MOLECULAR AND CELLULAR MECHANISMS OF HUMAN HYPERSENSITIVITY AND AUTOIMMUNITY

Organizer: Edward Goetzl
April 17-23, 1988

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Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

Keynote Address: Triggering Events and Consequences (joint)

R 001 Protein Kinase C: Structure, Function and Mechanism of Regulation by Lipid Second Messengers, Robert M. Bell, Duke University Medical Center, Department of Biochemistry, Durham, N.C. 27710.

Protein kinase C is activated by sn-1,2-diacylglycerol (DAG) produced from phosphatidylinositol-4,5-bisphosphate and glycerolipids and is inhibited in vitro and in cells by sphingosine and lysosphingolipids, breakdown products of cellular sphingolipids. The goals of our studies are to understand the mechanism of protein kinase C regulation by phospholipids, Ca^{2+}, DAG (phorbol-esters) and the physiological significance of sphingosine inhibition/pharmacology. The structure of protein kinase C isoenzymes (80 kDa) revealed by cDNA cloning and sequencing and biochemical studies have provided knowledge of two functional domains, the catalytic fragment (51 kDa) and the lipid binding (32 kDa) fragment. Mixed micellar techniques were employed to investigate phospholipid and DAG cofactor specificity and stoichiometry and to deduce oligomeric state. The proposed model, a 4PS-Ca^{2+}-DAG-PKC complex, was tested by preparation of 17 phosphatidylserine derivatives. These analogues support and extend the model. Studies on sphingosine inhibition in vitro were studied by autophosphorylation and with histone substrates. Those in cells, platelets, neutrophils, HL60 cells, and A4B1, demonstrated protein kinase C inhibition occurred without blocking other regulatory pathways, thus suggesting specificity. The available data for sphingosine/lysosphingolipids functioning as second messengers and for a sphingolipid cycle will be presented. Emphasis will be on the relationship between sphingolipids and tumor formation. The role of protein kinase C-I transfection of 3T3-NIH cells in altering cell growth and enhancing tumorigenicity is consistent with protein kinase C involvement in critical events of carcinogenesis. The detailed model of regulation has lead to the discovering of several inhibitors, many of which are anti-tumor agents. Supported by CA46738 and GM38738.

R 002 MUTATIONS TEST A STRUCTURAL AND FUNCTIONAL MODEL OF THE α CHAIN OF G_S (α_S). H. R. Bourne, R. T. Miller, S. B. Masters, and K. A. Sullivan. Departments of Pharmacology and Medicine and the Cardiovascular Research Institute, San Francisco, CA 94143, USA.

We have proposed a structural model of the G protein α chain and predicted specific locations for its functional domains (Masters et al., *Protein Engineering* 1:47, 1986). Now we have tested the model using mutations in the α_S polypeptide chain of S49 mouse lymphoma cells. The *unc* and H21a mutations resulted from selective pressure that abolished interactions of G_S with receptors or adenylyl cyclase, respectively. cDNA sequences indicate that the *unc* mutation encodes proline in place of arginine at the sixth position from the carboxy terminus of α_S. This mutation causes the observed change in charge of the *unc* polypeptide and could kink a predicted carboxy-terminal α helix. Pertussis toxin-catalyzed ADP-ribosylation of the α chains of G_i, G_O, and transducin, at a site near the carboxy terminus, uncouples these G proteins from their receptors. In parallel, the *unc* mutation strongly suggests that the carboxy terminus of α_S, like that of other G protein α chains, interacts with receptors. The H21a mutation substitutes alanine for glycine at a hinge region in the presumptive GDP-binding domain (residue 208). We propose that normal movement at this presumed hinge is required for GTP-dependent changes in conformation. This proposal is supported by the observation that binding of GTP analogs (but not of GDP) prevents tryptic cleavage of wild type α_S, but not of H21a α_S, at a site six residues from the mutated amino acid. Thus the H21 mutation appears to prevent α_S from stimulating adenylyl cyclase by preventing it from distinguishing between GTP and GDP. A third test of the model utilizes expression of a chimeric α chain nucleotide sequence encoding the amino terminal 60% of α_i linked (in frame) to the carboxy terminal 40% of α_S. The α_i/α_S chimeric polypeptide, expressed *via* a retroviral vector in S49 *cyc*$^-$ cells (which lack α_S mRNA and polypeptide), couples β-adrenoceptors to stimulation of cAMP synthesis, a phenotype similar to that produced by normal α_S in wild type S49 cells. This phenotype suggests that the substantial differences in amino acid sequence between the amino terminal portions of α_i and α_S do not affect coupling either to the β-adrenoceptor or to adenylyl cyclase itself.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 003 INOSITOL(1,3,4,5)TETRAKISPHOSPHATE - THE OTHER SECOND MESSENGER FROM INOSITOL LIPIDS? Robin F. Irvine, Department of Biochemistry, AFRC Institute of Animal Physiology & Genetics Research, Babraham, Cambridge, CB2 4AT, U.K.

The concept is now generally accepted that inositol(1,4,5)trisphosphate is an intracellular second messenger whose function is to mobilize Ca^{2+} from intracellular stores. However, more recent investigations have revealed that Ins(1,4,5)P_3 is not alone metabolically, and furthermore it is now emerging that it is not alone functionally either. Some Ins(1,4,5)P_3 is rapidly phosphorylated to Ins(1,3,4,5)P_4 (refs. 1,2) and this novel inositol phosphate can synergize with Ins(1,4,5)P_3 in some tissues to regulate Ca^{2+} entry, and probably also Ca^{2+} mobilization (refs. 3,4). Our current understanding of how Ins(1,3,4,5)P_4 works is that it controls the Ca^{2+} content of the intracellular Ca^{2+} store which is mobilized by Ins(1,4,5)P_3, and thus the two inositol phosphates work as a duet to regulate intracellular Ca^{2+} levels.

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Regulation of Production and Cellular Recognition of Antibodies

R 004 REGULATION OF IgE SYNTHESIS, Kimishige Ishizaka, Subdepartment of Immunology, The Johns Hopkins University, School of Medicine, Baltimore, MD 21239.
The IgE synthesis is regulated by T cell-derived IgE-binding factors in an isotype-specific manner. The IgE-potentiating factor and IgE-suppressive factors may share a common structural gene, therefore a common polypeptide chain, and their biologic activities are determined by post-translational glycosylation process. Under the physiological conditions, the carbohydrate moieties in the IgE-binding factors formed by a subset of T cells are determined by the ratio between two T cell factors, i.e., glycosylation enhancing factor (GEF) and glycosylation inhibiting factors (GIF) in their environment. GIF is an immunosuppressive lymphokine. Repeated injections of this lymphokine into antigen-primed mice facilitated the generation of antigen-specific suppressor T cells and suppressed both IgE and IgG antibody responses. This effect of GIF was reproduced in vitro. Activation of antigen-specific T cells by antigen-pulsed macrophages, followed by propagation of the antigen-activated T cells by IL-2 in the presence of GIF resulted in the generation of suppressor T cells which produced antigen-specific GIF upon antigenic stimulation. Some of the T cell hybridomas constructed from the antigen-specific suppressor T cells formed antigen-specific GIF upon antigenic stimulation. The antigen-specific GIF formed by the T cell hybridoma share several common properties with antigen-specific suppressive factor (TsF) and suppressed the antibody response of syngeneic mice in a carrier-specific manner. The results obtained with mouse lymphocytes suggest a maneuver to suppress the IgE antibody formation to an allergen in atopic patients.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

Major Histocompatibility Complex and T-Cell Associated Control of Immune Responses

R 005 Immunity to Insulin: Lessons in Self-tolerance. Judith A. Kapp and Phyllis Jonas Whiteley, Jewish Hospital of St. Louis at the Washington University Medical Center, St. Louis, MO 63110 and Richard Selden, Massachusetts General Hospital, Boston MA 02114.

Murine antibody responses to heterologous insulins are controlled by H-2-linked genes and our studies of nonresponder strains have demonstrated that nonimmunogenic species of insulin prime helper T cells but fail to induce antibody synthesis because dominant suppressor T cells also are stimulated. By contrast, immunogenic variants of insulin stimulate helper T cells but not suppressor T cells. The helper T cells and suppressor T cells primed by nonimmunogenic variants of insulin cross-react with autologous insulin; moreover, autologous insulin elicits functionally identical helper and suppressor T cells. T cells that recognize autologous insulin have been cloned from the lymph nodes of mice primed with nonimmunogenic insulin; they are MHC-restricted, L3T4 positive T cells that produce lymphokines and provide help to B cells. These cloned T cells are inhibited by suppressor T cells. Thus, classically defined helper T cells are stimulated by apparently nonimmunogenic species of insulin and these T cells recognize antigen complexed with Ia antigens of the nonresponder strain. These observations support the hypothesis that H-2-linked genetic nonresponsiveness occurs because the nominal antigen looks like a self-antigen to the host. They also show that T helper cells that recognize autologous proteins need not be clonally deleted to maintain self-tolerance.

Development and maintenance of self-tolerance is an important, but poorly understood, feature of the immune system. Currently, we are studying the induction of self-tolerance to a foreign antigen using transgenic mice that express physiological levels of human insulin. Transgenic mice did not produce antibody to human insulin or pork insulin, whereas their normal counterparts responded to both. Thus, expression of the human insulin gene has not only rendered these mice tolerant to human insulin, but it also has altered the T cell response to another, normally immunogenic insulin. Studies are in progress to address the mechanisms responsible for tolerance to autologous proteins using this new model system.

R 006 MOLECULAR GENETIC ANALYSIS OF MHC ASSOCIATIONS WITH AUTOIMMUNE DISEASES, John A. Todd, John I. Bell, Hans Acha-Orbea and Hugh O. McDevitt, Department of Medical Microbiology, Stanford University, Stanford, CA 94305.

Our aim was to analyze the association of HLA-D encoded antigens, DR and DQ, with the autoimmune disease insulin-dependent diabetes mellitus (IDDM) by sequencing these genes from patients. We have used the rapid method of Klenow enzyme-catalysed in vitro DNA amplification to isolate and sequence gene segments, starting from RNA. HLA class II coding sequences from IDDM patients are identical to those found in healthy individuals. However, the distributions of certain DQ_β alleles in patients and controls indicates that DQ directly contributes to IDDM susceptibility. Both positive and negative IDDM associations of these alleles are strongly correlated with amino acid 57 of the β-chain. DQ_β genes encoding Ala, Ser or Val at residues 57 are positively associated with diabetes. In contrast, DQ_β genes encoding Asp at residue 57 confer a marked decrease in disease susceptibility. This correlation is consistent with the distribution of HLA-class II antigens in IDDM patients from different ethnic groups. Residue 57 and other DQβ-chain and α-chain residues are probably critical for the autoimmune response to islet cell antigen(s). Disease-associated epitopes for rheumatoid arthritis in the third hypervariable region of DRβ-chains (residues 65-75) will also be presented.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

Immunological Effector Mechanisms

R 007 LYMPHOTOXIN: CYTOTOXIC FUNCTION AND REGULATION. N.H. Ruddle, K. McGrath, C.Bergman, W.L. Tang, S. Fashena and F. Clark, The Yale University Medical School, New Haven, CT. 06510 and The University of Connecticut Health Center, Farmington, CT. 06032.
Lymphotoxin (LT, TNF-β), originally described as a T cell-derived mediator of bystander killing, is now known to have several additional activities. A clearer understanding of its biological role may come from the identification of cells that produce it, the conditions under which it is elicited, and the molecular basis of this regulation. With the aid of biological assays, antibodys, and cDNA probes, we have studied the control of LT and the structurally related, functionally identical, genetically linked molecule, tumor necrosis factor (TNF, TNF-α, cachectin). LT is produced by most class I restricted, $CD8^{+}$ and class II restricted, $CD4^{+}$ cytolytic T cells after induction with specific antigen in the context of self MHC. Many T cell clones produce both LT and TNF after activation, and these factors alone can account for the killing effect of supernatant fluids derived from such cytotoxic cells. Since LT is produced by a wide variety of T cell clones that produce inflammation in vivo in response to ovalbumin, purified protein derivative, or myelin basic protein, it may contribute to that process.

A molecular analysis of the LT gene has been carried out in order to understand the basis of its regulation. Investigation of the 5' flanking region of the gene has resulted in the functional identification of the promoter and has revealed sequences that contribute to its positive and negative regulation in T and B cells.

R 008 BIOCHEMISTRY OF LYMPHOCYTE-MEDIATED KILLING: PERFORIN-DEPENDENT AND -INDEPENDENT KILLING PATHWAYS. Chau-Ching Liu, Pedro M. Persechini and John D.-E Young, Lab of Cellular Physiology and Immunology, The Rockefeller University, New York, N.Y. 10021
Cell-mediated killing represents an important immunological defense barrier against tumor cells, virus-infected cells and other foreign agents. This killing is thought to involve a contact-dependent mechanism and the exocytosis of granule contents from the effector cells.

A pore-forming protein (PFP, perforin or cytolysin) has been isolated from the cytoplasmic granules of murine CTL and human NK cell lines and from human peripheral blood NK cells. PFP has an apparent Mr of 70-72 kD (reducing conditions) and 60-66 kD (non-reducing), and is structurally/functionally/immunologically related to the ninth component of complement (C9). It is actively secreted from effector cells that have been stimulated with calcium ionophore A23187 and phorbol ester. Following secretion, it forms functional channels in planar bilayers and rapidly polymerizes to form ring-like structures with an internal diameter of 160 Å in target membranes. Both binding to and insertion into the target membrane by PFP are calcium-dependent. The purified PFP lyses a variety of tumor cells and sheep red blood cells efficiently.

Although the PFP has been clearly shown to mediate, at least in part, tumor cell killing in vitro, the relevance of this polypeptide in killing mediated by primary CTL in vivo is currently unknown. The role of other granule proteins in the cytolytic event has not been examined to date. Moreover, it is also unclear how lymphocytes induce DNA breakdown in target cells. Recently, we have been able to identify a novel cytotoxin from murine CTL cell lines. This cytotoxin is enriched partially in the granules but is also present abundantly in the cytosol. It cross-reacts immunologically with tumor necrosis factor and lymphotoxin, but also bears significant biochemical and functional differences. The affinity-purified TNF-like cytotoxic protein has an apparent Mr of 50 kD under reducing conditions. It kills both TNF-sensitive and -resistant tumor cell lines with slow kinetics and causes a step-ladder DNA fragmentation in several targets. It is secreted by CTL that have been stimulated with calcium ionophore and phorbol ester. Its lytic function can be neutralized by either anti-TNF or anti-LT antibodies. However, cDNA probes specific for TNF and LT do not hybridize with any CTL-specific messages, indicating that the isolated species from CTL is distinct from both TNF and LT. Unlike PFP, this cytotoxin is stable in the presence of calcium and at 37° C. It does not cause functional ion channel formation in target membranes. Results shown here suggest that CTL-mediated cytolysis may involve multiple mechanisms and/or cytotoxic mediators.

Multisystem Immune Networks

R 009 THE F ALLOTYPE OF HUMAN CR1 CONTAINS THREE TANDEM C3b/C4b BINDING SITES AND THREE COPIES OF THE EPITOPE FOR THE MONOCLONAL ANTIBODY YZ-1

Lloyd B. Klickstein, John A. Smith and Douglas T. Fearon. Program in Cell & Developmental Biology and Depts. of Molecular Biology and Pathology, Mass. General Hospital and Harvard Medical School, Boston, MA 02115 and Div. of Molecular and Clinical Rheumatology, Johns Hopkins Hospital and Johns Hopkins Univ., Baltimore, MD 21205.

The extracellular region of the F allotype of human CR1 is comprised of 30 short consensus repeats (SCRs), 28 of which are contained within long homologous repeats (LHRs). The plasmid piABCD directs the transient expression of cell surface recombinant human CR1 in 20%-50% of COS cells transfected via DEAE-dextran, DMSO shock and 0.1mM chloroquine. The recombinant protein comigrates at 235 kD with the F allotype of erythrocyte CR1 upon reduction and SDS-PAGE analysis, has cofactor activity for C3 cleavage by factor I and confers upon transfected COS cells the ability to rosette with C3b or C4b coated sheep erythrocytes (EAC3b or EAC4b).

Sixteen deletion mutants were constructed and transfected into COS cells. Each clone preserved the reading frame and SCR substructure of CR1. Deletion of all three NH2-terminal long homologous repeats, LHR-A, LHR-B and LHR-C, created a clone, piD, containing only LHR-D, that directed synthesis of an Mr 90 kD protein immunoprecipitable by polyclonal anti-CR1. However, this deletion mutant did not contain the YZ-1 epitope or mediate rosette formation with EAC3b. All clones created by deletion of LHR-A, -B or -C singly or pairwise, piBCD, piACD, piABD, piAD, piBD and piCD, directed synthesis of membrane proteins capable of mediating rosettes with EAC3b and EAC4b and having YZ-1 epitopes. The deletion mutants, piE1, piE2, piP1, piU1, piE-2 and piU-2, where the deletions spanned the LHR boundaries also had C3b/C4b binding functions. The clone piA/D, a fusion of the NH2-terminal four SCRs of LHR-A with the COOH-terminal two SCRs of LHR-D, was able to confer rosetting ability on COS cells; however no YZ-1 epitope was present. Thus, LHR-A contains a C3b/C4b binding site within the NH2-terminal four SCRs and a YZ-1 epitope within the COOH-terminal three SCRs. LHR-B and -C also contain a C3b binding site and a YZ-1 epitope, probably in the corresponding locations, whereas LHR-D does not. Furthermore, these results imply that the S allotype of CR1, which contains an additional LHR resembling LHR-B, may possess a fourth C3b/C4b binding site.

R 010 THE ROLE OF T CELL DERIVED LYMPHOKINES IN THE GROWTH, DIFFERENTIATION AND TRANSFORMATION OF HEMATOPOIETIC CELLS, James N. Ihle, Kazuhiro Morishita, David Askew, Chris Bartholomew, Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701.

During the past several years a variety of T cell derived hematopoietic growth factors have been purified to homogeneity and the genes cloned. Among these factors Interleukin 3 (IL3) has been shown to be a 28 kd glycoprotein which supports the proliferation and differentiation of early hematopoietic progenitors as well as cells committed to a variety of hematopoietic lineages. IL3 has been shown to be uniquely produced by activated T cells and is produced with kinetics comparable to other T cell derived lymphokines including IL2, IL4 and GM-CSF. Normally there are undetectable levels of circulating IL3 and the frequency of IL3 responsive progenitors is low. With immunological stimulation there are demonstrable increases in the frequency of IL3 responsive cells and with extreme immune stimulation detectable, circulating levels of IL3 can be transiently detected. Associated with chronic immune stimulation, leukemias are induced which are IL3 dependent for growth, have phenotypes of early hematopoietic progenitors but fail to terminally differentiate. Using long term IL3 dependent cell lines which have been isolated from primary leukemias we have examined their phenotypic properties and identified genes which may be involved in their transformation. The results of these studies will be described.

This research was supported by the National Cancer Institute under contract N01-CO-74101 with Bionetics Research, Inc.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 011 MEMBRANE PROTEIN REGULATORS OF EXPRESSION OF COMPLEMENT ACTIVITIES,
Victor Nussenzweig, Dept of Pathology, NYU Medical Center, New York, NY 10016.

During activation of the complement cascade, complement fragments C3b and C4b bind covalently to surfaces of cells. This event might lead to their destruction either through progression of the cascade and the assembly of the C5-9 membrane attack complex, or through the recognition of the bound C3b and C4b fragments by phagocytes. Two membrane proteins inhibit the progression of the cascade at this stage: complement receptor type 1 (CR1) and decay-accelerating factor (DAF). DAF binds reversibly to C3b or C4b, and competitively inhibits the uptake of C2 or factor B. The assembly of the C3 and C5 convertases of both classical and alternative pathways is thus inhibited. The importance of DAF in protecting cells from damage by autologous complement is vividly illustrated by the finding that DAF is absent from cells of patients with paroxysmal nocturnal hemoglobinuria, a disease characterized by intravascular complement-mediated hemolysis.

DAF is part of a growing number of proteins which are found to be anchored to the cell membrane by a complex glycosylphosphatidylinositol (GPI), which shares common features with the GPI anchor of the variable surface glycoprotein (VSG) of trypanosomes. Possible advantages of this type of anchor are a higher degree of lateral mobility in the bilayer and the potential for the selective removal of the protein from the membrane by specific phospholipases. The mean diffusion coefficient of DAF, measured by fluorescence photobleaching recovery, was ten times faster than most cell surface proteins. This may explain its ability to move with the speed which is necessary to encounter the randomly deposited complement fragments and prevent the binding and activation of C2 and factor B.

A phospholipase D capable of cleaving the anchors of purified DAF and VSG has been detected in human serum. The enzyme is GPI-specific and does not cleave other phospholipids. The physiological role of the enzyme is unknown, but if it acts on the GPI-anchored proteins it would generate membrane-associated phosphatidic acid, which has growth factor effects.

R 012 RECEPTORS FOR SUBSTANCE P ON IMMUNOLOGICALLY ACTIVE CELLS AND OTHER TISSUES,
Donald G. Payan and Joseph P. McGillis, Howard Hughes Medical Institute and Departments of Medicine and Microbiology-Immunology, University of California Medical Center, San Francisco, California 94143-0724.

Substance P (SP) stimulates receptor-mediated activities of human and murine lymphocytes, the secretory function of salivary glands, and the contraction and proliferation of smooth muscle. SP receptor diversity is suggested by the differential activities of its C- and N-terminal fragments, and by the relative potencies of different SP analogs in specific systems. SP and the molluscan tachykinin physalaemin are more potent than kassinin in contracting smooth muscle in the guinea pig ileum (SP-P prototype) but far less potent in their action on the vas deferens or bladder (SP-E prototype). Substance K (SK) and neuromedin K resemble kassinin in their preference for the SP-E receptor. A third tachykinin receptor in the mammalian nervous system, SP-N, preferentially binds the agonist succinyl[Asp^6,Me-Phe^8]SP$_{6-11}$. Analysis of a bovine SK receptor cDNA (SP-E) reveals a high homology to a family of "seven-transmembrane domain" proteins which include the muscarinic and α and ß adrenergic receptors. Following SP binding, cellular responses in various tissues include phosphatidylinositol turnover, an increase in cytosolic Ca^{+2}, release of amylase, and membrane depolarization, some of which are inhibited by guanyl nucleotides. In the anterior pituitary SP inhibits gonadotropin releasing hormone (GRH) induced release of luteinizing hormone and the level of expression of SP-P receptors is inversely related to GRH receptors.

Using [^{125}I]SP and a fluorescent conjugate of SP (SP*), specific receptors for SP have been identified on human (HL) and murine lymphocyte subsets. Two-color fluorescence-activated cell sorter (FACS) analysis shows that approximately 20-30% of HL bind SP*. The K_D for SP binding to HL was 1-2 nM, with approximately 20,000 receptors/cell. Similar affinities and distribution of SP-binding cells were found in murine lymphocytes, although Peyer's patches have a greater number of SP*⊕ cells than spleen. The human lymphoblast line IM-9 has a single class of approximately 20,000 SP-P type receptors/cell with a K_D of 0.6 nM. Functional IM-9 SP receptors have been solubilized from IM-9 membranes and the receptor has been characterized biochemically. Affinity labelling of the SP receptor identified a ligand binding protein of 58,000 mw. Immunoaffinity chromatography was used to purify the receptor to apparent homogeneity and obtain partial amino acid sequence. This information is now being used to isolate a full length cDNA for the SP receptor. Further analysis of the lymphocyte SP-P receptor will be usefull for delineating the differences between tachykinin receptor subtypes.

R 013 MONONUCLEAR CELL-DERIVED GROWTH FACTORS, Sharon M. Wahl, Henry L. Wong and Nancy McCartney-Francis, Cellular Immunology Section, NIDR, National Institutes of Health, Bethesda, MD 20892

Mononuclear cells generate a variety of hormone-like proteins termed growth factors which are instrumental in the evolution and resolution of inflammatory reactions. Many of these growth-regulatory molecules have multi-functional properties. For example, the mononuclear cell-derived growth factors, platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β), are potent leukocyte chemoattractants. In addition, TGF-β, a product of platelets, T lymphocytes and monocytes appears to function as an inducing agent for other monocyte-derived growth hormones. In this regard, picomolar concentrations of TGF-β stimulate peripheral blood monocytes to transcribe mRNA for PDGF (c-sis), basic fibroblast growth factor (FGF), interleukin 1 (IL1), and tumor necrosis factor (TNF). Each of these monocyte products exhibits a plethora of biological activities on other cell types. Furthermore, levels of mRNA for TGF-β, which is constitutively produced by resting monocytes, are also increased by exogenous TGF-β. Since TGF-β is secreted by monocytes only upon activation, it may function as an endogenous inducer of other monocyte growth factors. The addition of an antibody which neutralizes TGF-β to endotoxin-stimulated monocyte cultures results in marked suppression of inducible IL1 mRNA within 60-90 min, suggesting that stimulation of monocytes causes a rapid release of TGF-β which then is involved in the induction of mRNA for IL-1 and possibly other growth factors. Whereas the control mechanisms for gene expression and secretion of TGF-β appear to be independently regulated, the control mechanisms for TNF, PDGF, IL1 and FGF appear to be located, at least in part, at the level of mRNA transcription. Once secreted, these growth-promoting molecules provide a network of signals which regulate fibroblastic proliferation, matrix protein synthesis, and angiogenesis as well as the functions and growth of other inflammatory cells. Although TGF-β induces IL1 production, at similar concentrations it is also a potent inhibitor of IL1-dependent lymphocyte proliferation. These observations suggest that TGF-β may provide a negative feedback mechanism to reverse the inflammatory response, yet enable IL1, TNF and other monokines to continue modulation of tissue repair. These apparently contradictory biological effects of TGF-β may serve to protect the host from prolonged inflammatory events and to promote the healing of inflamed tissues.

Genetic Definition and Therapy of Immunodeficiencies

R 014 NORMAL AND MUTANT HUMAN ADENOSINE DEAMINASE GENES, John J. Hutton, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

The human adenosine deaminase gene contains 12 exons and 11 introns. There are 32,040 base pairs from the transcription initiation site to the polyadenylation site. Exons range in size from 62 to 325 base pairs while introns are 76-15,166 base pairs in size. The gene contains multiple copies of Alu repetitive DNA. The promoter regions lacks TATA and CAAT sequences, but contains six G/C rich decanucleotide sequences that are highly homologous to sequences identified as functional binding sites for transcription factor Sp1.

We and our collaborators have identified the mutations responsible for hereditary ADA deficiency in seven ADA alleles from children with severe combined immunodeficiency. These alleles were cloned as either cDNAs or genomic fragments from B-lymphoblast cell lines established from patients. Mutations were as follows: (a) transition of C to T at cDNA position 396 altering amino acid 101 from Arg to Trp; (b) two examples of transition of G to A at position 727 altering amino acid 211 from Arg to His; (c) two examples of C to T at position 1081 altering amino acid 329 from Ala to Val; (d) transition of A to G at an invariant base of the splice junction between intron 3 and exon 4 with deletion of exon 4 from the mRNA; and (e) deletion of a large region of genomic DNA including the ADA promoter and exon 1 with consequent absence of ADA mRNA from cells.

To test the effect of these mutations on ADA catalytic activity, expression vectors containing normal and mutant ADA coding sequences under transcriptional regulation of the RSV-LTR were constructed and transfected into human fibroblasts. All transfected cells had levels of ADA mRNA 15 to 25 times higher than endogenous ADA message, yet the mutant ADA sequences, unlike the normal, did not encode functional protein.

R 015 DEVELOPMENTAL AND TISSUE SPECIFIC REGULATION OF ADENOSINE DEAMINASE IN MICE,
Jeffrey M. Chinsky[1,2], Viraraghavan Ramamurthy[1], Thomas B. Knudsen[3] and Rodney E. Kellems[1,2], [1]Baylor College of Medicine, Department of Biochemistry, Houston, Texas 77030. [2]Baylor College of Medicine, Institute for Molecular Genetics, Houston, Texas 77030. [3]East Tennessee State University, Department of Anatomy, Johnson City, TN 37614.

The tissue distribution and developmental regulation of the purine metabolic enzyme, adenosine deaminase (ADA), is being studied. Although this enzyme is ubiquitously expressed in all cell types, there are clearly defined differences in tissue specific levels of enzyme activity. Specifically, the tissues of the upper alimentary canal and oral cavity demonstrate the highest levels of ADA specific activity. The majority of enzyme activity is localized to the mucosal layer of the stratified squamous epithelium. In these tissues, a pronounced postnatal developmental regulation is observed. Similarly, in the developing embryo-placental unit, a striking elevation in enzyme activity is observed, starting at approximately 2 days postimplantation. This elevated level can be correlated with high levels detected in the placenta compared to low levels detected in the body of the embryo. Intermediate levels of ADA activity are detected in the thymus and splenic T-cells, with no dramatic changes in specific activity occurring during postnatal development. We are currently performing histologic studies in order to localize the specific cells with enzyme accummulation and to identify the possible changes in organ architecture which accompany the observed increases in enzymic activity.

We have previously defined the murine ADA promoter region in a cell line with amplified ADA genomic DNA and greatly enhanced enzyme activity (10,000 fold, compared to parental cells)[1]. In these cell lines, as well as in different murine tissues, the level of enzyme activity correlates well with the abundance of ADA mRNA. Transcription initiation sites are being determined to aid in our evaluation of the basis for tissue specific and temporal differences in ADA activity levels. In addition, we are attempting to identify those genomic sequences which may contribute towards regulating ADA expression.

[1]Mol. Cell. Biol. 6:4458-4466 (1987).

Summation: Receptor Functioning (joint)

R 016 REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR, Gordon N. Gill, Department of Medicine, University of California, San Diego, La Jolla CA 92093.

Cellular responses depend on the concentration of both growth factors and their cognate receptors. Under physiological conditions, both are extensively regulated to give graded proliferative and other responses. Constitutive expression of ligand EGF or over-expression of EGF receptor results in transformation and enhanced tumor growth. Measured biological responses to EGF, including Ca^{2+} transport, gene induction, and cell proliferation, depend on activation of the intrinsic protein tyrosine kinase activity of the receptor. Mutational inactivation of intrinsic protein tyrosine kinase activity and microinjection of monoclonal anti-phosphotyrosine antibodies inhibit ligand-induced internalization and consequent receptor desensitization, indicating that receptor metabolism also depends on intrinsic protein tyrosine kinase activity. An unanswered question is whether this requirement for ligand-induced internalization reflects self-phosphorylation as an essential part of the allosteric change in the receptor or phosphorylation of cellular substrates. Phosphorylation of the EGF receptor via protein kinase C results in loss of high affinity binding and decreased EGF-stimulated tyrosine phosphorylation. Expression of the EGF receptor gene is increased by ligand EGF, tumor promoters, cAMP and retinoic acid, and is decreased by glucocorticoids. The complex multilevel control of EGF receptor expression allows appropriate biological responses under a variety of physiological conditions. In response to ligand binding, the protein tyrosine kinase is activated via allosteric changes necessary for transmembrane signaling and involves self-phosphorylation of a regulatory/inhibitory carboxyl terminus.

R 017 HUMAN LEUKOCYTE RECEPTORS FOR LEUKOTRIENES, Edward J. Goetzl, Jeffrey W. Sherman, Jeanne P. Harvey, and Catherine H. Koo, Howard Hughes Medical Institute and Departments of Medicine and Microbiology-Immunology, University of California Medical Center, San Francisco, California 94143-0724.

Human polymorphonuclear leukocytes (PMNLs) express receptors of three distinct specificities for the potent leukotriene (LT) mediators of hypersensitivity and inflammation. PMNL receptors for LTB_4 are localized in plasma membranes and exist in two affinity states with respective mean equilibrium dissociation constants (Kds) of 0.3 nM and 61 nM. A mean of 4400 high-affinity receptors per PMNL transduce chemotactic and surface adherence responses to nM LTB_4, whereas a mean of 270,000 low-affinity receptors per PMNL signal the degranulation and oxidative burst elicited by higher concentrations of LTB_4. Guanine nucleotides convert high-affinity receptors to the low-affinity state reversibly and LTB_4 enhances the binding of guanine nucleotides to specific N proteins in PMNL membranes, demonstrating the functional relevance of the interactions of N proteins with LTB_4 receptors.

In contrast to receptors for LTB_4, PMNL receptors for LTC_4 and LTD_4 are each a single population of approximately 30,000 per PMNL with a mean Kd of 30 nM. The subsets of receptors for LTC_4 and LTD_4 are distributed identically with approximately 1/3 on membranes and 2/3 on lysosomal granules of PMNL. The PMNL receptors for LTD_4 transduce increases in the cytosolic concentration of calcium and in adherence to surfaces, without altering other cellular activities. LTC_4 has no effects alone on PMNL functions, so that receptors for LTC_4 are dedicated solely to specific uptake of LTC_4 for intracellular oxidative and peptidolytic degradation.

Affinity cross-linking of LTB_4 and LTB_4 analogs to receptors on PMNL and HL-60 cell-derived myelocytes labels one membrane protein of 60-80 kD. Purification of the solubilized binding proteins of PMNL and HL-60 myelocyte receptors for LTB_4 by affinity-chromatography with columns bearing LTB_4 and mouse monoclonal antibodies to framework determinants of the receptors has yielded a predominant 60-80 kD protein for each type of leukocyte. The purified receptor proteins bind $[^3H]LTB_4$ and are recognized by rabbit anti-idiotypic antibodies to the combining site of LTB_4 receptors. The cellular properties of leukotriene receptors thus are explained by the structures of the binding proteins and their association with guanine nucleotide-binding proteins.

R 018 THE FUNCTION OF GLYCOSYL-PHOSPHOINOSITIDES IN HORMONE ACTION,

Alan R. Saltiel, The Rockefeller University, New York, NY 10021.

It has recently been discovered that over thirty proteins of diverse origin and function are anchored to the plasma membrane by covalent attachment to glycosyl-phosphoinositides. In these novel structures, proteins are linked via an amide bond to ethanolamine, which is coupled to an oligosaccharide glycosidically linked to phosphatidylinositol(PI). We have found a structurally homologous form of this glycolipid that apparently is not attached to protein. This molecule undergoes a phosphodiesteratic hydrolysis catalyzed by a specific phospholipase C, giving rise to diacylglycerol and an inositol phosphate glycan. In liver, muscle and fat cells, this hydrolysis reaction is acutely stimulated by insulin. The resulting inositol-glycan modulates the activities of several insulin-sensitive enzymes involved in metabolic regulation, and mimics certain of the actions of insulin in intact cells Kinetic and mechanistic studies on the generation and action of the inositol glycan suggest a possible role as a second messenger for some of the acute actions of insulin. The glycosyl-PI precursor for this putative second messenger has been isolated and characterized. Glycosyl-PI synthesis can be detected in a liver microsomal fraction and its kinetic characterization is underway. The specific phospholipase C responsible for the insulin-sensitive hydrolysis of the glycosyl-PI has been purified from liver plasma membranes. New data suggest that certain glycosyl-PI anchored proteins may be released in response to insulin, perhaps due to the activation of one or more specific phospholipases. Elucidation of the functional role of glycosyl-PI in the generation of second messengers or the release of proteins from biological membranes may provide further insights into the pleiotropic nature of insulin action.

Late Addition

R 019 LIPOCORTIN, Roderick Flower, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

"Lipocortin" was the name given (see (1) for key refs.) to a protein(s) released from cells such as macrophages when stimulated by glucocorticoids. Crude extracts, or highly purified fractions containing this protein, exhibited steroid-like effects on cells including inhibition of eicosanoid and PAF-acether synthesis, and an acute anti-inflammatory effect *in vivo*. It was suggested that the release of this protein was responsible for the commonly observed ability of glucocorticoids to block eicosanoid production by cells and also for a part of the anti-inflammatory effects of these drugs.

Initially it was considered that glucocorticoids simply induced the de novo biosynthesis of lipocortin in cells, but subsequent work demonstrated that this was an over simplification and that two separate processes were at work: a comparatively rapid steroid-induced 'release' of lipocortin from the cell and a slower steroid-regulated re-synthesis of the protein.

Concerning the mechanism of inhibitory action, it was observed that glucocorticoids produced a down-regulation of phospholipase A_2 activity in cells and this effect seemed to be duplicated exactly by externally applied lipocortin. Because of this it was suggested that lipocortin was a phospholipase inhibitor and this seemed to borne out by subsequent experiments in cell free assay systems using the purified porcine pancreas enzyme as a model target and either labelled *E.coli* or mixed micelles of labelled phosphatidylcholine as a substrate. However, this conclusion has been challenged recently (2).

Recently one member of the lipocortin family has been cloned and sequenced (3) and sufficient recombinant material has been made available for biological testing. Human recombinant lipocortin 1 has properties which closely resemble the naturally occurring compound. It has a similar profile of anti-phospholipase activity *in vitro*, it suppresses the generation of eicosanoids by different cell types and has an acute anti-inflammatory effect *in vivo*. The gene for lipocortin 1 is induced by glucocorticoids and it seems that this protein can account for some of the biological properties of the anti-inflammatory glucocorticoids.

(1) Di Rosa, M. et al. (1984) Prostaglandins, 28, 441-442.
(2) Davidson, F. et al. (1987) J. Biol. Chem., 262, 1690-1705.
(3) Wallner, B. et al. (1986) Nature, 320, 77-81.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

Regulation of T and B Cell Functions

R 100 INTERACTION OF AN IMMUNODOMINANT EPITOPE WITH Ia MOLECULES IN T CELL ACTIVATION.
Luciano Adorini and Zoltan A. Nagy, ENEA Casaccia 00100 Roma, Italy, Sandoz, Ltd., 4002 Basel, Switzerland.
The amino acid sequence corresponding to residues 107-116 of hen egg-white lysozyme (HEL) has been identified as an immunodominant T cell epitope recognized in association with I-E^d molecules. Correlating with the restriction of T cell recognition peptide 105-120 binds to I-E^d but not to I-A^d molecules. Substitutions at positions 113 (Asn to Lys) and 115 (Cys to Ala) abrogate recognition by T cells but not the binding to I-E^d molecules whereas an Arg to His substitution at position 114 profoundly impairs the capacity of the peptide to interact with I-E^d molecules and to compete for antigen presentation. Residues involved in the interaction with the T cell receptor and with I-E^d molecules appear to be contiguous and to alternate in the C-terminal region of this epitope suggesting that it assumes an extended rather than an helical conformation after interaction with Ia molecules. The substitution at position 114 may prohibit the conformation favorable for binding of this sequence to I-E^d molecules. Immunization of H-2^d mice with peptide 105-120(K113) induces a T cell proliferative response specific only for the immunogen and immunization with peptide 105-120(H114) fails to prime for a T cell response demonstrating a precise correlation between the binding capacity of a peptide to Ia molecules and its immunogenicity.

R 101 ABNORMAL T-CELL RECEPTOR MODULATION BY AN ANTI-T3 MONOCLONAL AB IN LPR DOUBLE NEGATIVE CELLS. J.-L. Davignon, P.L. Cohen, R.A. Eisenberg, Department of Medicine, University of North Carolina at Chapel Hill.
The role of the double negative (DN) cells accumulating in lpr mice is unclear. Although they bear the receptor (T3/Ti) for the antigen (Ag), the lpr DN cells do not respond to alloAgs and the function of T3/Ti on these cells is unknown. With the aid of a monoclonal AB anti-murine T3ε, we have investigated the function of the T3 molecule on the DN cells. We have shown that this AB was not mitogenic for lpr DN lymph node cells (LNC), in contrast to +/+ LNC. Surprisingly, the T3 modulation induced by the anti T3ε mAB was much more rapid in the lpr DN cells and was significant even after only 5 minutes of incubation. By comparison, a similar modulation required at least 2 hours in +/+ cells. This phenomenon was independent of the anti-T3 antibody, since a phorbol ester, TPA, also provoked a faster modulation in lpr DN cells. Double staining experiments showed that coculturing +/+ and lpr DN LNC did not alter the respective rate of modulation of the two populations, suggesting an intrinsic property of the lpr DN cells. After modulation, the T3 molecule was found to be reexpressed at the surface of both +/+ and lpr DN cells during subsequent incubation of the cells without the anti-T3 AB. Moreover, in LNC from 6 week old lpr mice (before the appearance of DN cells), as well as in normal phenotype-bearing T cells (Lyt-2^+ or L3T4^+, 20%) from 6 month old lpr mice, the T3 modulation was similar to that of +/+ cells. By contrast, the T3 recycling was similar in +/+ and lpr DN LNC. Such abnormality may play an important role in the hyporesponsiveness of the lpr DN cells and in the accelerated autoimmunity associated with them.

R 102 REGULATION OF CLONAL GROWTH BY ANTI Vβ8 DIRECTED LYSIS, Piergiuseppe De Berardinis, Marco Londei, Stefan Carrel,* and Marc Feldmann, Charing Cross Sunley Research Centre Lurgan Avenue, London, W6 8LW, U.K., *Institute for Cancer Research, Lausanne Branch, Switzerland.
CD4 and CD8 T cell clones were generated using the Mx9 monoclonal antibody, which recognise the Vβ8 T cell receptor gene family. The interaction of these clones with the Mx9 antibody was analysed and all were found to be specifically stimulated to proliferate by plastic adherent Mx9. In the presence of Mx9 or its F(ab')2 fragment CD8+ Mx9+ clones were capable of specifically lysing the CD4+ Mx9+ T cell clones. No lysis was seen of Mx9- T cells, or in the absence of the antibody. Conversely CD4+ Mx9+ T cells did not have lytic function. These results indicate that cross linking of T cells via their antigen specific receptors may initiate a unidirectional killing. Unlike previously reported lytic systems involving anti T cell receptor antibodies eg anti CD3, these results suggest that this mechanism may have an important physiological role in immune regulation. Anti idiotypic antibodies have been shown to recognise T cell receptors. These may exert profound immunosuppressive effects by inducing the lysis of the helper cells or B cells.

R 103 HIGH FREQUENCY OF CYTOTOXICITY AMONGST CD 4^+ HUMAN T CELLS. Anthony R. Hayward and Peter C. Beverley. University of Colorado, Denver CO 80262 and University College London, England.

Previous studies indicate a high frequency of HLA-DR restricted, antigen specific, cytotoxicity by CD 4^+ T cells cloned from cultures stimulated with varicella zoster virus or herpes simplex virus antigens. To determine whether cytotoxicity by CD 4^+ cells was a general phenomenon or a special feature of responses to herpes viruses we sorted and cloned CD 4^+ and CD 8^+ cells from human mononuclear cells (MNC) by limiting dilution in multiwell plates. Stimulation was with the CD 3 producing hybridoma, UCHT1 or PHA in the presence of IL 2 and wells with clones were identified by ^{125}IUdR uptake and visually after 18 days of culture. Cloning efficiencies ranged from 30 to 70% and cytotoxicity was determined in the same wells by adding ^{51}Cr labelled UCHT1 cells. The target cells were lysed by 95 of 103 CD 4^+ clones and 139 of 143 CD 8^+ clones. Representative CD 4 and CD 8 clones were expanded and tested with CD 3 coated B lymphoblastoid targets which had DNA labelled by 24 hours incubation with ^{125}IUdR. All clones triggered DNA fragmentation in these targets as measured by alkaline elution. Our results indicate that cytotoxicity through CD 3 is a common property of rapidly proliferating CD 4^+ T cell clones and they suggest that a non-cytotoxic population must be small in number or non responsive to the CD 3 or PHA + IL 2 stimulating conditions used.

R 104 MEMBRANE GLYCOPEPTIDES OF LPR LYMPH NODE CELLS, Robert Hooghe, Sergio Di Virgilio, Muriel Rampelberg, Marcel Dolmans and Encarnita Montecino. Chimie générale I (Dr A.G. Schnek), U.L.Brussels, Belgium; Laboratoire d'Immunologie (Dr F.Loor), Univ. Pasteur, Strasbourg, France and Pathology section (Dr M.Janowski), S.C.K.- C.E.N. Mol, Belgium.

We have tested the hypothesis that some phenotypic characteristics of T lymphocytes from lpr mice could be explained by abnormal glycosylation of membrane proteins.

LN cells from normal C57BL/6 and from C57BL/lpr mice were labeled with tritiated sugars. Membrane proteins were released with trypsin, then with pronase. After complete pronase digestion, glycopeptides were separated on Biogel P-6 and on Con A-Sepharose. Fractions not binding to Con A were also separated on Lentil lectin-Sepharose. Marked differences between normal and lpr cells were noticed. First, there were more glucosamine-labeled peptides with very high molecular weight (eluting fast on Biogel P-6) on lpr cells than on normal lymphocytes. Second, the proportion of mannose-labeled peptides binding to Con A was smaller in lpr cells. Third, among the Con A-negative peptides, the proportion binding to Lentil lectin was higher in lpr cells. Thus, lpr cells seem to carry more α 1-6 fucosylated high mannose chains and more very large carbohydrates. There may be a primary change in glycosylation in lpr cells. Alternatively, the glycosylation pattern of lpr cells may be characteristic for a subpopulation of T-lymphocytes that is expanded in this disease, or for a certain stage of activation. A large proportion of Con A-negative, Lentil lectin-positive peptides is a rather unusual feature in murine cells and requires further investigation.
Supported by the Belgian National Lottery and NFGWO, the Radiation Protection Research Program of the E.C. and the "INSERM-Communauté francaise de Belgique" exchange program.

R 105 REGULATION OF T CELLS BY DENDRITIC CELLS. David R Katz, Angela Brennan and Philip King, Pathology Department, University College & Middlesex School of Medicine, LONDON W1P 8AA, United Kingdom.

Previous studies which examine dendritic cell (DC) regulation of T cell function have emphasised the role of class II MHC determinants on the surface of these cells, and that DC do not process antigen via a conventional phagolysosome pathway, but the precise mechanism of DC function has not been clarified.

We have been investigating the cellular mechanism of DC function using DC isolated from human tonsillar tissue. These cells have typical morphology and phenotype and are potent inducers in both syngeneic and mitogen responses. Using periodate-pulsed T cells in an oxidative mitogenesis assay, we have shown that both anti-class I and anti-class II MHC antibodies inhibit the response. A panel of monoclonal antibodies against CD4, CD11a and CD11c determinants inhibit the response. Antibodies against CD11b and CD14 are not inhibitory. Neither anti-IL-1α nor anti-IL-1β have an inhibitory effect, despite the fact that both antisera bind to the surface of the DC. Exogenous recombinant IL-1 will augment the response, but the same concentration has no effect on the periodate pulsed cells alone.

Taken in conjunction with our previous study, which showed that DC express receptors for the monocyte product dihydroxycholecalciferol (1,25DHCC) (the active metabolite of vitamin D3) our results add further support to the hypothesis (i) that synergy between DC and macrophages is due to DC binding of macrophage derived mediators rather than to uptake of processed fragments of antigen; and (ii) that the differential role of the these two types of presenting cell is a function of whether or not an associated inflammatory mediator is required in the particular immune induction process.

R 106 THE HUMAN T-CELL δ-CHAIN RECEPTOR: GENETIC STRUCTURE, DIVERSITY AND EXPRESSION, Elwyn Loh*, Louis Lanier†, Steve Cwirla†, Yueh-hsiu Chien*, and Mark M. Davis*, Stanford University*, Stanford, CA. 94305, Becton Dickinson Monoclonal Center, Inc.† Mountain View, CA. 94043.
The antigen receptor on T-cells is most commonly composed of an α-β heterodimer associated with the CD3 complex. A subset of T-cells has been found to have a distinct receptor composed of a γ-δ heterodimer also associated with the CD3 molecules. We have recently sequenced a cDNA from the Peer leukemia cell line, which expresses the γ-δ receptor. We have shown that this cDNA codes for the δ-chain by the use of an antisera raised against a peptide synthesized according to the predicted amino acid sequence (Loh, *et al.*, *Nature*, 330:569-572, 1987). We have now isolated a region of germline DNA which codes for a D region and two J regions. They utilize the same signal sequences for rearrangement that other T-cell receptors use. Northern analysis of RNA from populations of thymic T-cells that express γ-δ receptors shows that the Peer V-region is the predominant V-region. This is also true of the peripheral γ-δ T-cells of some but not all individuals. Thus in some populations of γ-δ T-cells V-region utilization may be restricted. Similarly, in the populations studied, we show through Southern analysis that one J-region appears preferentially rearranged. We have also found a second V-region which can be utilized by the δ-chain but only uncommonly in the cells studied. Even though the diversity of the δ-chain V-regions may be limited, we find through the comparison of cDNA sequences that the diversity of the chains as a whole can be extensive, with the concentration of the variation between the V and the J- region by the use of multiple D and N regions. These studies give some clues as to the immunological function of the γ-δ T-cell, which remains a major unanswered question.

R 107 COMPETITION BETWEEN SELF PEPTIDES AND FOREIGN ANTIGENS FOR PRESENTATION TO T CELLS, Z. Nagy and L. Adorini, Sandoz Ltd., CH-4002 Basel.
Synthetic peptides corresponding to sequences 46-62 and 51-62 of mouse lysozyme were used as competitors in T-cell responses to 6 different antigens: hen egg-white lysozyme (HEL), peptide 46-61 of HEL, lactate dehydrogenase B, bovine insulin, GLT15, and tetanus toxoid, recognized together with 7 different class II restriction elements: IA^b, IA^d, IA^k, IE^d, IE^r, and DR 5,7. Within a range of 30 to 360 uM concentration, the inhibitors blocked antigen-specific proliferation of most primed T cells, exhibiting little or no selectivity with regard to either the antigen or the restriction element involved. Only the anti-GLT+ IE^d response and mitogen responses were not inhibited. Competition experiments with HEL-specific T-cell hybridomas have revealed that responses restricted by the poorest binder IE^d molecule require 10 fold higher peptide concentration than those restricted by the strong binder IA^k molecule to achieve 50 % inhibition. Totally nonbinder class II molecules could not be identified. The susceptibility of T-cell lines to peptide-blocking was found to decrease with time. Lines maintained by repeated restimulation with antigen acquired resistance within weeks. The data suggest that ubiquitous self peptides may compete with foreign antigen for presentation, and that this competition may select T cells with high affinity receptors for the antigen.

R 108 SIGNAL TRANSDUCTION VIA THE CD4 MOLECULE. S Neudorf, B McCarthy, M Jones, J Harmony. Children's Hospital Medical Center and the Univ. of Cincinnati, Cincinnati, OH
The CD4 molecule is expressed on 60% of T cells, some monocytes and in brain. One hypothesis for the function of the CD4 molecule is the transmission of biochemical signals affecting lymphocyte function. This hypothesis was tested by studying the effects of a monoclonal anti-CD4 termed 6B10 on mobilization of intracellular Ca in CD4+ leukemic cell lines and normal PBMC. Cells were loaded with 2 μM Indo-1 AM prior to addition of 5 μg/ml affinity purified 6B10, control IgG, PHA, OKT3 or OKT4. Cells were analyzed using a spectrofluorimeter. 6B10 caused a 66% increase in Ca in HPB-MLT (n=3) and a 98% increase in Ca in the CD3 negative cell line CEM (n=5). In contrast, OKT4 which binds to a different epitope of CD4 caused only an 11% increase in Ca (n=2) which is no different than that of the isotype control. 10 μg/ml of 6B10 Fab caused a 66% increase in Ca in HPB-MLT (n=3) and a 46% increase in Ca in CEM as well. Since anti-CD4 has been shown to inhibit the lymphocyte response to mitogens, we studied the effects of 6B10 on OKT3 and PHA-induced Ca mobilization. PBMC incubated with 10 μg/ml OKT3 showed a 42% increase in Ca (n=4). Preincubation with 10 μg/ml 6B10 resulted in only a 7% increase in Ca induced by OKT3 (n=4). 6B10 also caused a 76% inhibition of Ca mobilization by PHA (n=4). 6B10 did not inhibit A23187 induced Ca mobilization. The effect of 6B10 on PHA-induced phosphoinositol (PI) turnover was also studied. PB T cells were stimulated with PHA and 6B10, incubated with P^{32} for 2 hours and labeled phospholipids were extracted. 6B10 inhibited 71% of P^{32} incorporation in PI, PIP and PIPP. 6B10 had no effect on PI metabolism in the absence of PHA. These results suggest that CD4 is involved in signal transduction as defined using Ca mobilization and PI metabolism assays. We hypothesize that CD4 may function to down-regulate lymphocyte function by affecting early events in mitogen-induced activation.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 109 MODULATION OF THE IN VITRO HUMAN NEONATAL B CELL RESPONSE TO POLYSACCHARIDES BY 8-MERCAPTOGUANOSINE, Ger T. Rijkers, Ineke G. Dollekamp and Ben J.M. Zegers, University Hospital for Children and Youth "Het Wilhelmina Kinderziekenhuis", P.O. Box 18009, 3501 CA Utrecht, The Netherlands.

The concept that antibody responses to T cell indpendent type 2 (TI-2) antigens in mice are confined to Lyb 5^+ B cells was recently challenged by Ahmed et al. (J. Immunol., 136, 1223) who showed that B cells from CBA/N mice could be induced to respond to TI-2 antigens by addition of 8-mercaptoguanosine (8mGuo) to _in vitro_ cultures. We have shown previously that pneumococcal polysaccharides behave as human TI-2 antigens. When cultured _in vitro_ with type 4 pneumococcal polysaccharides (PS4), human neonatal B cells do not or only marginally respond. Limiting dilution analysis of neonatal B cells polyclonally activated by a combination of phorbol esters, calcium ionophore and T cells and T cell factors, however, showed that antigen reactive B cells are present in cord blood. The frequency of anti-PS4 reactive B cells in cord blood is comparable with that of adult peripheral blood. In order to obtain more insight in the activation requirements of these PS4 reactive neonatal B cells, 8mGuo was added to _in vitro_ cultures. Addition of 0.5-1.0 mM 8mGuo resulted in a 3 to 10-fold amplication of the anti-PS4 response. The effect of 8mGuo was most prominent when added 3 days after initiation of the culture. Based on experiments in which T cells or monocytes (constituent cells of the _in vitro_ culture system) were treated separately with 8mGuo and kinetic studies, we propose that (_in vitro_ activated) B cells are target for 8mGuo. These data further indicate that neonatal unresponsiveness to polysaccharides is not likely to be the consequence of different activation requirements as compared with adult B cells.

R 110 EPITOPE SPECIFICITY AND IMMUNOLOGICAL FUNCTION OF MURINE ALLERGEN SPECIFIC HUMAN T CELL CLONES. G.Gurka, B. McDonald, A. Kalluri, P. Feigelson, J. Ohman, and L.J. Rosenwasser, NEMC, Tufts Univ., Boston MA, Columbia Univ., N.Y., N.Y.

Mouse allergen 1 (MA-1) is a soluble antigen concentrated from mouse urine that induces proliferation of peripheral blood mononuclear cells or T cells from a population of test subjects. From antigen specific MHC restricted T cell lines, we were able to clone T cells that were antigen specific by limiting dilution. It was found that EBV transformed B cells as well as monocytes could function as APC and that antigen specific EBV B cells enjoyed a great advantage over nonselected transformed EBV B cells or monocytes as APC. Since MA-1 may be related to rat alpha 2U globulin (RA2UG) purified RA2UG was used as a stimulant for the allergen specific T cell clones. A cDNA for RA2UG was transcribed and translated in vitro and the resultant translation product also could stimulate cloned T cells. Finally, truncated peptides derived from the RA2UG cDNA were expressed and overlapping peptides suggested that the major T cell epitopes recognized by 9 separate T cell clones from 4 separate individuals resided between amino acid position 80 and 108 of the sequence of RA2UG. In addition to mapping the epitope recognized by the allergen T cell specific clones, the specificity and function of the clones were tested in a number of other systems. One clone, LJA5, a CD4+ clone required soluble IL-1 for optimal activation. Another clone MM-2, a CD8+, class II restricted allergen specific clone was shown to produce factors that augmented autologous B cell Ig but presumably interfered with Ig synthesis in vitro on a cellular interaction basis. These clones may be helpful in modeling immunotherapy for allergens in humans with hypersensitivity disorders.

R 111 SUPPRESSOR INDUCER (NAIVE) AND HELPER INDUCER (MEMORY) PHENOTYPES REPRESENT FUNCTIONAL MATURATIONAL STAGES OF A SINGLE T LYMPHOCYTE LINEAGE

M. E. Sanders, M.W. Makgoba, S.O. Sharrow, D. Stephany, T.A. Springer, H.A. Young, and S. Shaw, NIH, Bethesda, MD 20892, Dana Farber Cancer Institute, Boston, MA 02115, and Frederick Cancer Research Facility, Frederick, MD 21701.

Differences in expression of seven cell surface molecules defines two major subsets of adult human T lymphocytes. One subset expresses enhanced levels of three adhesion molecules (LFA-3, CD2, and LFA-1), enhanced levels of a molecule reported to identify murine memory T cells (Pgp-1), and enhanced levels of two molecules reported to identify subsets of T cells with "helper inducer" function for immunoglobulin production, i.e. 4B4 (CDw29) and UCHL1. The reciprocal subset expresses low levels of each of these molecules and has high expression of 2H4 (CD45R), a marker of "suppressor inducer" cells. Neonatal T cells are essentially all of the "suppressor inducer" phenotype. PHA activation of neonatal T cells leads to uniform conversion to the "helper inducer" phenotype, with enhancement of expression of LFA-3, CD2, LFA-1, CDw29, and UCHL1, and a gradual decrease in expression of CD45R. Adult T cells sorted for LFA-3 show proliferative response to soluble antigen only in the LFA-3+ subset, while proliferation to PHA was better in the LFA-3- subset. The LFA-3+ subset made five-fold more gamma interferon than the the LFA-3- subset in response to PHA. These studies demonstrate that when activated, cells of the "suppressor inducer" phenotype convert to the "helper inducer" phenotype. These phenotypes represent functionally different maturational stages, i.e. memory and naive T cell subsets respectively, rather than separate T cell lineages.

R 112 ACCESSORY PROTEIN-CLASS II MHC ANTIGEN COMPLEXES, Christopher H. Sorli and Robert E. Humphreys, University of Massachusetts Medical School, Worcester, MA 01655.
To understand the mechanisms by which class II antigens and associated proteins process and present foreign antigen, we examined microsomal membrane complexes of these proteins through the use of a reducible, bifunctional, crosslinking reagent. Proteins were labeled with ^{35}S-methionine and ^{35}S-sulfate for various times or under pulse-chase conditions, immunoprecipitated and analyzed by 2D SDS (non-reducing/reducing) gels. Class II antigens were present in at least three specific, crosslinked compleses. One lower molecular weight complex consisting of the class II alpha and beta chains associated with the invariant chain glycoprotein (I_i). Two other high molecular weight complexes could be separated by their ability to be immunoprecipitated by antibodies directed to different I_i epitopes. One high molecular weight complex, recognized by the anti-I_i monoclonal antibody, VIC-Y1, consisted of the class II alpha and beta chains, I_i, and the chondroitin-sulfate proteoglycan form of the invariant chain (CS-I_i). The second large complex, recognized with anti-I_i peptide (183-193) serum, contained the class II MHC alpha and beta chains, I_i, and an unidentified, discretely migrating protein of approximately 70 KD (p70). Kinetic studies, with Raji cells and various metabolic labeling times, showed that formation of these complexes with CS-I_i or p70 required at least a 6 hr label. p25, recognized by anti-I_i antibodies, accumulated within Raji cells at longer labeling times and remained uncomplexed.

R 113 REGULATION OF CYTOTOXIC T CELL (CTL) GENERATION BY SUPPRESOR T CELL (T_S) AND ANTI-SUPPRESSOR T CELL (T_{AS}). Chou-Chik Ting and Myrthel E. Hargrove. National Cancer Institute, NIH. Bethesda, MD. 20892. Our study demonstrated that a new class of immunoregulatory cells, the T_{AS}, were involved in the regulation of alloreactive CTL generation. It has been shown by various laboratories that CTL generation is subjected to the regulation by helper T cell (T_H) and T_S. During our study on the function of T_H in lymphokine production, we found that in addition to interleukin 2, a T cell differentiation factor (TCDF) was also involved in promoting CTL generation. TCDF not only was involved in the induction of CTL, it also prolonged the cytotoxic activity of CTL in bulk MLC (mixed lymphocyte culture). In the absence of exogenous TCDF, CTL cytotoxic activity usually terminated after 6-7 days in primary MLC. Adding conditioned medium containing TCDF (CM-TCDF) could prolong the CTL activity for at least 20-30 days. This effect was found to be mediated through the generation of T_{AS} which abrogated the T_S activity. T_S were generated in MLC and TA_S were generated in MLC which was supplemented with CM-TCDF. Both T_S and T_{AS} were allospecific, T_{AS} against H-2^b specifically abrogated the T_S activity against H-2^b, and had no effect on T_S against H-2^s. The reverse was also true that T_{AS} against H-2^s specifically abrogated T_S against H-2^s. T_{AS} were Thyl$^+$ and L3T4$^+$ cells and anti-Thyl or anti-L3T4 mAb blocked their generation. However antibody agaisnt MHC class II determinats had no effect on T_{AS} generation and thus was different from T_H. Generation of T_{AS} did not require Lyt2$^+$ cells and thus was different from contrasuppressor cells. Therefore, T_{AS} may represent a new class of immunoregulatory cells whose function is to provide an "off" signal to T_S which down regulate CTL.

R 114 MOLECULAR BIOLOGY OF THE T-CELL RECEPTOR IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, James Urban, Vipin Kumar, Dwight Kono, Christopher Gomez and Leroy Hood, California Institute of Technology, Pasadena, CA 91125.

We have cloned and sequenced the T-cell receptor (Tcr) genes of ten T-cell hybridomas reactive with a myelin basic protein (MBP) nonapeptide known to be a potent encephalitogen in PL/J mice. Six of ten α chain genes employ the same variable region (V) gene segment, Vα14, and seven of ten β chain genes employ the same Vβ gene segment, Vβ8.2. Each α/β gene combination utilizes at least one of these two conserved V gene segments. Usage of joining region (J) gene segments is also highly restricted to two predominant Jα gene segments and one predominant Jβ gene segment. Northern and Southern blot analyses of five additional cloned T-helper cell lines mediating experimental autoimmune encephalomyelitis in PL/J mice indicate a similar conserved usage of these V and J gene segments. Prominent restriction of the Tcr in its response to a major encephalitogenic epitope of MBP indicates strict requirements for T-cell recognition of this self-epitope and suggests that elimination of lymphocytes expressing certain Tcr may be a rational approach to treatment of certain autoimmune diseases.

R 115 IL-2 and IL-4 PRODUCING MURINE T CELL CLONES UTILIZE DIFFERENT TRANSMEMBRANE SIGNALS IN RESPONSE TO MITOGEN, Megan E. Williams, Philip M. Rosoff*, Andrew H. Lichtman, and Abul K. Abbas, Departments of Pathology Harvard Medical School and Brigham and Women's Hospital Boston, MA 02115 and *Departments of Pediatrics (Division of Hematology-Oncology, New England Medical Center) and Physiology Tufts University School of Medicine, Boston, MA 02111.

The T cell mitogen Concanavalin A (Con A) induces lymphokine secretion in both IL-2 and IL-4 producing murine, CD4-positive T cell clones. We have examined a panel of five IL-2 producing (TH1) and three IL-4 producing (TH2) clones and have demonstrated differences in transmembrane signal generation in response to Con A. Con A treatment of TH1 cells results in a rapid rise in intracellular free Ca^{++}. This is accompanied by increases in phosphatidyl inositol (PI) turnover as reflected by increased production of diacylglycerol and inositol phosphates. In TH2 cells treatment with Con A failed to produce an increase in intracellular free Ca^{++} and did not stimulate PI turnover. This lack of responsiveness was observed over a 2-log concentration range of mitogen. These results suggest that a subset of CD4-positive T cells utilizes a pathway of signal transduction that is independent of Ca^{++} and PI turnover, and that the pattern of generation of intracellular signals may correlate with the lymphokines produced by the cells.

Immunodeficiency

R 200 ANTIBODIES TO hnRNP CORE PROTEIN A1 IN CONNECTIVE TISSUE DISEASES. GCB Astaldi Ricotti[1], M. Bestagno[1], A. Cerino[1], F. Cobianchi[1], M. Longhi[2], R. Caporali[2], C. Montecucco[2]. 1 Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R. Pavia, 2 Istituto di Patologia Medica IRCCS Policlinico S. Matteo, Pavia Italy.

Heterogeneous nuclear ribonuclear proteins (hnRNP) have been described to be recognized by sera of certain patients affected by connective tissue diseases. However the specific polypeptides involved in anti-hnRNP autoimmunity have not yet been identified. We investigated the specificity of circulating autoantibodies to a hnRNP particle core protein (A1 protein) in different rheumatic diseases: 35 Systemic Lupus Erythematosus (SLE), 33 rheumatoid arthritis, (RA) 27 scleroderma, 14 primary Sjogren's syndrome, 10 idiopathic Raynaud, 3 MCTD and 25 healthy donors. All sera were tested by ELISA on hnRNP A1 protein. Optical density (OD) mean control values was 152 $\pm$ 54. Values $>$ 260 were obtained in 37% SLE, 29% scleroderma, 20% idiophatic Raynaud, 33% MCTD and 70% RA patients. RA patients also exhibited the highest titers of circulating antibodies. These patients were all seropositive RA and 5 of them showed extra articular manifestations. Therefore hnRNP A1 may be recognized by sera of various rheumatic disorders, however high titer antibodies to this protein appear mainly associated with seropositive rheumatoid arthritis.

R 201 AUTOIMMUNE MARKERS IN GLOMERULONEPHRITIS, Per Bygren, Nils Rasmussen and Jörgen Wieslander. Department of Nephrology, University Hospital, Lund, Sweden, Statens Seruminstitut, Copenhagen, Denmark and BioCarb AB, Lund, Sweden.

Almost any form of glomerulonephritis may be complicated by the formation of glomerular crescents, that is an obliterative proliferation of cells in Bowmans space. A rapid deterioratior of the renal function is observed and early diagnosis and treatment is therefore mandatory. In our laboratory we have developed specific ELISA:s for autoantibody markers in glomerulonephritis. These tests include a specific assay for Goodpasture antibodies, a specific assay for anti-neutrophil cytoplasmic antibodies (ANCA) present in sera of patients with Wegener's granulomatosis, non-Goodpasture anti-glomerular basement membrane antibodies, circulating fibronectin-IgA immunecomplexes present in sera of patients with IgA-nephropathy and some other nuclear and non-nuclear antibodies. In a study of all patients undergoing renal biopsy that were admitted to our department in the years 1974-1986 75 patients had more than 50% crescents morphologically. Of these 20% had classical Goodpasture syndrome with smooth linear IgG deposits along GBM and circulating antibodies against the globular domain of collagen IV. 60% of the patients had ANCA associated with Wegener's granulomatosis and 20% of the patients had miscellaneous other autoantibodies. Our data thus indicate that a specific serological diagnosis can be obtained by ELISA in a majority of patients with rapidly progressive crescentic glomerulonephritis.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 202 VIRUSES AND CYTOKINES: MULTIPLE ROLES IN PANCREATIC BETA CELL DESTRUCTION, Iain L. Campbell, Walter and Eliza Hall Institute of Medical Research, Vic, Australia.
Insulin-dependent (type-1) diabetes is due to the selective autoimmune-mediated destruction of pancreatic beta cells possibly initiated by viruses. We have examined the effects of reovirus (RV) and the cytokines IFN-γ and TNF-α on beta cell major histocompatibility complex (MHC) protein expression, viability and function. Infection of human beta and RlN-M5F (rat insulinoma) cells with RV was associated with: (i) cytopathic effects by 48 hr, (ii) enhanced expression of class 1 MHC proteins and mRNA and (iii) no detectable class II MHC protein expression. Stimulation of class 1 MHC protein expression in RV-infected RlN-M5F cells inhibited by antiserum to RV or by virus depletion of supernatants but not by antiserum to IFN-γ/β. IFN-γ and TNF-α also stimulated expression of class 1 MHC proteins and mRNA by beta cells, viz IFN-γ + TNF-α $\geq$ IFN-γ > TNF-α. While IFN-γ and TNF-α alone were ineffective, in combination these cytokines induced class II MHC protein expression on 30-40% of human or murine beta cells. Murine islets cultured for 3 d with IFN-γ and/or TNF-α had a decreased insulin response to glucose: 25% with IFN-γ or TNF-α; 90% with IFN-γ + TNF-α, the latter being irreversible. Murine islets cultured for 6 d with IFN-γ + TNF-α underwent degenerative changes associated with an 80% depletion of insulin content.

These findings suggest that RV and the cytokines IFN-γ and TNF-α contribute directly (inhibition of function and/or cytotoxicity) and indirectly (stimulation of MHC protein expression and subsequent enhanced immunogenicity) to the pathogenesis of beta cell destruction in type-1 diabetes mellitus.

R 203 REGULATION OF CLASS II ALLOANTIGEN EXPRESSION ON OCULAR ENDOTHELIAL AND EPITHELIAL CELLS: ROLE IN OCULAR IMMUNE AND AUTOIMMUNE RESPONSES
John J. Donnelly, Ph.D., University of Pennsylvania, Philadelphia, PA 19104.
The dynamics of Class II alloantigen expression on corneal endothelium and stromal fibroblasts, and retinal pigment epithelium (RPE), were studied in humans and in murine and rabbit models. In the normal adult human eye, these cells express only Class I alloantigens. The expression of HLA-DQ and DR, or of Ia, may be induced in primary cultures of all 3 cell types by γ-interferon (γ-IFN). In addition, RPE cells maintained in continuous log-phase growth in the absence of γ-IFN spontaneously express HLA-DR but not HLA-DQ. Local intraocular production of Ia-inducing factors occurs during immunogenic ocular inflammation, and Ia is expressed on corneal endothelium during immunogenic inflammation and during corneal allograft rejection. Inferferon-pretreated corneal endothelial cells stimulate proliferation of allogeneic lymphocytes, and are preferentially killed by sensitized CTL, in vitro. In contrast, γ-IFN-pretreated corneal fibroblasts do not stimulate proliferative responses of allogeneic lymphocytes. Interferon-stimulated corneal endothelium with enhanced Class II alloantigen expression may participate actively in corneal allograft rejection as afferent stimulus and as a target of CTL. Repair/metaplasia of RPE, with concurrent self-regulation of HLA-DR expression, may enhance the antigen-presenting capability of RPE and facilitate induction of autoimmunity to retinal antigens.

Supported by USPHS NIH Grants EY06616 and EY03984.

R 204 T CELL RECEPTORS IN AUTOIMMUNE DISEASE, Jörg T. Epplen, Johanna Chluba, Viktor Steimle and Ari Hinkkanen, Junior Research Unit, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, FRG.
Autoantigen-activated T lymphocytes are thought to play a crucial role in the pathogenesis of certain autoimmune diseases. We have determined the complete nucleotide sequence of a human autoreactive α/ß-T cell receptor from genomic and cDNA libraries. The autoaggressive T cell clone had been isolated from the synovial fluid of a postinfectious reactive, inflammed joint. The variable gene elements used to generate this receptor have not been described so far in T cell receptors with known antigen-specificity. The same or similarly rearranged variable elements are used, however, in other autoreactive T cell clones from the same patient indicating preferential usage at least of the ß chain elements as demonstrated by oligonucleotide hybridization experiments. The antigen recognized by the autoaggressive T cell clone is most likely the maternal HLA-DRw11 molecule; the nucleotide sequence of the polymorphic DRw11-ß chain has been determined and shows two amino acid changes in the hypervariable positions when compared to the known DR5ß-chains.
In a related approach the T cell receptor ß chain genes of autoaggressive rat T cell clones were molecularly cloned and sequenced. These clones are specific for myelin basic protein (MBP) and cause experimental autoimmune encephalitis. The V_{β} element common to the clones is homologous to a member of the mouse $V_{\beta 8}$ family. The amino acid sequence of the particular rat V_{β} element and the $V_{\beta}D_{\beta}$-junction (though not the J_{β} element) seem to be necessary for MBP recognition as deduced from independent T cell clones. The screening of a large panel of corresponding T cell clones is currently under way. The meaning of distinct receptor idiotypes for the recognition of autoantigens and the pathogenesis of autoimmune phenonema will be discussed.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 205 AN EXPERIMENTAL MODEL OF AUTOIMMUNE MYOSITIS. Michael N. Hart, Univ Iowa, Iowa City, IA 52242; D. Scott Linthicum, Univ Texas Health Science Center, Houston, TX 77025.

Splenic cells from two inbred murine strains (BALB/c and SJL/J) are each activated (immunized) in vitro by co-culture with syngeneic skeletal muscle myotubes. Subsequent injection of the activated splenocytes with or without B. pertussis into respective syngeneic hosts results in inflammatory myopathy in 80% of the SJL/J mice but never in the BALB/c mice. The muscle inflammation is very similar in appearance to human autoimmune inflammatory myopathies. The myositis is not effector cell-skeletal muscle specific because splenocytes activated by co-culture with smooth muscle will also elicit skeletal muscle lesions in a few animals. Skeletal muscle from both strains appears to express class II (Ia) antigens in vitro and the splenocytes from both strains appear to be equally activated. Thus we postulate that the difference in the expression of myositis between the two strains is in the effector phase of the disease. Since SJL/J mice have vasoactive amine-sensitive vascular systems and BALB/c do not, it is possible that activated splenocytes are able to emigrate from muscle microvessels in the SJL/J strain whereas they cannot do so in the BALB/c strain. Other studies in our laboratory indicate that the difference between SJL/J and BALB/c in terms of vasoactive amine sensitivity may be at the endothelial cell level. The most significant contribution of this model may be in its potential for addressing a sine qua non of cellular autoimmune disease--lymphocyte migration from the vascular compartment into the target tissue.

R 206 COMMON ANTIGEN - AN ANTIGEN COMMON TO A WIDE RANGE OF BACTERIA. FUNCTION AND ROLE IN PATHOGENESIS OF EXPERIMENTAL ARTHRITIS, Peter Hindersson, Jette Bangsborg, Klaus Hansen, Department of Treponematoses, Statens Seruminstitut, Amager Boulevard 80, 2300 Copenhagen.

Common antigen is a highly conserved antigen, expressed by more than 60 different bacterial species. We have cloned the Common antigen of three remotely related bacterial species (Treponema pallidum, Borrelia burgdorferi, Legionella micdadei). It has been shown that Common antigen has aminoacid homology with the so-called asm gene product of Escherichia coli which controls the stability of mRNA. We will present evidence that Common antigen belongs to the family of heat shock proteins. Recently it was shown that Common antigen of Mycobacterium bovis BCG stimulates a rat T-cell clone obtained from Lewis rats with adjuvant arthritis (induced by injection of Freunds complete adjuvant). This T-cell clone can transfer adjuvant arthritis to normal rats. Another T-cell clone recognizes the same T-celle epitope on Common antigen, but is able to protect against arthritis. The detailed investigation of Commmon antigen from other species will help to understand the pathogenic mechanisms underlying the adjuvant arthritis model and hopefully also help to understand human autoimmune arthritis.

R 207 $CD4^-CD8^-$ MYCOBACTERIA REACTIVE T-CELL CLONES FROM SYNOVIAL FLUID IN RHEUMATOID ARTHRITIS, Joseph Holoshitz and Samuel Strober, Stanford University, Stanford, CA 94305.

While synovial fluid (SF) T lymphocytes of rheumatoid arthritis (RA) patients respond poorly to T-cell mitogens such as PHA, Con-A or anti-CD3 antibodies, they show an increased proliferative response to mycobacterial antigens (Holoshitz et al. Lancet II: 305, 1986). In the present study, we isolated mycobacteria reactive T-cell clones from SFs of RA patients. A total of 34 clones were isolated from SF of two patients. These clones were found to be heterogeneous in respect to their proliferative responses, lymphokine secretion and surface phenotype. A subset of the mycobacteria reactive T-cell clones bore the CD2 and CD3 surface markers but were negative for both CD4 and CD8 and did not express the α/β heterodimer of the T-cell receptor, as judged by immunofluorescent staining with a series of monoclonal antibodies. They were also negative for CD5 (Leu 1) and Leu 8 surface markers. Although they did not secrete detectable levels of IL-2, they expressed IL-2 receptors upon activation with mycobacteria or mitogens. These clones showed a significant proliferative response to mycobacterial antigens, PHA, and anti-CD3 antibodies (Leu 4).

$CD4^-CD8^-$ cells have been recently implicated in the pathogenesis of murine autoimmune models. Experiments to study the fine antigenic specificity, HLA restriction, and the ability to help B-cells to produce antibodies will potentially indicate whether or not $CD4^-CD8^-$ mycobacteria reactive T-cell clones play a role in the pathogenesis of RA.

R 208 JOINT INFLAMMATIONS INDUCED BY A MURINE T CELL CLONE WITH THE HELPER PHENOTYPE

Ina S. Klasen, Renée M.T. Ladestein, Wim B. van den Berg* and Robbert Benner
Department of Cell Biology, Immunology and Genetics, Erasmus University Rotterdam, and
*Department of Rheumatology, University Hospital St. Radboud, Nijmegen, The Netherlands.

The antigen induced arthritis model (AIA) is a model for human reumatoid arthritis in which experimental animals (f.e. mice or rabbits) are immunized with an appropriate, positively charged, antigen and are challenged with the antigen in the knee joint. To examine the role of T cells in this model we have exploited a murine $MT4^{+}$, $Lyt\text{-}2^{-}$ T cell clone directed against the positively charged antigen methylated bovine serum albumin (mBSA). We were able to induce an arthritis by local or intravenous injection of this T cell clone and local injection of the antigen. Waned joint inflammations were capable of showing flare up phenomena after readministraiton of the antigen (J. Immunology 139: 3275 (1987)). It was also possible to induce these joint inflammations and flare up reactions in nude mice indicating that the T cells of the recipient mouse are not responsible for the inflammations measured. Moreover we have shown that the cloned T cells can, at least for some time, remain their functional capacity in a knee joint in the absence of the antigen. These data indicate a pivotal role for T cells in the induction as well as in the exacerbations observed in reumatoid arthritis.

R 209 THE T-CELL RECEPTOR α CHAIN IN AUTOIMMUNE ENCEPHALOMYELITIS.

Vipin Kumar, Jim Urban, Dwight Kono and Leroy Hood
California Institute of Technology, Pasadena, Ca 91125

MHC class II (Ia) restricted murine T-lymphocytes specific for the N-terminal peptide of myelin basic protein (MBP) mediate autoimmune encephalomyelitis in genetically susceptible animals. We have investigated the T-cell receptor repertoire against the N-terminal(9 amino acids) peptide of MBP in the PL/J ($H\text{-}2^{u}$) recombinant mouse strain. Full length cDNA clones were sequenced from a number of T-cell hybridomas. A majority of these hybridomas (80%) use a new subfamily of the variable region gene (Vα14). Both of the member of this subfamily (Vα14.1 & Vα14.2) are used . Northern blot and Southern blot analysis of the clones also revealed a predominant usage of the Vα14 gene. Surprisingly the junctional diversity of the T-cell receptor is also highly restricted in the autoimmune response.

R 210 CLONING OF ACTIVATED T CELLS FROM RHEUMATOID ARTHRITIS JOINTS: DETECTION OF COLLAGEN TYPE II SPECIFIC CELLS

M. Londei*, C.M. Savill+, P. de Berardinis*, A. Verhoef, F. Brennan, V. Duance++, R.N. Maini**, and M. Feldmann.
*Charing Cross Sunley Research Centre, London, W6 8LW, **KIR, London, W6 7DW. + ICI plc, ++ AFRC Bristol.

Rheumatoid arthritis (RA) is an autoimmune disease characterised by T cell infiltration of the synovium of joints. Analysis of the phenotype and antigen specificity of the infiltrating cells may provide insight into the pathogenesis of RA. In this study the phenotype of infiltrating T cells in the synovial membrane of an individual with RA was compared to those in blood. This revealed an imbalance of T cell subsets in the membrane, with a predominance of CD4 CDW29 (helper inducer) cells, a lack of CD4 CD45R (suppressor-inducer) cells, and an increase in $CD3^{+}CD4^{-}8^{-}$ $Leu19^{-}$ cells, which use the γδ T cell receptor. T cells were cloned using a procedure which selects for in vivo activated cells, using Interleukin-2. All clones had the CD4 CDW29 phenotype. Using a panel of candidate auto antigens, the antigen specificity of the clones was tested, and 4 of 17 reacted against autologous blood mononuclear cells. Two clones proliferated in response to collagen type II. After two years, another set of clones was derived from synovial tissue of the same joint, 1 of 8 clones tested showed a strong proliferative response against collagen type II. These data indicate that in the synovial membrane there is an imbalance of the T lymphocytic populations and suggest that collagen type II could be one of autoantigens involved in perpetuating the inflammatory process in RA.

R 211 THE ROLE OF THE IDIOTYPIC NETWORK IN THE INDUCTION OF EXPERIMENTAL SLE.

Edna Mozes[1], Stefan Brocke[1], Yehuda Shoenfeld[2] and Shlomo Mendlovic[1], [1]The Weizmann Institute of Science, Rehovot 76100, [2]Ben-Gurion University, Beer-Sheva, Israel.

We have recently reported the induction of systemic lupus erythematosus (SLE) in a murine strain (C3H.SW) that normally does not develop any immune disorders. The disease was induced by the immunization of the mice with a human monoclonal anti-DNA antibody, that bear a common idiotype-16/6 Id. A study performed on different mouse strains indicated that whereas SLE could be induced in most strains, C3H/HeJ and C57BL/6 mice did not respond to the 16/6 Id and were resistant to the induction of the disease. In order to study whether anti-idiotypic antibodies are involved in the induction of the disease, a murine monoclonal antibody against the 16/6 Id was prepared and injected into C3H.SW mice. Following immunization, high titers of antibodies to ssDNA, dsDNA, poly(I), poly(G), RNP, SS-A (Ro) and SS-B (La) were detected. Murine antibodies that bear the 16/6 Id were also obtained. Four months after immunization, significant proteinuria, leukopenia and elevated erythrocyte sedimentation rate were observed. The histological examination of the kidneys disclosed hypercellularity in the glomeruli and fusion of the visceral and parietal layers of the Bowman's capsules. It should be noted that the kidney pathology in the anti-16/6 Id injected mice was more pronounced than that observed in the 16/6 Id immunized mice. None of the above signs could be found in mice injected with a control monoclonal anti-idiotypic antibody against antibodies to the synthetic antigen (T,G)-A--L. These results suggest the involvement of the idiotypic network in the pathogenesis of this disease.

R 212 HLA-RESTRICTED RECOGNITION OF THE PRIMARY IN VITRO HUMAN ANTIBODY RESPONSE.

Bromberg, Jonathan S., Thurtle, Phillip S., and Nepom, Gerald T. Immunology Program, Virginia Mason Research Center and Department of Surgery, University of Washington, Seattle, Washington.

We have developed an assay for a primary, in vitro human antibody response to the T dependent antigen TNP-KLH in order to analyze genetic and regulatory signals in a primary response. PBLs in microculture are stimulated with antigen for 4 days and TNP specific antibody is collected in subsequent culture in the absence of antigen. The TNP specific response is dependent on a CD3+4+ T-cell population which can be depleted with specific antibody plus complement. Anti-CD3 without complement is also able to block the response suggesting that the T-cell antigen receptor is important for the function of this putative helper T-cell. Response is demonstrable in over 75% of a large population of normal HLA-typed PBL donors. Correlations between HLA specificity and responder/nonresponder phenotype are being investigated. Evaluation of MHC restrictions in a variety of individuals with a large number of mono- and polyspecific anti-HLA antibodies suggests that [1] the response is MHC class II restricted primarily by DR (in a codominant fashion) and to a lesser extent by DQ subregions, and [2] that a complex hierarchy of class II presentation for TNP-KLH exists with the involvement of other regulatory systems active in the polyclonal antigen-specific response such as Ir and Is gene mediated help and suppression and antibody mediated activation or tolerance.

R 213 T CEL AUTOREACTIVITY IN PSORIASIS

E.P. Prens, K. Benne, Th. van Joost and R. Benner; Departments of Dermatology and Immunology, Erasmus University Rotterdam, The Netherlands.

Psoriasis is a skin disease, with unknown aetiology and pathogenesis, affecting approximately 2% of the Western population. Recently activation and proliferation of T lymphocytes upon stimulation with autologous epidermal cells from psoriatic skin has been described (Schopf et al, 1986). We reproduced these studies and investigated the cellular requirements for the autologous mixed epidermal cell - T lymphocyte reaction (MECLR). Methods: A single epidermal cell suspension (ECS), of unvolved and univolved psoriatic skin, was made by enzymatic digestion and next passed through 100u and 30u mesh nylon gauzes. T lymphocytes were isolated by rosetting twice with AET treated sheep red blood cells. The ECS was depleted of Langerhans cells by complement lysis and by immunomagnetic separation. Monocytes were obtained by plastic adherence. Enrichment for dendritic cells was performed by panning the monocyte fraction on human IgG coated dishes. Results: Peripheral blood mononuclear cells as well as purified T lymphocytes from psoriasis patients showed a clear proliferative response to autologous epidermal cells from involved as well as uninvolved skin. In a control group of healthy persons and patients with contact allergy no significant T cell proliferation was observed in the autologous MECLR. Antigen presenting cells in the skin contributed most to this (HLA-DR restricted) response, peripheral blood monocytes and dendritic cells had an additive effect. Depletion of (T6+) Langerhans cells from the ECS did not result in a significantly decreased proliferation. Conclusion: These data are in favor of a T cell mediated autoimmune response in psoriasis.

R 214 "AUTOREACTIVE" T CELL CLONES SPECIFIC FOR CLASS I AND CLASS II HLA ANTIGENS ISOLATED FROM A HUMAN CHIMERA.
Maria Gracia Roncarolo[1], Hans YsseL[1], Jean-Louis Touraine[2], Hervé Betuel[3], Jan E. de Vries[1], Hergen Spits[1]. [1]UNICET, Laboratories for Immunological Research, 69570, Dardilly, France. [2]INSERM U 80, Hôpital Ed. Herriot, 69374, LYON, France. [3]Blood Transfusion Center, 69342, Lyon, France.

T cell clones of donor origin which specifically react with recipient cells were obtained from a SCID patient successfully reconstituted by allogeneic fetal liver and thymus transplantation performed ten years ago. The majority of these clones displayed both cytotoxic and proliferative responses towards peripheral blood lymphocytes (PBL) and an EBV transformed B cell line derived from the patient. In addition, these T cell clones had proliferative and cytotoxic responses towards parental PBL, EBV transformed B cell lines and PHA blasts. Blocking studies with anti-class I and anti-class II HLA mAbs indicated that the activity of the $CD4^+$ T cell clones was specifically directed against class II HLA antigens of the recipient. On the other hand, the cytotoxic and proliferative responses of the $CD8^+$ T cell clones were specific for class I HLA antigens which are widely expressed on the recipient cells.
Thus the establishment of transplantation tolerance observed in this stable human chimera is not due to the elimination of host-reactive T cells from the repertoire and suggests the presence of a peripheral autoregulatory suppressor mechanism.

Effector Pathways of Immunity and Hypersensitivity

R 300 MODULATION OF IL-4 INDUCED HUMAN IgE PRODUCTION IN VITRO BY IFN-γ AND IL-5:THE ROLE OF SOLUBLE CD23 (S-CD23), Jan E. de Vries[1], Jérome Pène[1], Françoise Rousset[1], Francine Brière[1], Jean Y. Bonnefoy[1], T. Yokota[2] and K. Arai. [1]Unicet Laboratory fo rImmunological Research, Dardilly, France, [2]DNAX Research Institute, Palo Alto.

IL-4 specifically induces IgE production by peripheral blood lymphocytes from healthy donors. This IL-4 induced IgE synthesis is T cell and monocyte dependent and is blocked by IFN-γ, IFN-α and prostaglandin E-2 (PGE-2).These substances also inhibited IL-4 induced CD23 expression and subsequent release of soluble CD23 (S-CD23). In addition, IgE production was blocked by $F(ab)'_2$ fragments of a Mab against CD23. In contrast, IL-5 enhanced IL-4 induced IgE production provided Il-4 was added at non-saturating concentrations. The increase in IgE production correlated quantitatively with an enhanced release of S-CD23. These results indicate that there is a correlation between S-CD23 release and IgE production. However, S-CD23 fractionated from supernatants of the lymphoblastoid cell line RPMI-8866 were ineffective in inducing IgE production in the absence of IL-4, but synergized with suboptimal concentrations of IL-4. Collectively our findings indicate that IL-4 induced IgE production involves complex interactions of T cells, B cells an monocytes and is positively or negatively modulated by IL-5 and S-CD23 or IFN-γ, IFN-α and PGE-2, respectively.

R 301 ANTI-CD3-STIMULATED RELEASE OF ARACHIDONIC ACID FROM T LYMPHOCYTES: COMPLEXING WITH Gc PROTEIN IN THE LIPID BILAYER AND AFTER RELEASE, R.M. Galbraith, M.H. Williams, and G.M.P. Galbraith, Medical University of SC, Charleston, SC 29425.
Modulation of the CD3-Ti antigen receptor Induces phospholipase-mediated hydrolysis of arachidonic acid (AA), and several eicosanoid metabolites have potent effects on T cells. Although activities of cyclo-oxygenase and lipoxygenase in T cells are extremely low, released AA can be metabolized by other cell types eg monocytes. One factor of probable importance is protein-binding, since fatty acids do not generally occur in free form. Normal peripheral blood lymphocytes depleted of monocytes were labeled with [^{3}H]-AA or [^{14}C]-AA and stimulated with either anti-CD3, or Ca^{2+} ionophore A23187 - a prototypic phospholipase activator. Thin layer chromatography (TLC) showed hydrolysis of AA predominantly from phosphatidylcholine (PC). SDS-PAGE of cell supernatants showed two radiolabeled proteins, MW 56K and 68K, identified by Western blotting as Gc protein and albumin, but the majority of released [^{3}H]-AA (60-70%) co-precipitated with Gc. When detergent solubilized extracts of unstimulated cells were immunoprecipitated with anti-Gc, TLC analysis identified 40-50% of [^{3}H]-AA brought down in phospholipid spots (PC, PI AND PS), indicating interaction of Gc with AA in situ prior to hydrolysis. Finally, complexing of [^{3}H]-AA with Gc inhibited cellular uptake by 80-95% in T cells, monocytes and neutrophils, even at 0.4 μg ml^{-1} (1000-fold below physiological). Thus, in T cells AA is largely complexed with Gc, both in the lipid bilayer and following release. This interaction could modulate secondary uptake and metabolism of AA.

R 302 PRODUCTION OF BSF-2/IL-6 BY SYNOVIOCYTES AND ITS MODULATION BY OTHER CYTOKINES,
Pierre-Andre Guerne, John H. Vaughan, Dennis A. Carson, Martin Lotz. Research Institute of Scripps Clinic, La Jolla, CA 92037.
Interleukin-6 (IL-6) has been recently characterized as a mediator of multiple inflammatory and immune responses. In addition to its effects on differentiation of B lymphocytes, it induces acute phase protein synthesis in hepatocytes. We have shown that IL-6 is also involved in T cell activation and have identified hepatocytes and endothelial cells as sources of IL-6. In this study, we examined the presence of IL-6 in synovial fluids and its production by synoviocytes. The results showed that inflammatory synovial fluids (rheumatoid arthritis [RA], Reiter's disease, psoriatic arthritis, seronegative arthritis), as well as osteoarthritis (OA) synovial fluids, all contain high levels of IL-6 activity as measured by the induction of IgG secretion in the EBV-transformed B cell line CESS. Furthermore, RA and OA synoviocytes spontaneously release IL-6 *in vitro*. This release decreases with the number of passages. IL-1, tumor necrosis factor-α and lymphotoxin all are able to increase IL-6 production, even at late passages. Monosodium urate, inorganic pyrophosphate, and hydroxyapatite crystals also are able to increase the release of IL-6 into synoviocyte supernatants. These results clearly demonstrate that synoviocytes, which are composed of fibroblast-like and macrophage-like cells, are a potent source of IL-6 and may contribute to the high level of this cytokine present in joint fluids. Locally, this IL-6 activity is likely to play an important role in activation of T and B lymphocytes; systemically, IL-6 probably contributes to the hypergammaglobulinemia and increased acute phase protein synthesis that are characteristic of patients with inflammatory arthropathies.

R 303 THE DEFECTIVE MACROPHAGE IN LEPROSY : EVIDENCE OF A DECREASED RESPONSE TO TUFTSIN CORRELATES WITH AN ABERRANT DIFFERENTIATION PROFILE. Ravi R. Iyer*, H.Krishna Prasad, D.Nageshwara Rao* and Indira Nath, Departments of Biochemistry* & Biotechnology, All India Institute of Medical Sciences, New Delhi, INDIA.
Defective macrophage function has been observed in lepromatous leprosy & various attempts are underway to activate these cells. We studied the phagocytic & microbicidal functions of blood monocyte/macrophages(Mø) from normal,tuberculoid(BT/TT) & lepromatous(BL/LL) subjects after varying periods(6h-14days),of in vitro culture against S.aureus, M.tuberculosis(H37Ra) & M.leprae. Both radiometric & conventional Microscopic/CFU assay systems were used & the experiments were designed to test the capability of the Mø to be stimulated by the Mø-stimulator, TUFTSIN. Validity of the stimulation seen was confirmed by elimination of non-specific opsonic effects and documentation of morphologic changes by SEM. The results indicate that BL/LL Mø exhibit an aberrant differentiation profile characterised by a decreasing response to Tuftsin stimulation with increasing age of in vitro culture such that 14day cultures were totally refractory at upto 10 x the optimal dose of Tuftsin(0.8 uM), for both test parametres. Normal & BT/TT Mø exhibited an increasing phagocytic response with increasing age while the microbicidal boost begins to taper off in the older culture The basal rates of both functions, however, appear to remain adequate in all 3 group irrespective of culture age. BL/LL Mø however, were able to be stimulated to inhibit M.leprae DNA synthesis when pulsed daily with 0.8 uM Tuftsin for 11-12 days. This effect was seen even in 14day-Mø & ranged from 80-140% inhibition (controls= 0%).

R 304 ALTERNATIVE PATHWAYS IN ACTIVATION OF CYTOTOXIC T-LYMPHOCYTE HYBRIDOMAS.
Yael Kaufmann and Tali Ozeri, Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel 52621.

The possible existence of alternative pathways for cytotoxic T-lymphocytes (CTL) activation was examined, using memory-like CTL-hybridomas with inducible cytolytic activity. These hybridomas are induced by antigen (Ag)-presenting cells to secrete IL-2 and to lyse specific target cells. Proliferation of the hybridomas, which do not express receptors for IL-2, is partially blocked during Ag-stimulation. Several inducers were tested for their ability to trigger clones of the CTL-hybridomas to express their functional reactivities. Two groups of activators were detected. The first type, which includes Ag, T-cell mitogens (Con-A, PHA) and the anti-Thyl Ab G-7; induced both cytolytic activity and IL-2 production. The second type, which includes interferon (IFN)$\alpha\beta$ and IFNγ, induced specific killing activity but not IL-2 secretion. Both types of inducers inhibited the proliferation of the CTL-hybridomas. The data suggest that induction of cytotoxicity in memory-CTL can be triggered by cell surface molecules other than the T-cell receptor (TcR), such as Thyl and the two IFN-receptors. The two modes in which cytotoxicity can be triggered, with or without concomitant induction of IL-2 secretion, points to a possible alternative pathway in which IFN$\alpha\beta$ and IFNγ operate in activating cytotoxicity.

R 305 CYTOTOXIC CELL AND LYMPHOTOXIN INDUCED RELEASE OF HISTONES IN TARGET LYMPHOMA CELLS, Marc C. Levesque, Nancy H. Ruddle, Yale University Medical School, New Haven, CT 06510.
Studies on the mechanism of cytotoxic cell (CTL,NK cell) and lymphokine (LT,TNF) mediated target cell killing have shown that DNA fragmentation is an early event in this type of cell death. This mechanism of killing is distinctly different from that mediated by complement and antibody, in which DNA fragmentation does not occur. The length of the DNA fragments produced after CTL or LT induced killing is similar to that segment of DNA surrounding the nucleosome core. Heretofore, little was known concerning release of other cellular components during this type of cell death. We have shown release of histones following cytotoxic cell and LT mediated killing using mouse lymphoma cells (BW5147.3,YAC-1) as targets. The kinetics of histone release are similar to those previously observed when DNA release was studied. Complement and antibody treatment does not show a similar pattern of histone release. Our objective is to further characterize release of histones in conjunction with DNA fragmentation, and test for other nuclear antigen release during the cytotoxic process. We are interested in the relationship that this mechanism of killing may have to the generation of autoimmune responses, particularly in systemic lupus erythematosus (SLE), in which autoantibodies to histones are prominent.

R 306 REGULATION OF CLASS II EXPRESSION AND ACCESSORY CELL FUNCTION IN A THYROID EPITHELIAL CELL LINE, Robert B. Levy, Robert J. Sigillito, Steven Miller, Jean C. Zegaldo and Arthur Alamo, Univ. of Miami, Miami, Fl. 33101.
Some thyroid epithelial (TE) cells from Grave's patients have been found to express MHC class II molecules. In the present experiments MHC expression (indirect immunofluorescence followed by flow cytometric analysis) and accessory function of a well characterized rat TE line, FRTL(5) were examined. Several treatments with recombinant lymphokines and thyroid stimulating hormone (TSH) were found to increase MHC class I and induce MHC class II (Ox-6) expression. Class II was induced most to least efficiently by the following: high dose gamma interferon (rIFN-G) + tumor necrosis factor (rTNF); high dose rIFN-G + TSH ; low dose rIFN-G + TSH, low dose rIFN-G + rTNF and high dose rIFN-G alone (the latter three induced approximately equivalent expression). Neither low dose rIFN-G, TSH, rTNF nor rIL-4 alone induced class II expression although the latter is known to be inductive for these products on other cell types. Accessory cell function was examined using MHC matched rat lymph node cells following nylon and double G10 column passage. Class II negative as well as rIFN-G +/- rTNF treated FRTL cells functioned efficiently to reconstitute ConA responses. The findings indicate that regardless of class II expression TE cells can provide the required accessory function to enable polyclonal T-cell activation by mitogen. The ability of various reagents together with TSH to induce class II expression on TE may, together with other factors, enable such cells to play a role in antigen presentation.

R 307 LYMPHOTOXIN PRODUCTION BY ENCEPHALITOGENIC T CELL CLONES, Marianne Broome Powell, Jennifer Lederman, Dennis Mitchell, Scott Zamvil, Nancy Ruddle* and Larry Steinman, Stanford University, Stanford, CA and *Yale University, New Haven, CT.
We are investigating the possibility that lymphotoxin (LT or tumor necrosis factor-β) is important in initiating & perpetuating the autoimmune disease, experimental allergic encephalomyelitis. EAE caused by immunization with the autoantigen, myelin basic protein (MBP) is often cited as a model for multiple sclerosis. Both share such features as relapsing paralysis and demyelination within the CNS. Recent studies have shown that L3T4+ T cell clones specific for MBP can mediate EAE. These clones recognize the N-terminal 11 amino acid residues of MBP in association with I-A^u class II molecules. When injected back into PLJ or PLJxSJL F1 mice, the T cells cause disease. Activation of the encephalitogenic clones in vitro with peptide plus syngeneic feeder cells or with concanavalin A results in the production of significant amount of LT (4-256U) as well as interleukin-2 (15-500U). The presence of these two lymphokines as well as TNF-α and interferon-γ are detected using both biological assays and gene expression. In EAE, LT can act at two different stages; as an effector molecule abeiting the pathogenesis or as as afferent molecule facilitating passage of the T cells into the CNS.

R 308 STRUCTURE-ACTIVITY ANALYSIS OF MODIFIED AND TRUNCATED FORMS OF THE TAC RECEPTOR PROTEIN. SITE-SPECIFIC MUTAGENESIS OF CYSTEINE RESIDUES. Cynthia M. Rusk, Michael P. Neeper, Li-Mei Kuo, Rusty M. Kutny and Richard J. Robb. E. I. du Pont de Nemours and Co., Glenolden Laboratory, Glenolden, PA 19036.
Receptors for interleukin 2 (IL 2) on activated lymphoctyes contain the Tac protein. As part of an effort to characterize this molecule, we examined the structure-activity relationship for each of its 12 Cys residues. A preliminary map of intramolecular disulfide bonding was derived by analysis of cystine-linked enzymatic fragments of the Tac protein. The results indicated that disulfide bonds linked Cys 3 with Cys 147, Cys 131 with Cys 163 and Cys 28,30 with Cys 59,61. The contribution of the Cys residues to an active protein conformation was tested by site-specific mutagenesis, followed by expression of the modified molecules in murine L cells. The results indicated that Cys 192 and 225 could be replaced without affecting ligand binding. In contrast, modification of any of the other 10 Cys residues, either singly or in combinations corresponding to the predicted disulfide bonds, greatly reduced the ability of the expressed protein to bind IL 2 or either of two monoclonal antibodies (anti-Tac and 7G7/B6) which recognize the Tac protein. Each of the latter mutations also partially interfered with the molecule's posttranslational modification and cell-surface expression. Consistent with these findings, transfection of the L cells with vectors containing truncated Tac cDNA inserts resulted in secretion of Tac fragments capable of ligand binding when the polypeptide chains terminated after Cys 163 (the tenth Cys residue in the full-length molecule), but resulted in inactive fragments of Tac which were poorly secreted when they terminated prior to Cys 163. These findings thus emphasize the remarkable sensitivity of the active conformation of the Tac molecule to intramolecular disulfide bonding.

R 309 T SUPPRESSOR FACTOR EFFECTS ON EARLY EVENTS OF T CELL ACTIVATION, L. A. Schwarz and S.S. Rich, Baylor College of Medicine, Houston, TX 77030.
Mixed leukocyte response T suppressor factor(MLR-TsF) is an immunoregulatory lymphokine produced by alloantigen-restimulated murine Thy1$^+$ CD8$^+$ lymphocytes. MLR-TsF suppressed proliferation of MLR as well as of the IL2-dependent HT2 cell line is not overridden by exogenous IL2. Using HT2 cells as a model, MLR-TsF effect on IL2 receptor expression and post-receptor events was examined. MLR-TsF reproducibly decreased high affinity IL2 receptor expression but this decrease failed to exclusively account for the degree of MLR-TsF-suppressed HT2 proliferation. Phosphotidylinositol(PI) hydrolysis, releasing 1,4,5-inositol tris phosphate (IP$_3$) and diacylglycerol, occurs post-IL2-receptor interaction. Calcium ionophore, ionomycin and/or phorbol ester, PMA, which mimic signals initiated by IP$_3$ and DAG, respectively, could not override MLR-TsF-induced suppression, indirectly suggesting that MLR-TsF does not interfere with PI turnover. Activation of adenylate cyclase (AC) through cAMP agonists and analogs also regulates IL2-induced proliferation. MLR-TsF effect on cAMP accumulation and on proliferation were compared in HT2, and in cAMP-sensitive S49 wild type (WT) thymoma, as well as cAMP-agonist insensitive S49 cyc$^-$ and kin$^-$ mutants, which lack G$_s$ required for stimulation of AC and effective cAMP-dependent kinase, respectively. cAMP accumulation was not induced in MLR-TsF-treated cyc$^-$ cells, through G$_s$ dependent or independent mechanisms. However, MLR-TsF dose-dependently suppressed proliferation of cyc$^-$ and kin , as well as S49 WT. These results suggest that MLR-TsF-induced suppression of proliferation occurs independently of cAMP modulation. Supported by NIH grant AI21420.

R 310 SUPPRESSION OF MURINE IFN-γ RNA MESSAGE BY MLR T SUPPRESSOR CELL FACTOR, J.G. Smith, K.J. Hardy, and S.S. Rich, Baylor College of Medicine, Houston, TX 77030.
Mixed leukocyte response T-cell suppressor factor (MLR-TsF) is the product of alloantigen primed Thy-1+, CD8+ lymphocytes. MLR-TsF suppresses proliferation in an MLR, and recently it has been shown to suppress the proliferation of Con-A treated splenocytes in a dose dependent manner. This suppression is not reversed by the addition of exogenous recombinant IL-2, suggesting that MLR-TsF does not directly affect IL-2 or IL-2 receptor binding. Rather, MLR-TsF appears to regulate proliferation through post IL-2 receptor initiated activation events. The present studies have been directed to the analysis of the effects that MLR-TsF may also impose on lymphokine production. As an initial approach, the effect of MLR-TsF on the steady state levels of RNA message of IFN-γ, IL-2, as well as IL-2 receptor has been examined in Con-A activated splenic T-cells. MLR-TsF decreases the amount of message for IFN-γ essentially to baseline levels. In contrast, message for IL-2, as well as for IL-2 receptor and actin, was unaffected. This inhibitory effect on IFN-γ message is dose dependent and maximal after 24 hours of culture in the presence of MLR-TsF. This is the first observation of a natural product which demonstrates independent down regulation of the amount of steady state message for IFN-γ in the absence of coordinate regulation of message for IL-2 and its receptor. Supported by NIH grant AI 21420.

R 311 HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) ENHANCES THE RESPIRATORY BURST RESPONSE BY HUMAN MONOCYTES. P.D. Smith, C.L. Lamerson, L.M. Wahl and S.M. Wahl. NIH, Bethesda, MD.

GM-CSF regulates the in vitro proliferation and differentiation of hemopoietic progenitor cells, and its administration to patients improves hematopoiesis and reduces the rate of infections. To determine whether the latter effect of GM-CSF might be due to increased production of respiratory burst microbicidal products, we investigated the effect of recombinant human GM-CSF (rhGM-CSF) on spontaneous and stimulated superoxide anion (O_2^-) production by human monocytes. Monocytes purified by countercurrent centrifugal elutriation were incubated in suspension overnight with varying doses (5-500 units/ml) of rhGM-CSF and then assayed for O_2^- production by cytochrome c reduction in the presence of media or optimal doses of phorbol myristate acetate (PMA), gamma interferon (γIFN), f-met-leu-phe (fMLP) or lipopolysaccharide (LPS). Monocytes preincubated with rhGM-CSF spontaneously secreted five-fold greater amounts of O_2^- than control monocytes (2.8 vs 0.5 nmoles O_2^- /1x10^5 monocytes, p<.005). Similarly, monocytes preincubated with rhGM-CSF exhibited significantly greater secretion of O_2^- following stimulation with PMA, γIFN, FMLP or LPS (p<.005). Enhanced O_2^- secretion was dose-dependent and specific for rhGM-CSF and macrophage-CSF but not granulocyte-CSF or interleukin-3. In addition, monocytes preincubated with rhGM-CSF exhibited a two-fold increase in cytotoxic activity toward Candida albicans. These studies are the first to document that rhGM-CSF enhances the respiratory burst activity of human monocytes and suggests that GM-CSF-stimulated monocytes have enhanced fungicidal potential.

R 312 *Abstract Withdrawn*

Inflammation, Clinical Hypersensitivity and Autoimmunity

R 400 GENETIC ASSOCIATION OF GRAVES' DISEASE AND IMMUNOGLOBULIN GENES. B. Blomberg, J. Chao, M. Zakarija and J. M. McKenzie, Departments of Microbiology/Immunology and Medicine, University of Miami School of Medicine, Miami, FL 33101.

The effector molecule in the autoimmune disorder, Graves' disease, is an immunoglobulin of limited heterogeneity. We and others have previously determined that the thyroid stimulating antibody (TSAb) is IgG and almost always (about 90 %) of the λ light chain type. For these reasons we decided to look for genetic association of either the heavy chain or λ light chain with Graves' disease by restriction fragment length polymorphism (RFLP) for these loci. Southern blots of peripheral blood monocyte DNA from Graves' patients and control subjects were probed with either a heavy chain "switch" region DNA fragment which detects several polymorphic regions within the heavy chain gene complex or with a λ constant region fragment hybridizing to all λ light chain genes. The λ probe also detects an RFLP but the polymorphism is not as extensive or informative as in the case of the heavy chain. In a preliminary study we found that 17 of 24 patients (71%) and only 3 of 14 (21%) controls showed a 6.8 kb fragment with the heavy chain probe (P<0.001). No genetic association was found for the λ light chain locus. We are currently analyzing sex-, race- and age- matched controls, patients with other thyroid abnormalities (e.g., Hashimoto's disease, thyroid nodule) and families with Graves' disease.

Molecular and Cellular Mechanisms of Human Hypersensitivity and Autoimmunity

R 401 IMMUNOMODULATION BY BROMOCRIPTINE. Joan M. Chapdelaine, Thomas C. Warren, Janey D. Whalen, Peter M. Grob and Paul H. Wooley, Wyeth-Ayerst Laboratories Research Inc. Princeton, NJ 08540.
Several groups have reported that occupancy of prolactin receptors on lympocytes by prolactin is necessary for immunocompetence. Bromocriptine (BC), a dopamine agonist that specifically blocks the release of prolactin from the pituitary gland, has been shown to lessen the severity of adjuvant arthritis, experimental autoimmune encephalitis, and autoimmune uveitis in rats. Lymphocytes obtained from mice treated with BC exhibited a diminished proliferative response in mixed lymphocyte cultures and to Con A. Spleen cells from the BC treated mice were also found to effectively suppress the response of normal spleen cells to Con A. In addition, when thymocytes from the treated mice were tested in the LAF assay, it was found that the response to IL-1 was markedly reduced while the response to IL-2 was normal. BC was also effective in preventing collagen-induced arthritis in mice. Treatment of DBA/1 Lac J mice with 100 mg/kg/day reduced the incidence of disease from 57% in the control group to 17% in the drug treated group ($p < 0.01$). BC also induced suppressor cells in vitro without pituitary gland involvement. Furthermore, there was no correlation between lowered prolactin levels and decreased immune response suggesting that BC is acting via a non-prolactin mediated mechanism.

R 402 SOLUBLE INTERLEUKIN-2 RECEPTOR IN MULTIPLE SCLEROSIS, Steven J. Greenberg, Luisa Marcon, Barrie J. Hurwitz, Robert H. Christenson, Thomas A. Waldmann, and David L. Nelson, NCI, National Institutes of Health, Bethesda, MD 20892 and Duke University, Durham, NC 27710.
The immunopathologic process leading to inflammatory demyelination of the CNS in multiple sclerosis bears some attributes of a viral infection. Human T cell leukemia virus type I (HTLV-1), associated with an aggressive T cell malignancy, adult T cell leukemia (ATL), is also linked to a progressive spastic paralytic condition, tropical spastic paraparesis (TSP). The Tac peptide, a subunit of the interleukin-2 receptor (IL-2R), is released in soluble form and its level is elevated in the supernatants of HTLV-1 infected T cell lines and the serum of patients with ATL via trans-activation by the viral tat protein. Sera and CSF were evaluated by an ELISA technique to determine whether elaboration of soluble Tac peptide was similarly induced in MS. The level of soluble Tac peptide in serum from 28 MS patients (mean:465 U/ml) was significantly elevated above normal controls (n=17, mean:257 U/ml) and HTLV-1 seropositive healthy carriers (n=8, mean:281 U/ml), $p<0.01$, but significantly different from the vastly elevated levels in ATL (n=6, mean:100,083 U/ml), $p<0.005$. Elevated soluble Tac peptide levels were found in serum but not in CSF of MS patients and may reflect peripheral activation of IL-2R expression.

R 403 *IN VITRO* **EFFECTS OF PLASMA NUCLEIC ACIDS ISOLATED FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE), Martin Herrmann, Werner Leitmann, Friedel E. Krapf and Joachim R. Kalden, Institute for Clinical Immunology and Rheumatology, University of Erlangen-Nürnberg, D-8520 Erlangen, FRG.**
Coincubation of a human EBV-immortalized B-cell line (B62) was performed with nucleic acids (PNA) isolated from plasma of SLE patients, which could not be gained from various other patients'plasmas. The DNA part of the PNA, migrating as a 20 kbp molecule, was previously characterized by molecular cloning and shown to partially contain retrovirus-like sequences as could be demonstrated by a strong homology of PNA clone E6 to the 5'-terminal pol-region of HIV-1. In cells treated with PNA (B62/SLE), and not in controls (B62/-), syncitia formation and blastoid vacoulization occured. An additional nucleic acid species, as compared to B62/- controls, comigrating with 20 kbp linear DNA was detected in B62/SLE cells. The molecule was visualized by gel electrophoresis and autoradiography after 4 h growth of cells in medium supplemented by ^{14}C-uridine and proved to be alkali labile. Probing with PNA clone E6 showed homologous DNA targets only in B62/SLE nucleic acid extracts. Distinctively B62/SLE cells could be stained by an antiserum against feline leukemia virus. A population of approx. 1/500 of B62/SLE cells showed a levamisole-resistant alkaline phosphatase activity located in the cytoplasm. A myrystilation pattern of cellular proteins was determined showing distinct bands, reminiscent of HIV-1 myrystilated products, present only in B62/SLE cells. The clonal integrity of both cell types was confirmed by an IgM- and an anti-idiotype ELISA, which, in addition, revealed reduced levels of IgM synthesis by B62/SLE cells in comparison to controls. In summary, the results suggest the association of PNA from SLE patients with the observed cell alterations; the possible involvement of a retroviral agent in pathogenetic events of SLE will be discussed.

R 404 INTERFERON γ AND ADJUVANT ARTHRITIS. INSIGHT INTO RHEUMATOID ARTHRITIS PATHOGENESIS? Chaim O. Jacob, Joseph Holoshitz, Samuel Strober, and Hugh O. McDevitt, Stanford University, Stanford CA 94305.

The role of IFN-γ in rheumatoid arthritis (RA) is unclear. In an attempt to evaluate the role of IFN-γ in autoimmune arthritis, we tested the effect of IFN-γ and monoclonal anti-IFN-γ antibody (DB1) in various phases of arthritis development in a rat model for RA, the adjuvant arthritis (AA) model, induced by complete Freund's adjuvant (CFA). In addition, the effects of IFN-γ were tested in vitro on T-cell clones derived from rats afflicted with AA. T-cell clone A2b which has been shown to be arthritogenic (Holoshitz et al.J.C.I. 1984) secreted very low amounts of IFN-γ and its antigen specific proliferation was slightly inhibited by IFN-γ. In contrast,clone A2c which can inhibit the development of AA produced high amounts of IFN-γ and its proliferation was slightly increased by IFN-γ. In vivo administration of IFN-γ 24 h prior to CFA caused a marked enhancement of arthritis, while giving IFN-γ 24 to 48 h after CFA suppressed the disease. Administration of IFN-γ between day +4 to+12 increased the severity of the first phase of the disease but had no effect later. Administration of DB-1 two days before CFA or between day +4 to +8 substantially decreased the disease while DB1 given from day +12 to +21 significantly enhanced it. These results illustrate the heterogeneity of the effects of IFN-γ in autoimmune arthritis and may explain the conflicting reports regarding the effects of IFN-γ in autoimmune diseases.

R 405 CYTOKINE-STIMULATED PROLIFERATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBM) FROM NEW-ONSET TYPE I DIABETICS. Douglas J. Kawahara, Bruce Buckingham, Christy Sandborg, Monique Berman and Ann Kershnar. Childrens Hospital of Orange County, Orange, CA 92668, and University of California, Irvine, CA 92717.

Peripheral blood mononuclear cells (PBM) were obtained from 54 individuals: 24 new-onset type I DM; 3 during remission; 11 long-term type I DM, 8 pediatric controls, 5 type II DM and 3 adult controls. The proliferation of PBM in the presence of exogenous human 30K MW interleukin 1 (30K IL-1) was significantly higher in new-onset type I DM than in age-matched controls. Both new-onset type I DM and controls were stimulated to the same degree by 17K MW recombinant human IL-1β (rIL-1). The 30K IL-1 responsiveness did not correlate with the presence or absence of islet cell cytoplasmic antibodies (ICA), or the metabolic status of the new-onset type I DM at the time of diagnosis. Because 2 of 5 type II DM were insulin-dependent, it is unlikely that insulin therapy has an effect on the IL-1 proliferative response.

These results indicate the presence of PBM, presumably T cells, in type I DM at the time of diagnosis and during remission that are in a very early stage of activation. This would be consistent with the active induction of newly responsive T cells during stages of DM when islet cells are present. This response is not the result of the metabolic aspects of DM but a reflection of the immunologically activated state in type I DM.

R 406 INFLAMMATORY ACTIVATED HUMAN SYNOVIAL FLUID AND GLYCOGEN ACTIVATED RABBIT PERITONEAL FLUID POLYMORPHONUCLEAR LEUKOCYTES (PMN) SYNTHESIZE AND SECRETE THROMBOSPONDIN, Monique La Fleur, Christophe Kreis, Claire Ménard and André D. Beaulieu, Inflammation and Immunology-Rheumatology Research Unit, Université Laval, Quebec, Canada.

Proteins synthesized and secreted by activated PMN have not been well characterized. We have previously shown that inflammatory activated PMN from synovial fluid of patients with Rheumatoid and Psoriatic Arthritis synthesize increased amounts of fibronectin when compared to non-activated PMN (J. Biol. Chem. 262:2111-2115, 1987). In the present study, we have discovered that synovial fluid PMN also synthesize increased amounts of thrombospondin as do glycogen activated rabbit peritoneal fluid PMN. Thrombospondin production was identified in human and rabbit PMN by immunoisolation using mouse anti-human monoclonal antibodies and protein A-sepharose. Thrombospondin was further characterized by ^{35}S-methionine metabolic labelling and 1- and 2- dimensional electrophoresis. Thrombospondin accounted for over 50% of the *de novo* synthesized and secreted proteins from these activated PMN whereas under identical experimental conditions little thrombospondin synthesis was observed when studying non-activated PMN obtained from peripheral blood. The isoelectric points of human and rabbit PMN thrombospondin were different from published results. Our results show that: 1) *in vivo* activated PMN synthesize and secrete increased amounts of thrombospondin and fibronectin coordinately, when compared to non-activated PMN from peripheral blood; 2) glycogen activated rabbit peritoneal fluid PMN appear relevant in the study of protein synthetic events by PMN in human inflammation; 3) thrombospondin synthesized by activated PMN may be different from platelet derived thrombospondin (post-translational modifications) suggesting a slightly different role in inflammation than in clot formation. The precise role(s) of thrombospondin in PMN physiology as well as in inflammation remains to be established.

R 407 **The immunomodullatory effect of anti-T cell monoclonal antibodies in experimental autoimmune disease in the rat.** Per Larsson,R. Holmdahl , K. Strigård , T. Olsson & L. Klareskog Dep. of Medical Chemistry, University of Uppsala and Dep. of Neurology, Huddinge Hospital, Karolinska Institute, Sweden

Anti -T cell monoclonal antibodies (mAbs) were used to study the immunoregulation of adjuvant arthritis (AA) and experimental allergic neuritis (EAN) in rats. It was demonstrated that 1) Anti-pan T cell mAbs (W3/13) *in vivo* prevents both AA and EAN although anti-CD8 mAbs (OX8) exerts no effect on the diseases. 2) Tolerance to EAN, induced by injection of low doses P2 (50 mg) peripheral myelin basic protein, was broken using anti-CD5 (OX19) mAbs *in vivo*. Anti-CD8 treatment did not change the tolerance of the rats. 3) Tolerance to AA, induced by injection of a low dose (10 mg) of *mycobacterium tuberculosis* , could be partially reversed with either anti-CD5 and anti-CD8 mAbs. Flow cytometry analysis after *in vivo* mAb treatment revealed a complete elimination of staining for CD8 positive cells in lymph nodes at day 1 after administration of OX8 mAbs. CD8 positive cells were detectable at day 5 after termination of mAB treatment in peripheral lymphoid organs. Anti-CD5 mAb treatment caused a partial elimination of staining for CD5 positive cells as well as a downregulation of CD5 receptor density from the cell surface of CD5 positive cells. In conclusion, these results speaks in favor for a regulatory role of both CD5- and CD8 positve cells in maintenance of tolerance to AA. Whether CD5 positive cells are the only cells responsible for maintenance of tolerance to EAN still needs more favourable experimental evidences.

R 408 THE START OF AN AUTOIMMUNE DISEASE: IDIOTYPIC NETWORK DURING DEVELOPMENT OF MYASTHENIA GRAVIS, Ann Kari Lefvert, Karolinska instit, Stockholm, Sweden

Anti-idiotypic antibodies and anti-receptor antibodies of IgG and IgM class were determined in the sera of myasthenic patients in early and late stage of the disease. Patients with a disease-duration of less than one year had a high prevalence of anti-idiotypic antibodies (31/32) as compared to patients who had had the disease for more than 5 years (49/79). The concentration of anti-idiotypic antibodies decreased concomittant with an increase in IgG receptor antibodies during evolution of the disease in all patients followed with serial determinations of antibodies. A switch from IgM to IgG receptor antibody production was also found.
Two patients who developed myasthenia gravis after bone marrow grafting had both anti-idiotypic and anti-receptor antibodies before start of clinical disease. During this asymptomatic period anti-idiotypic antibodies appeared before the anti-receptor antibodies.
The high prevalence and high concentrations of anti-idiotypic antibodies in early disease indicate that the development and expression of anti-idiotypic antibodies are critically important in the induction phase of myasthenia gravis.

R 409 **MOLECULAR CHARACTERIZATION OF DNA ISOLATED FROM PLASMA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS,** **Werner Leitmann, Martin Herrmann, Friedel E. Krapf and Joachim R. Kalden, Institute for Clinical Immunology and Rheumatology, University of Erlangen-Nurnberg, D-8520 Erlangen, FRG.**
DNA in the plasma of patients suffering from systemic lupus erythematosus (SLE) is thought to play an essential role in etiopathogenetic events during the induction of the disease. Yet until now, poor experimental data have been acquired concerning molecular features of such DNA capable of immune complex formation, supposed to be a central feature of SLE. Recently we have demonstrated a formerly undefined high molecular weight DNA species (20 kbp) associated by RNA fractions of at least 60 b as determined by HPLC analysis. After molecular cloning of randomly selected plasma DNA fragments originating from six different SLE-patients we sequenced 13 different clones and screened them for base distribution and sequence comparisons. One cloned DNA (E6) proved to be strongly homologous to the pol-region of HIV-1, besides various other stretches of similarity comprising endogeneous and exogeneous pol and env genes. Additional evidence was provided for all clones that plasma nucleic acids (PNA) are not derived from human cellular DNA, since CpG frequency (6.1% by average) and overall dG/dC content (50%) is reminiscent of 'HTF-like' regions, but not of common bulk DNA. Applying PNA as the agent in an *in vitro* transfection assay using an EBV-immortalized line (B62) as target cells, several cythopathic effects including syncitia formation and enhanced vacuolization in blastoid cells were detected in cells treated with PNA (B62/SLE), not in controls (B62/-). These and further observations indicate the transfective potency of DNA isolated from SLE patients' plasma and suggest the involvement of a retroviral agent in the pathogenesis of the disease.

R 410 IGG SUBCLASS ALTERATIONS IN PATIENTS WITH ULCERATIVE COLITIS AND CROHN'S DISEASE,
Richard P. MacDermott, Washington Univ. Med. Ctr., St. Louis, MO 63110.
Spontaneous IgG subclass secretion patterns by isolated intestinal mononuclear cells (MNC) from control and inflammatory bowel disease (IBD) specimens revealed increased spontaneous IgG secretion by ulcerative colitis intestinal MNC primarily due to markedly increased production of IgG_1. In contrast, Crohn's disease intestinal MNC exhibited increased spontaneous secretion of all the IgG subclasses, with IgG_2 being predominant. We next examined sera IgG subclasses. Ulcerative colitis patients exhibited a significantly increased mean concentration of IgG_1, while Crohn's disease patients had normal mean IgG_1 levels. In contrast, Crohn's disease sera had significantly increased mean IgG_2 levels. Systematic lupus erythematosus patients, like those with ulcerative colitis, had markedly elevated serum IgG_1 levels. These data show that alterations in immunoglobulin G subclass concentrations occur in inflammatory bowel disease patients. These observations lend further support to the concept that ulcerative colitis has immunologic characteristics consistent with a primary autoimmune disorder.

R 411 IMPAIRED T CELL RESPONSES IN OLD MICE ARE DUE TO A VARIETY OF DYSFUNCTIONS.
Lex M. Nagelkerken, Christine S. Vissinga, Anita M.A. Hertogh-Huijbregts and Jan Rozing, TNO Institute for Experimental Gerontology, Rijswijk, The Netherlands.
The ability of CBA/Rij spleen cells to respond to BALB/c spleen cells by a DTH reaction declines with age. This is also true for DTH cells generated in vitro. The underlying causes of the impaired T cell responses were further analyzed in an allogeneic MLR. In this system, T cells from old (> 120 weeks) mice were found to exhibit a five-fold lower response than T cells from young (< 20 weeks) mice. The low proliferative responses are in part due to the fact that $CD4^+$ T cells from old mice produce ten times less IL-2 than $CD4^+$ T cells from young mice. This difference remained after the addition of human rIL-1. After the addition of exogenous IL-2, the responses of $CD4^+$ and $CD8^+$ T cells from old mice were still 3 to 8 times lower than those obtained with their "young" counterparts. These differences were in part explained by a diminished frequency of $CD4^+$ as well as of $CD8^+$ alloreactive T cells in old mice. Moreover, we found that the expression of IL-2 receptors (IL-2R) on $CD8^+$ T cells after allogeneic stimulation was lower in old mice. The expression of IL-2R on "old" $CD4^+$ T cells appeared comparable to that by the "young" counterparts. The inability of "old" $CD4^+$ T cells to produce IL-2 may be due to an inadequate signal transduction: after stimulation with phorbol-13-myristate-12-acetate (PMA, 2ng/ml) and ionomycin (300 nM) IL-2 production by "young" and "old" $CD4^+$ T cells was about the same. Still proliferative responses of these cells were twofold less in old mice. In the case of "old" $CD8^+$ T cells the stimulation with PMA and ionomycin did not result in enhanced IL-2R expression.

R 412 NUCLEOTIDE SEQUENCE OF THE cDNA AND THE GENE OF HUMAN NEUTROPHIL ELASTASE (MEDULLASIN), AND DEDUCED PRIMARY STRUCTURE OF THE PRECURSOR OF THE ENZYME,
M. Naruto, K. Okano, H. Nakamura, M. Kajitani, T. Sakurai, T. Shimazu, S. Kanai, Y. Aoki+, and H. Shimizu, Toray Ind., Inc., 1111 Tebiro, Kamakura 248, Japan
Medullasin is a serine protease found in human bone marrow cells, granulocytes and erythroblasts. It was suggested that medullasin plays an important role in the bio-defense mechanism including the development of inflammation and the activation of LGL into natural killer cells. We cloned and sequenced the cDNA and the gene of medullasin from the information of the amino acid sequence of the protein. The "pre-pro" form of the protein is considered to consist of 267 amino acids which contains a possible leader sequence of 29 amino acids. The cDNA we cloned (1) encodes the whole amino acid sequence of human neutrophil elastase (2) which has been determined by the Edman degradation method. Like a human cathepsin G, which is also a serine protease in human neutrophils, the leader sequence contains a hydrophobic region, characteristic for signal peptides, a potential signal peptidase cleavage site, Ala-Leu-Ala, and a proposed activation peptide, Ser-Glu.
The genomic sequence is composed of five exons and four introns, and follows a typical promoter-like sequence.
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+ Institute of Public Health, Minato-ku, Tokyo 108, Japan

R 413 A HIGHLY DIABETOGENIC SUBSET OF HLA-DR4 HAPLOTYPES. Michael J. Sheehy, James R. Rowe and Barbara S. Nepom, American Red Cross Blood Services , Madison, WI 53705, and the Virginia Mason Research Center, Seattle, WA 98101.

Many studies have shown HLA-DR4, an allele of the HLA-DRβI locus, to be associated with insulin-dependent diabetes mellitus (IDDM), particularly in combination with HLA-DR3. It is now clear, however, that either (a) only certain subtypes of DR4 are diabetogenic, or (b) DR4 is merely a marker for a diabetes allele at a nearby locus (HLA-DQβ being the primary candidate locus). The present study was designed to test whether the primary IDDM association is with certain DR4 subtypes or with a certain DQβ allele. We show that neither locus is primary and the other secondary; when one controls for alleles at either locus, the IDDM association of the other locus becomes stronger rather than disappearing. In the Southern Wisconsin population studied, relative risks for IDDM were: DR3, 3.8; DR4, 4.2; Dw4 and/or Dw10 (two subtypes of DR4), 7.6; DQ3.2 (a DR4-associated DQβ allele), 6.3. The highest relative risk is conferred by haplotypes having either Dw4+DQ3.2 or Dw10+DQ3.2 (combined RR=14.0). These last two DR4 haplotypes were found in 32/40 DR4-positive IDDM patients but only 7/30 DR4-positive control subjects (P=1.6 x10^{-6}). In the population studied, persons heterozygous for a DR3 haplotype plus one of the diabetes-associated DR4 haplotypes (Dw4,DQ3.2 or Dw10,DQ3.2) have an absolute risk of approximately 1/12 of acquiring IDDM. Thus population-level testing for IDDM susceptibility is approaching the predictive power attainable in patients' first-degree relatives.