs. In the present study, we have analyzed whether this retroviral gene product can be presented in the context of human MHC class II proteins to murine T cells. Positive results were obtained with the DR isotype, but a vast range in the activity of the various DR alleles was observed. Interestingly, the same pattern of TCR Vβ restriction was seen as with presentation of Mls-1 in the context of murine class II molecules, suggesting that the TCR Vβ specificity is uniquely determined by the retroviral SAG. Transfection experiments with reciprocal exchange constructs between the second exon of the DR1 and DRw53 β gene revealed the importance of the membrane proximal domain of the human class II β chain in Mls-1 binding and presentation to T cells. Recent experiments indicate that human T cells respond in a TCR Vβ specific manner to Mls-1 in the context of DR1. It, therefore, appears that the T cell recognition of retroviral SAGs is conserved through the evolution of the two species.

Role of Major Histocompatibility Complex; Retroviruses in Autoimmune Diseases

EZ 100 T CELL RESPONSES TO CARTILAGE PROTEOGLYCANS: EFFECTS OF MHC HAPLOTYPE AND ANTIGEN CONFIGURATION.

John A. Goodacre, Stephen Lynn*, Shirley Middleton, Dawn Ross and Jeff Pearson*. Departments of Medicine (Rheumatology) and Physiology*, The Medical School, Newcastle upon Tyne, England, NE2 4HH.

Cartilage proteoglycan aggregates (PG) may contain MHC binding/T cell recognition sites involved in the pathogenesis of rheumatoid arthritis (RA). We have studied the properties of PG as class II-restricted T cell antigens in mice, using monomers (intact and deglycosylated) purified from human articular cartilage and synthetic peptides of the chondroitin sulphate attachment repeat region, CS1. T cell proliferation assays were used to compare the magnitude of primed lymph node cell (PLNC) responses to intact monomers among BALB and B10 congenic strains. There was a significant effect of MHC haplotype, with PLNC responses in H-2 k>d>b. Intact and deglycosylated monomers were cross-recognised by T cell lines but these lines did not recognise the CS1 region 19-residue peptide. Also, CS1 peptide-specific lines did not respond to monomers. Using smaller peptides it was found that the CS1 peptide contains at least two T cell recognition sites but that recognition of the C-terminal site was dependent upon whether or not the site formed part of the entire 19-residue peptide. These results show that the CS1 repeat region contains cryptic T cell recognition sites. T cells with specificities for otherwise hidden epitopes in PG might be activated in RA joints as result of the antigenicity of small fragments released through degradation of PG by extracellular proteases.

EZ 101 PURIFICATION OF ENDOGENOUS PEPTIDES EXTRACTED FROM HLA-DR4 ISOLATED FROM CULTURED CELLS AND SYNOVIAL MEMBRANES,

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HLA-DR4 is set of polymorphic glycoproteins present on the surface of antigen presenting cells. Susceptibility to Rheumatoid Arthritis has a positive association with the DR4 and to a lesser extent DR1 phenotypes. There is considerable evidence to suggest that T lymphocytes recognise antigenic peptides bound to HLA-DR molecules. Due to the tight binding to and slow off rate from the HLA DR molecule, we have been able to isolate peptides from purified HLA-DR4 molecules derived from a B-cell line (JAH cells) and from insect cells transfected with HLA-DR4. These peptides were purified by RP-HPLC, and sequenced by Edman degradation. In some cases the peptide molecular weight could be confirmed by FAB-MS. The average length of the peptides was 11 and 14 amino acid residues long respectively. In a similiar manner we were able to purify HLA-DR4 specific endogenous peptides from human synovial membranes derived from 3 patients with sero positive rheumatoid arthritis. Two patients were HLA-DR4Dw4 homozygotes. The synovium was obtained at the time of total knee joint replacement and snap frozen in liquid nitrogen. We found that fragments of HLA-DR4 dominated the pattern of peptide fragments although other peptides were present, with the average length of the peptide being 13 amino acid residues. This may either be due to proteolysis of the HLA-DR4 during preparation or may well be due to antigen processing. We have been unable to identify a sequence motif common to the peptide sequences. We have however been able to show that the length of the characterised peptides bound to HLA-DR4 is shorter than that reported for mouse MHC systems.

EZ 102 *Abstract Withdrawn*

EZ 103 UPREGULATION OF HLA-DQ EXPRESSION BY GM-CSF, OR IL-3, WITH DEXAMETHASONE ON HUMAN MONOCYTES BUT NOT ENDOTHELIAL CELLS,

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Three major histocompatibility complex (MHC) class II antigens, HLA-DR, DP and DQ, have been described in man. The majority of antigen specific human T cells responses described are restricted by HLA-DR, with few examples of HLA-DQ restricted responses. The latter antigen is expressed at low levels in the periphery and is not readily upregulated on the surface of human monocytes *in vitro* even by IFNγ. We have observed that the cytokines GM-CSF and IL-3 are much weak inducers of all three class II determinants in comparison to IFNγ. However when used in combination with the glucocorticoid dexamethasone, stimulation with these cytokines leads to a very marked increase in HLA-DR, DP and DQ expression. GM-CSF and dexamethasone induce strong HLA-DQ expression on purified monocytes from all donors tested (n=15) which is generally higher than the levels induced by IFNγ. Dexamethasone did not enhance IFNγ induced MHC class II expression. High levels of HLA-DQ antigen are observed *in vivo* on endothelial cells and other synovial cells in the inflammed joint where elevated levels of GM-CSF have also been documented. However we have observed no induction of any of these three MHC class II antigens on cultured human umbilical vein endothelial cells following stimulation with GM-CSF or IL-3 and dexamethasone. These observations suggest firstly that IFNγ and GM-CSF induce MHC class II expression by distinct mechanisms and secondly, that monocytes and endothelial cells differ in the control of MHC class II expression.

EZ 104 THE ROLE OF THE MHC CLASS I REGION AND THE CONFORMATION OF THE ANTIGEN IN RESISTANCE TO COLLAGEN-ARTHRITIS IN RHESUS MONKEYS, Margreet Jonker, Ronald E. Bontrop, Bert A. 't Hart, and Koos P.M. Bakker, Department of Chronic and Infectious Diseases, ITRI TNO, PO Box 5815, 22280 HV Rijswijk, the Netherlands.

Rhesus monkeys imunized with collagen type II (CII) develop arthritis (CIA) in 70% of randomly selected animals. Animals that did not develop arthritis shared one particular MHC class I antigen: MhcMamu-A26. This suggests that this MHC class I allele, or a closely linked gene, determines resistance to the disease. Resistance to CIA is reflected by low responsiveness of T cells reactive with CII, indicating a role of the MHC class I region in the regulation of the immune response to CII. Susceptible animals showed a T cell response to CII and the humoral response was dominated by anti-CII antibodies of the IgM type while resistant animals predominantly had IgG type anti-CII antibodies. The importance of T cells in the development of arthritis was further stressed by the observation that treatment of animals with Cyclosporin-A could prevent arthritis development. Denaturation of CII by heating resulted in the loss of arthritogenic properties of CII in otherwise susceptible animals (Mamu-A26 negative). Animals immunized with denatured CII produced only IgG anti-CII antibodies suggesting that IgM antibodies play an important role in triggering of CIA. Furthermore, immunization with denatured antigen resulted in a permanent resistance to CIA. In conclusion, resistance to CIA in rhesus monkeys is determined by a MHC class I allele and can also be achieved by preimmunization with denatured CII. This CIA model in an outbred population of genetically (MHC) well characterized rhesus monkeys offers a suitable model for the investigation of the role of MHC and relevant autoantigens in the immunoregulation of autoimmune arthritis in primates.

EZ 105 **IDENTIFICATION AND CHARACTERIZATION OF EPITOPES ON HUMAN TYPE II COLLAGEN**, Christopher J. Krco, Gerald H. Nabozny, Marie Griffiths[a], Harvinder S. Luthra[b] and Chella S. David, Depts. of Immunology and Rheumatology[b], Mayo Clinic, Rochester, MN 55905 and Dept. of Internal Medicine[a], Univ. Utah, Salt Lake City, UT 84132

Rheumatoid arthritis is a chronic, inflammatory disease of unknown etiology which primarily affects the joints. Type II collagen comprises the majority of the total collagen content of hyaline joint cartilage. Animal models of rheumatoid arthritis have established the arthritogenicity and immunogenicity of type II collagen. Using purified human type II collagen (HII) we have begun investigations directed towards establishing a complete characterization of serological and cellular epitopes expressed on type II collagen. We have determined that DBA/1 mice are very susceptible to developing arthritis following immunization with HII while B10.Q mice develop less severe disease and B10 mice are resistant. DBA/1 immunized with a cyanogen bromide (CB) digest of HII respond to in vitro challenge (day 4) with the HII digest (Δ cpm 15,488). Results of challenging T cells with individual CB fragments are underway. In addition to CB fragments overlapping, synthetic peptides of HII are also being utilized. DBA/1 T cells primed to CB fragments respond strongly to a peptide spanning residues 250-270. This peptide stimulates DBA/1 T cells primed to the HII digest (Δ cpm 19,859). In addition, peptide 250-270 itself can prime for in vitro responses (Δ cpm 39,359). Interestingly, peptide 250-270 is not immunogenic in moderately susceptible B10.Q or resistant B10 mice (Δ cpm 's < 4,000). Hybrid (DBA/1 x B10.Q)F1 mice exhibit intermediate in vitro responses (Δ cpm 8,115). Efforts are underway to determine T cell receptor Vβ usage in response to this peptide. In addition the effectiveness of peptide 250-270 in arthritis ameliorating immunotherapies is being explored.

EZ 106 **POLYMORPHISM IN AN HLA-LINKED PROTEASOME GENE INFLUENCES PHENOTYPIC EXPRESSION OF DISEASE IN HLA-B27 INDIVIDUALS,** **Walter P. Maksymowych, Andreas Wessler, Maria Suarez-Almazor, Jara Pazderka, Anthony S. Russell. Department of Medicine, University of Alberta, Edmonton, Canada T6G 2S2.**

The association of HLA-B27 with ankylosing spondylitis (AS) is well recognized although the greatly increased prevalence of disease in family members of AS patients when compared to the B27 background population implicates additional genes in disease susceptibility. Two polymorphic genes in the HLA class II region encode proteins demonstrating homology to subunits of the proteasome, a cytosolic proteinase complex implicated in antigen processing. Such genes may be implicated in disease through their potential influence on the processing/presentation of self-peptides by HLA-B27 to autoreactive T cells. A polymorphic CfoI restriction enzyme site in the coding region of one HLA-linked proteasome gene was evaluated in 69 genomic DNA samples from B27 individuals with well documented AS, 24 samples from B27 individuals with acute iritis, and 40 samples from normal, ethnically matched B27 blood donors where AS was excluded. One allele of this bi-allelic locus, allele A, designating absence of the CfoI restriction site, was significantly less prevalent in B27 AS patients (21.7%) than B27 controls (36.3%) (x^2 = 5.4; P < 0.05). Analysis of B27 individuals with iritis also revealed a significantly decreased prevalence of this same allele (15.2%) when compared to B27 controls (x^2 = 7.0; $P_{corrected}$ < 0.02). Furthermore, homozygosity for the second allele, allele B, was found significantly more frequently in iritis patients (70.8%) than in B27 controls (40.0%) (x^2 = 5.7; $P_{corrected}$ < 0.04). These findings support the involvement of additional HLA-linked genes in the phenotypic expression of disease in B27 individuals and suggest a role for the non-B27 HLA haplotype in susceptibility to iritis.

EZ 107 COLLAGEN INDUCED ARTHRITIS (CIA) IN T CELL RECEPTOR (TCR) V_β DELETION CONGENIC MICE. **Gerald H. Nabozny*, Harvinder S. Luthra#, Marie M. Griffiths@ and Chella S. David*; *Departments of Immunology and #Rheumatology, Mayo Clinic, Rochester, MN 55905; and @Department of Medicine, University of Utah, Salt Lake City, UT 84132.**

CIA is induced by injecting type II collagen (CII) and adjuvant into susceptible mouse strains of the H-2^q and H-2^r haplotype. Subsequent to immunization, an inflammatory polyarthritis develops which bears similarities to human rheumatoid arthritis. Previous studies suggest that certain V_β genes of the TCR may be important in CIA development; strains bearing a partial deletion of V_β TCR genes, like SWR (H-2^q, V^a_β) and RIIIS/J (H-2^r, V^c_β), are resistant to CIA despite a susceptible H-2 haplotype. Offspring of B10 (H-2^b, V^b_β) x SWR or RIIIS/J matings can develop CIA thereby inferring a role for TCR genes deleted in V^a_β or V^c_β mice in CIA susceptibility. However, the potential of other genes, in addition to the V^b_β locus, contributing to the experimental results cannot be excluded. Therefore, to directly examine the influence of V_β TCR gene deletions in CIA, we have produced CIA-susceptible B10.RIII (H-2^r) mice which are congenic for the V^a_β and V^c_β TCR deletion. Following immunization with porcine CII, B10.RIII-V^a_β animals developed an incidence of arthritis similar to control B10.RIII mice (84% vs. 96%, n=65). However, the onset of CIA was significantly delayed in the B10.RIII-V^a_β strain (36 d vs. 26 d, p = 0.02) and these animals showed a reduction in the severity of arthritis vs. B10.RIII controls. Certain TCR V_β's, such as $V_\beta 4$, showed an increased frequency in the V^a_β congenic mice. Thus, in the B10.RIII (H-2^r) strain, TCR V^a_β gene deletions can influence the induction and development but not the incidence of CIA. The effect of the V^a_β deletion in a congenic B10.Q-V^a_β (H-2^q) line and the V^c_β deletion in B10.Q-V^c_β and B10.RIII-V^c_β strains are currently under investigation and will be reported.

EZ 108 PRESENTATION OF MYCOBACTERIA TO γδ T CELLS IS MEDIATED BY NON-MHC CELL SURFACE MOLECULES,
Nadine C. Romzek, Yifeng Jia, Lynn E. Wagner, Luis M. Vila, Shu-Jen Chen, James M. Wilson, David R. Karp, and Joseph Holoshitz, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109-0531, and Simmons Arthritis Research Center, University of Texas Southwestern Medical Center, Dallas, Texas 75235-8884
The majority of human peripheral γδ T cells express antigen receptors using the Vγ9 and Vδ2 gene products. Cells of this subset have been previously shown to uniformly recognize mycobacteria regardless of their nominal antigenic specificity. This antigenic recognition superficially resembles recognition of bacterial superantigens by αβ T cells. Presentation of classical superantigens to αβ T cells is thought to be mediated by monomorphic regions of major histocompatibility complex (MHC) class II molecules. It is not known whether presentation of the mycobacterial antigen to γδ T cells is also mediated by class II MHC molecules. In this study we have examined the role of class II MHC molecules in presentation of the mycobacterial superantigen AP-MT to Vγ9/Vδ2 clones. Activation of γδ T cells by AP-MT required direct contact with antigen presenting cells, indicating that an interaction with cell surface molecules on the antigen presenting cells is required. Class II MHC molecules were neither sufficient nor necessary for effective presentation of the mycobacterial antigen to the γδ T cells, since transfectants expressing class II MHC molecules were unable to present, whereas cell lines lacking expression of MHC class II molecules could present the mycobacterial antigen. Based on these results, and our previous observation that presentation of AP-MT is independent of class I MHC molecules, we conclude that presentation of the mycobacteria to human Vγ9/Vδ2 cells can be mediated by non-MHC cell surface molecules.

EZ 109 CHARACTERIZATION OF TYPE II COLLAGEN ANTIGENIC DETERMINANTS PRESENTED BY A CIA SUSCEPTIBLE CLASS II ALLELE.
Edward F. Rosloniec, Linda K. Myers, David D. Brand, and John M. Stuart, Depts. of Medicine and Pediatrics, University of Tennessee, and VA Medical Center, Memphis, TN 38104.
The immunization of genetically susceptible mice with chick type II collagen (cCII) elicits an autoimmune polyarthritis that resembles rheumatoid arthritis. Susceptibility to collagen induced arthritis (CIA) is restricted by the class II molecule I-A. Mouse strains expressing I-A^q are highly susceptible to CIA when immunized with chick or bovine CII. In contrast, strains expressing I-A^p are resistant to CIA, yet I-A^p differs from I-A^q by only 4 amino acids. When DBA/1 mice (I-A^q) are immunized with chick CII, a major component of the T cell immune response is directed to antigenic determinants contained within the CII amino acid residues 245-270 and 181-210. We have characterized in detail the antigenic determinant within the CII 245-270 peptide and have identified key residues that control the binding of this peptide to I-A^q and those that interact with CII 245-270 specific T cell receptors. The core determinant encoded in this peptide lies within residues 260 to 270. Substitution of residues 260, 261, and 263 with corresponding residues of type I collagen prevent the recognition of this peptide by T cell hybridomas but do not affect the ability of the peptide to bind to I-A^q. In contrast, substitution of residues 266, 267, and 269 disrupt the ability of this peptide to bind to I-A^q. Mice expressing I-A^p also mount an immune response to CII 245-270, but do not appear to recognize the determinant within CII 181-210. We are currently investigating the effect of these peptide substitutions on I-A^p restricted presentation to determine if I-A^p utilizes the same interactions for presentation. These data support the hypothesis that peptide specific interactions with class II molecules play a major role in determining the genetic susceptibility to CIA.

EZ 110 DIFFERENTIAL EXPRESSION OF HUMAN NICOTINIC ACETYLCHOLINE RECEPTOR α SUBUNIT VARIANTS IN MUSCLE AND NON-MUSCLE TISSUES: POSSIBLE RELATION TO MYASTHENIA GRAVIS,
Sohel Talib, *Vanda A. Lennon, Thomas B. Okarma and Jane S. Lebkowski, Applied Immune Sciences, Inc., Menlo Park, CA, 94025, *Mayo Clinic, Rochester, MN 99505.
The α subunit of nicotinic acetylcholine receptor carries a binding site for cholinergic ligands and appears to be a prime target for pathogenic autoantibodies in the neuromuscular disease myasthenia gravis (MG). Two isoforms of the α subunit differing in the P3A exon arise from alternate RNA splicing. Analysis of genomic DNA from humans and primates revealed that the P3A exon sequence is present in new as well as in old world primates, and is thus evolutionary conserved during the last 30 million years. No evidence of P3A exon sequence was found in genomic DNA or cDNAs from mouse, rat, bovine and dog. The $P3A^+$ isoform of the α subunit appears to be constitutively expressed in skeletal muscle, brain, heart, kidney, liver, lung and thymus, while the $P3A^-$ isoform is differentially expressed only in skeletal muscle. Thus it appears that the $P3A^+$ variant is generated by "default" selection by the splicing machinery, while expression of the $P3A^-$ isoform is regulated by tissue-specific factors in the skeletal muscle. Mechanisms regulating differential expression of α subunit isoforms may play an important role in the pathogenesis of myasthenia gravis.

EZ 111 IDENTIFICATION OF AN EIGHTY FOUR RESIDUE FRAGMENT OF TYPE II COLLAGEN CAPABLE OF INDUCING ARTHRITIS IN DBA/1 MICE.
Kuniaki Terato, Jerome M. Seyer, Linda K. Myers, Michael A. Cremer, John M. Stuart, and Andrew H. Kang. VA Medical Center and The Department of Medicine, The University of Tennessee, Memphis, TN

Collagen-induced arthritis is mediated by autoimmunity to type II collagen. We have shown that the major epitopes for inducing arthritis are located within a fragment of type II collagen generated by CNBr digestion (CB11, amino acid residues 124-402). To further localize the major arthritogenc epitopes, chick CB11 was digested by endopeptidase LysC into 3 fragments: LyC-1 (residues 124-290), LyC-2 (residues 291-374) and LyC-3 (residues 375-402). Sera from DBA/1 mice immunized with intact CII were analyzed for reactivity with each renatured LyC fragment by ELISA. Reactivity with all three fragments was present, however, reactivity to LyC-2 predominated for autoantibodies purified by affinity chromatography using mouse type II collagen. LyC-1 reacted stronger with chick specific antibodies.

We have previously shown that monoclonal antibodies (mAb) reactive with CB11 are capable of inducing arthritis. Eight of 11 mAb specific to CB11 reacted with LyC-2; 3 mAb reacted with LyC-1 indicating that LyC-2 contains major immunogenic and possibly arthritogenic epitopes. To test this possibility, we chose 4 clones of autoreactive mAb which react with LyC-2 and were able to successfully induced arthritis in naive mice. In addition, we immunized DBA/1 mice with each peptide fragment. Arthritis developed only in those mice immunized with LyC-2. These data support the hypothesis that LyC-2 contains the major arthritogenic epitopes for CIA in DBA/1 mice.

EZ 112 RHEUMATOID ARTHRITIS IS NOT PRIMARILY ASSOCIATED WITH A VARIANT OF THE TAP-2 GENE, B. Paul Wordsworth, Kevin D. Pile, Katy Gibson and Steven H. Powis*, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU and *Imperial Cancer Research Fund Laboratories, London WC2A 3PX, United Kingdom
Rheumatoid arthritis (RA) is one of the group of multifactorial conditions known as autoimmune diseases to which there is a polygenic component. Much has been learnt in recent years about the physiology of antigen processing and presentation to T lymphocytes. In addition to the HLA molecules, several important components of these pathways are encoded in the MHC. They include several genes in the HLA class II region that may be important in the processing of complex antigens and the transmembrane transport of small peptides to compartments of the cell where they can associate with HLA molecules. Two of these transporter-associated peptides (TAP-1 and TAP-2) are encoded at loci centromeric to HLA-DQ. We have investigated the possibility that these genes may influence susceptibility to RA by analysing a dimorphism in the coding sequence of the TAP-2 gene in 90 patients with RA, 73 race-matched controls and a panel of 59 DR-4 positive healthy controls. The major allele frequencies (i.e. threonine at position 665) were very similar in the patients with RA (0.79) and controls (0.74). Although this frequency rose to 0.87 in the DR4+ patients with RA (n=65) this was not significantly different from the DR4+ controls (0.83), indicating linkage disequilibrium between the "threonine 665" TAP-2 allele and DR4. This strong linkage was confirmed in those subjects homozygous for DR4: 18/19 DR4 homozygotes with RA and 13/15 DR4 homozygous controls were also homozygous for the "threonine 665" TAP-2 allele. These results indicate that any association between the TAP-2 locus and RA is secondary to linkage with the DRB1 locus.

Other Environmental Factors in Autoimmune Diseases; T Cell Repertoire in Autoimmune Diseases

EZ 200 T CELL RECEPTOR β CHAIN VARIABLE REGION REPERTOIRES OF HUMAN T CELLS THAT MIGRATED TO HUMAN SYNOVIAL XENOGRAFTS IMPLANTED IN SEVERE COMBINED IMMUNE DEFICIENT (SCID) MICE. TS Barry, KE Rendt, RE Ware, DM Jones, CB Richer, and BF Haynes. *Duke University Arthritis Center, Duke Univ. Med. Ctr.*, Durham, NC 27710.

To develop an *in vivo* model of human synovial inflammation, we have engrafted human HLA-DR4+ rheumatoid (RA) synovium under the kidney capsule of C.B-17 *scid/scid* (SCID) mice. Histologic and phenotypic components of the preimplantation synovium (fibroblasts, vessels, macrophages, T cells, cell adhesion and activation markers) are maintained in the synovial xenografts. Following engraftment, untreated 50 x 10^6 autologous peripheral blood (PB) mononuclear cells were injected intraperitoneally into synovial-engrafted animals. After 7 days, human T cells selectively migrated to engrafted human synovium and to murine abdominal lymph nodes (LN). No CD45+ human cells were detected in peripheral blood or other murine organs; kidney, liver, spleen, thymus, or appendix. Following short-term (3 weeks) expansion *in vitro* (IL-2, PHA, and autologous EBV transformed feeder cells) RA PB T cells that had selectively migrated to human synovium were found to be CD3+, TCRα/β+ T cells with a predominance of CD8+ (74-92%) versus CD4+ (8-26%) cells. Only rare TCRγ/δ (0.5-2%) T cells were present. Analysis of T cell receptor β chain gene usage of human T cells that have selectively homed to the implanted synovial microenvironment by reverse polymerase chain reaction showed all Vβ gene families (Vβ1-20) were represented and found at frequencies similar to fresh RA PB T cells prior to injection. The development of this *in vivo* organ-specific animal model of human autoimmune disease holds promise as a system for analyzing cellular and molecular events that occur upon selective lymphocyte homing to human rheumatoid synovium. Moreover, the ability of human T cells to migrate to and colonize murine LN provides an *in vivo* model for the study of T cell-LN interactions.

EZ 201 INCREASE IN THE FRACTION OF MUTANT T CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS. Sheldon M. Cooper, Karen D. Roessner, J. Patrick O'Neill, Ralph C. Budd and Richard J. Albertini, University of Vermont College of Medicine, Burlington, VT 05405
Mutations at the hypoxanthine-guanine phosphoribosyl transferase gene (*hprt*) can be detected by cloning T cells in the presence and in the absence of thioguanine. This assay has been used to demonstrate increased fractions of mutant T cells (Mf) in populations exposed to mutagenic agents. Since random mutations occur more frequently in dividing cells, we reasoned that in rheumatoid arthritis, a disease that is thought to be T cell mediated, the Mf may be increased as a function of enhanced T cell proliferation. We therefore compared the Mf of peripheral blood T lymphocytes from 22 patients with RA and normal controls. While there was considerable overlap between these groups, patients with RA had a mean Mf of 20 (±15)x10^{-6}, while the Mf of controls was usually <10x10^{-6}. There was no obvious correlation between disease duration or disease severity and elevated Mf. Although there appeared to be no apparent correlation between therapeutic regimen and elevated Mf, we prospectively studied the effect of methotrexate (MTX) on 7 patients who were beginning MTX therapy. The Mf of these patients were determined prior to receiving MTX and at 6 month intervals for 1 to 2 years while on MTX. MTX did not result in an increase in Mf, and in fact there was little variation in the Mf of these individuals over time. The data indicate that some patients with RA have an elevated fraction of mutant T cells. A similar finding has also been reported in patients with SLE and MS. This supports the concept that autoimmune diseases, that are thought to be T cell mediated, are associated with increased T cell proliferation that is reflected in an increased fraction of mutant T cells. If the expansion of mutant T cells is occurring in response to specific antigens, these cells can be used to detect which antigens may be relevant to the pathogenesis of the disease.

EZ 202 INCREASE IN Vβ17⁺ T CELLS IN BLOOD AND SYNOVIAL FLUID FROM PATIENTS WITH RHEUMATOID ARTHRITIS.
Mary K. Crow, Gary Zagon, Joseph Tumang, David N. Posnett, Steven M. Friedman. Department of Rheumatic Disease, The Hospital for Special Surgery, New York, NY 10021.

An important role for T cells in the pathogenesis of rheumatoid arthritis (RA) is supported by the amelioration of clinical arthritis after T cell depletion, both in patients with RA and in murine models of inflammatory arthritis. The selective expansion of T cells bearing the products of particular T cell receptor (TCR) Vβ genes would support the hypothesis that microbial superantigens (SA), products of certain bacteria, mycoplasma species, and retroviruses, may trigger the activation of T cells important in RA. To investigate this possibility, we utilized our recently generated monoclonal antibody (mAb), C1, reactive with $V\beta17^+$ T cells, along with previously characterized mAb specific for Vβ6.7 (OT145) and Vβ12 (S511). We have performed indirect immunofluorescence analysis on peripheral blood T cells from normal subjects, and from patients with RA or systemic lupus erythematosus (SLE), and on synovial fluid (SF) T cells from patients with RA or non-rheumatoid inflammatory arthritis. Both blood (n=19) and SF (n=26) T cells from RA patients showed a statistically significant increase ($p<0.05$) in $C1^+$ T cells (x = 6.9 and 7.9, respectively) when compared with blood T cells from normal subjects (4.6%) or SLE patients (5.1%), or with SF T cells from non-RA controls (5.3%). There was no significant difference in the percentage of $OT145^+$ or $S511^+$ blood or SF T cells in RA patients and controls. 7/26 RA SF T cell samples contained >10% $V\beta17^+$ cells. Vβ17 is a member of subgroup IV of TCR Vβ gene families, whose products are selectively reactive with defined SA, including the *M. arthritidis* SA MAM. The generation of additional mAb reactive with Vβ products encoded by the other members of this gene family may identify additional RA patients bearing skewed populations of T cells that may have been activated by a SA.

EZ 203 T-CELL RECEPTOR VARIABLE β REPERTOIRE EXPRESSED BY RESIDENT CD4+ T-CELLS FROM RHEUMATOID SYNOVIUM.
Michael P. Davey, Department of Medicine, Oregon Health Sciences University and Department of Veterans Affairs Medical Center, Portland, OR 97207

T-cells are thought to play an important role in the etiology of rheumatoid arthritis (RA). Several T-cell receptor (TCR) variable (V) β genes have been identified (Vβ-3, -14 and -17) that are over-represented at sites of inflammation and appear to represent T-cell clones based on sequence analysis of PCR amplified transcripts. Our previous studies have shown that the TCR repertoire expressed by peripheral blood T-cells varies between CD4 and CD8 subsets. Furthermore, since MHC associations and immunophenotyping studies imply an important role for CD4+ T-cells in RA, this study compares the Vβ repertoire expressed by CD4+ T-cells from the peripheral blood and synovium of 5 patients with RA. CD4-enriched populations were prepared from both compartments by negative selection. Vβ levels were determined by PCR using 22 different Vβ primers. Amplified products were quantitated by HPLC and the data expressed as the percentage of the total repertoire amplified from blood or synovium. All Vβ primers were capable of amplifying DNA from the peripheral blood, indicating that T-cells expressing these genes were not deleted from the periphery. Three patients showed a higher percentage of Vβ-2 and -3 amplified from the synovium compared to peripheral blood. Four patients showed this feature for Vβ6. These three Vβ genes are usually detected at a high level in the peripheral blood thus sequence analysis is underway to further understand the significance of these observations.

EZ 204 ANTIBODIES TO TCR α/β AND CD28 REVERSE FUNCTIONAL ANERGY OF lpr AND gld B220⁺ DN T CELLS.
Wendy F. Davidson, James P. Allison and Thomas Giese, Laboratory of Genetics, NCI, National Institutes of Health, Bethesda, MD 20892 and Cancer Research Laboratory, Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720

Mice homozygous for lpr and gld develop severe lymphadenopathy. The predominant cell population in enlarged LN is TCR α/β^+ $CD4^-$ $CD8^-$ double negative (DN) T cells. These cells also express high levels of CD28, Ly-6C and CD45(B220) and 60-75% are $CD69^+$. $B220^+$ DN T cells are refractory to a wide variety of stimuli including crosslinking of TCR α/β, Ly-6C and CD69. For normal T cells, CD28 functions as an alternative, TCR-independent activation pathway and treatment of cells with Ab to CD28 enhances proliferative responses and IL-2 production induced by a range of stimuli including TCR crosslinking. To determine if the CD28 pathway was operational in lpr and gld T cells, we treated control, lpr and gld LN cells and sorted lpr and gld $B220^+$ DN T cells with PMA and anti-CD28 Ab or with combinations of PMA, anti-CD28 Ab and mAb to TCR α/β, Ly-6C and CD69. All unfractionated LN cells responded strongly to stimulation with PMA and anti-CD28 Ab, and both agents significantly enhanced the proliferative responses and IL-2 production induced by crosslinking TCR α/β, Ly-6C and CD69. DN T cells responded weakly to PMA and anti-CD28 Ab and strongly to PMA and cross-linking of CD28 and TCR α/β. No synergy was observed when DN T cells were treated with combinations of PMA, anti-CD28 Ab and Ab to Ly-6C or CD69. In keeping with its effects on normal T cells, cyclosporine A had little effect on the proliferative response of lpr or gld LN or DN T cells to PMA and anti-CD28 Ab but partially inhibited the response induced by Ab to CD28 and TCR α/β. These data suggest that lpr and gld DN T cells may have a substantially higher threshold for stimulation than control, lpr or gld $CD4^+$ and $CD8^+$ T cells. The different effects of engagement of CD28 may be indicative of an uncoupling of the TCR α/β, Ly-6C and CD69 activation pathways in DN T cells.

EZ 205 T-CELL RECEPTOR Vβ-GENE USAGE IN COLLAGEN INDUCED ARTHRITIS IN DA-RATS,
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Collagen-induced arthritis (CIA) is a T-cell mediated autoimmune disease that can be induced in rats and mice. So far it is unknown if the auto-reactive T-cell in CIA express a restricted T-cell-receptor repertoire. To investigate this, we have analyzed the TcR-Vβ-usage in inflammed synovial tissues from DA-rats with CIA, by PCR. DA female rats were immunized with 150 ug autologous CII emulsified in 100 ul of Freunds incomplete adjuvant. Two days after the onset of the disease, the inflammed synovial tissue was dissected and total RNA prepared. cDNA was then prepared with a oligonucleotide primer complimentary to a sequence found in the constant region of both β_1 and β_2-mRNA. Subsequent PCR-reactions were carried out with primers for 22 different Vβ-genes and a Cβ-primer, internal to the one used in cDNA-synthesis. 2% of the Cβ primer was labelled with ^{32}P-ATP and the PCR-products were separated by polyacrylamidgel-electroforesis and visualised by exposure to film. The expressed Vβ-bands were excised and quantitated by liquid scintillation counting. Our preliminary data of Vβ-expression in the infiltrating T-cells of the inflammed synovial tissues indicate a much more restricted Vβ-expression in the synovial tissue than in normal lymph nodes; only 6-8 Vβs are expressed in the synovia with Vβ6, Vβ8.2 and Vβ8.5 representing over 70% of the repertoire.

EZ 206 THE ROLE OF GAMMA DELTA T CELL RECEPTOR OLIGOCLONALITY IN RHEUMATOID ARTHRITIS,
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Many studies support the importance of T cells in the chronic inflammation of synovial joints that characterises RA. Most attention has been given to the $\alpha\beta$ T cells because they both dominant the rheumatoid synovium and clearly have the capacity to interact with the rheumatoid motif encoded by Class II MHC. Studies of $\alpha\beta$ T cells including our own have been variable and we have concentrated upon $\gamma\delta$ T cells which have been demonstrated in the rheumatoid synovium which have been shown by some others to express predominantly TCR Vδ1 and Vδ2 chains together with the Vγ9. We have studied the clonality of the TCR Vδ1, Vδ2 and Vγ9 by sequencing the V-J junctional regions of the rearranged TCR V-C chain CDNA. Our findings demonstrate a strong bias towards the use of the same V joining combination and junctional sequences although the specific sequences were unique for each patient with both δ and γ. In contrast, in the synovium the gene usage was polyclonal. The peripheral blood oligoclonality persisted with time. Our data support the important potential role of $\gamma\delta$ T cells in RA and are consistent with a model for the role of $\gamma\delta$ T cells in RA in which the cells are activated outside the joint and migrate to the joint where they recruit other cells in a non specific manner.

EZ 207 DIFFERENTIAL RESPONSE OF C3H-*lpr* T CELL SUBSETS TO STIMULATION WITH S. Enterotoxin B *in vitro* AND *in vivo*,
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Mice homozygous for *lpr* develop lymphadenopathy, splenomegaly, T and B cell activation and hypergammaglobulinemia and secrete autoantibodies. The predominant cell type in enlarged lymph nodes (LN) is a unique subset of $CD4^-$, $CD8^-$, $B220^+$ double negative T cells that share some phenotypic characteristics with previously activated T cells but are refractory to stimuli *in vitro* . The absolute numbers of $CD4^+$ and $CD8^+$ T cells also are increased in *lpr* LN and a high proportion of these are immunocompetent memory T cells. The mechanisms leading to the activation and accumulation of *lpr* T cells are poorly understood. Here we report on the response of LN Vβ8+ T cells from diseased C3H-*lpr* mice to stimulation with the bacterial superantigen Staphylococcal enterotoxin B (SEB). DN T cells were unresponsive and $CD4^+$ and $CD8^+$ T cells responded poorly to SEB *in vitro* and the response was not enhanced with accessory cells, IL-2 or anti-CD28 Ab. In contrast *lpr* LN T cells responded strongly to SEB *in vivo*. Evidence for this included a significant increase in the proportions and absolute numbers of Vβ8+$CD4^+$, $CD8^+$ and DN T cells and incorporation of BrdU by Vβ8+ T cell subsets. As reported previously for normal cells, T cells from SEB-treated *lpr* mice were unresponsive to restimulation with SEB *in vitro* . These data indicate that *lpr* DN T cells have an abnormally high threshold for activation that can be exceeded *in vivo* but not *in vitro*. The *in vivo* responsiveness of DN T cells also suggests that the activation and accumulation of these cells could be antigen-driven. The poor response of *lpr* $CD4^+$ and $CD8^+$ T cells to SEB *in vitro* provides another example of the recently reported difference in the responsiveness of naive and memory T cells to stimulation with SEB.

EZ 208 LIMITED HETEROGENEITY OF REARRANGED T-CELL RECEPTOR Vα- AND Vβ-TRANSCRIPTS IN SYNOVIAL FLUID T-CELLS IN RHEUMATOID ARTHRITIS,
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Rheumatoid arthritis (RA) is a disease of unknown etiology which, in the course of a chronic inflammatory process, leads to destruction of the joints. The identification of activated T-cells in the synovial fluid and the synovium in conjunction with the linkage of RA to specific class II major histocompatibility complex restriction elements indicate that these T-cells are critical in the etiology and pathogenesis of RA. Several attempts have been made to demonstrate clonality in synovial T lymphocytes. Conflicting results have been obtained in these studies. Whereas in most of these studies synovial T-cells have been expanded in vitro prior to analysis we have used the polymerase-chain-reaction (PCR) to amplify T-cell antigen receptor Vα- and Vβ-transcripts of synovial T-cells without prior culture of T-lymphocytes. The heterogeneity of the T-cell receptor repertoire was analysed by hybridization analysis and sequencing of PCR products. Evidence for a limited heterogeneity of Vα- and Vβ-T-cell antigen receptors in synovial T- cells has been obtained.

EZ 209 $CD4^+$ $CD45RO^+$ T CELLS ARE THE SOLE SUBSET OF PERIPHERAL BLOOD MONONUCLEAR CELLS THAT PRESENT MYCOBACTERIA TO HUMAN $\gamma\delta$ T CELLS.
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T cells of the Vγ9/Vδ2 subset constitute the vast majority of peripheral $\gamma\delta$ T cells, and have been found in increased numbers in inflammatory sites such as the joint of patients with rheumatoid arthritis. These cells respond uniformly to mycobacteria, regardless of their nominal antigenic specificity, and undergo extrathymic expansion throughout life. *In-vitro* non-clonal activation of these cells by the mycobacterial mitogen AP-MT requires interaction with antigen presenting cells. The cellular and molecular mechanisms by which AP-MT is presented to Vγ9/Vδ2 cells are completely unknown.

To address this, we have undertaken to characterize the phenotype of the naturally occurring antigen presenting cells in the peripheral blood. Using immunomagnetic separation techniques and various purification methods we have studied highly purified subpopulations of peripheral blood mononuclear cells. While no presentation of the mycobacterial antigen AP-MT to Vγ9/Vδ2 cells could be achieved with professional antigen presenting cells such as noncytes, B cells, or dendritic cells, a CD4+ subpopulation expression $\alpha\beta$ TCRs, and theRO isoform of CD45 was identified as the sole subset that presents the antigen to Vγ9/Vδ2 clones. Presentation could be achieved with AP-MT-pulsed and subsequently fixed APCs, however APCs fixed prior to the AP-MT pulse could not present, indicating that antigen processing, or another metabolically dependent step is involved in presentation of the mycobacterial antigen to Vγ9/Vδ2 cells. This is the first identification of the physiologic APCs for recognition of mycobacteria by the Vγ9/Vδ2 subset. Furthermore, this is the first evidence for differential antigen presentation ability by T cells bearing distinct CD45 isoforms. These results shed new light on the mechanism by which bacteria activate the Vγ9/Vδ2 subset of peripheral $\gamma\delta$ T cells, resulting in their selective expansion throughout life.

Both $\gamma\delta$ T cells and $CD4^+CD45RO^+$ T cells have been previously found to accumulate in the inflamed rheumatoid joint. It is therefore conceivable that the cellular interaction reported here is relevant to the pathogenesis of rheumatoid arthritis.

EZ 210 GENETIC CONTROL OF THE TCRβ AND TCRγ GENE REPERTOIRES IN RHEUMATOID ARTHRITIS MONOZYGOTIC TWINS, Hitoshi Kohsaka, Atsuo Taniguchi, Pojen P. Chen, William E.R. Ollier* and Dennis A. Carson, University of California San Diego, La Jolla, CA 92093-0663 and *University of Manchester, UK.
Rheumatoid arthritis (RA) is characterized by T lymphocyte infiltration into hyperplastic synovial tissues. Either genetic or environmental factors could affect RA incidence or severity, by altering the T cell repertoire. The aim of the present experiments was to determine the effects of genes and the environment on the expression of T cell receptor (TCR) variable region β and γ genes in RA. A novel quantitative PCR-ELISA method was developed that made it possible to assess quantitatively the expression of 24 different TCR Vβ subgroups and 4 different TCR Vγ subgroups in small aliquots of lymphocytes. This technique was then used to analyze TCR V gene expression in peripheral blood lymphocytes from five pairs of monozygotic twins who are discordant for RA, in three twin pairs who are concordant for RA, and in one normal pair. In each sample, total RNA was extracted from unmanipulated lymphocytes, converted to first strand cDNA, polyG-tailed, and amplified with a TCR constant region primer and an anchor primer. The resultant double stranded cDNA products were captured onto microtiter wells of ELISA plates. After washing away sense-strand DNA, the residual single-stranded DNA products were hybridized with TCR V family specific sense-oligonucleotide probes linked to digitoxigenin. After the addition of anti-digitoxigenin antibody, the expression of each V gene was quantitated by simple colorimetric assay. For TCR Vγ gene analysis, multiple clones from each gene family were sequenced so as to determine the ratio of functional transcripts in total transcripts. Non-parametric statistical analyses of the TCR V gene repertoires showed that Vβ gene repertoires are significantly more alike in monozygotic twins than in unrelated subjects ($p<0.05$). In contrast, TCR Vγ gene repertoires were divergent among twins. There were no significant differences in TCR V gene usage in the RA affected twins that could be attributed to the disease alone, independent of genetic variables. These results show that the expressed TCR Vβ repertoire, but not TCR Vγ repertoire, in humans is genetically regulated, and is not grossly altered as a consequence of RA.

EZ 211 A FAMILY OF STREPTOCOCCAL SUPERANTIGENS REPRESENTED BY RHEUMATOGENIC SEROTYPES OF M PROTEINS SHARING SPECIFICITY FOR HUMAN TCR-Vβ4 ELEMENTS. Malak Kotb, Rika Ohnishi, and Mark Tomai, Department of Surgery, Microbiology and Immunology, from the V.A. Medical Center and The University of Tennessee, Memphis, Memphis, Tennessee 38163
Rheumatic fever and rheumatic heart disease are human *autoimmune* disorders triggered by infection with certain serotypes of *Streptococcus pyogenes*. Although the mechanisms by which these organisms trigger autoimmunity is unclear, it is known that disease is only associated with eleven out of the 80 serotypes of *S. pyogenes*. Differences in serotypes is related to antigenic variations among the surface M protein molecule. We have previously reported that the rheumatogenic type 5 M protein is a superantigen for human T cells. We have now extended our studies to examine other M protein serotypes for their superantigenic properties. The results indicate that rheumatogenic serotypes of M protein represent a family of streptococcal superantigens with distinct patterns of Vβ specificities. All serotypes tested share specificity for human TCR Vβ4 elements. The highly homologous M5 and M6 proteins exhibit the same Vβ specificity with the exception of Vβ1 which is expanded by M6 and not M5 protein. The pattern of Vβ specificity for M5 and M6 is quite distinct from that found for the M24 protein which only shares few epitopes with these two other serotypes. We hypothesize that the Vβ specificity of the M proteins may be determined by the primary structure of the various serotypes and that their shared specificity for Vβ4 may be related to their α-helical coiled-coil conformation. Supported by funds from the US Veteran's Affairs and funds from NIH-G 38530 (MK).

EZ 212 SHARED PROPERTIES OF T CELLS INVOLVED IN THE PATHOGENESIS OF LYME AND REACTIVE ARTHRITIS, Riitta Lahesmaa*, Marie-Claude Shanafelt#, Hans Yssel$, Carol Soderberg#, Lawrence Steinman*, and Gary Peltz#. *Department of Neurology, Stanford University Medical Center, Stanford, CA 94305; Institute of #Immunology and Biological Sciences, Syntex Research, Palo Alto, CA 94303; and $DNAX Research Institute, Palo Alto, CA 94305.
Our characterization of pathogen-reactive T cell clones, isolated from patients with Lyme and reactive arthritis, indicates that these arthritogenic pathogens use similar mechanisms to selectively modulate the cellular immune response of arthritis patients. The antigenic specificity, TCR V-gene segment usage, profile of lymphokines secreted, and cell surface adhesion molecules on $CD4^+TCR\alpha\beta^+$ T cell clones reactive with B. burgdorferi or Y. enterocolitica antigens, isolated from patients with Lyme arthritis or HLA-B27+ reactive arthritis, was characterized. Although the T cell clones recognized a number of distinct antigens, and expressed different TCR V-gene segments, they all produced a Th1-like profile of cytokines upon activation. Similarly, both pathogens also appeared to express Vβ-selective factors which influenced the cellular immune response. Analysis of a large panel of antigen-reactive T cell clones, isolated from one patient with reactive arthritis and another from a Lyme arthritis patient, indicated preferential, though not exclusive, utilization of a particular TCR Vβ family. Furthermore, two T cell clones utilizing this TCR Vβ gene segment recognized an unidentified spirochetal antigen in an HLA unrestricted manner, and the percentage of T cells utilizing this TCR Vβ segment increased after in vitro incubation of this patient's PBMC with B. burgdorferi antigens. Adhesion molecules expressed on the surface of the arthritogen-reactive Th1 like T cell clones did not differ from those of allergen-reactive Th2-like T cell clones isolated from atopic individuals. In conclusion, our results indicate that selective activation of Th1-like T cells and influence of host cellular immune response by pathogen's Vβ-selective factors are common features likely to play an important role in the pathogenesis of reactive arthritis and Lyme disease.

EZ 213 HETEROGENEITY OF SUPERANTIGEN RESPONSES IN INFLAMMATORY TISSUES OF ARTHRITIS. Shu Guang Li♦♥, Malak Kotb#, Alison J. Quayle♦, Jorn E. Thoen$, O Vinje*, Jaco B. Natvig♦ and Øystein T. Førre*. ♦Institute of Immunology and Rheumatology, University of Oslo, Fr. Qvamsgt. 1, N-0172 Oslo 1, Norway; ♥Current address: Specialty Laboratories Inc., Santa Monica, CA 90404-3900; #Departments of Medicine and Microbiology, University of Tennessee, Memphis, Tennessee 38163, USA; $Det Norske Diakonhjem, Oslo, Norway; *Oslo Sanitetsforenings Rheumatism Hospital, Akersbakken 27, N-0172 Oslo 1, Norway.
The profile of T cell responses to superantigens *Staphylococcus* enterotoxin A (SEA), *Staphylococcus* enterotoxin B (SEB) and *Streptococcal* M type 5 protein (M5) was examined in seven rheumatoid arthritis (RA) patients, two psoriatic arthritis patients, two reactive arthritis patients and one ankylosing spondylitis patient. The limiting dilution analysis results showed heterogeneities of the frequencies of proliferating E^+ responder cells isolated from peripheral blood and synovial membrane (SM) or synovial fluid (SF) of these patients. Elevation of all three superantigen-reactive precursor T cell frequencies was found in SM/SF in comparison with autologous peripheral blood precursor T cell frequencies from three out of five RA patients tested. This phenomena was not seen in other types of arthritis patients. However, all patients tested showed increased SM/SF precursor T cell frequencies against BCG. Proliferation of T cell lines generated with superantigens was detected when superantigens were presented by human MHC class II transfectants. Heterogeneities of proliferation and cytotoxicity against those superantigens were observed in these cell lines. Furthermore, two of three mycobacteria heat shock protein 65-kD specific T cell clones generated from a RA patient could lyse themselves or each other when SEA was used. The T cell receptor V gene usage of those clones were different, i.e. Vß1, Vß6 and Vß17. Heterogeneities of specific lysis were also demonstrated by a few T cell clones from another RA patient generated with human IgG fragment or collagen type II fragment. These results suggested that activation by ubiquitous microbial superantigen(s) may be an initiator for an autoimmune response in susceptible individuals.

EZ 214 SEGREGATION OF TCRβ CHAIN HAPLOTYPES IN MULTIPLEX RHEUMATOID ARTHRITIS FAMILIES.

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The different concordance rates for Rheumatoid Arthritis (RA) in monozygotic and dizygotic twins indicates a genetic component to the disease but places an upper limit on the penetrance of these gene(s) to 10-20%. There is a well established association between possession of HLA DR 4 and/or HLA DR1 and RA in most populations but calculations based and on MZ twin concordance and HLA sharing in sibling pairs have suggested that the HLA contribution to the genetics of RA is less than 50%. We therefore choose the T cell receptor β chain locus as a candidate genomic region for RA susceptibility. We initially investigated this association in 26 families multiplex for RA (ARA criteria). Each family contained at least two siblings with RA; the proband in all cases was either DR4 or DR1. We first used RFLP markers within and flanking the TCRB on chromosome 7q32. The three probes were Cβ in the TCR locus, D7S111 (CR1-524) centromeric to TCRB and D7S22 (PλG3) telomeric to the TCRB locus. Using linkage package version 5.10 and multipoint linkage analysis we obtained a lod score of 1.02 for a single gene model of RA and a lod score of 1.3 using the two gene model developed by Jurg Ott. We have subsequently extended the number of families to 33 (7 families from University of Toronto) and further defined TCR β haplotypes in these families using Vβ polymorphic markers spanning the TCRB locus (Vβ 7, Vβ6.7 and Cβ). On completion of these analyses we will re-enter the data and re-evaluate whether the initial lod scores obtained disappear or whether there is indeed an association between susceptibility to RA and and particular TCRB haplotypes.

EZ 215 T-CELL RECEPTOR Vβ2 SEGMENT CODING POLYMORPHISM IS NOT ASSOCIATED WITH RHEUMATOID ARTHRITIS (RA)

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T-lymphocytes may be involved in the pathogenesis of RA, with the synovial fluid T-cell repertoire of a patient with RA showing enrichment of Vβ2 and Vβ3 sequences (Uematasu et al. Proc Natl Acad SciUSA 88:8534). We recently found a coding allelic polymorphism for the Vβ2 segment, that can be typed by single-strand conformation polymorphism analysis of Vβ2 PCR products. In order to investigate whether this polymorphism is implicated in RA susceptibility, we typed 136 caucasoid patients with erosive seropositive RA and 150 caucasoid controls.

	CONTROLS	RA PATIENTS (n=272)		
ALLELE	(n=300)	ALL	DR4+(n=272)	DR1+/DR4-(n=48)
TCRBV2S1*1	145	136	87	22
TCRBV2S1*2	108	104	61	22
TCRBV2S1*3	46	29	22	3
TCRBV2S1*4	1	0	0	0
TCRBV2S1*5	0	3	2	1

The allele frequencies of the 3 common variants were not significantly different in RA patients compared to controls (p=0.12), neither in DR4+ (p=0.25) nor in DR1+/DR4- RA (p=0.27). Two new alleles were identified. TCRBV2S1*4 was observed only once in a control; DNA sequencing revealed that it contains a frameshift mutation resulting in a nonfunctional segment. TCRBV2S1*5 was found in three RA patients, but this frequency was not significantly different from its absence in the controls (p=0.11). This data allows us to conclude that the Vβ2 polymorphism is not implicated in RA susceptibility.

EZ 216 TCR VARIABLE GENE USAGE IN RECENTLY INFLAMED SYNOVIAL TISSUE OF RHEUMATOID ARTHRITIS PATIENTS,

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T-cell infiltrates are found in the joints of rheumatoid arthritis (RA) patients and are thought to be important in the pathogenesis of the disease. Studies on the TCR variable gene repertoire expressed by these cells have led to conflicting results. Both oligoclonality and lack of oligoclonality have been reported. We have used a PCR assay with 18 Vα and 22 Vß family specific primers to analyze the T-cell repertoire. The synovia that we used for this study were from RA patients with longstanding disease. However, the T-cell populations that we studied were isolated from synovial tissue of joints that were no longer than 6 months involved in the disease process. Different stimulation protocols were used including PHA, anti-CD3 and IL-2. Peripheral blood lymphocytes of the patients were also stimulated with IL-2 and used as reference T-cell populations. In addition T-cells were selected for expression of CD4 and/or CD25. Even in this relatively early stage of the inflammatory process expression of all tested Vα and Vß gene families was observed. The different stimulation protocols had a significant influence on the T-cell repertoire. In a study of 6 RA patients it appeared that in individual patients significant higher levels of expression of 1-3 Vß gene families are found in the IL-2 stimulated synovial T-cells compared with IL-2 stimulated T-cells from the peripheral blood. The Vß9, Vß13.1 and Vß14 gene families were frequently found to be involved. The Vß repertoire of the selected T-cell populations was also determined. It did not appear to be more restricted than in the other unselected lines.

A number of V-D-J rearrangements were sequenced for Vß14, Vß17 and Vß13.1 gene families from synovial T-cell lines in which enhanced expression of these V-gene families was observed by PCR. In the Vß14 and Vß17 but not the Vß13.1 gene families evidence was found for clonal expansion of T-cells in the inflamed synovia.

EZ 217 T-CELL RECEPTOR V GENE SEGMENT USAGE IN T-LYMPHOCYTE SUBSETS FROM SYNOVIAL TISSUE OF RHEUMATOID ARTHRITIS PATIENTS,

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T-lymphocytes are playing an important role in the pathogenesis of rheumatoid arthritis (RA); in synovium T-lymphocyte infiltrates can be observed.

In this study the frequency of usage of the T-cell receptor (TCR) Vα and Vß gene segments in T-lymphocyte subsets of synovial tissue T-cell infiltrates have been analyzed. The $CD4^+/CD45RO^+$ (the memory, activated $CD4^+$ T-cells), $CD4^+/CD45RA^+$ (the naive $CD4^+$ T-cells), $CD8^+/CD45RO^+$ (the memory, activated $CD8^+$ T-cells) and $CD8^+/CD45RA^+$ (the naive $CD8^+$ T-cells) T-cell populations from peripheral blood mononuclear cells and synovial tissue of 3 RA-patients and one not full blown RA-patient have been analyzed for their TCR V-gene usage via MoAb-stainings and semi-quantitative PCR-analyses. These analyses have been performed on PHA/IL2 stimulated T-lymphocytes. The results of this study are that 1) almost all TCR V gene segments are present in the different subsets, 2) the frequencies of usage of the TCR V gene segments are different for each patient and 3) no overexpression of a particular TCR V gene segment can be observed in the different subsets of all patients investigated.

EZ 218 DEFINITION OF IMMUNOGLOBULIN HEAVY CHAIN AS A TARGET OF JOINT-DERIVED T CELLS IN RHEUMATOID ARTHRITIS, Wim C.A. van Schooten, Deirdre Devereux, Charles Ho, Jeanette Quan and Chantal J.J. Rust, ImmuLogic Pharmaceutical Corporation, Palo Alto, California 94304

Distinct T cell clones derived from the knee joint of a rheumatoid arthritis patient proliferated in response to autologous synovial fluid and plasma. The stimulating antigen was identified as immunoglobulin by the reaction of T cells to the eluate, but not the flow through, of a protein A column. Definition of the antigen as immunoglobulin heavy chain was established by T cell recognition of a 50 kD species isolated from the protein A eluate by preparative SDS-PAGE. The ability of immunoglobulin to serve as an autoantigen suggests an effective mechanism for the perpetuation and exacerbation of joint destruction, namely rheumatoid factor positive B cells in the synovium present immunoglobulin epitopes to autoreactive T cells.

EZ 219 ACTIVATION OF ANTI-TUMOR CYTOLYTIC ACTIVITY OF γδ T CELLS BY A HEAT SHOCK PROTEIN-DERIVED PEPTIDE, Lynn E. Wagner, Nadine C. Romzek, Govindashwamy Panchmoorthy, Hamid Band, and Joseph Holoshitz. Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109-0531, and Department of Rheumatology and Immunology, Harvard Medical School, Boston, MA 02115.

Previous studies have found that cells of the Vγ9/Vδ2 subset are capable of promiscuous cytolytic activity against a large variety of tumor targets. This cytolytic activity seems to be unrelated to the reactivity of these cells to soluble antigens, and unlike the latter, does not seem to be mediated by the T cell receptor (TCR). Other studies have found that both murine and human γδ T cells can recognize heat shock proteins (HSP). It is currently unclear whether these two functional activities are related.

In the present study we have addressed the role of HSP recognition in the anti-tumor cytolytic activity of human γδ T cells. Mycobacterial HSP65 expressed in a human mammary tumor line by cDNA transfection, or added in a soluble form, consistently induced augmented cytolytic activity (up to 40% above the baseline cytolysis of this target in the absence of the protein) by one Vγ9/Vδ2 clones. Other malignant targets and EBV-transformed B cell lines did not show this augmented susceptibility. Control T cell clones expressing Vγ9/Vδ2 TCR, Vδ1 TCR, or αβ TCR did not show any augmented cytolytic activity. The cytolysis could not be inhibited by anti-CD3, or anti-Vδ2 monoclonal antibodies at a concentration which could effectively block antigen specific proliferative responses of these cells. Anti-LFA-1α monoclonal antibodies inhibited the cytolysis. By using recombinant truncated forms of the HSP65 protein, and synthetic peptides, we mapped the active region on the HSP65 to amino acids 180-187.

Our results directly implicate HSP in the anti-tumor cytolytic activity of γδ T cells. It is noteworthy that the region 180-187 of the mycobacterial HSP65 has been previously shown to be recognized by arthritogenic rat αβ T cells, and by murine γδ T cell hybridomas. Unlike the presumed role of the TCR in recognition of the peptide by those cells, however, our data do not provide evidence that the enhanced cytolysis reported here is mediated by the TCR.

EZ 220 A NOVEL T CELL RECEPTOR (TCR) α CHAIN VARIABLE REGION ASSOCIATED WITH RHEUMATOID SYNOVITIS. William V. Williams, Dimitri Monos, Qiong Fang, Joan M. VonFeldt, David B. Weiner. Institute of Biotechnology and Advanced Molecular Medicine and the University of Pennsylvania, Philadelphia, PA 19104.

Recent reports indicate a potentially restricted usage of TCR β chains in RA synovial fluid and tissue. We previously reported a restricted heterogeneity of TCRs in RA synovial tissue which appeared the most pronounced for the TCR α chain (J. Clin Invest. 90:326, 1992). Here we report the identification of a novel TCR α chain which is frequently expressed in RA synovium. This chain belongs to the Vα 28 family (Vα 28.2), and is characterized by a highly charged motif in the fourth hypervariable region. Expression of Vα28.2 was detected in 8/8 RA synovial T cell populations and 4/11 RA synovial tissue specimens. Clonal restriction was apparent on sequence analysis in 5/7 specimens where three or more clones were sequenced. Vα 28.2 was not detected in 8 non-rheumatoid synovial tissue specimens, and was present in peripheral blood mononuclear cells (PBMCs) of only 1/8 unselected individuals. In contrast, Vα 28.2 was detected in PBMCs from 5/5 HLA-DR4+ individuals. Further analysis of this potential genetic linkage is underway. In addition, Vα 28.2 was detected in synovial tissue biopsies of 3/5 patients with early (.6 months duration) disease, and was the only Vα detected in more than one biopsy. This suggests that Vα 28.2 is present in early rheumatoid synovitis, persists in chronic disease, and may be normally restricted by MHC Class II antigens.

Adhesion Molecules in Cell Migration; Adhesion Molecules in Cell Activation

EZ 300 THE EFFECT OF CYTOKINES ON THE INTERACTION OF HUMAN MONOCYTES WITH VASCULAR ENDOTHELIAL CELLS,

Henry Beekhuizen, Jan A.M. Langermans and Ralph van Furth, Department of Infectious Diseases, University Hospital, Leiden, The Netherlands.

Cytokines produced at sites of inflammation affect the interaction of monocytes with vascular endothelial cells (EC) and as such can regulate the margination and extravasation of monocytes. The aim of our study was to investigate the effect of a variety of cytokines on the expression of adhesion molecules on the surface of EC and the adhesiveness of EC for human monocytes. Incubation of cultured monolayers of human venous EC with rIL-1α or rTNF-α for 4 h induced marked expression of E-selectin on EC, increased their adhesiveness for monocytes and increased the percentage of EC-bound monocytes with a stretched morphology, i.e. monocytes that migrate over the surface of EC. Incubation of EC with rIL-1α, rTNF-α or rIFN-γ for 24 h induced surface expression of ICAM-1 and, exept for rIFN-γ, VCAM-1, enhanced the binding of monocyte to EC and promoted stretching of EC-bound monocytes. Stimulation of EC with rIL-4 for 24 h induced expression of VCAM-1 but not ICAM-1 on EC, increased monocyte binding to EC but did not promote stretching and migration of bound monocytes. Studies with monoclonal antibodies (mAb) revealed that the mechanism underlying the initial binding of monocytes to nonstimulated EC involves the CD11/CD18-integrin molecules on monocytes. However, the increased binding of monocytes to cytokine-stimulated EC is not dependent on the CD11/CD18 molecules and, in part, is mediated by CD14 molecules on monocytes. Both E-selectin and VCAM-1 contribute slightly to the enhanced binding of monocytes to cytokine-stimulated EC, whereas ICAM-1 plays an essential role in the morphological changes and migration of monocytes on the surface of EC after their initial CD14-dependent binding. A similar interaction of monocytes with ICAM-1 on cytokine-stimulated EC was found when monocytes were preincubated with murine anti-CD14 mAb and subsequently with $F(ab')_2$-anti-mouse Ig, which caused crosslinking of CD14 on the surface of monocytes. Such crosslinking of CD14 induced activation of monocytes as measured by a rise in intracellular free calcium concentration and increased protein kinase activity. Taken together the results indicate that cytokines affect the adhesiveness of EC for monocytes; the degree of increased adhesion being dependent on the type of cytokine used to stimulate EC and the duration of cytokine treatment, and that different mechanisms underly the adherence of monocytes to nonstimulated and cytokine-stimulated EC. It is speculated that the binding of circulating monocytes to the surface of EC at sites of inflammation may occur via interaction of CD14 on monocytes with counterstructures expressed on cytokine-stimulated EC. Such interaction generates an intracellular response in monocytes and triggers an adhesion mechanism which allows monocytes to bind to ICAM-1 on EC and facilitates their transendothelial migration.

EZ 301 HUMAN CORD BLOOD DENDRITIC CELLS (DC) ARE DEFICIENT IN THE ABILITY TO CLUSTER WITH T CELLS AND TO SUPPORT T CELL MITOGENESIS,

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Primary antigenic and mitogenic responses in resting T cells are initiated by cluster formation, the physical interaction of T cells with DC. We have compared the proliferation (as measured by the incorporation of ^{3}H-thymidine), and cluster formation (as observed microscopically in tissue culture) of human cord and adult blood T cells cultured with highly purified autologous DC in the presence of a sub-optimal (2 ug/ml) concentration of concanavalin A (Con A). Con A-induced proliferation of cord blood T cells in the presence of cord blood DC (n=20 individual experiments) was consistently <20% of that observed for the corresponding cultures of adult cells (n=10). T cell-DC (≥ 3 T cells/DC) clustering was similarly reduced in the cord blood cultures (0.7 clusters/100 DC), compared to the equivalent adult cultures (4.5 clusters/100 DC) following the first 24 hours of incubation. Proliferative responses and clustering by cord blood T cells was greatly enhanced by using a higher Con A concentration (10 ug/ml), increasing the concentration of DC in the cultures, supplementing the cultures with interferon-τ, or replacing cord blood DC with adult blood DC. Cord blood DC functioned poorly as accessory cells in the response to Con A by adult T cells and as stimulators in the allogeneic mixed leukocyte reaction for both adult and cord blood mononuclear responder cell populations. The factors which contribute to the observed relative inefficiency of cord blood DC are not currently understood. Since adult and cord blood DC displayed comparable levels of cell surface MHC Class I and II molecules, the altered expression by cord blood DC of other molecules that are involved in T cell-DC clustering is suggested. Cord blood may represent a useful system for the study of factors which regulate human DC function.

EZ 302 T CELL ADHESION TO RHEUMATOID SYNOVIOCYTES RESULTS IN PARTIAL LOSS OF CD3 AND CD4, AND MARKED ENHANCEMENT OF IL-6 SECRETION,

Herbert B. Lindsley, Donald D. Smith, and Christopher B. Cohick. Department of Medicine, University of Kansas Medical Center, Kansas City, KS 66160

We postulated that differential binding of T cell subsets may occur at sites of synovial inflammation and result in cell activation. In preliminary studies of resting monocyte-depleted peripheral blood mononuclear cells (PBMC) binding to IFNγ-stimulated (100 U/ml, 48 hr) rheumatoid synoviocyte monolayers, CD4 was minimally detectable by cell ELISA (96 well plate). Positively selected CD4+ PBMC demonstrated CD3 expression, and minimal CD4 expression following adherence. Thus studies were expanded to six-well plates to allow recovery of adherent cells and assessment of surface markers by flow cytometry. Resting PBMC were allowed to adhere to the monolayers for 1 hr at 37° and nonadherent cells removed by gentle washing. Adherent cells were then either removed with dilute trypsin and transferred to wells containing no synoviocytes, or left on monolayers for an additional 23 hr. Cells were recovered and stained with T cell MAb (OKT3, OKT4, or OKT8). Dilute trypsin (0.125%) had no effect on CD3, CD4, or CD8 PBMC detection.

Preadherent PBMC consisted of 91% CD3+ cells (range 86-97%), 57% CD4+ cells (range 48-63%) and 25% CD8+ cells (range 12-31%). Post-adherent T cells from both time points showed lower percentages of CD3+ cells (91→59%) and CD4+ cells (57→25%), but no change in CD8+ cells (25→26%). IL-6 secretion over 24 hr was measured before or after adhesion. Basal IL-6 secretion did not exceed 5.3 ng/ml. After 24 hr of PBMC, synoviocyte adherence, IL-6 secretion markedly increased, to a mean (3 expt) value of 137.4 ng/ml, and in 1 expt, after 96 hr, to 430 ng/ml.

We concluded that both CD4+ and CD8+ cells bound to synoviocyte monolayers, and that subsequent partial loss of CD4 or CD3 expression was likely due to T cell activation, triggered by cell to cell adhesion. Marked enhancement of IL-6 secretion presumably reflects synoviocyte activation.

EZ 303 THE ROLE OF CD45 IN MODULATING HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL ADHESION AND T-LYMPHOCYTE RESPONSIVENESS.

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We demonstrated that triggering human PBMCs with mAb to CD45 or one of its isoforms can cause cellular aggregation through LFA-1/ICAM-1 interactions. Additionally, we found that the protein kinase inhibitor, H8, blocked CD45 induced cellular adhesion at concentrations which had no effect on PMA induced aggregation. This suggested that the CD45 signal cascade involves a cyclic nucleotide dependent protein kinase other than PKC. We also showed that CD45 signals can increase intracellular cAMP levels and directly activate PKA. Interestingly, the cAMP response is highly dependent on continued LFA-1/ICAM-1 interactions; i.e. in highly pure T-lymphocytes, CD45 mAb induced neither clumping nor increased cAMP. Additionally, we were able to block the cAMP response in a T-lymphocyte/monocyte mixture with mAb against LFA-1 and/or ICAM-1. Finally, we compared the effect of CD45 triggering versus direct stimulation of PKA with dibutyryl-cAMP, on CD3 induced T cell activation. We found nearly identical responses with regard to inhibition of T cell proliferation and IL-2/IL-2R (but not IL-4) mRNA production. Our *in vitro* studies thus demonstrate that the CD45 antigen is actively involved in modulating both T-lymphocyte responsiveness and adhesion, via PKA related pathways. We propose a mechanistic model by which CD45 and its downstream transduction pathways are capable of modulating such responses in human peripheral blood mononuclear cells.

EZ 304 ADHESION OF B LYMPHOCYTES OF CHILDREN WITH ARTHRITIS HUMAN UMBILICAL VEIN ENDOTHELIUM (HUVEC). Kiem Oen*, Glenys Danell*, Stephen Stewart**, Karen Tazumi**, John Wilkins*, University of Manitoba*, and Canadian Red Cross Blood Transfusion Centre**, Winnipeg, Canada.

To characterize the binding of circulating B lymphocytes of children with arthritis to endothelium, adhesion of peripheral blood lymphocytes to tumor necrosis factor alpha-activated HUVEC was studied in children with juvenile rheumatoid arthritis (JRA) (n=12), seronegative spondyloarthropathies (SSA) (n=7), and pediatric controls (CTL) (n=6).

In all subject groups CD4 T cells were selectively decreased in cells adherent to activated (A/A) HUVEC compared with the original (O) or non-adherent cell populations (NA). In contrast CD19 B cells were selectively increased or unchanged. This increase was higher in patients with JRA than in controls. There was no preferential adhesion of CD5CD19 B cells. CD49dCD4 cells and CD29CD4 increased in A/A cells while increases in CD49dCD19 or CD29CD19 cells were nil or minimal. Both Leu-8CD4 and Leu-8CD19 cells were decreased in adherent compared to original cells.

The results suggest that B lymphcytes have a great capacity for binding to activated endothelium, enhanced binding by B cells of patients with JRA, and possible differences in adhesion molecules utilized by B and CD4 T cells in this system.

	CD19 % bound	CD19 % in A/A / % in O	CD4 % bound	CD4 % in A/A / % in O
JRA	45	177 ($P<.05$)	15	57
SSA	32	122	16	62
CTL	39	129	14	54

Cells	CD29CD19 JRA	CD29CD19 SSA	CD29CD19 CTL	CD49dCD19 JRA	CD49dCD19 SSA	CD49dCD19 CTL
	% Positive					
O	37	55	51	39	37	39
A/A	38	49	45	55	54	52
NA	36	41	45	48	31	44

EZ 305 B7/BB1 AND LFA-3 AS ACCESSORY MOLECULES IN HUMAN T CELL ACTIVATION David M Sansom, Martina Boshell, John Lewis, Anne Wilson and Nicolas D Hall, Bath Institute for Rheumatic Diseases, Trim Bridge Bath BA1 1HD and School of Pharmacy and Pharmacology, University of Bath, Avon UK.

Understanding of the signals required for the activation of T cells is of critical importance in autoimmune disease. Current models suggest that both engagement of the TCR and the delivery of accessory or costimulatory signals are required for activation. In the absence of such costimulatory signals, clonal anergy or apoptosis may result. We have investigated two molecules, B7/BB1 and LFA-3 (CD58), for their ability to provide costimulation for resting and previously activated human T cells.

Using Chinese hamster ovary cell transfectants which express B7, LFA-3 and HLA-DR4 in various combinations, the ability of these cells to augment proliferation of T cells was determined. Anti-CD3 antibodies and DR4 transfectants pulsed with the superantigen SEB were used as T cell stimuli. These studies demonstrated that B7 but not LFA-3 transfectants are capable of costimulating proliferation of highly purified resting T cells. In addition we found that B7 alone was capable of stimulating proliferation in previously activated T cell blasts but not resting cells. Using SEB-pulsed DR4 expressing cells, costimulation was provided by B7-transfectants acting as a "third-party" cell. This situation is the first demonstration that B7, but not other accessory ligands such as LFA-3, can provide costimulation in a manner consistent with the model proposed by Schwartz. In control SEB-stimulation experiments, both DR4-LFA-3 and DR4-B7 double transfectants were considerably more potent stimulators of proliferation than DR4 transfectants alone. These data suggest that LFA-3 is only effective on the same cell as the T cell ligand and may physically be part of the TCR complex during activation.

Our data support the concept that the B7/CD28 interaction is unique in its costimulation abilities and are consistent with the role of B7 in the avoidance of anergy induction in T cells.

EZ 306 ACTIVATION OF Th1-LIKE T CELLS IN LYME DISEASE Marie-Claude Shanafelt*, Riitta Lahesmaa#, Hans Yssel*, Carol Sodeberg*, John Anzola*, and Gary Peltz*. *Institute of Immunology and Biological Sciences, Syntex Research, Palo Alto, CA 94303, and #Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

We have characterized T cell clones isolated from patients with Lyme arthritis which recognized antigens of the arthritogenic pathogen, *Borrelia burgdorferi*. The T cell clones recognized a number of distinct spirochetal antigens including the outer surface proteins A and B, flagellin and heat shock proteins of 60 and 70 kDa. Although they recognized different spirochetal antigens and utilized distinct V-gene segments in assembling their TCRs, they all produce the same restricted Th1-like profile of cytokines upon activation. The cell surface expression of costimulatory molecules on our panel of Th1-like arthritogen-reactive T cell clones was quantitatively the same as that on allergen-reactive T cell clones isolated from atopic donors, with a Th2-like profile of cytokine production. The ability of diverse T cell surface adhesion molecules to provide co-stimulatory signals for proliferation and cytokine production by human Th1 and Th2 T cell clones is presently under investigation using functional assays.

EZ 307 INDUCTION OF T CELL ADHESION BY PROTEOGLYCAN-IMMOBILIZED MACROPHAGE INFLAMMATORY PROTEIN-1β (MIP-1β) Yoshiya Tanaka, David H. Adams, Stefan Hubscher, Hiroyuki Hirano, Ulrich Siebenlist and Stephen Shaw, The Experimental Immunology Branch, NIH, Bethesda MD 20892

T cell migration from circulation into tissue is essential for immune responses and involves a co-ordinated sequence of events which results in strong adhesion via integrins and migration. Since T cell integrins cannot bind well until functionally activated, adhesion requires not only integrin ligands on the endothelium but also activation signals. Cytokines are good candidates for physiologic activation signals since they are released at sites of inflammation, act at short distance and induce leukocyte infiltration in vivo. We propose that a chemotactic cytokine, macrophage inflammatory protein-1β (MIP-1β) which is a member of the 'intercrine' family, plays an important role in T cell 'adhesion cascades'. We demonstrate that MIP-1β is chemotactic for T cells and also induces T cell adhesion to VCAM-1 and fibronectin via the α4β1 integrin. These effects are seen preferentially with CD8+ T cells. We reasoned that since cytokines released in the vessel lumen will be rapidly washed away in vivo, MIP-1β might be bound to endothelial surfaces via its GAG-binding site and mediate adhesion-induction in an immobilized form. In support of this theory, we have constructed an in vitro model, wherein the MIP-1β is captured by artificial heparin-BSA conjugate and thereby induces T cell adhesion to coimmobilized VCAM-1. We also show that MIP-1β induces adhesion to co-immobilized VCAM-1 when captured by CD44 proteoglycan, which is expressed on endothelium and is implicated in T cell adhesion to endothelial cells. Immunohistologic studies demonstrate that MIP-1β is present on the luminal surface of endothelium in lymph nodes and tonsil, suggesting the in vivo relevance of this model. We propose that 'pro-adhesive' cytokines such as MIP-1β produced in inflamed tissue and lymphoid tissue will bind to and be presented by endothelial proteoglycans to selectively trigger adhesion not only of lymphocyte subsets but also of other cell types.

Inhibition of IL-1 Action; Inhibition of TNF Action

EZ 400 EXPRESSION OF SURFACE P55 AND P75 TNF RECEPTORS IN RHEUMATOID ARTHRITIS,

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We previously proposed the hypothesis that TNFα plays a pivotal role in the pathogenesis of RA, based on our observation that it is the dominant inducer of IL-1 and GM-CSF in RA. As TNFα mediates its action by binding to one of two specific cell surface receptors (p55, p75) we investigated the expression of these molecules on RA synovial tissue. Using flow cytometric techniques both receptors were found to be significantly increased on mononuclear cells (MNC) isolated from synovial membrane of RA patients compared to normal or RA peripheral blood. Interestingly the p75 TNF-R was increased both on large monocytic-type cells and CD3+ lymphocytes. These results were confirmed by immunohistology, where 90% of cells in the lining layer of RA synovium were positive for both p55 and p75 TNF-R. Furthermore, cells in lymphoid aggregates expressed both TNF-R, with a predominant expression of p75. Cells at the cartilage-pannus junction, particularly those invading the cartilage, expressed both p55 and p75 TNF-R and many of these were also positive for TNFα. These results indicate that in RA synovial joint tissue there is upregulation of both p55 and p75 TNF-R, and that a wide variety of cells are potential targets for TNFα action - supporting our hypothesis that TNFα is of critical importance in the pathogenesis of RA.

EZ 401 ANALYSIS OF CYTOKINE GENE EXPRESSION IN RHEUMATOID ARTHRITIS USING QUANTITATIVE POLYMERASE CHAIN REACTION

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Cytokines have been implicated as important mediators of inflammation and joint destruction in rheumatoid arthtritis (RA). IL-1, IL-6, TNFα and GM-CSF have previously been detected in the synovial compartment at the protein as well as at the mRNA level. However the expression of cytokines produced by T-cells , IL-2, IL-4 and IFNγ, is still elusive. We have developed a highly sensitive assay based on polymerase chain reaction (PCR) for detection of cytokine mRNA. The relative amount of target mRNA could be evaluated by co-amplification of β-actin or the constant region of T-cell receptor α chain. Co-amplification of an internal mutated standard (an endonuclease cleavage site inserted in the experimental target cDNA) have also been employed. Following the PCR reaction, amplified cytokine cDNA was separated from the internal standard by chromatography on an anion exchange column (Mono Q). The amount of amplified target DNA and internal standard was evaluated by integration of the chromatografic peak area using the FPLC-manager computer program. Analysis of cytokine mRNA expression is feasible both in freshly isolated tissue and in cell culture samples. In addition, the methodology is applicible when elucidating the *in vitro* and *in vivo* effects of anti-rheumatic drugs. The down regulation of IFNγ, IL-2 and IL-4 but not IL-6 by cyclosporine A was demonstrated *in vitro*. We have used this assay for evaluation of the expression of IL-1, IL-2, IL-3, IL-4, IL-6, IL-10, IFNγ, TNFα, GM-CSF and G-CSF in synovial fluid/tissue and peripheral blood from RA patients.

EZ 402 INHIBITION OF TNF MEDIATED CYTOTOXICITY IN HUMAN AND MOUSE CELLS BY MONOMERIC SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR p55 AND A CHIMERIC RECEPTOR-IgG.

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Tumor necrosis factor α (TNFα) is considered to have a major role in rheumatoid arthritis (RA), promoting the release of inflammatory mediators and other pro-inflammatory cytokines. The inhibition of TNFα is therefore, of therapeutic interest in this disease.

Soluble forms of the extracellular domains of both TNF receptors have been reported in a broad range of biological fluids and are known to inhibit TNFα action. We have expressed a cDNA fragment corresponding to the sequence of the soluble form of the human p55 receptor (sp55) in chinese hamster ovary cells. A cDNA sequence encoding for a sp55-IgG fusion protein (sf2) has also been constructed and expressed. In this molecule, sp55 receptors have been grafted onto the first constant domains of a chimeric IgG, lacking the variable domains.

In this study a comparison between the ability of sp55 receptor, sf2 and the anti-TNFα chimeric monoclonal antibody (cA_2) to inhibit human TNFα, lymphotoxin (LT) and mouse TNF *in vitro* was determined. For these experiments two TNF sensitive cell lines were used in cytotoxicity assays, the WEHI-164 mouse fibrosarcoma line and the human rhabdomyosarcoma line KYM-1D4. WEHI-164 cells only express the mouse p55 receptor and are more sensitive to mouse TNF than human TNFα. KYM-1D4 cells express both human p55 and p75 TNF receptors and interestingly are more sensitive to human TNFα than LT.

In vitro, on a molar basis sf2 was found to be 10 fold more effective at inhibiting cytotoxicity than cA_2, and was 1,000 fold more effective than the monomeric p55 receptor, suggesting that a bivalent receptor structure has a higher avidity for TNFα. In the WEHI-164 assay the human cytokines were equally cytotoxic, however, both sp55 and sf2 were more potent at inhibiting TNFα action than LT, suggesting that they have a higher affinity for TNFα. This observation will be discussed in relation to the potential therapeutic use of chimeric fusion proteins in RA.

EZ 403 TISSUE-SPECIFIC REGULATION OF IL-6 PRODUCTION BY IL-4: DIFFERENTIAL EFFECTS OF IL-4 ON MONOCYTES AND FIBROBLASTS,

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IL-4 inhibits production of certain proinflammatory cytokines, including IL-1β, TNF-α and IL-6, in activated human monocytes. Although monocytes are a potent source of IL-6, other cell types such as fibroblasts and endothelial cells can also express this cytokine. To determine whether IL-4 inhibits IL-6 expression in non-hematopoietic cells, we investigated the effects of IL-4 on IL-6 production in both primary human fibroblasts and fibroblast lines. Rheumatoid synovial fibroblasts were used as target cells in these studies because, like monocytes, they produce high levels of IL-6 when stimulated with IL-1. Although peripheral blood monocytes did not constitutively express IL-6 mRNA or protein, stimulation with IL-1 or LPS induced IL-6 expression *de novo* in these cells. In contrast, synovial fibroblasts displayed a significant basal level of IL-6 production which was markedly increased following stimulation with IL-1. IL-4 suppressed IL-6 expression in monocytes, but did not inhibit IL-6 production in synovial fibroblasts. The inability of IL-4 to suppress IL-6 synthesis was not unique to rheumatoid synovial fibroblasts because IL-4 also failed to inhibit IL-6 production in normal fibroblast lines derived from other tissues. Synovial fibroblasts expressed a significant number of high affinity IL-4 receptors (approximately 2000 receptors/cell, Kd = 225 pM). Therefore, the lack of an inhibitory effect of IL-4 on IL-6 production in fibroblasts was not attributable to an absence of IL-4 receptors. These findings demonstrate that IL-4 does not inhibit IL-6 production in all IL-4R-positive cell types, and suggest that IL-4 may activate tissue-specific factors which differentially regulate IL-6 expression.

EZ 404 DEREGULATED EXPRESSION OF EARLY GROWTH RESPONSE TRANSCRIPTION FACTOR GENES IN RA SYNOVIOCYTES.

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In rheumatoid arthritis synovial lining cells play an important role in pannus formation, in tissue destruction, and in inflammation. In normal fibroblasts, the early growth response transciption factor genes c-fos and egr-1 are expressed at very low levels. Their gene products control a wide variety of cellular processes like cell proliferation or the expression of growth factor receptor and protease genes. Characteristically, their transcription is rapidly induced upon stimulation by growth factors. It ceases again due to mRNA instabilitz and because of the feedback regulation or repression by c-fos. In fibroblasts isolated from RA patients, however, we found constitutive co-expression of the c-fos and egr-1 genes at levels comparable to the induced state found in normal fibroblasts isolated from the same patients or from the synovial membrane of osteoarthritic controls. We further analyzed whether the constitutve expression of both early growth response genes depends on an autokrine or parakrine induction mechanism. However, the addition of RA synoviocyte supernatants to control fibroblasts did not change their normal egr-1 and c-fos expression pattern. Our results therefore suggest, that the impaired expression of these early growth response genes is an intrinsic defect of RA synoviocytes that may be an important molecular mechanism underlaying pannus formation and tissue destruction.

EZ 405 TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TNF-α: SAFETY, CLINICAL EFFICACY AND CONTROL OF THE ACUTE-PHASE RESPONSE.

Michael J. Elliott, Ravinder N. Maini, Marc Feldmann, Richard O. Williams, Fionula M. Brennan, Cong Qiu Chu, Clinical Immunology and Sunley Divisions, Kennedy Institute of Rheumatology, Bute Gardens, London, W6 7DW UK and Hanny Bijl, Centocor Inc (Europe), Martin Sanders, Centocor Inc, Malvern, PA 19355

Previous communications from our group have demonstrated the production of tumor necrosis factor α (TNF-α) and the expression of the TNF-α receptors, p55 and p75, in synovial tissue and pannus in rheumatoid arthritis (RA). In synovial explant cultures, we showed that anti-TNF-α reduced expression of pro-inflammatory cytokines. These findings have led us to identify TNF-α as a prime therapeutic target in this disease. We now report significant modulation of disease in the murine type-II collagen arthritis model of RA using a monoclonal anti-TNF-α antibody, and the results of a Phase 1 clinical trial of the treatment of severe refractory RA in patients, using a chimeric mouse / human neutralising monoclonal anti-TNF-α antibody (cA2). We treated 9 patients with active disease, each with a total of 20 mg/kg cA2, administered as 2 or 4 infusions over a 2 week period. The treatment was exceptionally well tolerated, with no hemodynamic, febrile or infective episodes, nor other significant adverse events. All patients showed rapid clinical responses. At day 29 of the trial, mean values (+/- SD) for each clinical measurement, expressed as a percentage of baseline values, were: Ritchie Articular Index: 24 +/- 13%, Swollen Joint Count: 28 +/- 17%, Pain Score: 29 +/- 19% and Morning Stiffness: 11 +/- 14%. Functional capacity, as measured by grip strength and HAQ scores also showed improvement. Serum CRP declined rapidly, with a mean value at day 9 of only 13 +/- 16% of baseline, and reached the normal range in 7 of the 9 patients. The ESR fell more slowly, with a mean value at day 29 of 58 +/- 18% of baseline, reaching the normal range in 4 of the 9 patients. These findings support our hypothesis that TNF-α is pivotal in the pathogenesis of RA, and provide a novel therapeutic approach in this disease.

EZ 406 DIFFERENTIAL REGULATION OF TUMOUR NECROSIS FACTOR RECEPTORS BY IMMUNOSUPPRESSIVE CYTOKINES

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the presence of autoreactive T cells and the proinflammatory cytokines, interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α). These inflammatory cytokines can be inhibited by immunosuppressive cytokines such as interleukin-4 (IL-4), interleukin-10 (IL-10) and transforming growth factor -β (TGF-β). As cytokines are known to act by binding to specific receptors we investigated the effect of these immunosuppressive cytokines on the expression of the TNF receptors (TNF-R) in RA which we have previously shown to be elevated. IL-4, IL-10 and TGFβ caused a dose dependent decrease in the TNF-R expression on the macrophage population in peripheral blood although an increase in the T cell TNF-R expression was also induced by IL-4. TGFβ and IL-10 caused a similar downregulation on RA mononuclear cells (MNC) although in this case IL-4 caused an overall increase in the TNF-R expression. These results suggest that although these immunosuppressive cytokines exhibit similar properties, the effect on TNF-R expression is both cell type and activation state specific. The mechanisms involved are currently being investigated and will be discussed with reference to the ability of these molecules to downregulate both cytokine and cytokine receptor expression as well as their ability to induce soluble receptors which are known to act as specific inhibitors.

EZ 407 EXPRESSION OF TNF-RECEPTORS, TNF-α AND TNF-ß IN RHEUMATIC DISEASES,

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Tumor Necrosis Factor (TNF) plays a central role in the maintenance of the inflammatory events in rheumatoid arthritis. We evaluated the expression of p75 and p55 TNF receptors (TNFR) and of TNF-α and TNF-ß on the surface of synovial fluid mononuclear cells in patients with rheumatoid arthritis (RA) (n=9), spondylarthropathy (SpA) (n=11), and traumatic effusions (n=3). Synovial T-lymphocytes from RA patients express in all cases the p75 TNFR on the cell-membrane, in 4/9 cases also a weak expression of the p55 TNFR is detectable, both mRNAs can be detected by polymerase chain reaction (PCR). Synovial macrophages also express the p75 TNFR and low amounts of the p55 TNFR. Patients with active RA also have circulating p75 TNFR positive T-lymphocytes in their blood. High concentrations of soluble TNFR (sTNFR) are found in the joint effusions of RA patients: up to 40 ng/ml of p75 sTNFR and up to 54 ng/ml p55 sTNFR. Significantly lower sTNFR levels are found in SpA effusions. Both receptors are also more elevated in the serum of RA patients (2.59 ± 0.28 ng/ml p75 sTNFR and 4.49 ± 0.55 ng/ml p55 sTNFR) as compared to SpA patients (1.41 ± 0.09 ng/ml p75 sTNFR and 1.78 ± 0.08 ng/ml p55 sTNFR, $p < 0.001$). TNF-α could be detected in the synovial fluid of RA patients (up to 140 pg/ml), but not in the serum. The soluble TNFR are biologically active and neutralize the effects of TNF-α in a cytotoxicity assay. The high levels of soluble TNFR in the inflammatory effusions may reflect TNF neutralizing activity in an environment where enhanced TNF synthesis has occured. We have generated several anti-sense-TNF-α oligonucleotides (as-TNF), in order to down-regulate TNF biosynthesis at the mRNA level. With as-TNF-3 we could achieve more than 90% inhibition of TNF secretion in PHA-stimulated peripheral blood or synovial fluid lymphocytes. The effects of as-TNF-3 on the expression of TNFR and on the synthesis of other cytokines are currently being investigated.

EZ 408 HIGH-LEVEL PRODUCTION AND CHARACTERIZATION OF FUNCTIONAL HUMAN INTERLEUKIN-1β CONVERTING ENZYME IN *BACULOVIRUS* AND *E.coli* EXPRESSION SYSTEMS.

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IL-1β converting enzyme (ICE) is a unique heterodimeric cysteinyl protease required for the activation of the potent inflammatory cytokine IL-1β in human monocytes. Purification, microsequencing, and cDNA cloning indicates that active ICE is composed of two nonidentical subunits of 20 (p20) and 10 (p10) kDa derived from an inactive primary translation product of 45 kDa. To better our understanding of the role of ICE in the production of active IL-1β, a thorough grasp of its structure and catalytic mechanism is required. Since ICE is only expressed naturally at low levels, we employed two heterologous systems to over produce catalytically-active ICE. In both *Baculovirus*-infected insect cells and *E.coli*, large quantities of active recombinant ICE (rICE) were generated by cleavage of the p45 proenzyme to p20 and p10 and purified to homogeneity utilizing affinity chromotography. Human rICE is identical to the native enzyme with regard to substrate specificity, kinetic constants for selected inhibitors, subunit structure, immunoreactivity and amino acid sequence. In addition, attempts to reconstitute ICE activity by combining the individually expressed p20 and p10 subunits or by co-expression were unsuccessful suggesting that folding of the proenzyme determines the active conformation of the enzyme.

EZ 409 INTERLEUKIN-6 INDUCTION IN HUMAN MACROPHAGE CELL LINE U937 BY ALEUTIAN MINK DISEASE PARVOVIRUS,

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Aleutian mink disease parvovirus (ADV) infects mink macrophages, and produces a disease characterized by hypergammaglobulinemia, plasmacytosis, and immune complex glomerulonephritis. After infection with virulent ADV-Utah I, serum gammaglobulin begin to increase around 3 weeks after infection; however, as early as 10 days after infection, marked lymphoadenopathy and plasma cell infiltration of liver, lung and kidney are observed. These findings suggest that the late phases of B cell differentiation are abnormally stimulated by ADV infection even early after infection. The cytokine Interleukin-6 (IL-6) induces the maturation of B cells into plasma cells, and is implicated in autoimmune states and plasma cell proliferation disorders, similar to Aleutian disease. We have characterized an in vitro infection system of the human macrophage cell line U937 with ADV-Utah I. ADV infection of U937 cells induced IL-6 mRNA and immunoreactive IL-6. This result suggested that ADV induced IL-6 expression in macrophages might play a role in triggering the immune disorder, characteristic of ADV infections.

EZ 410 PROINFLAMMATORY CYTOKINE PRODUCTION BY RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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A recently standardized semiquantitative RT-PCR method was used to evaluate the cytokine gene expression in rheumatoid arthritis synovial fibroblasts (RASF) and human skin fibroblasts (HSF). Cytokine message levels were evaluated during 4th or 5th passage of the cells. Cells were cultured for 48 hr with media alone or with IFN-γ (100 U/ml) and activated for 18 hr with either TNF-α or bacterial superantigens SEA, SEB or MAM. The levels of IL-6 and IL-8 mRNA were equal and high in both cell types without activation. Low levels of IL-1α, IL-1β, as well as IL-1R-antagonist transcripts were detectable in unactivated cells, but increased at least 16 fold after activation with IFN-γ and TNF-α. Message levels of IL-1RA also increased after culture with IFN-γ alone. The mRNA levels of IL-1α and IL-1β were higher in HSF than in RASF under all conditions tested. IL-1RA transcript levels were higher in HSF than in RASF before activation, but were comparable in activated cells. Other proinflammatory cytokines, TNF-α and IFN-γ, were not detectable in either cell type, unless they were activated with both IFN-γ and TNF-α, whereupon each cell type produced equal levels of these messages. The maximum difference in cytokine transcript was observed for TGF-β : activated RASF produced about 250 times less transcripts than corresponding HSF. IL-10 or IL-12 (40 kDa) mRNAs were undetectable under all conditions tested. The superantigens did not influence mRNA level of any cytokine in either cell type, although Class II MHC antigens (cell surface receptors for superantigens) were detectable on fibroblasts after culture with IFN-γ.

EZ 411 INTERLEUKIN 10 AND RHEUMATOID SYNOVITIS,

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IL 10 was first described as a mouse TH2 product, for its ability to inhibit the production of IFN g by TH 1 cells and that of proinflammatory cytokines by monocytes. Since the production of T cell derived cytokines such as IFN g is defective in rheumatoid synovium, the production of IL 10 in rheumatoid arthritis was investigated using a specific ELISA with a lower detection limit of 50-100 pg/ml. IL 10 could not be detected in the synovial fluid of rheumatoid arthritis patients. When pieces of rheumatoid synovium were used as an ex-vivo model of RA synovitis as described (A&R, 1992, 35: 874-883), no IL 10 could be detected in the culture supernatants, a result contrasting with the high levels of proinflammatory cytokines such as IL 1, TNF a and IL 6. When these synovium cultures were incubated with recombinant human IL 10, reduction of proinflammatory cytokine production was found. This effect was observed as early as 2 days and remained stable over a 10 day culture duration. This inhibitory effect could be reversed with a neutralizing anti-IL 10 monoclonal antibody.

These results extend the anti-inflammatory properties of IL 10 to an in vivo situation such as RA synovitis and suggest that the reduced production of IL 10 might play a role in the increased production of proinflammatory cytokines leading to joint degradation.

EZ 412 PATHOGENIC MECHANISMS IN LYME ARTHRITIS: STUDIES IN A MOUSE MODEL, Markus M. Simon, Manuel Modolell, Crisan Museteanu and Ulrich E. Schaible, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, Germany
Arthritis is one of the a major clinical features of Lyme disease, a tick-born infection caused by the spirochete Borrelia burgdorferi (B.b.). The mouse model of B.b. organisms infection has revealed that viable B.b. induce chronic arthritis, carditis, myositis and hepatitis in severe combined immunodeficient (SCID) mice but not or only to much lesser extent in various immunocompetent inbred strains of mice. Histopathological alterations in the joints are characterized by inflammatory infiltrations of articular and periarticular tissues and proliferation of synovial lining cells associated with erosion and destruction of cartilage and/or bone. In SCID and most notably also in normal mice, the majority of inflammatory cells in the affected joints were found to be $Mac\text{-}1^{+}$. T-lymphocytes are rarely seen and, if at all, are only found in joint lesions of infected normal mice. No B lymphocytes have been found in any of the tissue sections analysed. Infective spirochetes can be isolated from blood, joints and other organs of SCID mice but only occasionally from normal mice. In vitro studies have shown that viable B.b. organisms are able to induce inflammatory mediators such as oxygen-radicals, nitric oxide and TNF in naive bone-marrow derived macrophages (BMMØ). The generation of these molecules is regulated by IFNγ. BMMØ are also able to phagocytose and kill the spirochetes. The presence of viable spirochetes within synovial tissue and the preponderance of macrophages in inflammatory infiltrates together with their ability to produce bioreactive molecules in response to B.b. organisms in vitro therefore indicates that the joint pathology observed in SCID mice and probably also in normal mice is initiated by non-immunological processes. The fact that similar findings have been made in patients with Lyme disease suggests that the pathogenesis observed in mice may have direct relevance to the human situation.

EZ 413 EFFECT OF INFLAMMATORY CYTOKINES ON TNF RECEPTOR SHEDDING FROM RHEUMATOID SYNOVIAL FIBROBLASTS, David J. Taylor, Department of Medicine, Manchester University, U. K. M20 8LR.
Increased levels of TNF binding proteins (TNFBP) are found in the plasma of rheumatoid patients, and even higher levels are observed in the synovial fluids of affected joints. However, the cellular origin and the factors controlling TNFBP generation remains uncertain. We have recently shown that both TNF receptor types (p75 and p55 giving rise to type A and type B TNFBP, respectively) are expressed by rheumatoid synovial fibroblasts (RSF); the expression of p75 being markedly increased by certain inflammatory cytokines. Using the assay of Porteu et al., the effect of these cytokines, IL-1, IL-4 and IFN-γ has subsequently been examined on receptor shedding over 48h by RSF. Unstimulated RSF released more type B TNFBP than type A, reflecting the greter expression of p55 receptor on these cells. IL-1 and IL-4 both caused a 2-3fold increase in the amount of type A TNFBP within the media, whilst IFN-γ did not. Increased levels of type B TNFBP were only achieved with IL-4. The combination of IL-1 with IL-4 caused a synergistic increase in the release of type A TNFBP representing an 8fold increase compared to control; the type B level was no greater than with IL-4 alone. These studies suggest that RSF, in response to specific inflammatory cytokines, could make a substantial contribution to both types of TNFBP found within rheumatoid synovial fluid. Moreover, since more type B TNFBP was released into medium than type A, even in cells treated with cytokines such that much more p75 receptor was expressed on the cells than p55, it would appear that differences exist in the regulatory mechanisms for the shedding of each receptor type. Furthermore, the cytokine-stimulated shedding of the p75 receptor was shown not to be merely a function of cell surface expression.
Drs M. Brockhaus and M. Morris are thanked for antibodies and rheumatoid synovium, respectively. Supported by the ARC.

EZ 414 TRIPTERINE (RPR 102194), A POTENT IL-1 SYNTHESIS INHIBITOR FROM TRIPTERYGIUM WILFORDII HOOK F (T_2), Kin-Tak Yu, Navin Jariwala, Gail Owens, Aniello Pennetti, Wan Chan, Fu-Chih Huang, De-Ching Zhang, Michael Chang, Ronald Burch and Asher Zilberstein, Rhône-Poulenc Rorer Central Research, Collegeville, PA 19426
An extract from Tripterygium Wilfordii Hook f, T_2, has been widely used in China for the treatment of rheumatoid arthritis (RA). As IL-1 is postulated to be involved in the pathogenesis of RA, we have investigated the action of T_2 on cytokine and mediator syntheses by LPS-stimulated human monocytes. In a concentration dependent fashion, T_2 preferentially inhibited the syntheses of IL-1α and IL-1β, IC_{50} = 1 μg/ml. The potency of T_2 against the production of other cytokines (IL-6, IL-8 and TNF-α) and mediators (TxB_2 and PGE_2) is 4-8 fold lower than that against IL-1 synthesis. In the rat streptococcus cell wall (SCW) induced arthritis model in which IL-1 receptor antagonist is efficacious, T_2 dose dependently suppressed the recurrent joint swelling with oral ED_{50} = 20 mg/kg. As tripterine is a component in the T_2 extract, the action of the pure compound on IL-1 synthesis by human monocytes was investigated. Tripterine, in a concentration dependent manner, inhibited the LPS-stimulated IL-1α and IL-1β syntheses with IC_{50} about 30 nM. Selectivity studies indicated that tripterine was three or more fold less active at inhibiting synthesis of IL-8, TNF-α, PGE_2 and TxB_2. In vivo, oral administration of tripterine suppressed the recurrent joint swelling in SCW challenged rats with an ED_{50} = 6 mg/kg. Taken together, our results indicate that tripterine represents an active principal in the T_2 extract against IL-1 synthesis. Conceivably, the anti-inflammatory effects of tripterine and T_2 in the IL-1 mediated rat SCW arthritis model may be attributed to inhibition of IL-1 synthesis in the afflicted joints.

Abnormalities in Autoantibody Production

EZ 500 GLYCOSYLATION NETWORKS IN RHEUMATIC DISEASE John S Axford & Azita Alavi Academic Rheumatology Unit, Division of Immunology, St George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, UK.

Oligosaccharides are an integral component of the immune system, and glycosylation changes may occur both normally or in association with disease. We have previously reported that serum IgG galactosylation and lymphocytic galactosyltransferase (L.GTase) are reduced in rheumatoid arthritis (RA), and that a humoral regulatory component may be associated with these changes. To investigate the potential mechanisms controlling these glycosylation changes we have studied the interrelationship between:-

1- **L.GTase and agalactosylated IgG (Go).** This was found to be positive and linear ($p<0.05$) in the healthy individuals (n=18) and those with non-autoimmune arthritis (n=14), and negative and linear ($p<0.05$) in the RA population. The difference between these two correlations was found to be significant ($p<0.01$), suggesting a positive feedback homeostatic mechanism regulating Go level in the normal population, which is disrupted in the RA population.

2- **L.GTase and IgG anti-GTase antibodies (Abs).** In a longitudinal study of 13 patients with RA, we found a significant association between increased IgG anti-GTase Ab levels and reduced L.GTase activity (B cell $p<0.05$ and T cell $p<0.01$). These data suggest that IgG anti-GTase Abs are functionally related with the down regulation of GTase and may be part of an aberrant glycosylation network associated with RA disease pathogenesis.

These data indicate the presence of a network of regulatory elements involved in IgG galactosylation, which are of relevance to the pathogenesis of RA, and may be an integral component of a glycosylation mechanism controlling the carbohydrate content of other immunologically pertinent molecules.

EZ 501 SEQUENCE ANALYSIS OF VκIII GENE SEGMENTS EXPRESSED IN RHEUMATOID ARTHRITIS (RA) SYNOVIUM AND PERIPHERAL BLOOD LYMPHOCYTES (PBLs) S. Louis Bridges, Jr., Soo Kon Lee, John Lavelle, William J. Koopman and Harry W. Schroeder, Jr. University of Alabama at Birmingham, Birmingham, AL 35294-3300

In order to gain insight into the forces shaping B cell differentiation in the diseased synovium of a 62 year old patient with RA, we previously analyzed randomly isolated κ transcripts from an unrestricted synovial cDNA library. We found preferential utilization of three gene segments (Humkv325, Humkv328, and Vg) that belong to the VκIII family. These κ transcripts contained extensive N region addition and numerous somatic mutations. Two sets of clonally related Humkv325-derived sequences were detected. In order to compare the extent of somatic mutation and N region addition in this patient as compared to normal tissues, we used PCR to amplify VκIII trancripts from: 1) the synovial tissue of the original patient, 2) PBLs from this patient, 3) PBLs from a healthy 32 yo WM, and 4) splenic lymphocytes from a 49 yo WM without autoimmune disease. Sequence analysis revealed that 4 of 12 synovial, 7 of 13 RA PBL, 8 of 12 normal PBL, and 9 of 10 splenic transcripts were Humkv325-derived. All others were derived from Humkv328 or Vg. One of the Humkv325-derived synovial clones was a member of the clonally related set first identified by cDNA library analysis. On average, the extent of somatic mutation and N region addition was greater in synovium and PBL of our patient, than in spleen and normal PBL. The CDR 3 region contributes to the center of the antigen binding site. More than 70% of the CDR 3 regions from the controls contained 9 amino acids. In contrast, due to N region addition and exonucleolytic nucleotide loss at the VJ junction, only 25% of the patient's transcripts contained this preferred CDR 3 structure. The increased rate of somatic mutation, enhanced N region addition, and unusual CDR 3 length distribution of the VκIII gene segments expressed by this RA patient suggest that there is a fundamental difference in the nature of her immunoglobulin repertoire when compared to normal controls.

EZ 502 RAPID INDUCTION OF THE AUTOANTIGEN PCNA BY A NOVEL MYCOBACTERIAL γδ T CELL MITOGEN, Yan Chang, Hilary Haftel, Samir Hanash, and Joseph Holoshitz, Departments of Internal Medicine, and Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI 48105-0531.

The acetone precipitable fraction of *Mycobacterium tuberculosis* (AP-MT) has been previously shown to stimulate synovial fluid cells from rheumatoid arthritis joints, in particular the subset of synovial T cells bearing the Vγ9/Vδ2 receptors (Nature 339:226,1989). Presentation of AP-MT could be mediated by peripheral blood mononuclear cells (PBMC) without restriction by MHC. In order to elucidate the molecular events involved in presentation of this novel γδ T cell mitogen, we have analyzed total cellular protein expression by PBMC following incubation with AP-MT, using two dimensional polyacrylamide gel electrophoresis. An increase in a 36 kDa polypeptide was observed and represented a major change in silver stained gels after only 2-4 hours incubation with AP-MT. The observed polypeptide was identified as PCNA by Western blot analysis. Incubation of PBMCs with known T cell mitogens such as PHA or anti-CD3 monoclonal antibodies was associated with late upregulation of this protein, consistent with its described induction late in G1, associated with its role in DNA synthesis. The increased intensity of the spot was contributed by a newly synthesized protein as indicated by ^{35}S methionine labeling studies which were undertaken in quantitative analysis and revealed 30-fold increase in PCNA in PBMC following incubation with AP-MT. Northern blot analysis showed that unlike the late induction of PCNA by PHA, the enhanced early expression of PCNA by AP-MT was not accompanied by mRNA synthesis, indicating that the upregulation of the protein by AP-MT was mediated at the postranscriptional level.

Our results point to a distinct activation pathway involving PCNA that is induced by AP-MT. γδ T cells have been implicated in autoimmunity, and PCNA has been found to be an autoantigen in human autoimmune conditions. It is therefore possible that this novel activation mechanism may represent a pathogenically relevant cellular event.

EZ 503 ANALYSIS OF THE IMMUNOGLOBULIN HEAVY CHAIN REPERTOIRE IN RHEUMATOID ARTHRITIS (RA) SYNOVIUM. Björn E. Clausen, S. Louis Bridges, Jr., William J. Koopman, and Harry W. Schroeder, Jr. University of Alabama at Birmingham, Birmingham, AL 35294-3300

In order to gain insight into the forces shaping B cell proliferation in a 62 year old patient with RA, we have been analyzing the antibody repertoire expressed in her synovium. Through analysis of an unrestricted synovial cDNA library, we have documented oligoclonal expansion of a limited set of B cell clones expressing a Vκ Humkv325-derived immunoglobulin. We have now analyzed a total of 62 randomly isolated $C\gamma^+$ transcripts from the same synovial cDNA library. Of these, 50 contained identifiable VH gene segments. The VH1 family contributed 14 (28%) gene segments, VH2 1 (2%), VH3 28 (56%), VH4 6 (12%), VH6 1 (2%), and the VH5 and VH7 families were not represented. JH4 was used in 50% of the sequenced transcripts, followed by JH6 in 30%, JH3 in 10%, and JH5 in 7%. Due to 5' exonucleolytic activity, the JH could not be identified in 3% of the sequenced clones. Analysis of the CDR 3 regions showed marked variability in the extent of N region addition. Although the origin of the DH gene segment could not be identified in 31% of the clones, there was a clear preference for members of the DH DXP (43%) and DH DLR (14%) families. Among the transcripts utilizing DH DXP gene segments, three clones had similar amino acid sequence in the CDR 3 region. These sequences were enriched for charged and hydrophilic residues. However, none of these newly sequenced transcripts shared CDR 3 identity with previously isolated clones. Based upon the frequency of clonally related κ gene segments in our library, we have calculated that as many as 5% of the κ producing plasma cells in this synovium may express the same κ light chain. We estimate that at least 100 VH gene segments may need to be sequenced in order to find the potential heavy chain partner of this κ chain.

EZ 504 A SLE NEPHRITIS-SPECIFIC MONOCLONAL ANTI-DNA ANTIBODY IDENTIFIES TWO CELL SURFACE GLYCOPROTEINS SYNTHESIZED BY HUMAN AND MOUSE PROXIMAL TUBULE KIDNEY CELLS,

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Anti-ds DNA antibodies are implicated in the pathogenesis of lupus nephritis, but it is uncertain whether anti-ds DNA antibodies are pathogenic as a result of binding DNA. A monoclonal anti-ds DNA antibody (mAb 3E10) was produced from MRL/lpr mice with lupus nephritis that had an idiotype conserved in murine and human lupus associated with nephritis. Previous studies performed have shown that inhibition of expression of the DNA idiotype suppressed nephritis and prolonged survival of the MRL/lpr mice, suggesting that mAb 3E10 may be pathogenic in lupus nephritis. In addition to binding DNA, mAb 3E10 specifically binds membranes of the tubular cells in human kidney by immunohistochemistry analysis. MAb 3E10 bound cell lines derived from mouse proximal kidney tubules and human embryonal kidney cells. Therefore, these cells were used to further characterize the specific antigen targeted by mAb 3E10 reactivity. Radiolabelled cell cultures (^{14}C-glucosamine or ^{35}S-Met/^{35}S-Cys) were immunoprecipitated with mAb 3E10. Two proteins of 200 kD and 43 kD were identified using SDS polyacrylamide gel electrophoresis and autoradiography. These molecules were not detected in other control cell lysates or in the culture media. These findings along with the preliminary immunofluorescence data suggests that one or both of the antigens are glycoproteins bound to the cell surface and are targets for mAb immunoreactivity. Affinity purification of the antigens for N-terminal sequence analysis is underway.

EZ 505 THE CHARGE SPECTRUM OF NORMAL HUMAN IgG: THE EFFECT OF ISOLATION TECHNIQUES.

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IgG is diverse in charge and cationic charge is an important determinant favoring deposition of antibodies and immune complexes in glomeruli. The antigens of autoimmune diseases are noted for their negatively charged regions which are capable of stimulating the formation of positively charged antibodies. Most purification techniques for IgG utilize charge to separate it from other serum or plasma proteins. Cohn Fraction II's from multiple sources, both research and therapeutic, were used in these studies. IgG was obtained from normal sera or plasma by saturated ammonium sulfate (SAS) or caprylic acid precipitation, and DEAE ion exchange chromatography. These were analyzed by isoelectric focusing (IEF) utilizing both protein and immunological stain for determining IgG net charge distribution. Cation exchange chromatography at pH 9 on carboxymethyl Sepharose (CM) in 0.01 M sodium borate buffer with a 1.5 M NaCl step elution was used to characterize the proportion of antibodies bearing sufficient regional cationic charge to bind under these stringent conditions. CM binding goat antibodies contain the population mediating the early deposition of immune complexes in mouse glomeruli. The population of normal human IgG containing cationic antibodies obtained by each isolation procedure was determined. SAS and caprylic acid fractionation (CM binding of 10.5 ± 0.55%) gave the most representative charge spectrum of IgG when compared to the IgG in whole serum by IEF (pI 4.5 to >9.3). Cohn fractionation (pI range 6 to>10 with 25% CM binding) and ion exchange chromatography with DEAE lacked a large neutral and anionic population of IgG. These preparations are maldistributed in relation to charge and should be used with caution in studies attempting to investigate activities involving electrostatic factors. Anticoagulation with lithium heparin, EDTA, or sodium citrate from the same normal gave similar patterns by both IEF and CM column analysis. The role of antibody charge on the clinical course of human autoimmune disease is unknown. Collection, isolation and storage techniques must be examined carefully in studies involving antibody charge or characteristics associated with antibody charge to avoid prejudicing the outcomes of studies.

EZ 506 CORRELATION OF SEROLOGICAL ABNORMALITIES WITH DISEASE EXPRESSION IN INTERSPECIFIC BACKCROSS lpr MICE.

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MRL-lpr mice develop arthritis and nephritis in association with autoantibody production. To define the genetics of these inflammatory manifestations and their relationship to serological abnormalities, we analyzed 182 interspecific backcross (MRL-lpr x *Mus castaneus*) x MRL-lpr mice , 27 parental mice and 17 F$_1$ mice. All mice were assessed pathologically for lymphadenopathy, nephritis, and arthritis and serologically for anti-DNA, total IgG3 and RF levels by ELISA. Of 182 backcross mice, 137 had moderate lymphadenopathy including 48 with profound lymphadenopathy, 73 had demonstrable nephritis and 34 had arthritis. None of the F$_1$ mice had demonstrable disease. In the backcross mice, renal disease was significantly correlated with lymphadenopathy by Chi square analysis; arthritis, in contrast, lacked this association. The levels of anti-dsDNA antibodies and renal disease correlated by rank sum analysis (ELISA OD for mice with renal disease-0.736±.576; without renal disease-0.248±.360). This was true even when considering only lpr/lpr genotype mice. In contrast, total IgG3 levels were similar in MRL-lpr, F$_1$, and backcross mice with or without renal disease, while levels of IgM or IgG RF showed no correlation with arthritis in backcross mice (IgM RF in mice with arthritis 0.289±.158; without arthritis 0.255±.117). These results indicate that, of serologic abnormalities of MRL-lpr mice tested, anti-dsDNA antibodies correlated with nephritis, while RF and IgG3 levels were not associated with any disease manifestations.

EZ 507 THE INFLUENCE OF THE IMMUNOGLOBULIN HEAVY CHAIN LOCUS ON AUTOANTIBODY PRODUCTION IN AUTOIMMUNE MICE,

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Mice that bear the *lpr* gene spontaneously produce a number of autoantibodies (autoAbs). We have investigated the influence of the Ig heavy chain (Igh) genetic locus on the production of autoAbs in *lpr* mice using Igh allotype markers. Serum IgG2a anti-chromatin Abs from MRL/Mp-*lpr/lpr* x C57BL/6-*lpr/lpr* (MB/*lpr*), [Igh$^{j/b}$]; C57BL/6-*lpr/lpr* x C57BL/6-*lpr/lpr*-Igh^{a} (B^{b}B^{a}/*lpr*), [Igh$^{b/a}$]; and MRL/Mp-*lpr/lpr* x MRL/Mp-*lpr/lpr*-Igh^{b} (M^{j}M^{b}/*lpr*), [Igh$^{j/b}$] were quantitated by allotype specific ELISAs. Allotype skewing was determined by dividing the μg/ml of *b* allotype by the μg/ml of *a* (or *j*) plus *b* allotype for each mouse. A ratio of .50 thus indicates equal use of the two allotypes, and *p* values refer to the significant difference of the calculated mean ratio from .50. Strikingly, anti-chromatin Abs were significantly skewed towards the *b* allotype in all cohorts of F$_1$ mice examined. The mean(SD) *b/(a+b)* or *b/(j+b)* for MB/*lpr* was .65(.23), n=46, $p \leq .01$; B^{b}B^{a}/*lpr*: .62(.40), n=68, $p \leq$ 05; M^{j}M^{b}/*lpr*: .59(.23), n=35, $p \leq .05$. IgG2a anti-histone Abs in MB/*lpr* mice (.60(.24), n=40, $p \leq .01$) and anti-Sm Abs in M^{j}M^{b}/*lpr* mice (.87(.14), n=8, $p \leq .001$) were also skewed toward the *b* allotype. However, the ratios calculated for IgG2a Abs directed against TNP (MB/*lpr*), DNA (MB/*lpr*, B^{b}B^{a}/*lpr*), or total IgG2a (all F$_1$s) were not significantly different than .50. Examination of the MRL/*lpr*-Igh^{b} strain showed 37/47 (79%) produced anti-Sm Abs at five months of age compared with 23/84 (27%) for the conventional MRL/*lpr* strain that is Igh^{j}. Of those mice that produced anti-Sm, the MRL/*lpr*-Igh^{b} strain had higher levels by ELISA (mean(SD) mg/ml 1.76(1.72), n=33) than the MRL/*lpr* strain (.76(.67), n=21). In addition, anti-Sm positivity segregated with the *b* allotype in MB/*lpr* mice backcrossed with MRL/*lpr*. Finally, an allotype recombinant locus was utilized to examine the genetic control of *b* allotype skewing of anti-chromatin. In two separate models, the V$_H$ region did not appear responsible for the preferential use of *b* allotype. These results indicate a contribution to autoimmunity by the Igh locus and raise the possibility that Ig allotype influences autoimmune disease by its effect on the production of certain autoAbs.

EZ 508 ANTI-MYELOPEROXIDASE ANTIBODIES AND NEUTROPHILIC VASCULITIS IN MRL-lpr MICE.

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The pathogenic role of circulating antibodies to a variety of neutrophil enzymes present in patients with systemic vasculitis is uncertain. We have identified anti-myeloperoxidase (MPO) antibodies in the sera of MRL-lpr mice who develop neutrophilic vasculitis and neutrophil tissue infiltrates. Non-depleting anti-CD4+ antibody YTS 177.1 inhibits the production of anti-MPO antibodies to undetectable levels without affecting the titre of anti-DNA antibodies.
A cross reactive anti-DNA monoclonal antibody has been raised from MRL-lpr mice which binds by Western blot to the 70kD heavy chain of human MPO, the site recognised by anti-MPO antibodies from patients with vasculitis.
The effect of this monoclonal antibody on TNFa-primed neutrophils will be examined *in vitro* and *in vivo*.

EZ 509 IMMUNISATION OF DBA/1 MICE WITH COLLAGEN TYPE II INDUCES SIMILAR CHANGES IN IgG GLYCOSYLATION TO THOSE FOUND IN PATIENTS WITH RHEUMATOID ARTHRITIS.

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Sequential analyses of the oligosaccharides on the Fc region of IgG have revealed that rheumatoid arthritis patients frequently lack terminal galactose and thus express lectin detectable N-acetyl glucosamine. The collagen- induced arthritis in DBA/1 mice shares many features with rheumatoid arthritis. We have investigated whether glycosylation differences are found on the IgG molecules from the collagen induced arthritic mice. We have found that the IgG from arthritic DBA/1 mice has lower amounts of lectin reactive galactose ($p=0.027$) and higher amounts of lectin reactive N-acetylglucosamine ($p=0.0001$) compared with that from non-arthritic mice. Interestingly the levels of galactosyl transferase, the enzyme responsible for the galactosylation of IgG molecules is significantly reduced in the peripheral cells of the arthritic mice when compared with control mice ($p= 0.0075$). The reduced galactosyl transferase enzyme found in peripheral cells of arthritic mice is not however found in the splenic cells from arthritic mice. Therefore there seems to be a regulation of glycosylation which occurs at specific local sites in the collagen induced arthritis. Rheumatoid arthritis patients are also known to have reduced levels of galactosyl transferase enzyme in their peripheral cells.The similarities in glycosylation changes in the collagen induced arthritis and in patients with rheumatoid arthritis suggests that common pathogenic mechanisms may be involved.

EZ 510 EFFECTS OF AUTOANTIBODIES TO RIBOSOMAL P-PROTEINS ON HUMAN HEPATOCYTES IN CULTURE

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The sera of patients with systemic lupus erythematosus contain autoantibodies to ribosomal P-protein in 12-16% of cases. These highly conserved proteins comprise P_0, P_1, and P_2 molecules corresponding to sizes of 38,19 and 17 kD, respectively. P-proteins are acidic phosphoproteins primarily associated with 60S ribosomal subunit in eukaryotic cells although their presence in ribosome free cytoplasm also has been reported. P-proteins play a role in protein synthesis serving as an anchor for the attachment of elongation factors to ribosomes. We have recently demonstrated on the surface of human hepatoma (HepG-2) cells, the presence of an epitope that is antigenically related to the immunodominant carboxy terminus of P-proteins. Detailed immunofluorescence microscopy studies revealed that anti-P autoantibodies bind to the surface and enter into the live HepG-2 cells under physiological conditions. These observations were confirmed by the use of quantitative, digital imaging fluorescence microscopy and confocal microscopy. Internalized anti-P autoantibodies appear to cause significant decrease in synthesis and secretion of apolipoprotein B within 4-6 hours as revealed by ELISA analyses of cell homogenates and cell supernatants. Longer term effects (18-24 hrs) include an increase in cellular lipid content, based on gas liquid chromatographic analyses and on microscopic analyses of intracellular lipid droplets. Anti-P autoantibodies appear to have direct cytotoxic effect on significant population of cell as well. The clinical significance of our findings lies in possible participation of anti-P autoantibodies in the expression of liver disease in SLE patients.

EZ 511 TOLERANCE TO NATIVE DNA CAN BE BROKEN BY IMMUNIZATION WITH DNA-PEPTIDE COMPLEXES

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Mice genetically predisposed to the autoimmune disease systemic lupus erythematosus develop autoimmunity to DNA including the production of autoantibodies specific for native, mammalian DNA (nDNA). Recent results from our laboratory as well as others have indicated that both early, IgM and the later appearing, IgG anti-DNA autoantibodies in autoimmune mice have the structural characteristics of secondary immune, selectively stimulated antibodies. Based upon the selection for particular antibody variable-region structures as the autoantibody response progresses from IgM to IgG, the most likely stimulus for this autoimmune response is native DNA or complexes containing nDNA. These findings are somewhat at odds with previous results demonstrating that mammalian nDNA either alone or in complexes with an immunogenic protein is generally not immunogenic. Likewise, mice transgenic for rearranged immunoglobulin genes that encode an anti-DNA antibody are tolerant to nDNA. In the present experiments mice were immunized with complexes of DNA and an immunogenic, DNA-binding peptide, Fus1. Mice immunized with such complexes produce IgG anti-ssDNA antibody after the second immunization and anti-nDNA antibody after the third or fourth immunization. The specificity and variable region structures of DNA-Fus1 induced monoclonal anti-DNA antibodies are similar if not identical to those of autoimmune monoclonal anti-DNA antibodies. Moreover, the immunopathological function of the induced anti-DNA antibodies is similar to those in autoimmune lupus. The difference in time interval between the appearance of IgG antibody specific for ssDNA versus nDNA may be a reflection of the time necessary for the generation of B cells specific for nDNA. This could occur either by somatic mutation of the immunoglobulin variable regions of B cells initially specific for ssDNA or the low frequency escape from tolerance of B cells initially specific for nDNA. These results suggest that tolerance to nDNA can be overcome by immunization with nDNA in an appropriately immunogenic form. Supported by NIH AI 26833, BRSG-RR05423, and AI07238.

EZ 512 CATION EXCHANGE RESIN BINDING CHARACTERISTICS OF AMMONIUM SULFATE PRECIPITATED IgG FROM NORMALS, ANA NEGATIVE PATIENTS, AND SLE PATIENTS. Angela Paez and V. Joyce Gauthier, Department of Medicine, University of Washington, Seattle, WA 98195

Electrostatic charge has been postulated to represent an important pathogenic factor in autoantibodies and autoantigens. The positive charge of antibodies to DNA in murine models of lupus and in lupus patients is under intense investigation. Cationic charge is an important determinant of glomerular localization of immune complexes in animal models of lupus nephritis. To determine if highly cationic antibodies exist in humans, saturated ammonium sulfate (SAS) precipitated fractions were obtained from 9 normal persons, 24 ANA negative patient sera, and 13 lupus patients in various stages of activity. Carboxymethyl-Sepharose (CM) cation exchange chromatography (pH 9 in 0.01 M borate with and without 6M urea followed by elution with 1.5 M NaCl) detects a subpopulation of IgG with sufficient cationic regions to bind to the gel. Similarly cationic goat antibodies are capable of initiating immune deposit formation in glomeruli in experimental models. In all sera except one, CM binding protein was seen to represent 12.4 ± 5.3% of the SAS precipitated protein . Analysis of samples from CM binding peaks revealed this CM binding material was IgG by SDS-PAGE, Western blot, and HPLC analysis. Under dissociating conditions some samples showed an increase in the cationic antibody peak. Remarkably, one SLE patient had a population of CM binding IgG representing 31 % of the SAS precipitable material, a value over 3.5 standard deviations above the mean of the whole population. The CM binding material persisted under dissociating conditions and was monomeric IgG by HPLC and Western blot. Further studies to investigate the distribution of antibody activity, subclass, and clonal heterogeneity of these antibodies relative to those not capable of binding CM gel may reveal important information into the origin and nephritotrophic characteristics of autoantibodies.

EZ 513 B1 (CD5+ B) CELLS DO NOT PRODUCE AUTOANTIBODIES IN CHRONIC GRAFT-VERSUS-HOST DISEASE, Elizabeth A. Reap, Eric S. Sobel, Philip L. Cohen, and Robert A. Eisenberg, Department of Medicine, University of North Carolina, Chapel Hill, NC 27599-7280.

Chronic graft-versus-host (GVH) disease induces in normal mice the production of SLE-like autoantibodies. Since B1(CD5+ B) cells are found in increased numbers in some autoimmune strains of mice and have previously been implicated as the important autoantibody B-cell subset, we examined the contribution of B1 cells to autoantibody production in GVH disease using C57BL/6Kh (B6: H-2^b, Igh^b), Igh allotype congenic B6.C20 (H-2^b, Igh^a) and Ia congenic B6.C-H-2^{bm12} (bm12: H-2^{bm12}, Igh^b) and B6.C-H-2^{bm12}-Igh^a (bm12-Igh^a: H-2^{bm12}, Igh^a) mouse strains. Two approaches were used to establish B cell chimeras. In one, we reconstituted sublethally irradiated mice with B1 cells of one allotype and bone marrow cells of the other. In the second method, we suppressed endogenous B cells in neonatal mice with allotype specific anti-IgM antibody and injected peritoneal cells of another allotype. After treatment was stopped, mice had B1 cells of the donor allotype and conventional B cells of the host allotype. An SLE-like syndrome was then induced in both chimeric models by transfer of bm12 or bm12-Igh^a allogeneic T cells. These T cells were of the same allotype as the recipient B1 cells in order to prevent allotype suppression and, thus, to maximize the chance of autoantibody production by the B1 cell subset. The B cell subpopulation that produced the autoantibodies could be identified by allotype-specific ELISAs. In one set of radiation chimeras, B6.C20 mice reconstituted with B6.C20 bone marrow cells and B6 peritoneal cells, had total serum IgM levels of both *a* and *b* allotypes present in substantial quantities. Flow cytometric analysis on peritoneal cells showed B1 cells that were all *b* allotype. After GVH induction, autoantibodies (IgG2a anti-chromatin and anti-ssDNA, and IgM RF directed against $IgG2b^b$) were derived entirely from the conventional (*a*) allotype source. B6 radiation chimeras reconstituted with B6 bone marrow and B6.C20 peritoneal cells showed parallel results with only *b* allotype autoantibodies, with one exception. (One mouse produced IgG2a ssDNA autoantibodies of the *a* allotype.) Similarly, *b* allotype conventional B cells made all the GVH-induced autoantibodies in the neonatal chimeras. These results indicate that conventional B cells and not B1 cells produce the autoantibodies characteristic of chronic GVH. This conclusion matches that of our previous experiments in the spontaneous autoimmunity of _lpr_ mice.

EZ 514 THE ROLE OF Ig LIGHT CHAINS IN BINDING Sm AND DNA IN Ig HEAVY/LIGHT CHAIN TRANSFECTOMAS. Marc W. Retter, Robert A. Eisenberg, Philip L. Cohen and Stephen H. Clarke, Curriculum in Genetics and Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Anti-Sm antibodies are diagnostic of systemic lupus erythematosus (SLE), yet these antibodies occur in only 25% of patients and autoimmune MRL/lpr mice at 5 months of age. Previous work has shown that a majority of MRL/lpr anti-Sm hybridomas bind single stranded DNA (ssDNA), and in some cases, also bind double stranded DNA (dsDNA). We have demonstrated that three anti-Sm/ssDNA hybridomas express germline (2-12) or mutant forms (1-8, 3-7) of the same V_H gene paired with three different V_K genes. A fourth hybridoma (34-8-1) selected for DNA binding expresses the same V_H gene and a different V_K gene, but does not bind Sm. It has recently been shown that the heavy chain is dominant in determining DNA binding specificity of anti-DNA selected B cells, and that the light chain plays a minor role. Thus, to understand the basis for the dual binding of Sm-selected hybridomas, we have combined the germline 2-12 V_H gene with a variety of V_K light chains by transfection. The 2-12 V_H rearrangement was cloned into a μ expression vector and transfected into light chain only cell lines or co-transfected with light chain genes into myeloma cell lines. Anti-Sm ELISAs showed that the transfectoma antibodies bound Sm with 5-5000 fold less avidity than the 2-12 V_H/V_K transfectoma control combination, whereas anti-ssDNA ELISAs showed that the transfectoma antibodies bound DNA at least as well as the 2-12 V_H/V_K combination, and often better. One germline V_H/V_K combination had a 200 fold increase in avidity for ssDNA over the 2-12 V_H/V_K transfectoma antibody, and had a 5000 fold decrease in avidity for Sm. Thus, this antigen binding data confirms that the heavy chain plays the dominant but not unique role in determining the specificity for DNA, and indicates that the light chain is critical for determining the binding to Sm.

EZ 515 CIRCUMSTANTIAL EVIDENCE THAT AGALACTOSYL IgG HAS A PATHOGENETIC ROLE IN AUTOANTIBODY-MEDIATED DISEASE. Graham A.W. Rook, Clarissa Pilkington, Pamela Taylor,[1] and Ann Kari Lefvert.[2] Department of Medical Microbiology, University College & Middlesex School of Medicine, London W1P 7PP, U.K.; Dept. Obstetrics & Gynaecology, University of Leeds, U.K.[1]; Department of Medicine, Karolinska Institute, Stockholm, Sweden.[2]

Agalactosyl IgG (Gal(0)) lacks terminal galactose from the oligosaccharide situated on the conserved glycosylation site in the CH2 domain of the γ chain. The %Gal(0) rises in rheumatoid arthritis (RA) and other conditions, but it falls during pregnancy both in normal and in diseased individuals, and Gal(0) tends not to be transported across the placenta, so babies of normal mothers have %Gal(0) even lower than that of their mothers at birth. In pregnant RA patients, a fall to normal levels is associated with remission, which could imply a pathogenetic role for Gal(0).

Neither the incidence of Neonatal Lupus, manifested as Congenital Heart Block (CHB) in babies of mothers with SLE, nor the incidence of *Myasthenia gravis* in babies born to Myasthenic mothers, correlates with maternal or neonatal levels of the relevant autoantibodies. Therefore we hypothesised that neonatal disease might correlate with the galactosylation status of the autoantibody passed from the mother, rather than with its titre. We assayed %Gal(0) IgG in paired maternal and cord blood samples and found that CHB occurs in babies born to SLE mothers with high %Gal(0) at parturition ($p=0.014$, compared to %Gal(0) of SLE mothers with healthy babies), and such babies have high neonatal %Gal(0) values ($p<0.01$, compared to healthy babies of SLE mothers), though lower than their mothers' values. In contrast, myasthenic neonates have %Gal(0) even higher than that of their myasthenic mothers ($p<0.0004$), and higher than that of non-myasthenic babies born to myasthenic mothers ($p<0.001$). The latter healthy neonates have %Gal(0) lower than their mothers ($p=0.017$). These findings are compatible with, but do not prove, an effector or B cell regulatory role for Gal(0) IgG.

EZ 516 THE NUCLEAR AUTOANTIGEN RA33 IS A COMPONENT OF THE HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN, Günter Steiner, Klaus Hartmuth, Karl Skriner, Eva Thalmann, Ingrid Maurer-Fogy, Wolfgang Hassfeld, Andrea Barta, and Josef Smolen, 2nd Dept. of Medicine, Lainz-Hospital, and Institute of Biochemistry, University of Vienna, Vienna, Austria.

RA33 is a newly described nuclear autoantigen with an apparent molecular weight of 33 kD. Autoantibodies against RA33 have been reported to occur characteristically in about 35% of sera from patients with RA (Arthritis Rheum 32:1515, 1989). In order to elucidate the primary structure of this antigen, RA33 was highly purified from nuclear extracts and partially sequenced. Sequence analysis of five tryptic peptides revealed that RA33 was apparently identical with the A2 protein of the heterogeneous nuclear ribonucleoprotein (hnRNP) particle. The N-terminal portion of this protein contains two conserved RNA binding domains which can be found in many other RNA binding proteins such as Ro/SSA, La/SSB, or the 70 K protein of U1-snRNP. The glycine rich C-terminus shares sequence homologies with several glycine-rich proteins, among those keratin and EBNA1. However, no crossreactivities between anti-RA33 and other autoantigens could be detected. In contrast, two other components of the hnRNP particle were highly crossreactive with anti-RA33. Their migration on two-dimensional immunoblots indicated that they were identical with the hnRNP proteins B1 and B2. When semi-purified antigen was used in immunoblotting studies reactivities against RA33/A2, B1, B2 were not only detected in one third of RA sera, but also in 40% of MCTD sera and in 17% of SLE sera. In most of the MCTD and SLE sera the occurrence of anti-RA33 was strongly associated with antibodies to U1-snRNP. This is particularly interesting since both hnRNP and snRNP particles are involved in processing (splicing) of pre-mRNA. In summary, these data strongly suggest that the hnRNP proteins A2, B1, B2 form a "cluster" of autoantigens which may turn out to be as valuable for the diagnosis of connective tissue disorders as other well-defined autoantigenic structures.

EZ 517 DNA ISOLATED FROM DNA/ANTI-DNA ANTIBODY IMMUNE COMPLEXES IN SYSTEMIC LUPUS ERYTHEMATOSIS ORIGINATES FROM THE PATIENT OWN LEUCOCYTES Zhu JiLing, Xu DeQing & Chen WeiXiong, Department of Dermatology ,Sun Yat-Sen Memorial Hospital,Sun Yat-Sen University of Medical Sciences, GuangZhou 510120, The People's Republic of China

DNA isolated in sera or plasma of patients suffering from SLE has been investigated by a lot of researchers . The size of these DNA was reported by different authors ranges between 20bp and 17.5kbp. Their possible origination however is still discussed.

We isolated the macromolecular DNA from plasma cryoprecipitates enriched with circulating immune complexes of 58 active and 12 inactive patients clinically. By 0.8% agarose gel electrophoresis and Ethidium Bromide staining. The DNA m.w. was close to 20kbp. The mean content of DNA ranged from 0.05 to 0.2 μg/ml in plasma. Hybridization experiments by Enchanced Chemiluminescence Assay indicated the DNA from CIC originated from the human leucocyte . DNA of all patients showed identical electrophoresis patterns but a little differences among DNA contents. The DNA marked as a probe from a patient could hybridized with the DNA of all cases and also the DNA from the health subjects. However no any hybridizations were observed with the leucocyte DNA from a rabbit, the HBV DNA and EBV DNA. Furthermore the probe from the leucocyte DNA of the health subject also could hybridized with the DNA of all patients. Our results showed that it is much likely that the DNA isolated from DNA/anti-DNA antibody immune complexes originates from the leucocyte of patient own.

Interaction Between Matrix Components, Cytokines and Cells; New Therapeutic Directions

EZ 600 EXPRESSION AND PURIFICATION OF A SINGLE-CHAIN POLYPEPTIDE CONTAINING THE α1 AND ß1 DOMAINS OF HLA-DRB1*0101, Joseph A. Affholter, Jessica Gauthier, H. Lynn Kan, Kathy M. Jackson and Shannon L. Gleason The Dow Chemical Company, Central Research-Bioproducts Lab, Midland, MI, 48674

The association of rheumatoid arthritis and other human and rodent autoimmune diseases with discrete subsets of class I or class II MHC molecules has been widely documented. Although the functional significance of these associations remains uncertain, it seems likely that they relate to the capacity of the disease-associated MHC markers to bind and present one or more autoantigens. This concept has been widely discussed in the literature and is backed by substantial data in animal model systems. Indeed, recent work has shown that administration of purified class II MHC-autoantigen complexes can suppress the induction of experimental autoimmune encephalomyelitis (EAE) in mice. Application of similar therapeutic approaches to humans would require the capacity to produce large quantities of purified, soluble HLA molecules. In this report, we describe the bacterial expression, affinity purification and initial biochemical characterization of a single-chain protein comprised of the α1 and ß1 domains of HLA-DR allele B1*0101. The molecule (scDRß/α) described in this report is generated by amplification of the α1 (residues 1-87) and ß1 (residues 1-107) domains from cDNA clones of known sequence. The amplified domains are then ligated into an E. coli expression vector. This vector allows synthesis of a protein containing the α1 and ß1 domains separated by a 25 amino acid linker sequence, the pectate lyase B (pelB) signal peptide and a carboxy terminal 8 amino acid FLAG® peptide (International Biotechnologies, New Haven, CT). The FLAG peptide allows for efficient purification of the expressed protein via an M2 (anti-FLAG; International Biotechnologies, Inc.) affinity column. Using this approach, the scDRß/α protein has been purified. The purified preparation contains a single major band at ~25 kD on reducing SDS-PAGE which constitutes >80% of the total protein in the purified preparation. In addition, the purified preparation contains a single M2-reactive band on immunoblots from SDS gels. Initial biochemical studies describing the binding of anti-HLA-DR antibodies and binding of a peptide derived from influenza hemagglutinin to purified scDRß/α will also be presented.

EZ 601 HISTOLOGIC EVALUATION AND IN VIVO STABILITY OF A POTENTIAL NEW AGENT FOR RADIATION SYNOVECTOMY: HOLMIUM-166 HYDROXYLAPATITE, James W. Brodack, Lori K. Chinen, Edward Deutsch, Karen F. Deutsch, Mallinckrodt Medical, Inc., St. Louis, MO 63134, and Sonya Shortkroff, A. Mahmood, Alun G. Jones, Clement B. Sledge, Harvard Medical School, Boston, MA 02115

Radiation synovectomy is a non-surgical procedure that utilizes beta-emitting radionuclides to functionally ablate the inflamed synovium of the rheumatoid joint. Previous clinical studies have indicated a therapeutic success rate equivalent to open surgical or arthroscopic synovectomy, but the radiopharmaceuticals currently in use have significant disadvantages: extra-articular leakage (Y-90) and limited availability (Dy-165). A new agent, holmium-166-labelled hydroxylapatite particles, has potential in the treatment of rheumatoid arthritis due to its short half-life (27 hours), a beta particle energy of 1.84 MeV, a maximum tissue penetration of 8.7 mm, and a biodegradable carrier. We investigated the in vivo stability of this radiopharmaceutical and the effect of the carrier on the synovium and cartilage. The right knees of 20 New Zealand white male rabbits were injected with 2 mgs of non-radioactive holmium-coated hydroxylapatite particles. Two particle size ranges were tested: 4-20 μm (average = 8 μm) and 4-38 μm (average = 14 μm). At one week, 2 weeks, 1 month or 2 months, the knee joints were dissected and the entire joint and a specimen from the infra-patellar fat pad were processed for histologic analysis. Gross inspection of the injected joint demonstrated a slight inflammatory response as compared to the opposite side. On histologic examination of the synovial tissue, incorporation of the particles into the lining, and recruitment of histiocytes to the lining were observed at 24 hours and at one week. The particles were slowly degraded with few remaining at one and two months. Leakage studies with the radioactive compound were performed in normal and antigen-induced arthritic rabbits. Gamma camera imaging and biodistribution analysis demonstrated minimal leakage from the joint (less than 0.5%). From these studies, holmium-166 hydroxylapatite appears to be a safe radiopharmaceutical with significant potential as a radiation synovectomy agent.

EZ 602 AN INVESTIGATION OF THE MECHANISM OF DEPLETION OF CD4⁺ CELLS BY A MOUSE/HUMAN CHIMERIC CD4 mAb. M.R. Dalesandro, C.S. Kinney, M.P. Happ, K.A. Siebert, J.I. Krieger, R.C. Fletcher, D. Healey*, P.E. Daddona, and M.E. Sanders, Department of Immunobiology, Centocor, Inc., Malvern, PA, 19355, *Department of Pathology, Cambridge University, Cambridge CB2 1QP, UK.

We have shown in binding experiments with both chimeric CD4 transfectants and anti-idiotypic antibodies that M-T412, a murine IgG2a CD4 mAb, defines a unique epitope in the V2 domain of CD4. Previous work in our laboratory revealed that a mouse/human chimeric (cM-T412 G1) version of M-T412 inhibited peripheral blood lymphocyte (PBL) proliferation in response to mitogens and antigens more effectively than did the murine parental mAb. The cM-T412 G1 mAb has shown promising results in rheumatoid arthritis and multiple sclerosis trials. Significant depletion of $CD4^+$ cells was observed, however, in patients treated with the mAb. Current efforts to determine the mechanism of depletion indicate that cM-T412 G1 efficiently mediates antibody dependent cellular cytotoxicity (ADCC) of human $CD4^+$ cells by PBL effectors. cM-T413 G1, a chimeric mAb binding an epitope in the V1 domain of CD4, produces 80% of the lysis observed with cM-T412 G1 while the murine M-T412 averages 25% of the ADCC seen with cM-T412 G1. In some assays, $CD4^+$ target cells were stimulated through the CD3/TCR complex and proved to be more susceptible to cM-T412 G1-mediated ADCC than were resting cells. Furthermore, antigen-specific $CD4^+$ clones were killed by a 100-fold lower concentration of cM-T412 G1 than was required for lysis of resting $CD4^+$ cells. The possible role of apoptosis in the lysis of activated $CD4^+$ PBL and clones is under investigation. In contrast with the ADCC results, the cM-T412 G1 mAb produced 20% of the complement dependent cytolysis (CDC) of $CD4^+$ cells observed in the presence of either the murine M-T412 or the cM-T413 G1. These *in vitro* results indicate that ADCC may be more important than CDC in the *in vivo* depletion of $CD4^+$ cells by cM-T412 G1.

EZ 603 INTERLEUKIN-6 INDUCED SYNTHESIS AND SECRETION OF α1-ANTITRYPSIN IN HUMAN ARTICULAR CHONDROCYTES, **Dagmar-C. Fischer*, Lutz Graeve, Hans-D. Haubeck*, Monika Günther, Eddy van de Leur*, Peter C. Heinrich and Helmut Greiling***
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In chronic inflammatory and degenerative joint diseases cartilage is destroyed by the action of proteinases. Proteinases are not only released by inflammatory cells but also by chondrocytes and synovial fibroblasts. Synthesis and release of these proteinases is stimulated by inflammatory cytokines such as interleukin-1ß (IL-1ß) and TNF-α for metalloproteinases and IL-8 for PMN elastase. We and others have recently demonstrated that human chondrocytes produce IL-6 upon stimulation with IL-1ß, TNF-α and LPS. Here we demonstrate that IL-6 induces the synthesis and secretion of α1-antitrypsin, the major protease inhibitor of PMN elastase. Differentiated human chondrocytes in organ culture as well as in agarose culture, after stimulation with 100 U/ml IL-6, produce up to 200 ng/10^6 cells of α1-antitrypsin within 48 h. This represents an at least tenfold increase compared to controls. The identity of the secreted α1-antitrypsin was confirmed by immunoprecipitation. The induction of α1-antitrypsin synthesis has also been shown on the mRNA level by Northern blot analysis. These results suggest that IL-6 produced by human chondrocytes in response to pro-inflammatory cytokines induces a "local acute-phase response" in the joints.

EZ 604 IMMUNE MODULATION OF AUTOIMMUNE DISEASES: REGULATION OF EXPERIMENTAL AUTOIMMUNE NEURITIS BY TWO INDEPENDENT METHODS, ANTIGEN-INDUCED TOLERANCE AND TCR PEPTIDE THERAPY, DELINEATE DIVERSE REGULATORY PATHWAYS. Shahik K. Gregorian, Wyne P. Lee, L. Steven Beck, Abdolmohammad Rostami and Edward P. Amento, Genentech, South San Francisco, CA and University of Pennsylvania, Philadelphia, PA.

Experimental autoimmune neuritis (EAN), an autoimmune disease of the peripheral nervous system, is a model of human Guillain-Barré syndrome. It is induced in Lewis rats with myelin P_2 protein or a synthetic peptide (SP-26) corresponding to amino acid residues 53-78 of bovine P_2 protein. The disease is mediated by $CD4^+$, Ia^+ T-cells reactive to myelin P_2 protein or a synthetic peptide derived from the P_2 protein (SP-26). Two approaches were taken to dissect the regulation of immune response governing the expression of EAN. Tolerance to autoregulatory T-cells could be induced in rats by i.v. administration of antigen coupled splenocytes. Although disease progression was reduced markedly, the delayed type hypersensitivity (DTH) response to SP-26, remained intact. A novel means of control of immune regulation has arisen from the observation that limited T cell receptor (TCR) Vβ genes are operating in several autoimmune diseases. We have used the semiquantitative polymerase chain reaction (PCR) to analyze the TCR V-gene usage with 20 Vβ primers. T cells that mediate EAN preferentially utilize the Vβ8 gene in their TCR. We have used a synthetic peptide derived from the CDR2 region of rat Vβ8 TCR (residues 39-59) that, when given i.v. at the induction of clinical signs, reduced disease progression. In contrast to antigen-induced tolerance, however, the DTH response to SP-26 was profoundly decreased in Vβ8 TCR peptide treated rats. In addition, T cell proliferative responses to SP-26 in tolerized and peptide treated rats were decreased. The state of nonresponsiveness (anergy) that was generated with both approaches could be overcome by the addition of exogenous IL-2. Of particular interest, PCR analysis of the draining lymph nodes from tolerized and TCR peptide-treated rats showed the persistence of $V\beta8^+$ T cells, thereby indicating the change in disease outcome was not due to clonal deletion of $V\beta8^+$ T cells. Thus, two methods used to succesfully intervene in the progression of EAN demonstrate similarities in T cell proliferative response to SP-26 and differences in DTH response to SP-26. This approach may be used to further identify novel means of control of immunological processes as well as gain further insights into the mechanisms underlying the regulation of autoimmune disorders.

EZ 605 STRUCTURAL DOMAINS OF COLLAGENASE IMPORTANT FOR SUBSTRATE SPECIFICITY AND GENERAL CATALYTIC ACTIVITY. Karen A. Hasty, Tomohiko Hirose, John M. Stuart and Carlo L. Mainardi, Departments of Anatomy and Neurobiology and Medicine, University of Tennessee, Memphis, TN 38104.

Using the animal model of collagen-induced arthritis, we have described two phases of cartilage matrix destruction; initial loss of proteoglycan followed by erosion of the underlying collagen matrix. Loss of collagen is felt to be the irreversible step in degradation of cartilage matrix. We have undertaken study of the primary structure of collagenase, the matrix metalloproteinase responsible for degradation of interstitial collagens, to define the structural determinants important in recognition and cleavage of its collagen substrate. To address this question, we generated truncated mutants of neutrophil collagenase (TrNC) with varying deletions in the carboxy-terminal (C-terminal) domain and chimeric molecules between neutrophil collagenase (NC) and stromelysin (Strom), and assayed the expressed enzymes against type I collagen and the general substrate, casein. Our data suggest that substrate-specificity for interstitial collagen is determined by a 16 amino acid sequence in the C-terminal domain of NC and is dependent upon the integrity of a disulfide-defined loop at the C- terminus for maximal activity. It was found that a relatively large region of 62 amino acid residues influenced the relative efficiency of collagenolytic activity. In addition to the region which conferred this specificity, a site at the carboxyl side of the presumptive zinc binding locus was found to be necessary for general catalytic activity. Mutation of a critical aspartic residue at position 253 within this area resulted in complete loss of proteolytic activity, indicating that Asp^{253} might function as one of the ligands for divalent cations which are essential for enzymatic activity. Our data support the concept that the domain(s) governing substrate specificity are distinct from those governing general catalytic activity.

EZ 606 CARTILAGE DEGRADATION BY CO-CULTURES OF MOUSE TRANSFORMED MACROPHAGE AND FIBROBLAST CELL LINES: PARTIAL CHARACTERIZATION OF A PROTEOGLYCAN DEGRADING METALLOPROTEINASE, Michael J. Janusz and Michelle Hare, Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215

In rheumatoid arthritis the normally thin synovium becomes hyperplastic and infiltrated by inflammatory cells. This invasive inflammatory tissue which is composed of macrophage-like synovial A cells and fibroblast-like synovial B cells spreads into the joint space and erodes cartilage and bone. In an effort to develop a cell culture based model of cartilage degradation that would have some similarity to that which occurs in arthritis, a panel of human and mouse macrophage and fibroblast cell lines were examined for their ability to degrade cartilage. The mouse transformed macrophage cell line J774A.1 alone or in combination with the mouse transformed fibroblast cell line 10.ME.HDA5R.1 were the only cell lines that extensively degraded cartilage proteoglycan after seven days of culture. Macrophage/fibroblast co-culture supernatants were incubated with radiolabeled cartilage discs in the presence of serine, cysteine, aspartic acid and metalloproteinase inhibitors. Only inhibitors of metalloproteinases, including the endogenous inhibitor TIMP, were able to block cartilage degradation by co-culture supernatants. Gel filtration of the co-culture supernatant yielded an estimated molecular weight range of 20-55 kDa. Using western blot analysis, specific antisera to human and rat MMP-3 did not bind to this mouse metalloproteinase. The co-culture supernatant proteoglycan degrading activity did not bind to gelatin-sepharose but did bind and was eluted from dyematrex green and heparin-agarose columns. Identification of the proteoglycan degrading metalloproteinase will help to establish the utility of this model system.

EZ 607 PREVENTION OF COLLAGEN-INDUCED ARTHRITIS IN DIABETES-RESISTANT BB/WOR RATS BY TREATMENT WITH ANTI-RAT CD5 MONOCLONAL ANTIBODY. Fred R. Kohn, Robert Magee, Robert Peterson, Roger Little and Ada H. C. Kung, Department of Pharmacology and Toxicology, XOMA Corporation, Berkeley, California 94710.

Collagen-induced arthritis (CIA) in the diabetes-resistant Biobreeding (DR BB) rat is a particularly relevant animal model of human rheumatoid arthritis (RA), in that the DR BB rat RT1.Dβ gene encodes a nucleotide sequence homologous to the human HLA-DRβ gene associated with RA susceptibility. In the present investigation, we have evaluated efficacy of a mouse IgG1 monoclonal antibody (OX19 mAb) directed against the CD5 surface antigen on rat T cells in the DR BB rat CIA model. In preliminary studies, a single i.v. bolus injection of OX19 mAb (0.5 mg/kg) was found to saturate and subsequently significantly deplete T cells from blood, spleens and lymph nodes of normal Sprague-Dawley rats, as compared to treatment with an isotype-matched control mAb (H65 mAb; mouse IgG1 anti-human CD5). DR BB/Wor rats (obtained from the University of Massachusetts breeding facility; 8 per group) were administered OX19 mAb (0.5 mg/kg, i.v. bolus), H65 mAb (0.5 mg/kg, i.v. bolus) or buffer on day -7 and day -4 prior to bovine type II collagen (BII) immunization in incomplete Freund's adjuvant (day 0). Control (buffer- and H65 mAb-treated) rats developed severe, predominantly bilateral hindlimb arthritis between days 10 and 14 with high incidence (88% for both groups). Treatment with OX19 mAb completely prevented development of hindlimb arthritis (0% incidence). In contrast, OX19 mAb treatment did not prevent or lessen the severity of arthritis when animals were dosed on day 0 and day 3, *i.e.*, after BII immunization, even though OX19 mAb was fully capable of depleting T cells from lymphoid tissues of severely arthritic rats. These results indicate that T cells are required for initiation of arthritis, but are not required during the development of the disease, in the DR BB rat CIA model.

EZ 608 A SYNTHETIC PEPTIDE ANALOG OF A DETERMINANT OF TYPE II COLLAGEN PREVENTS THE ONSET OF COLLAGEN-INDUCED ARTHRITIS. Linda K. Myers*, Edward F. Rosloniec, Jerome M. Seyer, John M. Stuart, and Andrew H. Kang. From the Departments of Pediatrics* and Internal Medicine, the University of Tennessee, Memphis, Tennessee 38163.

Our laboratory has previously identified a region of type II collagen, (CII), CII 260-270, which contains a dominant T cell epitope, important in the murine model of collagen-induced arthritis. Five specific residues have been identified which are critical for T cell response and tolerance. Therefore, it was of interest to determine whether a synthetic peptide containing site-directed substitutions within these critical residues might be developed which could bind to critical antigen binding sites in I-A^q, resulting in reduction of arthritis via competitive inhibition. Such a peptide might provide specific anti-T cell therapy for autoimmune arthritis without itself being immunogenic. Four synthetic peptides containing substitutions in these five critical positions were developed. One peptide based on the sequence of CII 245-270, yet containing site-directed substitutions in positions 260, 261, and 263, competitively inhibited T cell responses to either CII 245-270 or CII. These data suggest that CII 245-270[s160,261,263] binds to I-A with greater avidity than the wild type peptide. This same peptide completely suppressed arthritis when administered simultaneously with CII into DBA/1 mice. Peptides designed using this approach could have therapeutic potential for human autoimmune arthritis.

EZ 609 SYNTHESIS AND DEGRADATION OF HYALURONATE BY SYNOVIA FROM PATIENTS WITH RHEUMATOID ARTHRITIS, Peter Prehm, Peter Schenck, Stefan Schneider and Rolf Miehlke, Institut für Physiologische Chemie, University of Münster, Waldeyerstr. 15, D-44 Münster, Germany

Hyaluronate degradation was analyzed in cultures of synovial tissue obtained from patients with rheumatoid arthritis. Arthritic tissue produced superoxide and hydroxyl radicals and degraded nascent or exogenous hyaluronate in contrast to normal synovial tissue. Radical scavengers protected hyaluronate from degradation in synovial tissue. Some protection was achieved by superoxide dismutase and catalase or by methionine and complete protection by DETAPAC (diethyltriaminepentacetic acid). It is concluded that hydroxyl radicals were the main degrading species and that the myeloperoxidase-H_2O_2-system also participated in degradation.

EZ 610 THE POTENTIAL OF BLOCKING PEPTIDES TO INTERFERE WITH MHC CLASS II LOADING AND T CELL STIMULATION, Corinne M. Savill, Vanessa C. Vickers, Steven J. Norris, Richard E. Shute, *Luciano Adorini & Eric J. Culbert, ICI Pharmaceuticals, Bioscience 1 and Chemistry 2 Departments, Alderley Park, Macclesfield, Cheshire, SK10 4TG, England and *Sandoz AG, Basle, Switzerland.

The use of non-stimulatory MHC class II binding peptides to block $CD4^+$ T cell stimulation offers a potential therapeutic strategy for autoimmune diseases such as rheumatoid arthritis. One of the key issues governing the feasibility of a peptide blockade approach is the ability of inhibitors to gain access to the site of MHC class II loading with antigenic peptide which has been suggested to occur within intracellular vesicles such as endosomes.

We have compared the ability of blocking peptides to inhibit the T cell response to antigen presented by either living B cells, or B cells fixed with glutaraldehyde to limit antigenic peptide loading to cell surface MHC class II molecules. The inhibition of two different T cell hybridomas 3DO.548, specific for ovalbumin 323-339, and 2C8.4, specific for hen eggwhite lysozyme 110-129, was investigated using 4 different blocking peptides. In no case was any significant difference observed in the amount of peptide blocker required for effective competition of living compared to fixed B cells. This implies either peptide blockers can effectively access intracellular sites of MHC class II peptide loading, or substantial antigenic peptide exchange occurs at the cell surface. Current investigations are aimed at differentiating between these two possible explanations.

EZ 612 EFFECTS OF INTRAMUSCULAR ADMINISTRATION OF CYTOKINE GENES TGFβ1 AND IL10 ON EXPERIMENTAL ARTHRITIS IN RATS. Ian P. Wicks, Eyal Raz, Martin Lotz, *Stephen M. Baird, and Dennis A. Carson, University of California--San Diego, La Jolla, CA 92093-0663 and *Department of Pathology, VA Medical Center, San Diego, CA 92161
In vivo transfection by intramuscular injection (IMI) of cytokine genes may provide a novel and convenient approach to the treatment of human systemic autoimmune disease. Adjuvant arthritis (AA) is an experimental model of human rheumatoid arthritis, which is induced in susceptible rats by the intradermal injection of heat-killed Mycobacterium tuberculosis (M.Tb). Weight loss is a feature of the inflammatory response in AA. We examined the effects of two cytokine genes - TGFβ1 and IL10 - administered by IMI prior to the induction of AA. Human TGFβ1 was expressed under the control of the RSV promoter in pRSV and human IL10 was expressed under the control of a SV40/MLV promoter in SRα. The vector p290 was used as a negative control. One hundred μg of purified TGFβ1/pRSV and IL10/SRα was administered separately into the right and left thigh muscles of 16 six-week old male Lewis rats weekly for three weeks. p290 was given in the same regimen to a control group of 16 animals. AA was induced on the day of the third cytokine gene injection by the intradermal injection of 1 mg of heat-killed M.Tb suspended in IFA. AA was monitored by weekly recordings of weight loss, clinical assessment of arthritis (arthritis score) and in some animals, by histological evaluation of the degree of joint destruction. T cell responses to M.Tb were measured by injecting purified protein derived from M.Tb (PPD) into the heel-pads of selected animals from each group and measuring the change in heel-pad diameter after 48 hours.

There was no clinical or histological evidence of significant side effects in the treated group. At 3 weeks post-adjuvant, control animals had lost a mean of 4.6 gm (S.E. ± 6.6) in weight, compared to a mean gain of 8.8 gm (S.E. ± 4.9) in the treated animals. There was a mean change in heel-pad diameter of 1.1 mm (S.E. ± 0.08) in the control group following the injection of 5 IU of PPD, compared to 0.5 mm (S.E. ± 0.15) in the treated group ($p = 0.005$). At 4 weeks post-adjuvant, 7 animals in the control group had developed arthritis, compared with 3 in the treated group. The cumulative arthritis score was 34 in the control group, compared with 15 in the treated group. There was a marked attenuation of the degree of joint destruction seen histologically in treated versus control animals. These data may indicate that cytokine genes such as TGFβ1 and IL10 can have beneficial effects on the course of AA in rats when administered by IMI.

EZ 611 A POTENTIALLY NOVEL MELANOMA GROWTH STIMULATORY ACTIVITY (MGSA)/GRO RECEPTOR ON RHEUMATOID SYNOVIAL BUT NOT NORMAL FIBROBLASTS MODULATES INTERSTITIAL COLLAGEN EXPRESSION IN RESPONSE TO MGSA/GRO, Elaine N. Unemori, Richard Horuk, Edward P. Amento, Genentech, Inc., S. San Francisco, CA 94080
In rheumatoid arthritis, a network of interactions among inflammatory cells and resident stromal cells is believed to result in extracellular matrix degradation and eventual joint dysfunction. These interactions are mediated in large part by cytokines present within the inflamed joint that are secreted by activated macrophages, T cells, and other cells. In response to these cytokines, synovial fibroblasts attain a phenotype consistent with destruction of cartilage, tendon, and bone, including the ability to secrete enzymes which degrade components of the matrix. MGSA/GRO has recently been detected within synovial fluid (Ref). In the present study, we show that MGSA/GRO induces a dose-dependent decrease in the expression of interstitial collagens by rheumatoid synovial but not normal dermal fibroblasts. This decrease is observed over a dose range of 0.6-6.0nM MGSA/GRO. This effect is specific, as MGSA/GRO has no demonstrable effect on the expression of collagen-degrading metalloproteinases nor does it affect expression of the collagenase inhibitor, tissue inhibitor of metalloproteinases. It also has no effect on the proliferation rate of these fibroblasts, unlike its mitogenic effect on melanoma cells. Direct binding experiments with ^{125}I-MGSA on synovial fibroblasts allow us to identify an MGSA/GRO receptor with a K_D of 10.lnM and approximately 75,000 binding sites per fibroblast. ^{125}I-MGSA binding is specific and is not displaceable by unlabeled IL-8. These data suggest that this receptor is different from the previously described MGSA/IL-8 receptors (Holmes, et al., Science 253:1278, 1991; Murphy and Tiffany, Science 253:1280, 1991) and is similar to the novel MGSA-specific receptor we have previously described on Hs294T melanoma cells (Horuk, et al., J. Biol. Chem., in press). MGSA binding is not detectable on normal dermal fibroblasts. These results suggest that rheumatoid synovial fibroblasts are different from normal fibroblasts in their expression of a specific receptor for MGSA/GRO and that down-regulation of collagen expression induced by MGSA binding may contribute to the overall rheumatoid synovial fibroblast phenotype of extracellular matrix destabilization.