LYMPHOCYTE ACTIVATION

Organizers: Charles Janeway, James Allison and Jonathan Sprent

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Signal Transduction Through Lymphocyte Receptors

V 001 THE B CELL ANTIGEN RECEPTOR COMPLEX: PHYSICAL BASIS OF ACTIVATION OF CYTOPLASMIC EFFECTORS, John C. Cambier, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Antigen receptors play a central role in the immune system, focusing antigen and transducing signals which lead to cell activation, anergy and deletion. Both T and B cell antigen receptors are representative of a family of multisubunit receptors which utilize src-family kinases as proximal cytoplasmic effectors in signal transduction. Recent studies have shown that distinct receptor subunits with the receptor complex mediate interactions with antigen (mIg/TCR$\alpha\beta/\gamma\delta$) and cytoplasmic effector enzymes (Igα, Igβ, TCRζ and η, CD3γ, δ and ε). Further, it has been shown that physical interaction with and activation of effectors is a function of an ~26 amino acid motif, termed ARH1 (or TAM or ARAM) found in multiple receptor subunits. Antigen receptor ligation induces tyrosine phosphorylation of this motif, and this phosphorylation in turn initiates SH2 mediated association with and activation of src-family. This association also triggers lyn and fyn SH3 mediated binding to PI3-k. The resultant conformational change leads to PI3-k activation. These findings allow construction of an integrated model of signal transduction which provides a structural basis for antigen induced activation of cytoplasmic enzymes which mediate signal propagation. Implications of these findings for the basis of receptor desensitization and cell anergy will be discussed.

V 002 T CELL ANTIGEN RECEPTOR SIGNAL TRANSDUCTION: REGULATION BY TYROSINE KINASES AND A TYROSINE PHOSPHATASE, Arthur Weiss, Makio Iwashima, Bryan A. Irving, David Straus, Dev Desai and Andrew C. Chan Howard Hughes Medical Institute, Depts. of Medicine and of Microbiology and Immunology, U.C.S.F., San Francisco.

Stimulation of the T cell antigen receptor (TCR) induces protein tyrosine phosphorylation. A 17 residue sequence motif, termed the antigen recognition activation motif (ARAM), contained in the TCR ζ and CD3 chains is responsible for coupling the receptor to cytoplasmic protein tyrosine kinases (PTKs). Two families of PTKs have been implicated, the Src and Syk/ZAP-70 families. Here, we provide genetic and biochemical evidence that, in the Jurkat T cell leukemic line, signal transduction is initiated by the Src-family PTK lck, when it phosphorylates of two critical tyrosine residues within an ARAM. In turn, a second PTK, ZAP-70, is recruited to a doubly-phosphorylated ζ ARAM via both of its SH2 domains where it is phosphorylated by lck. Thus, the TCR uses ARAMs to interact with two distinct PTKs in a sequential and coordinated manner.

In addition to PTKs, the induction of tyrosine phosphorylation by the TCR is also regulated by the protein tyrosine phosphatase CD45. In CD45 deficient cells, stimulation of the TCR fails to induce protein tyrosine phosphorylation or later events associated with receptor stimulation. This is associated with hyperphosphorylation of Lck at a negative regulatory site of tyrosine phosphorylation. Wild-type CD45 or chimeras, in which the CD45 extracellular and transmembrane domains have been replaced by those of the epidermal growth factor receptor (EGFR), can reconstitute TCR signaling function. These results demonstrate that the CD45 extracellular and transmembrane domains are not required for basal PTPase function. However, ligands added to the EGFR/CD45 chimera suggest that ligand-induced dimerization inactivates the function of the PTPase. Mechanisms responsible for this inactivation will be discussed.

Lymphocyte Receptor: Ligand Interaction

V 003 CRYSTAL STRUCTURE OF THE CLASS I MHC-RELATED FC RECEPTOR, Wilhelm P. Burmeister[1], Malini Raghavan[1], Louis N. Gastinel[2], Michael L. Blum[3], and Pamela J. Bjorkman[1], [1]Division of Biology 156-29, Caltech, Pasadena, CA 91125, [2](present address) Agouron, 3565 General Atomics Court, San Diego, CA 92121, [3](present address) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115.

Maternal immunoglobulin G (IgG) in milk is transported to the bloodstream of newborn rodents via an Fc receptor (FcRn) expressed in the gut. The receptor shows a striking structural similarity to class I MHC molecules, being composed of a related heavy chain and the identical light chain (ß2-microglobulin). FcRn binds IgG at the pH of milk in the proximal intestine (pH 6.0 - 6.5), and releases it at the pH of blood (~ pH 7.5). Because the structures of class I MHC molecules appear uniquely adapted to their peptide binding function, it is surprising to find a molecule with a similar domain organization and significant sequence similarity, but a completely different function in the immune system. The structural similarity between these molecules that function so differently in the immune system affords the opportunity to study which aspects of the class I structure evolved solely for its peptide binding function, how evolution has conserved this structural motif for different purposes, and to increase our understanding of the use of the class I MHC structural motif for immunological recognition. We have expressed a soluble form of the FcRn heterodimer that retains its physiological pH dependence of IgG binding. The counterpart of the class I peptide binding groove is empty in FcRn, as demonstrated by comparative acid elution experiments with purified FcRn and a purified class I molecule. Crystals of FcRn were obtained and the structure was determined to 2.2 Å resolution. The structure bears a close resemblance to class I molecules, with the same arrangement of the α1, α2 and α3 domains of the heavy chain with respect to the ß2-microglobulin light chain. Although the two α-helices that form the sides of the class I peptide binding groove are present in FcRn, they lie next to each other without an intervening space, such that FcRn does not contain a groove similar to the one that binds peptides in MHC molecules. Site-directed mutagenesis studies suggest that the binding site for Fc is far from the α1-α2 domains, being near the bottom of the α3 domain, close to the counterpart of the loop implicated in the CD8/class I interaction. The stoichiometry of FcRn/Fc interaction is two FcRn molecules per Fc. Interestingly, we find a dimer of FcRn molecules in three different crystal forms of FcRn, with the dimer interface at the bottom of the α3/ß2m domains. Although FcRn is a monomer in solution, the high concentration of protein within the crystals may favor dimerization even if the dimer affinity is weak. This dimer could represent a dimer formed in response to Fc binding, a suggestion supported by the fact that the same dimer is found in a preliminary low-resolution anaysis of crystals of an FcRn/Fc complex. The FcRn mutation in the α3 domain that affects Fc binding is near the dimer interface in a position such that it could be directly contacted by a single Fc molecule binding to the FcRn dimer. Further mutagenesis studies are in progress in order to map the interaction between FcRn and Fc, to establish the relevance of the FcRn dimer observed in the crystals, and to explore the molecular mechanism of the pH dependent binding and release of IgG.

V 004 THE INTERACTION OF A T CELL RECEPTOR WITH VARIOUS LIGANDS, Charles A. Janeway, Jr., Soon-cheol Hong, Sang-wook Yoon, and Derek Sant'Angelo, Section of Immunobiology, Yale University School of Medicine, and Howard Hughes Medical Institute, New Haven, CT 06510.

The initiation of an immune response requires the activation of CD4 T cells by specific recognition of a ligand comprising an MHC class II molecule binding a foreign peptide; the same T cell receptor (TCR) may also recognize non-self MHC molecules, and be stimulated by superantigens and by anti-TCR antibodies. In the present studies, we have used site-directed mutagenesis to characterize the interaction of a T cell receptor with its MHC:peptide ligands. We have found that a critical interaction site of the TCR with an MHC ligand maps to CDR1 and/or CDR2 of the TCR alpha chain, which contacts a site in the second polymorphic region of the I-A alpha chain, around residues 57-60. Further analysis of this system has shown that residue 51 in CDR2 of the alpha chain controls recognition of the amino terminus of a 13 amino acid foreign antigenic peptide, and is influenced by residues in the amino terminal end of the alpha helical portion of the I-A alpha chain and the carboxy terminal end of the I-A beta alpha helical region; these are thought to form a single site at one end of the peptide binding groove. A pair of corresponding mutations in CDR2 of the TCR beta chain disrupts recognition of 5 allogeneic MHC class II molecules by this TCR, but does not affect antigen recognition; its contact sites on MHC class II and peptide are being analyzed. From these data, a tentative map of TCR contacts with MHC molecules has been prepared. This map suggests an explanation for the association of V alpha usage with CD4 or CD8 T cells. The folding of the TCR is being explored using these point mutants and a collection of clonotypic monoclonal antibodies. When T cells bearing this TCR are stimulated with soluble anti-TCR antibodies and IL-1, they proliferate readily. This has allowed us to show that some antibodies are far more potent stimulators of T cell activation than others. The high potency anti-TCR antibodies all induce a physical association of CD4 and CD45R with the TCR. This association can be induced using the monovalent Fab fragment of such anti-TCR antibodies, implying that monovalent ligation of the TCR can either change its conformation or signal the T cell. Low potency antibodies do not have this capacity. Moreover, low potency antibodies can competitively antagonize activation by high potency antibodies. Low potency, high affinity antibodies can anatagonize activation by high affinity or low affinity, high potency antibodies, demonstrating that ligand antagonism of T cell activation is not affinity based. Rather, our data suggest that both cross-linking and conformational change are involved in T cell activation. The implications of this for T cell development will be discussed.

The MHC: Structure and Genetics

V 005 ORGANIZATION AND FUNCTION OF MHC CLASS Ib GENES Kirsten Fischer Lindahl, Elsy P. Jones, and Chyung-Ru Wang Howard Hughes Medical Institute, Departments of Microbiology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9050.

The proximal halves of the mouse and human MHCs show extensively conserved synteny over two megabases densely populated by genes, only some of which are involved in immune functions. In the distal halves, most of the known genes belong to the MHC class I family, but they do not exhibit discernible orthologies, suggesting that the more than 40 genes, pseudogenes, and gene fragments in the mouse have developed separately from the 15 or so in humans by independent gene duplications and deletions.

We have taken advantage of our collection of congenic mouse strains, derived from *castaneus* and *spretus* haplotypes by recombination in the distal part of the MHC, as well as yeast artificial chromosomes (YACs) containing more than two megabases of DNA from the *H-2M* region, to map orthologs of new genes identified in the distal part of the *HLA* complex. We have thus established correspondence between *HLA-C* and the *H-2Q* region (*S* gene), between *HLA-E* and *T24* in the *H-2T* region (*R1* gene), and between the *HLA-A* class I gene cluster and the *M4-M6* genes in the *H-2M* region (*B30.2* and *Mog*).

The *H-2M* YACs form two contigs. *M2* and *M3*, which are both expressed, are the only class I genes residing in more than 500 kb of DNA in the distal contig. The proximal contig contains 19 class I gene fragments, as detected with a probe for the conserved exon 4. Only the intact, silent genes *M1*, *M5*, and *M9*, the pseudogenes *M4*, *M6*, and *M7*, and the *M8* fragment have been characterized so far.

Mouse MHC class Ib molecules have recently gained attention, following the demonstration that they present peptide antigens. H-2M3, which selectively binds N-formylated peptides of bacterial or mitochondrial origin, plays a role in host defense against *Listeria monocytogenes* as a target for $CD8^+$ cytotoxic T lymphocytes (CTL) with $\alpha\beta$ receptors. TL and Q2 are expressed in the gut, and TL is recognized by T cells with $\gamma\delta$ receptors. Qa-1 can present a variety of peptides, not yet well defined, to both $\alpha\beta$ and $\gamma\delta$ T cells; Qa-2 requires nonamer peptides with histidine in position 7. In humans, CD1 may be more important than MHC class Ib molecules in host defense against mycobacterial antigens. Because of their mono- or oligomorphism, class Ib molecules may be superior targets for peptide vaccines.

V 006 MHC GENES INVOLVED IN ANTIGEN PROCESSING, John J. Monaco, University of Cincinnati, Cincinnati, OH. 45267-0524.

The class II region of the MHC contains two genes (*Tap-1* and *Tap-2*) encoding the subunits of a structure required for the transport of peptides from the cytoplasm into the lumen of the endoplasmic reticulum for binding to MHC class I molecules. We are analysing allelic differences in the sequence of these two genes in inbred strains of mice in an attempt to relate polymorphism with potential differences in the specificity of peptide transport. Closely linked to the two transporter genes are a pair of genes (*Lmp-2* and *Lmp-7*) that encode subunits of a large cytoplasmic protease called the LMP complex, or proteasome, which is believed to be required for the proteolytic generation of MHC class I-bound peptides. We are determining the molecular basis of polymorphism observed in these gene products, and the effect of these two subunits on the overall proteolytic specificity of the complex *in vitro*. Genetic mapping of other, non-polymorphic, proteasome subunits is also in progress. Multiple copies of some of these sequences are present in the mouse genome, and these are genetically unlinked either to one another or to the MHC. Data will be presented which demonstrate that the cellular pool of proteasomes consists of a collection of related protein complexes which differ both in subunit composition and in proteolytic activity. Biochemically distinct froms of the complex may be found in different tissues, and the subunit composition of these complexes may be altered by treatment of the cells with IFN-γ.

V 007 ORGANIZATION AND FUNCTIONS OF THE CLASS II REGION OF THE HUMAN MHC, John Trowsdale, Stephan Beck, Monica Belich, Richard Glynne, Amanda Jackson, Adrian Kelly, Stephen Powis, Frances Sanderson, Philippe Sanseau. Imperial Cancer Research Fund, 44 Lincolns Inn Fields, Holborn, LONDON WC2A 3PX, UK.

The MHC encompasses several clusters of genes with related functions in antigen presentation and processing. We have cloned the entire class II region in order to identify any new genes that may be involved in these processes. So far, we have found over ten genes which fit into one of three categories. First are genes which have no obvious relationship to the immune system. The *RING3* gene, for example, has homology with a *Drosophila* gene, *female sterile homeotic*. Second are a pair of genes, *DMA* and *DMB*, which encode a heterodimer very weakly related to other class II sequences. Finally, there is a cluster of genes involved in antigen procesing: *LMP2-TAP1-LMP7-TAP2*. The *LMP* gene products are proteasome components, which play a subtle role in antigen processing. We have cloned two *LMP*-related genes which are not in the MHC and it is proposed that their products can substitute for the MHC-encoded proteins. The *TAP* genes are traffic ATPases which transport peptides from the cytoplasm into the lumen of the endoplasmic reticulum. Other regions of class II are being mapped and sequenced to facilitate the search for genes which may help to understand antigen processing and presentation as well as MHC-linked disease associations.

Antigen Processing and Presentation

V 008 THE BIOCHEMISTRY AND CELL BIOLOGY OF MHC CLASS II ANTIGEN PRESENTATION, Ronald N. Germain, Flora Castellino, Austin G. Rinker, Jr., Scheherazade Sadegh-Nasseri, and Paola Romagnoli, Lymphocyte Biology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD USA 20892

MHC class I and class II molecules bind and transport to the cell surface peptides derived from self or foreign proteins. Each class is specialized for acquisition of peptides in distinct intracellular locations - class I for peptides present in the ER, class II for peptides in the endocytic pathway. Movement of class II dimers from their site of initial assembly in the ER to the cell surface involves a complex interplay between the class II molecules, peptide, and the invariant chain (Ii). Ii provides a chaperone-like function for class II in the ER, enhancing formation of properly folded αβ dimers and preventing aggregation and retention of newly synthesized chains. At the same time, class II association with invariant chain inhibits the function of the class II peptide binding site. Recent data suggest that these two activities of invariant chain - class II assembly and regulation of peptide binding, are coordinate activities of the same region of the invariant chain protein.

The path taken by class II to the endosomal pathway and the site(s) of peptide loading has been controversial. Our data, from both transfection models and analysis of normal antigen-presenting cells, suggest that class II-Ii complexes access multiple stations in the endosomal pathway. Ii can play an active role in modifying movement through the early endocytic compartment, retarding the flow of fluid phase and membrane material *en route* to late endosomes. Arrival of class II in later endocytic compartments with some of the characteristics of immature lysosomes is associated with extensive cleavage of Ii. In this site, substantial loading of peptide into the class II molecules takes place.

Peptide plays an important role in the structure of the class II dimer, as revealed by stabilization of αβ dimers *in vitro* against dissociation in SDS-containing buffers. A similar transition in class II structural stability can also be observed in living cells when newly synthesized, Ii-associated class II dimers are compared to those present in the cell several hours after synthesis. Ii-associated class II does not show evidence of associated peptide The proportion of class II molecules surviving for several hours increases following addition of antigen to the medium. An *in vitro* model of endosomal peptide loading indicates that peptide binding prevents class II that is free of intact Ii from undergoing prolonged interaction with unfolded proteins. We suggest that in the endosomal pathway, when Ii is cleaved and removed from class II, the exposed molecules have two possible fates: 1) to become associated with free peptide and move to the cell surface for T cell recognition, or 2) to remain associated / aggregated with partially denatured/degraded proteins, resulting in endosomal retention. This provides an intracellular editing mechanism that results in the selective surface expression of effectively loaded class II dimers.

These various features of the class II processing and presentation system will be discussed in the context of the structural features of the class I and class II molecules that differentiate the two peptide binding proteins and permit them to specialize in presentation of discrete families of antigenic peptides to the T cell immune system.

Lymphocyte Subsets and Immunological Memory

V 009 CELL INTERACTIONS NECESSARY FOR THE MAINTENANCE OF MEMORY, Per Dullforce[1], Polly Matzinger[2] and David Gray[1]. [1]Department of Immunology, Royal Postgraduate Medical School, London, UK. [2]NIAID, NIH, Bethesda.

The longterm persistence of memory cells within the immune system seems to be largely brought about by intermittent restimulation of these cells. Our experiments and those of others have illustrated the central role that antigen depots play in the continuance of this stimulation. For non-replicating antigens (ie. not viral) the most important sites of storage are on the surface of follicular dendritic cells (FDC), specialized cells that can keep antigen-antibody complexes on their surface for upto 1 year and possibly longer. Because the antigen stored on FDC is undegraded, presentation of this antigen to a memory T cell will require an intermediary APC (FDC are incapable of processing antigen themselves). As the only cells that can efficiently obtain antigen from FDC are antigen-specific B cells we have predicted that the longterm persistence of a memory T cell response will require the presence of antigen-specific memory B cells. As a corollary to this hypothesis it is logical to conclude that in the absence of an antibody response, and the subsequent failure of FDC antigen localization, the maintenance of T cell memory will be impaired. We have tested this hypothesis in a variety of systems (including SCID mice reconstituted with T cells but not B cells and in normal mice immunized with peptide-pulsed APC to elicit T cell responses in the absence of antibody production). The results so far suggest that indeed the lack of antigen-specific B cells or antibody impairs the efficient development of a longterm memory T cell response. The interaction between a memory T cell and a memory B cell seems likely to be mutually beneficial as the transfer of memory B cells into nude mouse recipients, with antigen-antibody complexes but without T cells, leads only to a relatively short term survival of the memory B cells. We are currently investigating the role of the CD40-CD40 ligand interaction (in addition to lymphokines) in the memory survival process. Data from experiments involving the in vivo treatment of mice with soluble CD40-Fc, indicating a central role of CD40-CD40 ligand interaction in memory B cell generation will also be discussed.

V 010 CD4 MEMORY-GENERATION AND PERSISTENCE, Susan L. Swain, Linda M. Bradley, Michael Croft, Laura Haynes and Xiaohong Zhang, Department of Biology and the Cancer Center, University of California, San Diego, La Jolla, CA 92093.

Immunization of mammals can result in the development of a state of memory such that re-encounter with Ag elicits a huge, fast and protective response. We have been studying the properties of memory CD4 T cells which contribute to systemic memory and have developed a model in which the development of memory can be studied, and the factors which regulate the generation and persistence of memory can be investigated.

In studies with a conventional Ag, KLH administered to mice, we have established that priming results in large increases in the frequency of Ag-specific cells, and confirmed that Ag-specific memory cells display many changes in cell surface phenotype when compared to "naive" counterparts. We show that memory cells recirculate differently from naive cells displaying an independence of L-selectin for their localization to lymph nodes.

We have also directly studied Ag-specific naive T cells using TCR transgenic mice with a Vα11/Vβ3 receptor specific for Pigeon cytochrome C (88-104) plus IE^k. The naive cells require stimulation with specialized Ag-presenting cells (especially dendritic cells), and do not produce cytokines unless they receive costimulation, such as that delivered through CD28. Memory cells can be stimulated by a broader range of APC and are less dependent of costimulation through CD28.

The function of CD4 T cells is largely dictated by the profile of cytokines they produce. Naive T cells produce only IL-2 with small amounts of IL-3, when first stimulated. The bulk of peripheral memory phenotype cells and the KLH memory cells also produce high titers of IL-2 with only small amounts of IL-4, IL-5, IFN-γ, etc.

To study memory generation, and to ask if memory cells can be subdivided into other more Th1- or Th2-like subsets, we generated polarized Ag-specific effector populations in vitro, transferred them to syngeneic ATXBM hosts and followed the phenotype and function of recovered cells. The effectors gave rise to substantial population of Th^+ CD4 T cells with memory phenotype which could secrete a profile of cytokines much like that of the particular effector population from which they were derived. The recovered cells also had appropriate memory function in assays of help.

These results indicate that (1) memory CD4 can be generated and studied in this model, (2) that they persist in the absence of specific Ag, and (3) that they can have polarized patterns of cytokine secretion.

Tolerance

V 011 MECHANISMS FOR ELIMINATING OR INACTIVATING SELF-REACTIVE B CELLS

Christopher C. Goodnow, Srinivas Akkaraju, Sarah E. Bell, Karen Canaan, Michael P. Cooke, Jason G. Cyster, Suzanne B. Hartley, Denise Leong, Kevan M. Shokat, Jeff Rathmell, Karolyn Zeng, Andrew Heath*, Maureen Howard*, William Ho, Mark M. Davis
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Transgenic mice expressing a model self antigen, hen egg lysozyme (HEL), in different forms, concentrations, and tissues, were used to explore mechanisms for preventing the production of autoantibodies. By mating these animals with transgenic mice carrying rearranged immunoglobulin (Ig)-genes, cellular and molecular events underlying the acquisition of tolerance can be studied in large homogeneous populations of self-reactive B cells in vivo. Distinct tolerance mechanisms were found to act at different points along the B cell developmental pathway. The first acts within the bone marrow as soon as surface IgM is expressed. Binding of multivalent membrane-bound self antigen at this stage triggers an unknown signaling pathway that reversibly arrests B cell development, preventing acquisition of many key molecules required for B cell survival, migration, and interaction with helper T cells. The second mechanism acts after B cell migration to the periphery, and involves selective inactivation of a tyrosine kinase cascade normally triggered by antigen binding to sIg. Additional mechanisms affecting follicular homing, survival, and germinal center formation will be discussed. B cells are not rendered tolerant if they bind systemic autoantigens with too low an affinity or too low an antigen concentration. Similarly, B cells fail to be censored against a thyroid-specific form of membrane HEL. Parallels with T cell tolerance to HEL in TCR-transgenic mice will be discussed.

V 012 NEGATIVE SELECTION, INDUCTION AND EXHAUSTION OF ANTIVIRAL T CELL RESPONSES, Rolf M. Zinkernagel, Hanspeter Pircher, Hans Hengartner, Institute of Experimental Immunology, Department of Pathology, University of Zürich, Switzerland

$CD8^+$ T cells may be negatively selected in the thymus upon encounter of self antigen presented by class I MHC, apparently on any cell. Whether this occurs, in the cortex or in the medulla of the thymus or else in the periphery, depends on relative concentrations of presented peptide, MHC, possibly some factors and on susceptibility of thymocytes to die. Localised and small, versus widely spread and high doses of antigen/peptide in the periphery may induce effector cells or induce and exhaust cytotoxic T cells. This results in immuno protection/immunopathology or in peripheral exhaustion/deletion. The exact rules of why and how induction or exhaustion results are not quite clear yet, but distribution, expansion and persistance as well as concentration of antigen/peptides, but also precursor frequencies of inducible T cells, propably interleukin levels and by-stander effects play a role. Examples of virus infection or immunization with peptide illustrate the various balances resulting in induction of effectors and of memory or no memory, or in exhaustion of the specific T cells. In view of these results and concepts, the nature of T cell memory must be revisited to find out whether it reflects a "special phenotype" (but there are no memory-markers that are different from markers of activated CD8+ T cells) or rather a low level stimulation by antigen depots. That relative doses, distribution and kinetics of antigen determine whether an antigen is causing tolerance (by exhaustion) or induces an effector T cell response and/or memory maybe the simplest concepts that fits most immunobiological findings.

Peptide: MHC Interactions

V 100 MHC CLASS II MOLECULES, BINDING PARTIALLY UNFOLDED PROTEINS IN THE ABSENCE OF THE INVARIANT CHAIN, FORM STABLE DIMERS AFTER PROTEOLYSIS, Gerald Aichinger, Lars Karlson, Per Peterson, Department of Imunology, The Scripps Research Institute, La Jolla, CA 92037

We have analyzed soluble HLA DR4 Dw14, Dw4 and Dw10 complexes, produced in Drosophila SC-2, HeLa and Ii chain deficient M1 cells. In the absence of the Ii chain, MHC class II complexes are heterogenous in size (M_r 55 to over 300 k) and charge (pI 7.5 to 5) due to binding of a heterogenous set of partally denatured proteins. The MHC class II molecules in these complexes display normal conformation and are functionally competent as demonstrated by binding of conformation-sensitive antibodies and peptide binding. Complexes, digested with papain represent SDS stable MHC class II αβ dimers of homogeneous size of 55 kd. We are now conducting experiments with constructs of influenza A hemagglutinin (HA), x31A$^-$, and Japan strain, PEMT, MHC class II α and β chains, and a construct of p31 invariant chain in HeLa cells and the M1 cell line (a cell line, that does not express the Ii chain). These experiments will show whether binding of HA to MHC class II does occur in the ER of mammalian cells and wheter it can be inhibited by coexpression of the Ii chain. Class II molecules binding partially denatured proteins in the absence of Ii chain might be retained in the ER and Ii chain may prevent this. MHC class II molecules, binding partially denatured proteins in the ER, may, however, be transported to the cell surface, endocytosed and processed in the endosomal compartment and present endogenous antigens. We will address these questions in our presentation.

V 101 *IN VITRO* FOLDING OF MHC CLASS II HETEORDIMERS IN THE ABSENCE OF A PEPTIDE, John D. Altman and Mark M. Davis, Department of Microbiology and Immunology and the Howard Hughes Medical Institute, Stanford University, Stanford CA 94305-5428.

Under controlled conditions, major histocompatability complex class I and class II proteins can fold *in vivo* and exist on the surface of cells without their normal peptide ligands. Class I molecules can fold *in vitro*, but the folding reaction seems to absolutely require peptides which are specific ligands of the class I protein. Class II α and β subunits produced in *E. coli* can also fold *in vitro* in the presence of a peptide, but the issue of a requirement for a peptide in the class II folding reaction has not been resolved. We now show that heterodimers can be isolated from class II I-E^k folding reactions which do not include peptides, and that these molecules will subsequently bind specific peptide ligands. The pH dependence of peptide binding for the "empty", *in vitro* folded I-E^k heterodimers is apparently identical to that of soluble "empty" I-E^k produced in a lipid-linked form in CHO cells. We conclude that at least some class II proteins contain all of the information in their primary sequences necessary for folding.

V 102 PRESENTATION OF OVA PEPTIDE BY K^b BM MUTANTS

Anders Brunmark*, Pierre Langlade Demoyen*, Michael R. Jackson*, Jeffrey E.W. Miller¶, William D. Huse¶ and Per A. Peterson*. *The Scripps Research Institute, Department of Immunology, 10666 N. Torrey Pines Rd., La Jolla, CA 92037 and ¶Ixsys Inc., 3550 Dunhill St., San Diego, CA 92121

The K^b mutant phenotypes are characterized by their inability to present certain peptide epitopes, e.g. ovalbumin$_{257\text{-}264}$ (OVA) to CTLs. In order to determine whether the mutant phenotype is a result of lack of peptide binding we have determined the dissociation constants for complexes between OVA peptide and K^b mutant molecules K^{bm1} or K^{bm8}. The K_D values determined did not differ more than a factor 5 from those measured for K^b, indicating that lack of binding of peptide is not the reason for the lack of presentation of this peptide. We are currently investigating wheter it is the structure of the K^{bm1} or K^{bm8}-OVA peptide complexes that results in the lack of recognition by T-cells.

Bulk CTLs from C57Bl/6, K^{bm1}, and K^{bm8} mice were stimulated with cells (either T2 or *Drosophila melanogaster*) transfected with K^{bm1} and K^{bm8} and pulsed with OVA. The CTLs generated were found able to kill OVA-pulsed K^{bm1} and K^{bm8}-transfected T2 cells as well as K^b-transfected T2 cells. However, limiting dilution assay revealed that K^b-mutant mice had a 20-fold lower frequency of OVA-specific CTL precursors. Furthermore, we found that CTLs from K^b mutant mice were more dependent on CD8 for killing, especially if the targets were not syngeneic. These results suggest that the lack of recognition of certain peptides presented by K^b mutant molecules is a result of conformational differences.

V 103 IDENTIFICATION OF A PEPTIDE BINDING MOTIF FOR THE MURINE CD1.1 MOLECULE. A. Raúl Castaño*, Jeffrey E.W. Miller†, Michael R. Jackson*, K. Fischer Lindahl°, Mitchell Kronenberg#, William D. Huse† and Per A. Peterson*.

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cDNAs encoding the extracellular portion of the murine non-classical class I molecules Tl (T13^c), HMT (M3) and CD1 (CD1.1) were generated by PCR and cloned into a pRMHa3 *Drosophila* expression vector. Schneider *Drosophila* cells were cotransfected with these cDNAs, a mß2m-pRMHa construct and a neo resistant plasmid. Stable transfectants were selected with G418 and cells that expressed soluble T13^c, M3 and CD1.1-ß2m complexes were obtained. The soluble CD1 heterodimers were used to screen a random peptide phage display library (see Miller et al. abstract) to identify peptides that bind the non-classical class I CD1 heterodimer. The sequences of the clones that bind to CD1 describe a peptide motif which is heterogeneous in length at the N-terminus and contains three anchor residues in a seven aminoacids long stretch. Using codon-based deletion mutagenesis on selected peptide phage display clones we have determined the importance of the N-terminal positions to CD1-peptide binding. Surface plasmon resonance studies allowed direct measurement of peptide binding in solution and the determination of affinity constants. Alanine scanning mutagenesis on synthetic peptides will be done to verify the relative contribution of the putative anchor residues to the binding to CD1.

V 104 NATURALLY PROCESSED PEPTIDES LONGER THAN 9 AMINO ACID RESIDUES BIND TO THE CLASS I MHC MOLECULE HLA-A2.1 WITH HIGH AFFINITY AND IN DIFFERENT CONFORMATION, Ye Chen[1], John Sidney[2], Scott Southwood[2], David Benjamin[1], Andrea L.Cox[3], Kazuyasu Sakaguchi[4], Robert A. Henderson[1], Ettore Appella[4], Donald F. Hunt[3], Alessandro Sette[2], and Victor H. Engelhard[1], Beirne Carter Center for Immunology Research and Departments of Microbiology[1], Chemistry[3], University of Virginia, Charlottesville, VA 22908, Cytel[2], 3525 John Hopkins Court, San Diego, CA 92121, Laboratory of Cell Biology[4], National Cancer Institute, NIH, Bethesda, MD 20892

An equilibrium binding assay was used to directly measure the affinities of naturally processed 9mer, 10mer and 12mer peptides for the human class I MHC molecule HLA-A2.1. The peptides exhibited a range of affinities, with IC_{50} values of 11-214 nM. In some cases the affinities of longer peptides were higher than or similar to that of a 9mer peptide containing the same core sequence. The mode of interaction between these peptides and HLA-A2.1 was examined using peptides in which Asp had been substituted for suspected anchor residues. Regardless of length, the previously identified Leu at position 2 relative to the amino terminus was critical for peptide binding. While the carboxyl terminal residue was also critical for the binding of a 9mer peptide, it was much less important in the binding of longer peptides. Additional residues close to the carboxyl terminus that contained aliphatic hydrocarbon side chains were of similar or greater importance in peptide binding. In addition, the residue at position 3 also appeared to be important for the binding the longer peptides. The data suggest that different naturally occurring longer peptides can bind in different conformations to class I MHC molecules. While one of these is similar to the kinked conformation described previously, another conformation appears to involve an extension of the carboxyl terminus out of the class I binding site. The ability of MHC molecules to accommodate the same peptide in different conformations would appear to have distinct advantages to the immune system. The significance of differences in the carboxyl terminal contribution to binding is being studied by computer modeling.

V 105 IDENTIFICATION OF NATURALLY PROCESSED PEPTIDES BOUND TO TYPE I DIABETES ASSOCIATED HLA-DQ MOLECULES, Roman M. Chicz*†, William S. Lane†, Renee A. Robinson†, Massimo Trucco§, Jack L. Strominger*, and Joan C. Gorga§, *Department of Biochemistry and Molecular Biology and the †Microchemistry Facility, Harvard University, Cambridge, MA 02138, and the §Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Genetic susceptibility to several autoimmune disorders is associated with the expression of MHC class II alleles. These membrane bound glycoproteins present processed foreign antigens as peptides to helper T cells, thus eliciting an immune response. Autoimmune reactions result from T cell recognition of naturally processed self-peptides bound to class II molecules. Treatment and eventual prevention of such diseases awaits the identification of the class II restriction elements and the bound pathogenic self-peptides. Naturally processed self-peptides bound to HLA-DQ1 and HLA-DQ8, alleles permissive toward susceptibility to type I diabetes, were characterized by high-performance liquid chromatography, mass spectrometry and Edman microsequencing analyses. Over 200 unique peptide masses were identified for each allotype. The most abundant M_r values were between 1,000 and 4,000 daltons. Although the chromatographic profiles and mass distributions of the peptide repertoires are unique, they exhibit general similarity to those reported for naturally processed self-peptides bound to HLA-DR. Thirty-one individual peptides representing thirteen sets nested at the amino- and/or carboxy-terminal ends were identified. Allotype-specific amino acid preferences were observed for the peptides and confirmed in binding assays with purified HLA-DQ protein. The promiscuous invariant chain peptide (98-119), which is presented by several HLA-DR allotypes, was identified bound to HLA-DQ8.

V 106 SITE-DIRECTED MUTAGENESIS OF HEL (HEN LYSOZYME) AND ALTERATION IN IMMUNODOMINANCE PATTERNS, Hongkui Deng, Jeff Ohmen*, Lisa Fosdick, Brian Gladstone, Hanju Bang, Diana Y-T Tsai, Jane Guo and Eli Sercarz, Dept. Microbiology and Molecular Genetics and *Dept of Medicine (Dermatology), UCLA, Los Angeles, CA 90024

To investigate how antigen processing might influence the immunodominance hierarchy in vivo, a set of site-directed mutants of HEL has been constructed. One type of mutation involves an amino acid change within the determinant at a residue known to be important for MHC class II binding. A second type of mutation involves regions that flank known determinants. A third type of HEL mutant bears a change at a putative enzyme cutting site, designed to either create new determinants or destroy existing ones. In BALB/c mice, HEL 106-116 E^d is the dominant determinant. R114H has been reported to profoundly impair 106-116/E^d interaction. As one example of the use of the mutants, when R114H HEL is used as an immunogen, it alters the determinant expression pattern by allowing an A^d restricted determinant, 74-88, to be expressed in BALB/c mice, which fails to make an A^d restricted response when HEL itself is used as the immunogen. Another example is F34K, creating a new cathepsin B site (33K34K), which is near determinant 23-33/A^d and dramatically enhances its expression over 20 fold. Examples with other mutants will be presented. (Supp. by a JDFI grant)

V 107 INTERFERENCE WITH THE BINDING OF A NATURALLY PROCESSED PEPTIDE TO CLASS II ALTERS IMMUNODOMINANCE OF T CELL EPITOPES IN VIVO. **Alison Finnegan, Kelly W. Nikcevich and Dianne McDonald, Depts. Internal Medicine and Immunology/Microbiology, Rush Presbyterian St.Luke's Med. Ctr. Chicago, IL 60614.**

T helper cell activation requires the recognition of peptides bound to the MHC class II molecule. T cells primed to an antigen are limited to recognition of one or a few peptides of the overall antigen. This phenomenon is referred to as immunodominance. T cells primed to staphylococcal nuclease (Nase) are limited to recognition of the 86-100 Nase peptide in association with the I-E^k molecule. Our data suggests that one possible mechanism for dominance of the 86-100 peptide over other Nase peptides is through competition for access to the I-E^k molecule. We show that single amino acid substitutions in the 86-100 peptide at residues 89 (leu to phe) and 91 (tyr to ser) blocked 86-100 peptide binding to the I-E^k molecule. Substitutions in the 86-100 peptide were based on a putitive peptide motif for binding I-E^k. We speculated that the same amino acid substitutions introducted into the Nase protein would block processed 86-100 peptide presentation to T cells. When mutant proteins were used to immunize mice, T cells were primed to Nase peptides in the 111-140 region but not to the dominant peptide 86-100. In binding studies peptides 111-131 and 116-135 bound to the I-E^k molecule with lower affinity than the 86-100 peptide. These data are consistent with the hypothesis that processed peptides compete for binding to the class II molecule. This may be one mechanism responsible for the immunodominance of peptides.

V 108 ASSEMBLY OF CLASS II HETERODIMERS IN VITRO, Mary Lynne Hedley, Robert G.Urban, and Jack L. Strominger, Harvard University, Department of Biochemistry and Molecular Biology, Cambridge, MA 02138

Class II heterodimers assemble in the endoplasmic reticulum (ER) with the help of at least one other protein, invariant chain (Ii). Ii is thought to prevent the class II pocket from acquiring peptide in the ER and furthermore, directs class II to the endosomal compartment of the cell. Here, Ii is released and peptides generated from endocytosed proteins bind to class II molecules. We have developed an in vitro system to study heterodimer formation and peptide binding. cDNAs encoding invariant chain, DR alpha and DR1 beta chains were independently cloned into RNA expression vectors. Minigenes encoding the DR alpha signal sequence followed by sequences coding for one of three peptides were generated by overlapping PCR. Two of the peptides encoded by the minigenes are known to bind DR1, whereas the third peptide does not bind. mRNA was transcribed from these constructs and was added to a rabbit reticulocyte lysate translation mix containing canine pancreatic microsomal membranes. Translation of each product was assayed by SDS-PAGE. Co-translation of alpha and beta chain mRNA followed by immunoprecipitation with LB3.1, a monoclonal antibody specific for alpha/beta heterodimers which does not react with single chains, demonstrated that DR alpha and beta assemble in the microsomal membranes in the absence of Ii. Co-translation of peptide minigene, DR alpha and beta mNRAs followed by immunoprecipitation suggest that certain peptides can interact with class II molecules in the absence of invariant chain.

The DA6.147 antibody can recognize the DR1 heterodimer/Ii complex and was used to determine if DR1 assembled with Ii in vitro. Co-translation of all three mRNAs (alpha, beta and invariant) followed by immunoprecipitation with DA6.147 demonstrated that the DR alpha/beta complex assembles with Ii in vitro. Data from additional experiments using the in vitro system suggests that at least some peptides can compete with Ii for binding to alpha/beta complexes in the ER.

V 109 FUNCTIONAL DIFFERENCES IN HLA-DR11 AND -DR13 MICROVARIANTS. Carolyn Katovich Hurley, Noriko Steiner, Hugo A. Araujo and Armead H. Johnson, Departments of Microbiology and Immunology and Pediatrics, Georgetown University, Washington, DC 20007

In the human, HLA-DR molecules can be clustered into groups based on shared common serologic determinants. Members of a group (microvariants) differ only subtly from one another in β chain amino acid sequence. One intriguing and complicated group of molecules bear both DR11 and DR13 serologic determinants. To study the effect of microvariation on function, 17 DR11- and DR13-expressing wild type and mutant murine L cell transfectants were generated. Mutant DR β chain cDNAs were created by altering amino acids in and around the antigen binding groove. The substitutions utilized amino acids found at the same position in other microvariants in the same group. Cells expressing DRB1*1101/*1303/*1304 bound HA 307-319 peptide at a higher level than cells expressing other wild type molecules. When DR(α,β1*1304) was mutated to alter position 57 or 58, HA binding was decreased 5 fold. Changing positions 32 and 37 in DR(α,β1*1304) to residues found in DR(α,β1*1301) reduced HA binding to the level observed for DR(α,β1*1301). Interestingly, changing these positions in the reverse direction (*1301 to *1304) also decreased HA binding. These results suggest that single amino acid residues in the groove can affect binding; however, peptide binding is also influenced by the milieu of the antigen binding groove of each DR molecule. The effect of microvariation on allorecognition (and self peptide binding) was assessed using alloproliferative T cell clones (TLC) (R:DRB1*1101, S:DRB1*1102 and vice versa). Some of these TLC recognize L cells expressing DRB1*1102 as well as cells expressing DR13 allelic products. The patterns of recognition suggest that recognition involves the shared α helical regions of these DR molecules as well as a peptide component. These observations demonstrate the power of apparently subtle evolutionary changes in creating this diverse repertoire of antigen binding molecules.

V 110 CARRIER-INDEPENDENT RECOGNITION OF TNP-PEPTIDES BY CLASS II MHC-RESTRICTED T CELLS. Jochen Kohler, Stefan Martin and Hans Ulrich Weltzien, Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-79108 Freiburg, Germany

A notable proportion of trinitrophenyl (TNP) specific, class I MHC-restricted T cells has been demonstrated by us, to react with MHC-associated, haptenated peptides largely independent of the carrier peptides' amino acid composition (J. Immunol. 149 (1992) 2569 and 151 (1993) 678). It was then argued that chemically trinitrophenylated cells might present an unusually repetitive pattern of TNP-epitopes to such T cells, and that this might relate to the hapten's pronounced allergenic properties. However, allergic responses such as contact sensitivity are widely discussed as being mediated primarily by T cells of the $CD4^+$, class II MHC-restricted compartment. We have therefore produced TNP-specific T cell lines and hybridomas of the $CD4^+$ phenotype, restricted to $I\text{-}A^b$, $I\text{-}A^d$ or $I\text{-}E^d$ encoded class II molecules. Our cells were selected for antigenic reactivity to TNBS modified irradiated syngenic spleen cells. 25 % of our hybridomas and 50 % of our T cell lines were reactive to syngenic spleen cells modified with TNP-protein digests. We also screened several synthetic peptides, known from the literature to bind to $I\text{-}A^b$, $I\text{-}A^d$ or $I\text{-}E^d$ in which lysine residues were ε-N-alkylated by TNP. The same peptides without TNP were not antigenic for our T cells and the recognition could be inhibited by TNP- or MHC class II-specific antibodies. Some of our T cells crossreactively reacted with TNP-BSA and one of our synthetic TNP-peptides. We computer-compared the sequence of this peptide with the sequence of BSA. The three most similar TNP-BSA-peptides revealed maximally 31 % identity (46 % similarity) to the synthetic peptide, but were not antigenic for our crossreactive T cells. The antigenic peptide within the TNP-BSA sequence must, therefore, be of still lower homology to our antigenic TNP-peptide than the three synthesized sequences. As for class I, also class II MHC-restricted TCR, thus apparently may interact with TNP-epitopes in a carrier-independent fashion. We take this to further support our hypothesis that the strong antigenic, and possibly the allergenic properties of haptens such as TNP may be influenced by the repetitive type of hapten-determinants on different carrier peptides resulting from chemical cell modification.

V 111 HLA-DRß-CHAIN RESIDUE 86 CONTROLS DRαß-DIMER STABILITY, Frits Koning, Frank A. W. Verreck, and Annemarie Termijtelen, Department of Immunohaematology and Bloodbank, University Hospital Leiden, The Netherlands

MHC class II molecules exist in two forms, which can be distinguished on the basis of their stability in Sodium Dodecyl Sulfate (SDS) as SDS-stable and SDS-unstable αß-dimers. The ratio of stable versus unstable αß-dimers varies between murine H-2 alleles and isotypes, but the molecular basis for this observation is unknown. We found that also human MHC class II molecules HLA-DR and HLA-DQ display allelic specificity in their SDS-stability. For the human HLA-DRB1 and HLA-DRB3 gene products it appeared that this stability ratio is controlled by the valine/glycine dimorphism at position 86. Haplotypes coding for DRß-chains with a valine at position 86 express higher numbers of stable dimers compared to similar haplotypes expressing DRß-chains with a glycine at that position. Reverse phase HPLC analysis of iodinated peptides, which were eluted from DR dimers with either a DRB1*1101 or a DRB1*1104 ß-chain which differ only at position 86, indicated that these DR dimers contain (partially) distinct sets of peptides.

The valine/glycine dimorphism is highly conserved, present in most HLA-DR alleles and influences peptide-binding. Analysis of the occurrence of the Val^{86} and the Gly^{86} gene products revealed that these are not equally present in the population. Depending on the DR specificity either the Val^{86} or Gly^{86} allelic variant is favoured. Thus, the natural, highly conserved dimorphism at HLA-DR beta-chain position 86 influences peptide selection. The dimorphism is therefore likely to influence antigen presentation and forms the molecular basis for the observed differences in SDS-stability of Val^{86} and Gly^{86} containing DR dimers.

V 112 IDENTIFICATION OF AN IMMUNOSUBDOMINANT NUCLEOPROTEIN DERIVED PEPTIDE PROCESSED BY H-2^b CELLS INFECTED WITH INFLUENZA A VIRUS. Konstadinos Kosmatopoulos, Mohammed Oukka, Jean-Pierre Cabaniols and Nicole Riché. Unité INSERM 267, Hôpital Paul Brousse, Villejuif, France.

Nucleoprotein (NP) from influenza A virus has two peptides, NP 366-374 and NP 55-63 that fulfil the prerequisits to have high binding affinity towards D^b molecule. NP 366-374 is an immunodominant peptide whereas NP 55-63 peptide does not participate to the antiviral CTL response in C57BL/6 (B6) (H-2^b) mice. The aim of our work was to study why NP 55-63 peptide is ignored by the immune system of B6 mice. Two hypotheses have been proposed : a) B6 mice are tolerant to the NP 55-63 peptide ; b) NP 55-63 peptide is either inefficiently processed or not processed at all by the virus infected H-2^b cells. Results we have got have shown that 1) B6 mice possess a NP 55-63 specific CTL repertoire since their immunization with NP 55-63 peptide itself recruits specific CTL ; 2) NP 55-63 peptide is naturally processed by the virus infected EL-4 (H-2^b) cells, but efficient presentation requires the presence of high amount of intracellular NP ; in fact NP 55-63 specific CTL killed EL-4 cells when they were heavily infected with virus ; 3) NP 55-63 peptide is naturally processed in the virus infected B6 mice ; the number of D^b/NP 55-63 complexes is sufficient to tolerize NP 55-63 specific CTL repertoire in B6 → B6 chimeras rendered tolerant by virus infection, but it is not sufficient to recruit NP 55-63 specific CTL in virus infected B6 mice. The choice of the NP 366-374 peptide to be efficiently presented by H-2^b cells seems to be due to the fact that its affinity for binding to the D^b molecule is higher than the affinity of the NP 55-63 peptide. This difference in the affinity for binding to the D^b molecule between these two peptides leads to an *in vivo* competition for inducing peptide specific CTL ; coimmunization of B6 mice with the two peptides recruits CTL specific for the NP 366-374 but not CTL specific for the NP 55-63 peptide. These results clearly show that NP 55-63 peptide, although it is not immunodominant, is naturally processed by influenza A virus infected H-2^b cells and suggest that its inefficient presentation is due to a competition with the immunodominant NP 366-374 peptide or other self peptides bound to the D^b molecule with an affinity as high as the affinity of the NP 366-374 peptide.

V 113 Demonstration of a highly stimulatory moiety in a dog hair allergen hyposensitisation vaccine using human T cell clones.

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To investigate the T cell response to dog hair allergens *in vitro*, we measured peripheral blood mononuclear cell (PBMC) proliferation to a dog hair hyposensitisation vaccine. Strong responses were made by all allergic (n=15) and non-allergic donors (n=15) tested, the maximal response occurring at day 6. T cell clones specific for the dog hair preparation were isolated from PB of two allergic donors. The response to dog hair was APC-dependent and MHC class II-restricted. Unexpectedly, a panel of non-HLA-DR matched EB-LCL could also present to the clones. To investigate the possibility that the T cell clones had been selected by a contaminating superantigen in the dog hair preparation, we analysed clones for T cell receptor Vβ usage and their pattern of response to a wide panel of common bacterial superantigens. Both analyses revealed heterogeneity among the clones arguing strongly against selection by superantigen. To identify the stimulatory moiety in dog hair, we used reverse-phase HPLC to fractionate the preparation and tested these fractions for recognition by PBMC and T cell clones. All cell populations responded strongly to a 64kD fraction which N-terminal sequencing showed to be dog albumin. However, a source of highly purified dog albumin failed to induce proliferation. We conclude that the dog hair preparation may contain a highly stimulatory peptide that co-elutes with albumin and is promiscuous in its ability to be presented to T cell clones by APC of differing MHC class II haplotypes.

V 114 H2-K^B ANCHOR MOTIF POINT SUBSTITUTED PEPTIDES: IMMUNOGENICITY DEPENDENT ON PEPTIDE INDUCED MHC CLASS I STABILITY, Grayson B. Lipford, Stefan Bauer, Herrman Wagner and Klaus Heeg, Institute for Medical Microbiology, Technical University of Munich, Munich 81675, F.R.G.

Class I molecules are conformationally sensitive to peptide binding, prolonging the complexes half-life on the surface of the cell. To date the concept of peptide-class I binding strength has been equated with a peptide's ability to stabilize class I surface expression. By making a series of H2-K^b anchor motif amino acid point substitutions to the OVA 257-264 octomer, we were able to analyze subtle changes in peptide binding, K^b stabilization and in vivo immunogenicity. Binding studies were conducted by titrating the peptides in a competition assay utilizing ^{125}I-SIINYEKL as the reporter peptide. Of sixteen conservative or nonconservative amino acid substitutions to position 3, 5 or 8 tested, only glycine at position 3 severely compromised peptide-K^b binding at 4°C. The cell line RMA-S was used to determine peptide-K^b stabilization. Surprisingly, K^b stabilization was much more susceptible than binding to amino acid changes in the OVA peptide, with several substitutions severely effecting K^b surface expression. When substituted peptides were used as immunogens to prime CTL in vivo, a peptide's ability to stabilize K^b directly correlated with the intensity of specific CTL activation. We therefore conclude that peptide-class I binding may be less limiting than peptide-class I stabilization on the pool of presented peptides and perhaps the phenomena of immunodominance can be related to cell surface presented peptide steady state levels.

V 115 ANALYSIS OF TCR RECOGNITION OF A CLASS I/PEPTIDE EPITOPE AIDED BY THE USE OF A SYNTHETIC, COMBINATORIAL PEPTIDE LIBRARY, Douglas J. Loftus, Kazuyasu Sakaguchi, Ye Chen*, Hiroshi Sakamoto, Alessandro Sette#, Victor H. Engelhard* and Ettore Appella, National Institutes of Health/NCI , Bethesda, MD 20892; #Cytel, San Diego,CA 92121; and *Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22908

Clone AHIII12-2 is an HLA-A2.1 -restricted, xenoreactive CTL clone isolated from a C57BL/6 (H-2^b) mouse. These CTL efficiently lyse EL4/A2.1 transfectants pulsed with the peptide ALWGFFPVL, corresponding to the naturally occurring epitope identified by mass spectrometric analysis (as AXWFFPVX) of peptides eluted from purified A2.1 molecules. Molecular modelling of the A2.1/ALWGFFPVL complex suggested that peptide residues P4 and P6 are appreciably solvent exposed, and might form important TCR contacts. To address the predictions of this model, we examined the ability of variously substituted derivatives of ALWGFFPVL to sensitize target cells for lysis by clone AHIII12-2. Unexpectedly, single substitutions at most positions only minimally affected target lysis, while substitutions Ala-P3 and Ala-P5, occurring at positions expected to be nearly or completely buried in the MHC groove, each gave rise to reduced cell lysis while retaining high affinity (IC_{50}~10nM) binding to A2.1. However, Phe-P3 and Tyr-P5 substitutions gave results comparable to the parent peptide. Guided by these initial results, we constructed a limited synthetic peptide library comprised of 1296 different peptides having 6-fold degeneracy at positions P3-P6, in order to examine an expanded array of potential epitopes. The HPLC-fractionated library was tested for its ability to sensitize target cells for lysis by clone AHIII12-2. This analysis revealed two prominent, late-eluting peaks of activity, one of which corresponds to the retention time of ALWGFFPVL, as well as lesser peaks at earlier retention times. We are currently characterizing these peaks. Our observations thus far indicate that TCR recognition of the class I/peptide epitope shows a non-rigid dependence on peptide sequence, and are consistant with recent observations of others who have suggested that TCR recognition may be sensitive to peptide-induced MHC conformation. However, it is equally plausible that residue substitutions alter the conformation of the MHC-bound peptide, consequently altering T cell recognition. Additionally, our results suggest the utility of synthetic, combinatorial peptide libraries as a means to identify immunologically active and potentially useful peptides for a given T cell/target system in the absence of information regarding the exact sequence of the naturally occurring epitope.

V 116 RAPID DETERMINATION OF CLASS I PEPTIDE BINDING MOTIFS USING CODON-BASED RANDOM PEPTIDE PHAGE DISPLAY LIBRARIES, Jeffrey E.W. Miller†, Tapio Haaparanta†, Anders Brunmark§, A. Raul Castaño§, Michael Jackson§, Per A. Peterson§, and William D. Huse†.
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Random peptide phage display libraries were screened with empty soluble class I molecules (purified from *Drosophila melanogaster* cells which were cotransfected with β2-microglobulin) following enrichment of candidate phage by panning. Sequencing of positive clones allowed rapid delineation of peptide motifs displayed by these phage. The peptide motifs described are consistent with data obtained from: crystal structures of class I peptide complexes, peptides eluted from cellular class I molecules, and class I-restricted peptide antigen studies. In addition to providing "anchor residue" information, this method provides important binding data concerning the contribution of amino acids which lie proximal to these anchor residue positions. Screening of murine H-2K^b-specific clones with empty class I molecules representing alternative alleles (H-2K^{bm1}, H-2K^{bm8}), yields information concerning the requirements for both shared and allele-specific discrimination in peptide binding. Surface plasmon resonance studies allowed direct solution competition measurements of various peptide motif analogs, and were utilized to quantify binding. Murine HMT, CD1 molecules, and several H-2K^b alleles are among the class I molecules successfully screened using this technique.

V 117 BINDING MOTIFS AND ANTIGENIC PEPTIDES IN HUMAN MHC CLASS I MOLECULES
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Class I molecules bind peptides based on the presence of anchor residues, whose position and identity depend on the individual class I molecule. We have recently determined peptide-binding motifs for HLA-A1, -A3, -B8, -B14 and -Bw44, and have used these motifs to identify new antigenic peptides. In order to increase the predictive power of these motifs, we have developed a mathematical model that we call the independent binding of sidechains (IBS) hypothesis to interpret peptide-binding data. According to the IBS hypothesis, each amino acid within the peptide contributes to binding in a fashion that is independent of the rest of the peptide. To test the IBS hypothesis, an equation is written out for each peptide for which binding data is available, in which the $t_{\frac{1}{2}}$ for dissociation is set equal to the product of coefficients that represent the quantitative importance of each residue. The coefficients are determined by solving the set of simultaneous equations consisting of all of the available binding data. Data on nonbinding peptides are also useful because they place an upper limit on the values of certain coefficients. When these calculations are performed on peptide-binding data for HLA-A2, it is found that the IBS hypothesis accounts for the behavior of the vast majority of the peptides, indicating that significant sidechain-sidechain interactions are unusual. Many antigenic peptides bind tightly to HLA-A2 compared to the predicted binding affinity of all other peptides from the same protein, indicating antigenic peptides are often among the best possible binding peptides. Occasional antigenic peptides are of rather weak relative affinity, suggesting that these peptides may be processed unusually efficiently, or alternatively, that certain T cells are originally activated by a higher affinity peptide, followed by cross-reactive recognition.

V 118 A SMALL NUMBER OF RESIDUES IN THE CLASS II MOLECULE I-A^u CONFER THE ABILITY TO BIND THE MYELIN BASIC PROTEIN PEPTIDE Ac1-11. C.I. Pearson, A.M. Gautam, I.C. Rulifson, R.S. Liblau, and H.O. McDevitt, Department of Microbiology and Immunology, Stanford University Medical Center, Stanford, CA 94305.

The N-terminal peptide, Ac1-11, of myelin basic protein (MBP) can induce experimental autoimmune encephalomyelitis (EAE) in PL/J and (PL/JxSJL/J)F1 mice, but cannot in SJL/J mice, since Ac1-11 binds to I-A^u, but not to I-A^s. We have studied the interaction of Ac1-11 and I-A^u as a model system for therapeutic intervention of the autoimmune response seen in EAE. In this study we have identified a major interaction between the arginine at position five in Ac1-11 and glutamic acid at position 74 in the beta chain of I-A^u. We have also identified two residues that differ between I-A^u and I-A^s that are critical for peptide binding to I-A^u. These two residues, positions 26 and 28, lie on the bottom of the peptide binding groove on the beta chain. Mutants that contain an Aα^u chain paired with an Aβ^s chain which has only these two residues changed to those of Aβ^u bind Ac1-11[4A], a high binding analog of Ac1-11, with much greater affinity than does the wild type I-A^u. Furthermore, when position 70 in the beta chain, which lies on the helix, is changed to an arginine, as is found in I-A^u, binding is reduced to wild type levels. Arginine at position 70 in the beta chain, however, is required for recognition by four of five Ac1-11, I-A^u-specific T cell hybridomas that we have tested. The requirement of arginine for the four T cells tested correlates with the presence of a *negatively charged residue* in the CDR3 region of the T cell receptors, suggesting that the CDR3 regions of the T cell receptors may recognize this residue as well as the peptide. Residues 26, 28, 70, and 74 of the beta chain of I-A^u lie in close proximity and may form a pocket that allows Ac1-11 to bind to I-A^u. These results have provided us with a better understanding of how Ac1-11 sits in the groove of I-A^u and will help us to design immunotherapeutic molecules that can specifically interfere with the interactions we have identified between the peptide and MHC.

V 119 PEPTIDES UTILIZE DIFFERENT DR CONTACT RESIDUES FOR BINDING TO THE DR3 MOLECULES, **Phillip E. Posch, Annemiek Geluk, Hugo A. Araujo, Armead H. Johnson, Tom Ottenhoff and Carolyn Katovich Hurley, Departments of Microbiology and Immunology and Pediatrics, Georgetown University Medical Center, Washington, DC 20007. University Hospital, Leiden, The Netherlands.**

Two naturally occuring DR3 molecules differ by 4 β-chain amino acid residues (positions 26, 28, 47 and 86) whose side chains point into the class II antigen binding groove. Previous analysis has shown that positions 26, 47 and 86 influence recognition by alloproliferative T cell clone suggesting that the functional differences between the DR3 microvariants are mediated primarily through the influence of the variant positions on peptide binding. Murine fibroblast cell lines expressing DR(α,β1*0301), DR(α,β1*0302) and 11 mutant DR3 molecules (created by exchanging the amino acid residues found at the variant DR3 positions) were examined for their ability to bind peptide antigens. The binding of HA 307-319 (DR(α,β1*0302)-specific) was affected only when position 28 was mutated in conjunction with position 26 or 86 suggesting a cooperative interaction between these positions for HA binding to DR(α,β1*0302). Two peptides (HSP 3-13 and SWM 132-151) that are DR(α,β1*0301)-specific utilize different combinations of variant DR3 amino acid residues for binding. Changing position 28 or 86 alone significantly reduced HSP 3-13 binding to DR(α,β1*0301) which suggests that both positions are equally important for binding HSP. All of the mutant molecules examined, including single residue mutant 0302 molecules, were able to bind SWM to some degree indicating that all four variant DR3 positions interact with SWM; thus, only by changing all four residues (0301 to 0302) is binding completely abrogated. These data suggest that different peptides utilize different combinations of DR contact residues for binding and may explain why not all peptides isolated from an individual DR molecule contain a similar binding motif. Finally, it is clear that subtle evolutionary changes which created such DR microvariants result in dramatic differences in the peptide repetoires that bind to these molecules.

V 120 EPITOPE MAPPING OF CD4+ T-LYMPHOCYTE LINES FROM SEROPOSITIVE PATIENTS ENROLLED IN A GP160 VACCINE TRIAL, Silvia Ratto*§, Karl V. Sitz‡, Lawrence D. Loomis*, Fabrizio Manca§, Robert R. Redfield‡ and Deborah L. Birx‡, *H. M. Jackson Foundation and ‡Department of Retroviral Research, Walter Reed Army Institute of Research, 13 Taft Court, Rockville MD 20850, §Department of Immunology, University of Genoa. San Martino Hospital 16132 Genoa Italy and the MMCARR.
Gp160 and gp120 specific CD4+ T lymphocyte lines were developed and evaluated from 11 HIV seropositive volunteers enrolled in a double blinded phase II gp160 vaccine trial. We were able to generate gp160 specific T cell lines in 8/11 and gp120 specific T cell lines in 11/11 volunteers after 3 cycles of stimulation. Each lines was started from PBMC collected 150 days from the beginning of the trial. We challenged the specific T cell lines with a panel of peptides covering constant and variable regions of gp160. We arbitrarily characterized the response as broad or narrow according to the number of peptide pools the antigen-specific T cell lines were able to recognize. All but one gp160 specific T cell lines were able to recognize V3 peptide pool. Moreover 3/7 narrow responders showed the presence of p24 in their culture supernatants demonstrating active viral replication and 0/4 broad responder had detectable levels of p24. Despite the lines being developed with IIIB antigens, all of the lines which were tested were able to recognize gp120(MN). Taken together these preliminary findings, observed in this unique cohort of patients, provide new insight into the character of gp120 peptide recognition by T lymphocytes. This information not only contributes to a better understanding of the functional capabilities of the cellular immune response, but will in the future, help determine the capacity of vaccines to modify this response.

V 121 FINE STRUCTURE OF PEPTIDE ANTIGEN RECOGNIZED BY FBL-3 TUMOR SPECIFIC TH CLONES, Takeyuki Shimizu[1], Hirohide Uenishi[1], Tokukazu Kondo[1], Yasufumi Teramura[2], Michihiro Iwashiro[2], Kagemasa Kuribayashi[2] and Hideo Yamagishi[1], [1]Department of Biophysics, Faculty of Science and [2]Institute of Immunology, Faculty of Medicine, Kyoto University, Kyoto, Japan.
When Friend murine leukemia virus (F-MuLV) induced tumor, FBL-3 is injected in syngenic mouse, the host immune system is elicited and the tumor will be regressed. While cytotoxic T cell (CTL) population play a major role in the rejection, $CD4^+$ helper T (Th) cells also participate as an effector cell population. Although most of $CD4^+$ cells from spleen of immunized animal are autoreactive, we have isolated four Th clones specific for FBL-3. Two of these Th clones (SB14-31, BL4L-23) were stimulated to proliferate by 20mer peptide, *env*122-141 (DEPLTSLTPRCNTAWNRLKL) in MHC I-A^b restricted manner. Even shorter peptide *env*125-137 showed stimulating activity. In order to identify the function of each amino acid residues, we synthesized variant peptides, in which one of each residue of *env*122-141 were replaced by alanine. By analyzing the activities of these peptides, we showed that five residues were necessary to stimulate clone SB14-31. When all but these five residues of *env*125-137 are replaced by alanine, the peptide still have an activity for SB14-31. This indicate that five residues are sufficient for MHC-binding and TCR recognition. Only three of five key replacements can compete the activity of *env*122-141. This indicates that T cell receptor (TCR) of clone SB14-31 recognizes three residues 128(L), 129(T) and 133(N) and that two residues 131(R) and 134(T) are necessary for interaction with MHC. By immunizing mouse with the peptide *env*122-141, some other Th clones were efficiently isolated. These clones, in addition to BL4L-23, showed different activities for alanine substituted or shortened peptides. For example, TCRs of clones BL4L-23 and BP1-3 don't necessitate the key residues of 129(T) and 128(L), respectively. 126(T) is necessary for recognition by clone BP1-2. These data suggest that TCRs of these clones interact with an antigen peptide, *env*122-141-MHC complex in different manner.

V 122 ANALYSIS OF THE INTERACTION OF PEPTIDE-FREE MHC CLASS I MOLECULES WITH IMMUNOGENIC PEPTIDES.
Luis J. Sigal and Dwane Wylie. Department of Biological Sciences, University of Nebraska, Lincoln, NE 68588
MHC class I molecules bind short peptides for cytotoxic T cell scrutiny at the cell surface. The peptide binds to a groove formed by the $\alpha1$–$\alpha2$ domains of class I molecules. Peptide binding occurs in the endoplasmic reticulum (ER) where class I molecules also associate with $\beta2$-microglobulin ($\beta2$m) through their $\alpha3$ domains. Although $\beta2$m is necessary for efficient transport of the complex to the cell surface, whether it is required for efficient peptide-class I binding is still in question. Moreover, the events that occur to form the trimolecular complex (i.e., class I-$\beta2$m-peptide) are not yet fully understood, since work with purified molecules has been hampered by technical difficulties, such as obtaining preparations of class I molecules free of peptides and developing an experimental system that reflects results obtained with intact cells or cell lysates. We have used purified, full-length, class I D^b molecules isolated from the cell line RMA-S (RMA-S-D^b) to obtain efficient, specific binding of peptides in the presence or absence of $\beta2$m. Peptide binding was determined by $\alpha1$ domain refolding, as measured with conformation-specific monoclonal antibodies (mAb) in an ELISA assay system. In a second set of experiments, D^b molecules isolated from RMA cells (RMA-D^b) and subjected to heat (H-D^b) or acid (A-D^b) treatment experienced unfolding of both $\alpha1$ and $\alpha3$ domains. A-D^b regained the $\alpha1$ domain conformation upon addition of specific peptides, but, surprisingly, reappearance of the native conformation of the $\alpha3$ domain also occurred only upon addition of D^b-restricted peptides. While H-D^b retained the ability to undergo peptide-induced $\alpha1$ refolding, the native conformation of the $\alpha3$ domain could not be rescued. These data indicate that: first, $\beta2$m is not necessary for peptide binding; second, acquisition of the native conformation of the $\alpha1$ domain of class I MHC is dependent on specific peptides, but not on the structure of the $\alpha3$ domain; and, third, specific peptide binding affects not only the tertiary structure of the $\alpha1$ domain but also affects folding of the more distal $\alpha3$ domain.

V 123 MURINE MHC CLASS 1 HEAVY CHAIN EXISTS IN MULTIPLE CONFOTRMATIONS ON THE CELL SURFACE, Gautam Thor and Richard W Dutton, Department of Biology and UCSD Cancer Center, University of California, San Diego 92093-0063.
Abstract : We have shown that cell surface radiolabelled murine MHC Class 1 exists in different conformations using various antibodies. In both, Dd transfected fibroblasts or from Spleen cells obtained from C57BL/6 mice, multiple conformations of the MHC heavy chains can be shown by sequentially clearing the lysates with one antibody and subsequent challenge with another.We also provide evidence that one of the antibodies, originally identified by us and called 7.2.14. recognizes a non phosphorylated, non ß2 microglobulin associated heavy chain. This set of heavy chains can be distinguished from those recognized by a pan specific monoclonal antibody called M1/42 believed to recognize all alpha 3 domains of the MHC heavy chain. In fact a more detailed analysis is indicating to us that multiple conformations of the MHC heavy chain exist on the cell surface.

V 124 THE CONFORMATION OF THE PEPTIDE-FREE CLASS II MOLECULE: INFORMATION FROM MUTANT STUDIES, Paul J Travers and Christina Zamoyska. ICRF Structural Molecular Biology Unit, Birkbeck College, Malet St., London WC1E 7HX England

The structure of the MHC class II molecules, recently revealed by crystallographic studies (Brown, J. H., *et al Nature* 364: 33-39, 1993) reflects the conformation adopted by the heterodimer after having bound peptide. However, it is clear from a number of analyses that the conformation of the peptide-free, or "empty" class II heterodimer differs from that of the "full" heterodimer. Since it is the "empty" conformation of the class II molecule that must initially bind peptide, we have been interested in determining how this conformation differs from that of the fully folded, peptide-associated class II molecule. Using a broadly DRβ specific antibody, TAL14.1, we have found that the determinant recognised is dependent on the conformation of the β chain but is also present in monomeric DRβ chains except that of the DRB5*0101 chain. The failure to bind to the monomeric DRB5*0101 chain is not simply as a result of the loss of the epitope, since TAL14.1 does bind to the peptide associated DRB5*0101 αβ heterodimer, but results from the conformational lability of this molecule compared to other DRβ chains. We have determined that the instability in the DRB5*0101 chain is a consequence of ionic interactions between aspartate residues, two of which are unique to the DRB5*0101 sequence. By mutating these residues we have increased the stability of the β chain and restored binding of TAL14.1. From the positions of the aspartate residues in the structure of the class II molecule we can conclude that at least the first three β strands and the short (β1) α helix are formed and must interact, suggesting that they are in an orientation similar to that in the native conformation.

V 125 THE EFFECT OF pH ON HLA-DR1 PREBOUND SELF-PEPTIDES, Robert G. Urban, Roman M. Chicz, and Jack L. Strominger. Harvard University, Department of Biochemistry and Molecular Biology, Cambridge, MA 02138

The predominant peptides bound to MHC class II molecules expressed on human B cells are derived from a relatively limited number of self proteins. We have performed a set of experiments to determine which, if any of these bound self-peptides are uniquely released at pH 4.0 and thus may be released during MHC class II recycling *in vivo* or more readily exchanged than others during pH binding experiments *in vitro*. To avoid the complications arising from the presence of detergent, intact immunoaffinity purified HLA-DR1 molecules were digested with papain to remove the cytoplasmic tail and transmembrane domain. These highly purified water soluble HLA-DR1 molecules were exposed to differing pHs in the presence or absence of high-affinity synthetic peptide. The resulting bound peptides were then acid extracted, and separated by reversed-phase HPLC. Using a combination of mass spectrometry, amino acid analysis, and ultraviolet spectroscopy the ratio of prebound self-peptides to newly bound synthetic peptide was determined. The results unequivocally demonstrate that most of the predominant self-peptides bound to HLA-DR1 are not appreciably released during extended exposure to acidic pH. However, some, but not all, forms of invariant chain derived peptides are substantially reduced after extended acidic exposure. These findings provide new insight into the processes of peptide exchange *in vitro* and imply that the prebound self-peptide repertoire would provide a formidable obstacle to productive recycling of human MHC class II molecules *in vivo*.

V 126 IDENTIFICATION OF AN HLA-DQ2 PEPTIDE BINDING MOTIF AND AN HLA-DPw3-BOUND SELF-PEPTIDE BY POOL SEQUENCING, Frank A. W. Verreck, Anja van de Poel, Annemarie Termijtelen, Reinout Amons*, Jan-Wouter Drijfhout, and Frits Koning.
Dept. of Immunohaematology and Bloodbank, University Hospital Leiden, and Department of Medical Biochemistry*, Sylvius Laboratories Leiden, the Netherlands.

Molecules of the Major Histocompatibility Complex (MHC) present antigenic peptides to T cells. Sequencing peptide pools eluted from MHC class I molecules has established allele-specific peptide binding motifs. We applied pool sequencing to analyze human MHC class II-bound peptides and found that HLA-DQ2-eluted peptides predominantly contained lysine, isoleucine, and phenylalanine at relative position i, i+3 and i+8 respectively. These residues putatively represent anchor residues for MHC binding. Analysis of a heterogeneous HLA-DPw3/DPw4-eluted peptide pool yielded a sequence matching an epitope from the endogeneous enzyme glyceraldehyde-3-phosphate dehydrogenase. This self-peptide and a partially identical, known allo-epitope bound specifically to DPw3- and DR13-molecules, suggesting the sharing of a binding motif. Thus, pool sequencing appears to be applicable for the analysis of MHC class II-eluted peptides. Furthermore, the results indicate that the peptide content of HLA-DP- and HLA-DQ-locus products is of similar size as HLA-DR-bound peptides, supporting the predicted structural similarity between these HLA class II-locus products.

V 127 A SYSTEMATIC STUDY OF T CELL RECOGNITION OF A MHC CLASS II RESTRICTED T CELL EPITOPE: INFLUENCE OF PEPTIDE LENGTH AND TERMINAL CHARGES

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MHC class II-restricted T cell recognition of systematically shortened synthetic peptides derived from a 13-residue T cell epitope was investigated. All 65 possible analogues with a size between 3 and 12 residues were prepared with uncharged termini and with free termini. When the peptides were tested for recognition by a panel of HLA-DR2 restricted T cell clones, a dynamic interaction of MHC and TCR contact residues was deduced. Sometimes, the addition of a C-terminal amino acid residue was found to compensate for the truncation of an important N-terminal residue, and *vice versa*. Occasionally, deletion of additional terminal residues from a peptide that was non-stimulatory for a T cell clone resulted in a peptide that was recognized again by the clone. To the charged analogues, very few positive responses were recorded. Finally, non-overlapping sub-regions of OMP(49-61) were identified, capable of activating a single T cell clone independently.

The results are discussed in the context of a three-dimensional image of the HLA-DR2 - OMP(49-61) complex. The DR2 antigen binding site was modeled departing from the recently resolved structure of the HLA-DR1 molecule. An autostereoscopic 3-D lenticular hard copy was created, based on several different optical angles rendered from the 3-D computer-generated model of the HLA-peptide complex.

V 128 COMPLETE DISSECTION OF THE IGk.1b(180-194) DETERMINANT, GENERATED DURING B CELL PROCESSING OF THEIR OWN IG RECEPTORS, Vitaly L. Yurin, Iren V. Ljadova, Olga G. Kulakova, Rimma A. Bobreneva, Laboratory of Immunology, Reserch Institute for Genetics and Selection of Microorganisms, Moscow 113545, Russia.

We recently showed that Ck domain peptide/s (p.1b) containing 3 (V184,E185,R188) out of 11 Igk.1b allotypic aa substitutions is formed during self Ig receptor processing by normal Igk.1b Bc (peripheral and thymic origin) and recognized by MHC class II (RT-1Bc)-restricted Tc clones from Aug.1a congeneic rats.It was demonstrated that MHC/p.1b-driven Tc-Bc interaction is a bidirectional cognitive event which induce Tc, Bc proliferation and Ig synthesis. Here we present a complete dissection of p.1b determinant using a panel of 14 Th clones to naturally processed Igk.1b, a series of 29 single-residue substitution analogues of p.1b (180-194) so as its N- and C-terminus truncated variants. By performing proliferation and MHC-peptide cell and cell-free competition assays we identified leading MHC and TCR p.1b contact residues for five main Tc recognition patterns. Results support the notion that the clonotypic TCR can see a single MHC class II/peptide structure in different ways. On the other hand, our observations give some grounds for the assumption that different structural aspects of p.1b product/s, generated during Bc processing of their own Ig, may be used by the same MHC restriction element. The current results which extend the analysis of microheterogeneity in MHC-p.1b-TCR recognition, describe the TCR agonist/antagonist action of p.1b analogues, so as influence of self Igk.1b epitope on TCR repertoire generation to p.1b and its single-residue substitution peptides are discussed.

V 129 MAPPING OF MURINE CTL EPITOPES IN HPV 16 E7.

X Zhu[1], E Sadovnikova[1], S Collins[1], C Thomas[1], H Stauss[1], K Vousden[2], L Crawford[3] and P Beverley[1]. [1]ICRF,TIU, 91 Riding House Street, London W1. [2]LICR St Mary's Hospital Medical School, Norfolk Place, London W2. [3]ICRF, TVG, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP.

Intracellular processing of viral proteins produces peptides which associate with class I MHC and are presented to host cytotoxic T cells (CTL). Such virus specific CTL are crucially important in the resistance to many virus infections, and are thought to be important in controlling human infection with papillomavirus type 16 (HPV 16), a virus associated strongly with cervical cancer. We have studied mouse CTL response to the transforming proteins E6 and E7 of HPV 16.

Mice were immunised with recombinant vaccinia E7 (vac E7) and T cells were boosted *in vitro* with E7 transfectants or vac E7 infected stimulators. H-2^b, H-2^d and H-2^k mice immunised with vac E7 developed E7-specific CTL which killed vac E7 infected targets. An epitope was mapped by CTL of H-2^b mice to a region between amino acids 46 to 65 using transfectants contain E7 N- and C- terminal half constructs and a set of overlapping peptides spanning E7. The boundary of the epitope in H-2^b mice was defined to E7 amino acids 49-57. This epitope binds to H-2D^b, but has only a weak H-2D^b motif. E7 peptide 21-28 contains a strong K^b binding motif, but is not recognized by E7 specific CTL from H-2^b mice.

We are currently mapping epitopes recognised by E7 specific H-2^d and H-2^k restricted CTL. To date, none of the overlapping peptides have been recognised, raising the possibility that a host cell protein is involved as a target antigen.

V 130 PEPTIDE BINDING TO DR4DW4 UNDER CONDITIONS THAT RESEMBLE THOSE IN ENDOSOMES. H. Zweerink, M. Gammon, J. Hawkins, B. Devaux*, D. Freed, P. Whiteley and D. Zaller. Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 and *Immulogic Pharmaceutical Corp., Palo Alto, CA 94304.

Binding of peptides to MHC class II molecules that are affinity purified or on the surface of cells has been studied. In neither case do the experimental conditions resemble those in endosomes where peptides bind to class II molecules after removal of the invariant (Ii) chain. We have developed an experimental system that allows us to more closely approximate conditions for peptide binding to DR4Dw4 as they occur in endosomes. CHO cells expressing DR4Dw4 (SE24 cells) were transfected with DNA coding for a truncated form of the Ii chain lacking the first 20 amino acids (SE24Δ20 cells). Similar to fibroblasts expressing DR1Dw1 and the truncated Ii chain (Roche et al. EMBO J. 11: 2841;1992), we observed that the Ii chain is expressed at the cell surface and that the majority of DR4Dw4 molecules is complexed with the Ii chain.

SE24 cells present peptides [two derived from the human immunoglobulin κ chain (amino acids 145-159 and 188-203) and one from the HLA-A2 heavy chain (amino acids 52-70)] efficiently to their corresponding DR4Dw4 restricted T cell hybridomas, whereas SE24Δ20 cells present peptides very poorly. After exposure of SE24Δ20 cells to pH5.5 in the presence of EDTA and cysteine they became more efficient in presenting peptides.

It was also found that this treatment preferentially removed the invariant chain from the cell surface and that Ii chain loss did not occur when a mixture of protease inhibitors was present during treatment. Data will be presented to show the effect of individual protease inhibitors on invariant chain removal, thus allowing the identification of the classes of protease responsible for this.

Adhesion and Accessory Molecules and Their Regulators

V 131 THE INTEGRIN $\alpha_4\beta_7$ DEFINES MATURE THYMOCYTES AND SUBSETS OF MEMORY CD4 LYMPHOCYTES. Andrew, D.P., Berlin, C., Hamaan, A. and E.C. Butcher. Department of Pathology, Stanford University School of Medicine, Stanford CA 94305.

In this report we examined expression and function of $\alpha_4\beta_7$ on lymphocytes using a panel of mAbs to this integrin. We looked at the blocking activity of this panel of anti-$\alpha_4\beta_7$ mAbs in several in vitro adhesion assays and also mapped the epitopes recognized by the panel of mAbs in competition studies. From the results we conclude that there are unique although overlapping epitopes on $\alpha_4\beta_7$ for each of its various adhesive interactions with MAdCAM-1, fibronectin, VCAM-1 and an as yet uncharacterized ligand on lymphocytes for $\alpha_4\beta_7$. Using a novel mAb to a combinatorial epitope on $\alpha_4\beta_7$ we further show that $\alpha_4\beta_7$ is the major homing receptor on lymphocytes for homing to mucosal lymphoid organs.

In terms of expression β_7 proved a useful marker in T cell development, been expressed on mature single positive CD4 and CD8 thymocytes but been absent from double positive $CD4^{+ve}CD8^{+ve}$ thymocytes, although a minor subset of double negative $CD4^{-ve}CD8^{-ve}$ thymocytes expressed β_7. The majority of β_7^{+ve} thymocytes expressed β_7 as $\alpha_E\beta_7$ while a minor non overlapping subpopulation of thymocytes expressed $\alpha_4\beta_7$ at low levels. In the bone marrow expression of β_7 could be used to subdivide the HSC population. Finally, in the periphery, β_7 was expressed at high levels on subsets of memory (activated) memory CD4 lymphocytes and using L-Selectin $\alpha_4\beta_7$ and $\alpha_4\beta_1$ expression it is evident that there are at least 4 subsets of memory CD4 lymphocytes which will differ in their trafficking properties due to the differences in the homing receptors that they express.

V 132 ISOLATION AND CHARACTERIZATION OF MCP-1 AS A T CELL CHEMOATTRACTANT. Michelle Woldemar Carr, Stephen J. Roth, Shayla S. Rose, Ed Luther, Timothy A. Springer, Harvard Medical School and The Center for Blood Research, Boston, MA.

Migration of lymphocytes into sites of inflammation is known to be mediated by adhesive interactions. Additionally, it has long been suspected that a chemoattractant secreted at sites of inflammation might also play a role in recruiting lymphocytes from the bloodstream into the inflammatory lesion. However, the role of chemoattractants in lymphocyte transendothelial migration into inflammatory sites has been poorly characterized. We have utilized a novel transendothelial lymphocyte chemotaxis assay to identify and purify a lymphocyte chemotactic factor in supernatants of mitogen-stimulated peripheral blood mononuclear cells (PBMC). The factor was purified by heparin-Sepharose, size exclusion, and HPLC chromatography. Amino acid sequence analysis revealed it to be identical to monocyte chemoattractant protein-1 (MCP-1), a chemoattractant of the C-C chemokine family that had previously been described as monocyte specific. We confirmed that recombinant MCP-1 is chemoattractive for purified T lymphocytes and for $CD3^+$ lymphocytes in peripheral blood lymphocyte (PBL) preparations. Furthermore, we showed that the majority of T lymphocyte chemotactic activity in mitogen-stimulated PBMC supernatants is neutralized by antibody to MCP-1. Phenotyping of chemoattracted T lymphocytes shows that they are an activated, memory subset, expressing the antigens CD26, CD45RO, and increased levels of CD29. The chemoattracted population is depleted of cells expressing L-selectin and CD45RA. The T lymphocyte response to MCP-1 is dose-dependent, with maximal chemotaxis occurring at 50 ng/ml. The response is chemotactic, rather than chemokinetic, as demonstrated in a checkerboard assay. Furthermore, the response to MCP-1 by T lymphocytes is not dependent on the endothelium present in our chemotaxis assay system since the response can be duplicated using uncoated filters. We conclude that MCP-1 is the major lymphocyte chemoattractant secreted by mitogen-activated PBMC. MCP-1 is capable of acting as a potent T lymphocyte, as well as monocyte, chemoattractant and this may help explain why monocytes and T lymphocytes of the memory subset are always found together at sites of antigen-induced inflammation.

V 133 THE AVIDITY BETWEEN VLAs AND THEIR LIGANDS IS DEPENDENT ON THE STAGE OF T CELL MATURATION, Andrew C. Chang, Scott Wadsworth, Myung-Joo P. Hong and John E. Coligan, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

VLA molecules (β_1 integrins) are cell-surface $\alpha\beta$ heterodimers that bind to extracellular matrix (ECM) proteins such as fibronectin and merosin. We demonstrate that the avidity between VLAs and their ligands is down-regulated during T lymphocyte maturation in the thymus. Mouse thymocytes were fractionated into immature and mature populations based on their expression of the heat stable antigen (HSA), recognized by the J11d mAb. Virtually all thymocytes expressed VLA-4, -5 and -6. However, predominantly immature (HSA^+) thymocytes exhibited ECM-binding capacity *in vitro*. This constitutive binding was down-regulated at the HSA^+ to HSA^- transition stage. The low binding of mature HSA^- cells was not due to decreased levels of VLAs on the cell surface or permanent protein modification since HSA^- cells were able to become adherent cells after stimulation with both PMA and ionomycin. Binding to fibronectin was inhibited by RGD and CS-1 peptides, suggesting that both VLA-4 and VLA-5 are thymocyte receptors for fibronectin. Binding to merosin was inhibited by antibodies recognizing VLA-α_6 or -β_1, suggesting that VLA-6 is a merosin receptor. The constitutive binding of immature HSA^+ thymocytes to ECM was inhibited by protein kinase C (PKC) inhibitors, indicating that PKC plays an important role on regulation of thymocyte adhesion during T cell development. We postulate that a down-regulatory mechanism is responsible for the decrease ECM-binding of mature HSA^- thymocytes, and that this mechanism may play an important role in allowing mature thymocytes to leave the thymus and emigrate to the periphery. The molecular mechanism involved in this down-regulation will be discussed.

V 134 β_1 INTEGRIN CHAIN CONFORMATION AND ANTI-β_1 ANTIBODY MEDIATED ENHANCEMENT OF FIBRONECTIN BINDING ARE DEPENDENT ON THE STAGE OF T CELL MATURATION AND ACTIVATION, John E. Coligan, Andrew Chang, Myung Joo P. Hong and Scott Wadsworth, Biological Resources Branch, Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Integrins are a superfamily of $\alpha\beta$ heterodimers, most of which serve as cell surface receptors for extracellular matrix (ECM) proteins. In this report, we demonstrate that the recently described anti-mouse β_1 integrin chain mAb KMI6 selectively recognizes a β_1 epitope on immature thymocytes and Con A-activated mature thymocytes and peripheral T cells. Surface expression of this epitope was previously thought to be limited to a subset of bone marrow stromal cells and fetal liver erythroblasts. Since virtually all cells examined express β_1 integrins on their surface, the KMI6 epitope is T cell maturation and activation stage-specific. Most day 15 fetal thymocytes were $KMI6^+$ and expression was increased concomitant with IL2R expression. In adults, KMI6 expression was highest on $CD4^-$, $CD8^-$ $CD3^-$ thymocytes and was down regulated at the $CD4^-$ $CD8^-$ $CD3^-$ to $CD4^-$ $CD8^+$ $CD3^-$ transition, with the exception of a subset of $\gamma\delta$ TCR^+ thymocytes which retained expression. $CD4^+$ $CD8^+$ and mature single positive thymocytes, as well as resting peripheral T cells, were $KMI6^-$. Immunoprecipitation analyses demonstrated that KMI6+ β_1 chains were paired with α_4, α_5 and α_6 chains. Addition of KMI6 to cell adhesion assays enhanced $CD4^-$ $CD8^-$ thymocyte, but not activated mature thymocyte or peripheral T cell, binding to fibronectin (via $\alpha_4\beta_1$ and $\alpha_5\beta_1$) while laminin binding (via $\alpha_6\beta_1$) was unaffected. The data show that β_1 integrin conformation is controlled by factors associated with T cell maturation and activation. Furthermore, the ability of KMI6 to enhance ligand binding appears to be integrin-specific and is also affected by factors related to T cell maturation. These complex changes in integrin conformation and ligand binding behavior suggest that regulation of integrin-ECM interactions may have important consequences in early T cell development and at later stages of peripheral T cell activation.

V 135 CTLA-4 IS A LIGAND OF THE LEUKOCYTE INTEGRIN CD11a/18 (LFA-1), Raymond T. Doty,* William A. Brady,‡ Peter S. Linsley,‡ and Edward A. Clark*§, Departments of *Immunology and §Microbiology, University of Washington, Seattle, WA 98195 and ‡Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

CTLA-4 and CD28 are both ligands for B7/BB1, a molecule found on APC. A soluble immunoglobulin fusion of CTLA-4 (CTLA4Ig) has a higher affinity for B7/BB1 than does CD28Ig. CTLA4Ig *in vitro* blocks the binding of CD28 to B7/BB1, and both *in vivo* and *in vitro* inhibits costimulation of T cell activation. We have found that although CTLA4Ig and CD28Ig both immunoprecipitate a 55 to 100 kD glycoprotein from B7/BB1$^+$ CHO cells, CTLA4Ig, unlike CD28Ig, immunoprecipitates additional proteins 100 kD and 180 kD in size from several B cell lines. Sequential immunoprecipitation demonstrated that the additional proteins recognized by CTLA4Ig were the CD11a/18 (LFA-1) heterodimer. Subsequently, CTLA4Ig was found to bind to the surface of, and immunoprecipitate CD11a/18 from, B7/BB1$^-$ cells, thus demonstrating that the binding of CTLA4Ig to CD11a/18 does not require concurrent binding to B7/BB1. Using a recombinant CTLA-4/CD28 fusion protein, we found that the region of CTLA-4 required for binding to CD11a/18 was within the extracellular Ig-like domain. In addition to CTLA4Ig binding to, and precipitating CD11a/18 from lymphocytes, we found that CTLA4Ig can inhibit CD18-dependent lymphocyte aggregation, and that this inhibition depends on CTLA4Ig binding to CD11a/18. This suggests that CTLA-4 may play a role in the immune response independent of its binding to B7/BB1.

V 136 IDENTIFICATION AND EXPRESSION OF MURINE CD27, A MEMBER OF THE NGF-R FAMILY, Loes A. Gravestein, Bianca Blom, Evert de Vries, Jannie Borst and Wil A.M. Loenen, Division of Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, THE NETHERLANDS

CD27 is a lymphocyte specific member of the nerve growth factor receptor family, that includes two TNF-Rs, CD40 and Apo-1/Fas, all molecules important in the regulation of cell survival. Also the CD27 ligand (CD27L) has considerable homology with the ligands of other family members like TNF and CD40L. Sofar CD27 has not been implicated in cell survival. Its expression pattern and the functional effects of CD27L suggest a role for CD27 during T cell activation and possibly during thymic differentiation.

To allow *in vivo* studies, which would provide more insight into CD27 function, we have identified murine CD27 (mCD27) at the cDNA and protein level. The mCD27 gene was mapped to mouse chromosome 6, tighly liked to Ly-4, Tnfr-2 and Hcph[1]. mCD27 is 65% identical to human CD27 (hCD27). Highest homology of 80% is found in the amino terminal cysteine rich, ligand binding domain, and the carboxy terminal part of the cytoplasmic domain. The structure and expression of mCD27 was compared to that of 4-1BB, another lymphocyte specific member of the NGF-R family. mCD27 and 4-1BB are 39% identical in the ligand binding domain and have a high degree of homology in the carboxy terminal part of the cytoplasmic tail. mCD27 mRNA was detected in spleen and thymus, in CD3$^{-/dull}$ as well as CD3^{bright} thymocytes, but not in non lymphoid tissues; mCD27 mRNA expression was found in resting T cells and was upregulated upon activation. 4-1BB was detected exclusively in activated T cells, where it was expressed with different kinetics than mCD27. Peptide antisera identified murine CD27 as a 45 kDa protein on thymocytes and activated T cells and 4-1BB as a 35-40 kDa protein on activated T cells.

[1]Neal G. Copeland et al, Science (1993) 262: 57-66

V 137 ANALYSIS OF β1 INTEGRIN STRUCTURE AND FUNCTION UTILIZING A β1-NEGATIVE HUMAN T CELL LINE. Estelle S. Harris, Nadine C. Romzek, Pamela J. Reynolds, Elizabeth Ennis, James L. Mobley, and Yoji Shimizu, Department of Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, MI 48109

The β1 or VLA integrins are αβ heterodimeric cell surface receptors that play an important role in T cell function by mediating adhesion to extracellular matrix proteins such as fibronectin (FN) and cell surface counter-receptors such as VCAM-1. A critical but poorly understood mechanism of β1 integrin regulation involves the rapid upregulation of the functional activity of β1 integrins by T cell activation without corresponding changes in β1 integrin cell surface expression. Although studies of β2 integrins have implicated the β2 cytoplasmic domain as a target for intracellular signals that upregulate β2 integrin activity, similar analysis of β1 integrin activity has not been conducted. Like peripheral T cells, activation of the Jurkat T cell line with PMA or mAb crosslinking of either CD3, CD2 or CD28 results in increased Jurkat adhesion to FN via the α4β1 (VLA-4) and α5β1 (VLA-5) integrins. Using γ-irradiation and multiple selections by panning for mutants that have lost the ability to bind to FN upon PMA stimulation, we have isolated a Jurkat derivative, designated A1, that lacks expression of the β1 chain. FACS analysis of mutant A1 reveals that loss of cell surface β1 expression also results in complete loss of cell surface expression of the α3, α5 and α6 chains. Compared to parental Jurkat cells, cell surface expression of α4 is reduced but not eliminated in mutant A1, presumably due to association of α4 with β7. Functional studies show that mutant A1, either unstimulated or PMA-stimulated, is completely unable to bind to either FN or VCAM-1. Immunoprecipitation studies using metabolically labeled lysates also show no expression of the β1 chain but normal levels of α4 expression. Northern blotting analysis reveals reduced levels of β1 mRNA in mutant A1 compared to parental cells. Reintroduction by DNA-mediated gene transfer of a full-length β1 cDNA results in re-expression of the β1 chain on the cell surface, although not to levels seen in parental Jurkat cells. However, adhesion studies show that these transfectants are now able to bind to FN or VCAM-1 and this adhesion can be upregulated by PMA stimulation or CD3 crosslinking. Thus, mutant A1 represents a unique cellular reagent for the analysis of T cell β1 integrin structure and function.

V 138 IDENTIFICATION AND ANALYSIS OF FUNCTIONAL DOMAINS OF MOUSE COMPLEMENT RECEPTORS 1 AND 2, V. M. Holers, Taroh Kinoshita and Hector Molina. University of Colorado Health Sciences Center, Denver, Colorado, Washington University School of Medicine, St. Louis, Missouri and Osaka University, Osaka, Japan.

Mouse complement receptor 1 and 2 (MCR1 and MCR2) are receptors for mouse C3 and are considered to be the B lymphocyte homologues of human CR1 and CR2. MCR1 also acts as a cofactor for factor I mediated cleavage of C3, a process necessary to generate the C3d ligand form of C3 which then binds CR2 with high affinity. As opposed to human CR1 and CR2, however, which are products of unique genes, MCR1 and MCR2 are the alternatively spliced products of a common mRNA. MCR1 shares with MCR2 the COOH-terminal 15 short consensus repeats (SCR) but has six additional NH_2-terminal SCR. To further determine the relationship between human and mouse CR1, and identify C3 binding and factor I cofactor activity sites, we analyzed mouse-human chimeras in which the C3 binding domain of human CR2 has been replaced by different regions within the first eight SCR of MCR1. Rosette analysis of our chimeras with erythrocytes bearing different mouse C3 fragments revealed a weak C3b binding site within SCR 1-2 of MCR1. There is no independent C3b binding domain within SCR 3-6, but their presence enhances C3b rosette formation to chimeras containing SCR 1-2. 8C12, a mAb which partially blocks C3b interaction with MCR1, binds only chimeras containing SCR 3-4. There is no C3d binding area within the first six SCR, but our data confirm previous studies demonstrating the presence of an additional C3b/C3d binding region within SCR 7-8 of MCR1 (SCR1-2 of MCR2). Cofactor activity for C3 cleavage is in the first four SCR of MCR1. In summary, like human CR1, MCR1 contains two independent C3b binding sites and has unique cofactor activity. These results further support our hypothesis that these two molecules are functional homologues of human CR1 and CR2 despite their pronounced structural differences.

V 139 ON THE INDUCTION OF APOPTOSIS BY THE HIV ENVELOPE GLYCOPROTEIN COMPLEX, E. Jacotot, C. Callebaut and A.G Hovanessian, Institut Pasteur, Unité de Virologie et Immunologie Cellulaire (UA CNRS 1157), 28, rue du Dr. Roux 75015 Paris France

The external or surface (SU) and transmembrane (TM) envelope glycoproteins of HIV, are involved in the mechanism of HIV entry into cells and when expressed on the membrane of infected cells have the capacity to trigger cell death by apoptosis through interactions with cell-surface CD4 molecules. Recently, we demonstrated that entry of HIV requires the presence and functioning of the T cell activation antigen, CD26. Here we suggest that CD26 may also be involved in the mechanism of initiation of apoptosis by the SU/TM complex in CD4 expressing cells.

Previous reports from several laboratories have indicated that CD26 may contribute to T cell activation, and that its dipeptidyl peptidase IV activity may be required during this process. Accordingly, stimulation of CD26 with a specific monoclonal antibody results in a comitogenic effect on T cell activation. This and the observation that CD26 is found to be associated with CD45, suggest that the CD26/CD45 complex through tyrosine kinase-dependent transduction pathways may play a regulatory role in T lymphocyte activation. The HIV envelope SU glycoprotein, through the interaction of its V3 loop with CD26, could probably interfere with the normal functioning of CD26 during the T cell activation process and thus result in an abnormal signaling which is associated with apoptosis. Several reports have shown a selective decrease in the proportion of CD26 expressing $CD4^+$ T lymphocytes in HIV-1 infected individuals. This latter is consistent with the requirement of CD26 for HIV entry, and the fact that HIV producing cells *in vitro* cultures die by apoptosis. Taken together, these observations favor the hypothesis that CD26 is implicated in the mechanism of triggering apoptosis in CD4 expressing cells by the SU/TM complex of HIV.

V 140 CHANGES IN L-SELECTIN EPITOPE EXPRESSION DURING B CELL ACTIVATION.
Gitte Jensen, Patricia Huerta, John Po, and Chaim Shustik, McGill University, Departments of Surgery and Hematology, Montreal, Quebec, Canada.

L-selectin is the principal homing receptor that directs the homing of blood lymphocytes to peripheral lymph nodes via high endothelial venules. It is involved to a lesser degree in lymphocyte homing to Peyer's patches. We have demonstrated rapid changes in L-selectin epitope expression early (10 minutes) after activation of blood B cells in vitro. Using three different monoclonal antibodies specific for the L-selectin molecule (TQ1, Leu8, and FMC46) we found that most freshly isolated blood B cells express TQ1 and FMC46, whereas only an average of 60% express Leu8. After activation with pokeweed mitogen in vitro we observed a rapid increase in the number of B cells expressing Leu8, almost to the same numbers as for TQ1 and FMC46. This suggests that some B cells express an alternative form of L-selectin, not recognized by the Leu8 antibody, and that this can be rapidly modified upon activation, possibly by a conformational change. The number of B cells positive for FMC46 and TQ1 remained almost constant at 95% during the first 24 hours post activation, whereas the number of B cells expressing the Leu8 epitope of L-selectin after 24 hours were only 40%. Thus, the activation-induced up-regulation of the Leu8 epitope of L-selectin is transient. Our data indicate that previously described functionally different subsets of B cells based on Leu8 expression are 1) not reflecting L-selectin expression and cannot be reproduced using other L-selectin antibodies, and 2) are reversible, and may reflect the activation status of a given cell.

V 141 LY-49 MEDIATES EL4 LYMPHOMA ADHESION TO ISOLATED CLASS I MHC MOLECULES, Kevin P. Kane, Deparment of Immunology, Univ. of Alberta, Edmonton, Alberta, Canada. T6G 2H7

Ly-49 is a recently identified cell surface molecule expressed on a subpopulation of NK cells and certain T lymphomas. It has been suggested, based on gene transfection and antibody blocking studies, that Ly-49 is a negative regulator of NK lytic activity, possibly through an interaction with target cell class I molecules. We have found that T lymphomas expressing Ly-49 bind isolated class I MHC molecules but not class II molecules immobilized on plastic. Adhesion to class I molecules was not found with T lymphomas lacking Ly-49 expression. The Ly-49 expressing EL4 lymphoma bound D^d and D^k but not K^d and K^k, demonstrating a restricted pattern of class I adhesion that is consistent with the specificities previously suggested for Ly-49 in NK lytic assays. The observed cell adhesion is class I density dependent and is extensively inhibited by the A1 monoclonal antibody directed against Ly-49. These results provide direct evidence for Ly-49 serving as a receptor for a subset of class I MHC molecules.

V 142 **ANTI-ICAM1 AUGMENTED IGE SYNTHESIS BY NORMAL B CELLS BUT NOT BY B CELLS FROM PATIENTS WITH WISCOTT-ALDRICH SYNDROME IN RESPONSE TO IL-4 PLUS ANTI-CD40,** Yoshinori Katada, Toshio Tanaka, Hiroshi Ochi, Masakazu Aitani, Shoji Hashimoto, Masaki Suemura and Tadamitsu Kishimoto, Department III of Int. Med., Osaka Univerity Medical School, Suita 556, Japan

It has been demonstrated that in purified B cells IgE synthesis is induced by a T-cell derived cytokine, IL-4 and CD40 ligand (or anti-CD40). Since these stimulants cause a strong aggregation of B cells, we examined the expression of adhesion molecules such as ICAM1(CD54), LFA-1(CD11α/CD18β) and CD43. The incubation of purified B cells from tonsils with anti-CD40 plus IL-4 strikingly enhanced the expression of ICAM1 and CD43 but modestly affected the expression of LFA-1 by FACS analysis. The addition of anti-ICAM1 inhibited its aggregation and, suprisingly, enhanced IgE synthesis by such stimulated B cells at 5-10 fold. This enhancing effect of anti-ICAM1 on IgE synthesis was achieved by the increase in the expression of germline transcript of Cε gene. However, the mechanism through which anti-ICAM1 affected the germline expression remained to be determined. Wiscott-Aldrich syndrome (WAS), is a X-linked immunodeficiency, in whose patients a decreased level of platelets and a defect of Ig production to T-independent antigen are charcteristic features. In the patients, a molecular disturbance of CD43, gp1b or cytoskelton perhaps due to an abnormal glycosylation has been shown. But how these defects lead to immune disfunction is unknown. Since in the patients with WAS generally serum level of IgE is increased, CD43 as well as LFA-1 are ligands to ICAM1 and since it is reported that the avidity change (an increase of the binding capacity) of LFA-1 to ICAM1 may occur by its interaction with cytoskelton, we tested the effect of anti-ICAM1 on IgE synthesis by B cells from two patients with WAS. In contrast to the enhancing effect of anti-ICAM1 on IgE synthesis by normal B cells, the addition of anti-ICAM1 could not enhance IgE synthesis nor augment the expression of germline transcript of Cε gene by B cells with WAS, suggesting that in vivo physical association of ICAM1 with CD43 or LFA-1 molecule may be defected and its interaction regulates IgE production. The binding capacity of LFA-1 and CD43 in lymphocytes from the patients to ICAM1 is now being determined and the molecular characterization of this defect will be discussed.

V 143 STRUCTURE-FUNCTION ANALYSIS OF THE INTERACTION BETWEEN MHC CLASS II MOLECULES AND THE T-CELL CO-RECEPTOR CD4

Rolf König, Sealy Center for Molecular Science and Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX 77555-1019.

Interactions between major histocompatibility (MHC) molecules and the T-cell co-receptors CD4 and CD8 are important in intrathymic T-cell selection and differentiation. Antigen recognition by mature T cells is associated with a class-specific bias in MHC molecule recognition, with CD4+ T cells responding to MHC class II- and CD8+ T lymphocytes to MHC class I-associated antigen.

We have recently identified a region in the MHC class II β-chain that is critical for functional interactions with CD4 (König et al., *Nature* 356: 796, 1992). Here, I show that the α-chain of MHC class II molecules participates in interactions with CD4, too. The three-dimensional structure of the class II MHC molecule HLA-DR1 (Brown et al., *Nature* 364: 33, 1993) suggests that HLA-DR1 exists as a dimer of class II αβ heterodimers. Also, mutational analyses of MHC class II interaction sites on CD4 reported by other laboratories suggest that CD4 may interact with two MHC class II molecules simultaneously. The data reported here corroborate this hypothesis.

V 144 IS CD8 DEPENDENCE A TRUE REFLECTION OF T CELL RECEPTOR AFFINITY FOR ANTIGEN? Gek E. Kwan-Lim, Tim Ong and Rose Zamoyska. Division of Molecular Immunology, NIMR, The Ridgeway, London NW7 1AA. U.K.

Cytotoxic T lymphocytes (CTL) are generally specific for class I major histocompatibility complex (MHC) proteins plus antigen and express CD8 co-receptor molecules. The effector function of some CTL can be blocked by antibodies to CD8 (CD8 dependent CTL), whereas that of others is resistant to blocking (CD8 independent CTL). This difference in sensitivity to antibody-mediated inhibition is assumed to reflect variations in affinity of particular T cell receptors (TCR) for antigen. However, with a panel of T cell hybridomas from CTL clones which have different susceptibility to blocking with anti-CD8 antibody, we have found that a major difference between CD8 independent and CD8 dependent T cells lies in their sensitivity to stimulation, the former responding to lower concentrations of anti-CD3 antibody than the latter. Thus the contribution to cell signalling provided by the co-association of $p56^{lck}$ and CD8 is particularly relevant for CD8 dependent cells. These data challenge the notion that the affinity of an individual TCR for antigen is related to the sensitivity of a cell to anti-CD8 antibodies. Indeed we can show that antibodies to co-receptor molecules have several effects on T cell activation, only some of which may be related to T cell affinity.

V 145 DIFFERENTIAL RESPONSES OF αβ CD8+ AND αα CD8+ αβ TCR+ MURINE INTESTINAL INTRAEPITHELIAL LYMPHOCYTES (iIEL) UPON STIMULATION VIA T CELL RECEPTOR, Nan-Shih Liao, Jen-Pey Su, and Vasily Gelfanov, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

Intestinal IEL locate between and beneth the epithelial cells covering the intestine lumen, and are probably the first group of lymphocytes that encounter antigens and pathogens passing through the intestine. The cellular composition of iIEL is more complicated than that of peripheral T cells, as shown by the presence of CD4+8+ and ααCD8+ cells. Among the ααCD8+ cells, one fouth to half are αβ TCR+ whereas the rest are γδ TCR+ cells. To understand function(s) of CD8 iIEL subpopulations, we have developed purification scheme to separate αβCD8+ and ααCD8+ iIEL, and characterized them in comparison with lymph node (LN) CD8 cells. We found that the expression pattern of Ly6C and CD44 molecules are different among freshly isolated LN and iIEL CD8 subpopulations. When stimulated with αβTCR-specific mAb and peritoneal macrophages in the presence or absence of IL2, αβCD8+ iIEL proliferate and produce IL2, interferon-γ, and tmor necrosis factor(s), whereas ααCD8+ iIEL are negative for these responses even with addition of anti-CD2 mAb. However, the expression patterns of Ly6C and Thy-1 molecules of ααCD8+ iIEL are different before and after stimulation.

V 146 CD38 IS FUNCTIONALLY ASSOCIATED WITH THE IG RECEPTOR COMPLEX AND EXHIBITS A SIGNALLING DEFECT IN TWO IMMUNODEFICIENT B CELL SUBSETS, Frances E. Lund, Michael Cooke*, Leopoldo Santos-Argumedo, Andrew Heath, Nancy Yu, Nanette Solvason, J. Christopher Grimaldi, Michael Parkhouse#, Chris Goodnow*, and Maureen Howard, DNAX Research Institute, Palo Alto CA 94304, *Dept. of Micro. and Immunol., Stanford, CA, and #Div. of Immunol., Inst. for Animal Health, Pirbright Laboratory, Surrey, England.

Murine CD38 is glycoprotein present on the surface of many different hematopoietic cell types. CD38 possesses a unique enzymatic activity which allows the conversion of NAD to ADP-ribose and cyclic ADP-ribose. In addition, Abs to CD38 induce upregulation of cell surface molecules as well as B cell proliferation in conjunction with additional costimuli.

We now demonstrate that signalling through CD38 is impaired in two immunodeficient subsets of B cells; XID B cells and anergic B cells derived from the double transgenic mouse model of tolerance developed by Goodnow *et al.* We have observed that while B cells from both of these mice are competent to receive and respond to signals from T cells, cytokines, and mitogens, they do not proliferate in response to triggering through either the Ag receptor or CD38. Although anergic B cells do not proliferate to these stimuli, these cells can be activated by CD38 and antigen as evidenced by the increases in class II, ICAM-1 or other activation markers. This is distinct from the XID B cells where the CD38 response is completely abrogated, suggesting that the block in CD38 signal transduction is at different points in B cells from these two types of mice. The expression and enzymatic activity of CD38 was examined in both types of B cells and appears normal. Proteins that were tyrosine phosphorylated after anti-CD38 triggering were examined. The profile of these phosphorylated proteins was found to overlap with the profile of proteins that are phosphorylated after Ig receptor crosslinking, suggesting receptor cross-talk between Ig and CD38. Tyrosine phosphorylation after CD38 stimulation was also analyzed in XID and anergic B cells. We have also examined proteins which are either directly or indirectly associated with CD38 in an attempt to understand CD38 signal transduction. Our results demonstrate that by a variety of criteria, the CD38 signalling pathway is functionally similar but not identical to the Ig receptor pathway, and therefore, CD38 may play an important role in a variety of Ag driven responses.

V 147 REGULATION OF IL-2 PRODUCTION BY Ly-6A/E INDEPENDENT OF ITS GLYCOSYL-PHOSPHATIDYLINOSITOL (GPI)-ANCHOR. Thomas R. Malek and Tony J. Fleming, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33136.

Cross-linking of Ly-6 molecules on T lymphocytes leads to IL-2 production that is dependent upon expression of the TCR ζ chain and the GPI-anchor of Ly-6A/E. More recently we found that co-stimulation of T cells via Ly-6A/E and the TCR inhibits IL-2 secretion by a pathway independent of TCR ζ chain expression. This effect was specific for Ly-6A/E in as much as anti-Thy-1 did not block anti-CD3-induced IL-2 production. The present study was initiated in order to determine whether there were unique structural requirements at the level of the Ly-6 molecule for its capacity to activate or block IL-2 production. Functional studies using EL4 cells transfected with various Ly-6 hybrid molecules demonstrated that direct activation of IL-2 secretion or inhibition of anti-CD3-induced IL-2 production was independent of whether anti-Ly-mAbs reacted with NH_2- or COOH-terminal epitopes of Ly-6. In addition, stimulation of mouse Ly-6 proteins expressed in Jurkat cells antagonized PMA/OKT3-induced IL-2 production suggesting that the Ly-6 inhibitory pathway is operative in human cells. EL4 cells were also transfected with a hybrid construct in which the GPI anchor of Ly-6E was replaced by the transmembrane and a portion of the cytoplasmic tail of H-$2D^b$. Anti-Ly-6A/E mAb also blocked anti-CD3ε-induced IL-2 production for these cells even through anti-Ly6E failed to directly induced IL-2 secretion. Thus, anti-Ly-6A/E blockade of IL-2 production is independent of the GPI anchor of Ly-6. This finding suggests that there may be aspects of signalling via Ly-6 that are solely dependent upon the extracellular amino acid sequence of this protein.

V 148 DISRUPTION OF THE LAG(LYMPHOCYTE ACTIVATED GENE)-3 GENE IN MICE, Toru Miyazaki, Christophe Benoist and Diane Matihis, U-184/INSERM-CNRS/LGME, Strasbourg, France.

LAG-3 is a membrane molecule belonging to the Ig superfamily, which is expressed selectively on activated T and NK lymphocytes. This molecule is closely related to CD4 in protein structure, sequence and chromosomal location, suggesting both these two molecules may have shared a common ancestor. Like CD4, LAG-3 molecules associate with class II MHC molecules, however the function of LAG-3 molecule is still unknown. Because this molecule is expressed not only on activated CD4+T cells but also on activated CD8+T cells, γδ T cells and NK cells, LAG-3 molecule may have several immunological roles in addition to the interaction with class II MHC molecule. Moreover, so far as we have examined the expression of LAG-3 molecule in mice, it is expressed also on some populations of thymocytes. This suggests that this molecule may be involved in T cell ontogeny in the thymus.

The best way to determine the exact function of LAG-3 molecule in vivo is to disrupt this molecule in mice by gene targeting. After cloning cDNA and genomic DNA of LAG-3 gene, homologous recombination was carried out in ES cells. The efficiency of homologous recombination was very high; about one in six *neo* resistant clones was targeted clone. Injected targeted clones were well transmitted into germ line.

As Littman et al reported recently, some helper-T cell function is saved in CD4 knock-out mice and it is possible that this helper function may be compensated by LAG-3 molecules. Therefore, in the mean time, we are disrupting both CD4 and LAG-3 molecules using double knock-out method.

We will present preliminary results of analysis of the knock-out mice.

V 149 FIBRONECTIN INDUCES PHOSPHORYLATION OF A 120KD PROTEIN IN T CELLS THAT IS ENHANCED AFTER STIMULATION THROUGH THE T CELL RECEPTOR, Hanne L. Ostergaard and Eileen Ma, Department of Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

T cell activation in response to antigen is a complex event involving a number of cell surface interactions between the T cell and the antigen presenting cell. The majority of studies on T cell signal transduction have been done using antibodies to the T cell receptor (TcR) complex to characterize the signalling pathways that may occur upon engagement of the TcR, however these reveal little about the contributions of accessory molecules to these signalling events. We are beginning to identify some of the signalling events that occur upon engagement of T cell accessory molecules and their contribution to T cell activation. Extracellular matrix proteins, specifically fibronectin (FN), have been shown to stimulate T cell activation in combination with suboptimal amounts of immobilized antibody to the TcR complex. We used this system to determine the contribution of FN to the activation of CD8 positive cytotoxic T lymphocyte (CTL) clones. Consistent with previous observations, we found that FN enhances degranulation in response to suboptimal levels of anti-TcR, but is itself insufficient to trigger degranulation. Also, FN induces a transient increase in phosphorylation of a protein of about 120 kd, peaking between 10-20 min. and decreasing by 40 minutes. A similar pattern is observed with substimulatory amounts of anti-TcR alone. Stimulation of cells with co-immobilized FN and substimulatory amounts of anti-TcR has a synergistic effect and prolongs the increased phosphorylation past 40 minutes. Preliminary experiments suggest that this increase in phosphorylation by FN is inhibited by RGD, a sequence found on many extracellular matrix proteins that forms the binding site for some integrin receptors. Consistent with this, Pronectin, a recombinant FN-like protein consisting of RGD repeats stimulates even higher, but still transient, levels of phosphorylation of p120. We are in the process of trying to identify this target molecule of phosphorylation. A likely candidate is focal adhesion kinase (FAK) which is a 125kd kinase induced in fibroblast cells after binding to fibronectin. The experiments to determine if p120 is FAK are in progress.

V 150 CORTICOSTEROIDS (CS) INHIBIT T CELL ADHESION BY DOWNREGULATING LFA-1 AND CD2 EXPRESSION, C Pitzalis, N Pipitone and GS Panayi, Rheumatology Unit, Division of Medicine, UMDS, Guy's Hospital, London SE1 9RT, UK.

Adhesion molecules play a major role in generating chronic inflammatory infiltrates by regulating leucocyte migration and local retention. First, they mediate adhesion to endothelium (EC). Second, they increase tissue retention by enhancing binding to extracellular matrix. Third, they promote homo/heterotypic intercellular adhesion which is crucial in antigen responses. Finally, they deliver costimulatory signals for cell activation. CS are potent immunosuppressants affecting many immune functions including cytokine production; the relative importance of these effects is unclear. Since CS induce blood leucocytosis in vivo and reduce cellular infiltration in inflamed tissues, an ability to inhibit cell adhesion and migration may be important. We examined whether preincubation of resting or PMA-activated MNC with CS could inhibit 1) adhesion to IL-1 stimulated EC, measured as % of ^{51}Cr-labelled MNC binding to an EC monolayer, 2) formation of cell clusters, assessed by visual scoring on an arbitrary scale of 0-5 (after Rothlein et al) and 3) expression of LFA-1 and CD2 adhesion proteins, measured as mean intensity of fluorescence (mif). First, pretreatment with CS was able to inhibit the binding to IL-1 stimulated EC of both unstimulated (22.8% vs 15.9%) and activated MNC (26.6% vs 14.1%). Second, although the initial PMA-induced aggregation was not affected by CS, CS-pretreated MNC had largely disaggregated by 72h (mean aggregation score 1 compared to 4 for non-CS-treated cells). Third, the expression of LFA-1 (155.7 vs 600.2) and CD2 (116.7 vs 261.3) was reduced in CS-pretreated compared to CS-untreated activated MNC. All these effects were mediated through the glucocorticoid receptor since they were inhibited by the receptor antagonist RU-486. In conclusion, CS-pretreated MNC were less able to adhere to EC and to form stable aggregates; consonant with this, LFA-1 and CD2 expression was decreased. We are currently investigating whether these effects are mediated by a direct action of CS on transcription of adhesion protein genes via their glucocorticoid regulatory elements (GREs) or indirectly, via decreased production of cytokines important for regulating adhesion.

V 151 CD54/ICAM-1 AND MHC II COMPLEMENT HELP PROVIDED TO B CELLS THROUGH CD40 LIGATION,

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We have examined the relative roles of CD40, CD54 and MHC II signalling in the induction of B cell proliferation and responsiveness to IL-2. We used a non-cognate Th1 helper system that is contact as well as IL-2 dependent. Paraformaldehyde-fixed, activated Th1 cells induced expression of IL-2Rα, IL-2Rβ and B7, and upregulated both MHC II and CD54 expression on B cells. Both the T-dependent induction of Ig secretion, and B cell phenotypic changes were inhibited by anti-CD54 and MHC II mAbs and by a CD8-CD40-L/gp39 construct. We compared this to the effects generated through crosslinking of these molecules on B cells. Crosslinking CD40 upregulated expression of MHC II, CD54 and B7, analogous to the effect of fixed Th1 cells. Co-crosslinking of MHC II and CD54 generated comparable effects. Crosslinking of MHC II and CD54 (in the presence of IL-5) induced expression of a functional IL-2R on B cells, but did not induce proliferation. By contrast CD40 ligation induced B cell proliferation, but did not induce IL-2R expression or IL-2 responsiveness. Our data confirm the importance of the CD40:CD40L/gp39 interaction during delivery of T help for B cells, but show that CD40 ligation is not sufficient for B cell differentiation. CD54 and MHC II signalling is complementary to that of CD40 in the generation of T-dependent B cell responses to IL-2. We are presently investigating Protein Tyrosine Kinase activation that accompanies CD54 and MHC II ligation with a view to defining biochemical signalling pathways critical for these events.

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V 152 SIGNALING THROUGH CD53 ON B LYMPHOCYTES

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CD53 is a pan leukocyte glycoprotein which is a member of the recently described Transmembrane 4 Superfamily (TM4SF) which span the lipid layer four times. The structure and functional characteristics of these molecules indicate that they may play an important role in transmembrane signaling in different cells. Monoclonal antibodies to members of this family, including CD9, CD37, CD63 and TAPA-1, have been shown to exert effects on activation of human platelets and leukocytes. Recently, it has been shown that cross-linking of CD53 in the membrane of human B cells, monocytes and granulocytes induce calcium fluxes. In order to investigate if CD53 could induce B cell proliferation and differentiation, we have measured responses following cross-linking of CD53 and compared these with signaling through sIgM. The CD53 mAb (MEM53) was not able to activate B cells alone, but increased proliferation was observed after cross-linking with sheep-anti-mouse H+L chain (SAM) in the presence of T cell supernatant (54 $\pm$ 5,4 percent of an anti-μ+T cell supernatant response). Stimulation of B cells with cross-linked CD53 or IgM promoted a clear shift in cellular volume distribution and a corresponding increase in CD69 and CD71 expression. Furthermore, stimulation of B cells with anti-CD53 cross-linked with SAM in the presence of T cell supernatant induced IgG and IgM production. Taken together, the data suggest that CD53 plays an important functional role in B cell activation and proliferation.

V 153 THE ROLE OF EXTRACELLULAR MATRIX-PROTEINS IN THE ACTIVATION PROCESS OF MURINE CD4+ T CELLS,

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CD4+ T cells are activated by signals transduced via the T cell receptor/CD3 complex in the presence of accessory cells which deliver necessary costimulatory signals. We investigated the costimulatory role of diverse extracellular matrix (ECM)-proteins such as various collagen types, fibronectin, laminin and undulin for resting and preactivated murine CD4+ T cells. Highly purified CD4+ T cells, treated with plate-bound anti-CD3 antibodies, were not induced to IL-2 secretion, IL-2 receptor upregulation and proliferation by coimmobilized ECM-proteins. However, a contamination of the CD4+ T cell population with small amounts of accessory cells resulted in enhanced IL-2 secretion and proliferation induced by coimmobilized collagens or fibronectin. Irradiated splenocytes induced a significantly higher level of T cell proliferation, suggesting that signals delivered by the ECM-proteins are not sufficient for full T cell activation. Antigen-specific cloned T cells reacted to contact with ECM-proteins by augmented IL-2 secretion and expansion. Our data suggest, that cell surface integrin receptors on resting T cells are not activated by stimulation of the T cells through the TCR-CD3 complex. Rather, preactivation of the T cells via accessory molecules in addition to TCR ligation is required. Thus only preactivated CD4+ T cells can profit from stimulatory signals delivered by ECM-proteins.

V 154 MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL MEMBER OF THE IMMUNOGLOBULIN GENE SUPERFAMILY EXPRESSED ON ACTIVATED HUMAN T LYMPHOCYTES, MONOCYTES AND GRANULOCYTES

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We have generated a monoclonal antibody, P1C5, which recognizes a cell surface antigen expressed on human monocytes, granulocytes and activated T lymphocytes. Expression is constituitive for monocytes and granulocytes and is inducible with allogenic and mitogenic stimuli for T lymphocytes. Immunoprecipitation experiments revealed a specific cell-surface labeled polypeptide of 135 kD detected on human monocytes and CD8+ T cells. The P1C5 mAb inhibited T cell activation as assessed by allogenic stimulation *in vitro*. We have used the P1C5 mAb to isolate a full-length cDNA clone which when transiently expressed in fibroblastoid cells results in cell-surface expression of the P1C5 epitope and a precipitable polypeptide of 135 kD, indistinguishable from that expressed by monocytes and activated T cells. DNA sequencing revealed a novel 3,340 bp insert containing a 1,021 aa open reading frame predicted to comprise a type I integral membrane protein. The extracellular domain contains seven variable-type Ig domains and exhibits moderate but significant sequence homology to several members of the Ig gene superfamily. Our results indicate that the P1C5 antigen is a novel member of the Ig gene superfamily that appears to play a role in T cell activation.

V 155 THE ROLE OF PROTEIN KINASES AND INTRA-CELLULAR CALCIUM IN LFA-1 ACTIVATION.

Mairi Stewart and Nancy Hogg, Leucocyte Adhesion Lab, Imperial Cancer Research Fund, London, WC2A 3PX, UK.

The leucocyte integrin LFA-1 (CD11a/CD18) and its major ligand ICAM-1 (CD54) are required for many adhesive functions of leucocytes. Regulation of this intermolecular interaction is controlled by LFA-1 which can alternate between adhesive and non-adhesive states. On T lymphocytes, triggering the TCR/CD3 complex with antibodies induces LFA-1 to bind to ICAM-1 in a transient manner. The activation of LFA-1 following cell surface receptor triggering occurs by unknown "inside-out" signals which are thought to modulate pre-existing cell surface LFA-1 receptors. Phorbol esters have been shown to cause a sustained activation of LFA-1 which suggests that the serine/threonine kinase PKC (protein kinase C) could be involved in the regulatory pathway. We have investigated the role of tyrosine kinases, PKC and intracellular calcium in the regulation of LFA-1 adhesion with emphasis on TCR/CD3 mediated activation. Using inhibitors specific for PKC and tyrosine kinases we have analysed their effects on the ability to stimulate LFA-1 activation. We have measured LFA-1 activation using an adhesion assay in which T cells are induced to bind to rICAM-1 immobilised on plastic. We have also studied the ability to induce the activation epitope on LFA-1 recognised by the mAb 24. Results have indicated a role for tyrosine kinases in a post receptor-occupancy signalling event which seems to be necessary for T cells to adhere to ICAM-1 in a more stable manner. The nature of this signalling event is currently under further investigation.

V 156 DETECTION OF IN VIVO DNA-PROTEIN INTERACTIONS AT THE PROMOTER OF THE CD20 GENE IN B CELLS,

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The CD20/B1 gene encodes a B lymphocyte specific protein involved in the regulation of B cell proliferation and differentiation. Transfection studies with 5' deletion CD20 promoter-CAT constructs have identified two regions of the promoter between bases -186 and -280 and bases -280 and -454 that contain positive regulatory elements. The proximal region has a diverged octamer binding site termed the BAT box that is likely important in maintaining transcription of CD20 in mature B cells and its induction in pre-B cells. Placement of this sequence element upstream of a heterologous promoter resulted in B lymphocyte specific activity of the construct. Deletion of the BAT box from the $CD20_{-454}$ promoter-CAT construct resulted in a 50-70% loss of activity compared to wild type. An in vivo footprint has been identified over this site in B cells. The distal region confers a lymphoid specific activity when placed upstream of a heterologous promoter. Three of five mobility shift assay probes spanning this region bound nuclear proteins. Two of the sites -450 to -432 and -376 to -355 have been studied in detail using mutant probes and competition experiments with known cis-elements. The first binding site has been identified as a NF-Y site and the second as a BSAP binding site. Evidence for an in vivo protein-DNA interaction at the BSAP site has been found. Deletion of the sequences between bases -455 and -410 resulted in a substantial increase in the activity of this construct compared to $CD20_{-454}$ promoter-CAT suggesting the presence of negative regulatory elements in this portion of the promoter. Preliminary results with reporter plasmids containing mutations of either the NF-Y or the BSAP sites suggest that both factors have negative regulatory effects on CD20 gene transcription. In vivo footprinting of other regions of the CD20 promoter has identified several additional regions likely important in CD20 gene transcription including a large footprint just proximal to the major transcriptional start sites. Identification of the proteins interacting with cis-elements in these regions is in progress. These studies are leading to a better understanding of the mechanisms controlling B lymphocyte specific expression of the CD20 gene.

V 157 SPATIAL DISTRIBUTION OF ACTIVATED LFA-1 DETECTED BY FLUORESCENCE RESONANCE ENERGY TRANSFER,

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ß-2 Integrins on cells exist in a resting and in an activated form. Upon activation LFA-1 undergoes a transient conformational change during which it switches to the high affinity state for binding to ICAM-1. However the biochemical correlate which causes LFA-1 activation is still unknown. We wanted to investigate whether the spatial distribution of LFA-1 on T cell surfaces alters upon activation. Fluorescence Resonance Energy Transfer detected with a FACScan provides a good means for determining the proximity of cell surface molecules. The transferred energy between a Fluorescein labelled donor antibody and a Rhodamin labelled acceptor antibody is proportional to the distance between the antibodies. We measured the energy transfer between activated LFA-1 and various other cell surface molecules and compared it to energy transfer between non activated LFA-1 and other cell surface molecules. We found that activated LFA-1 acting as an energy donor or acceptor revealed a much higher energy transfer than non-activated LFA-1. This suggests that LFA-1 molecules move closer to each other upon activation. Another finding was that higher energy transfer occurred using activated LFA-1 as a donor and CD3 as an acceptor than with non-activated LFA-1 as the donor and CD3 as the acceptor, suggesting that activated LFA-1 molecules are in closer range to CD3 than non-activated LFA-1 molecules. In keeping with this finding was the observation that CD4 and CD8 also revealed higher energy transfer with activated LFA-1 than with non-activated LFA-1. No difference in the energy transfer was found between activated LFA-1 and non-activated LFA-1 and other leukocyte proteins such as CD45RA or ICAM-1. These results provide evidence that upon activation, LFA-1 colocalizes with the T cell receptor/CD3 complex.

V 158 LIGATION OF CD7 BUT NOT CD5 T CELL SURFACE MOLECULES LEADS TO PHOSPO-INOSITIDE 3-KINASE ACTIVATION IN T CELLS.

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CD7 is the earliest T cell surface marker molecule to appear during T cell ontogeny which is believed to act as an accessory molecule in T cell activation. CD7 has previously been reported to associate with the TCR complex, CD45 and a protein tyrosine kinase. The CD7 cytoplasmic tail contains the consensus sequence for PtdIns 3-kinase binding (Tyr-X-X-Met) at tyrosine 197. This motif is also found in the cytoplasmic tail of the co-stimulatory molecule CD28 which has recently been reported to modulate D-3 phosphoinositide lipids, the products of PI 3-kinase activity. We have found that ligation of the CD7 molecule on the leukaemic T cell line Jurkat with a monoclonal antibody also leads to time-dependent elevation of D-3 phosphoinositide lipids, specifically $PtdIns(3,4,5)P_3$ and $PtdIns(3,4)P_2$. In contrast, monoclonal antibody ligation of CD5, another T cell surface molecule that associates with TCR and is tyrosine phosphorylated but lacks the consensus motif for PI 3-kinase binding, failed to elicit any change in D-3 phosphoinositide levels. These results imply that CD7 may influence T cell activation processes via modulation of D-3 phosphoinositides. The mechanism of CD7 coupling to PI 3-kinase to CD7 is under investigation.

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Antigen Presenting Cell Subsets

V 159 MRC OX-62: A MONOCLONAL ANTIBODY RAISED AGAINST RAT VEILED CELLS WHICH LABELS DENDRITIC CELLS AND INTRAEPITHELIAL LYMPHOCYTES. Mary Brenan and D Jasper G Rees. Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

Dendritic cells specialised to present antigen to T cells, include Langerhans cells and interstitial dendritic cells in non-lymphoid organs that migrate via afferent lymphatics as veiled cells, to become interdigitating cells in T dependent areas of lymphoid organs. As there are few dendritic cell restricted monoclonal antibodies, identification of dendritic cells has relied on dendritic morphology, constitutive expression of MHC class II and professional antigen presenting capacity. Recently we raised a monoclonal antibody (OX-62) against rat veiled cells obtained from thoracic duct lymph, which recognises a novel α integrin subunit that associates with the β_7. subunit. The monoclonal OX-62 antibody labelled veiled cells and cells with dendritic morphology in non-lymphoid and lymphoid sites where classical dendritic cells have been reported to occur. Unexpectedly, populations of OX-62$^+$, MHC class II$^-$ cells were identified in non-lymphoid tissues. Double immunofluorescence analyses of epidermal ear sheets revealed non-overlapping populations of $\gamma\delta$ dendritic epidermal T cells that were OX-62$^+$, MHC class II$^-$ and Langerhans cells that were OX-62, MHC class II$^+$ cells. This finding raises the question as to which cells migrate out of the skin into the afferent lymphatics to give rise to the OX-62$^+$, MHC class II$^+$ veiled cells. Experiments using the monoclonal OX-62 antibody are in progress to establish the predecessors of veiled cells and to delineate the minor subsets of cells with dendritic morphology in various tissues.

V 160 STIMULATION OF NAIVE CD4 T LYMPHOCYTES BY ACTIVATED B CELLS, Delanie J. Cassell and Ronald H. Schwartz, Laboratory of Cellular and Molecular Immunology, National Institutes of Health, Bethesda, MD 20892

Activation of primed, cloned CD4 T cells to produce IL-2 requires both TcR occupancy and as yet undefined signals mediated by interaction between costimulatory ligands on antigen presenting cells (APC) and CD28 on T cells. B7, a ligand expressed on certain APC subsets, binds CD28 and initiates a costimulatory pathway in T cells triggered via their TcR. CTLA4, a CD28 homologue, has high affinity for B7 and binds to potentially costimulatory ligands expressed on dendritic cells and activated but not resting B cells. In contrast to resting B cells, dendritic cells and activated B cells can serve as stimulators for primed T cells and clones.

Naive CD4 cells share with primed T cells a requirement for costimulation in addition to TcR occupancy in order to produce IL-2. Costimulatory signals can be delivered by ligation of CD28 on naive T cells. Despite high expression of ligands reactive with the CD28 homologue, CTLA4, there is disagreement in the literature as to whether activated B cells can stimulate naive T cells. Even in cases where activated B cells are stimulatory, they are usually inferior APC by comparison with dendritic cells. The reasons for their relative deficiency have not been determined.

We have coupled the use of highly purified APC subsets and Pgp-1^{low} naive CD4 cells with limiting dilution analysis to examine these issues. We find that activated B cells stimulate as high a frequency of naive CD4 cells as do dendritic cells. The amount of IL-2 produced by naive CD4 cells in response to activated B cells, however, is lower than that induced by dendritic cells. The lower lymphokine levels detected do not reflect toxicity caused by activated B cells or their consumption of IL-2 because the levels of IL-2 detected in response to dendritic cells are not affected by the addition of activated B cells to the cultures. Instead, although activated B cells express high levels of CTLA4 reactive ligands, their relative deficiency as APC is due to their suboptimal ability to provide costimulation, since direct ligation of CD28 restores the ability of activated B cells to stimulate naive CD4 T cells.

V 161 **Reexpression of MHC class II genes in MHC class II negative cells,** Cheong-Hee Chang and Richard A. Flavell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Terminally differentiated plasma cells and mouse T cells do not express Major Histocompatibility Complex (MHC) class II genes although class II gene expression is observed in pre B cells and mature B cells as well as in activated human T cells. Transient heterokaryons were prepared and analyzed to investigate the mechanisms of inactivation of MHC class II gene in mouse plasmacytoma cells and mouse T cells. The endogenous MHC class II genes in both mouse plasmacytoma cells and mouse T cells can be reactivated by factors present in B cells. This reactivation of class II gene is also observed by fusion with a human T cell line which express MHC class II genes but not with a class II negative human T cell line. It appears that the loss of MHC class II gene expression during the terminal differentiation of B cells or T cell lineage is due to absence of positive regulatory factor(s) necessary for class II transcription. To clone the factor plasmacytoma cells were transfected with cosmid DNA. MHC classs II positive cells were then sorted and cloned by limiting dilution. The cosmid DNA was rescued from the sorted transfectants and it is under characterization.

V 162 MODULATION OF MURINE MACROPHAGE FUNCTION BY INTERLEUKIN-13.

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Activated macrophages are important effector cells for immune responses to many parasites and immune responses are strongly modulated in part by the effect of Th cell-derived cytokines on macrophages. Th1-derived cytokines such as interferon-γ, are strong stimulators of macrophage activation, while cytokines produced by Th2 cells, including IL-4 and IL-10, have been shown under some conditions to inhibit macrophage activities associated with inflammatory responses. IL-13, a recently described cytokine produced by Th2 cells, is capable of downmodulating macrophage activity in a manner similar to, but more specific than that described for IL-4. Treatment of activated macrophages with IL-13 reduces the production of monokines in response to IFN-γ or lipopolysaccharide, both potent stimulators of these factors. The effect on the production of monokines (such as IL-12) by macrophages could greatly affect T cell subset development. We have also shown that IL-13 affects the production of nitric oxide, which has been implicated in both macrophage cytotoxicity and macrophage-associated immunosuppression. The suppression of nitric oxide by IL-13 leads to a decrease in parasitacidal activity by activated macrophages. However, our data indicate that IL-13 has plieotropic effects - while the inflammatory potential of activated macrophages is significantly reduced, the potential of other macrophage subsets is unimpaired. These data indicate that IL-13 could be a potent modulator of immune responses *in vivo*, with effects that embrace both macrophage suppressive and macrophage potentiating functions.

V 163 MORPHOLOGICAL CHANGES INDUCED IN T CELLS BY ANTIGEN-PRESENTING CELLS, Emmanuel Donnadieu, Georges Bismuth* and Alain Trautmann, Laboratoire de Neurobiologie, E.N.S., 46, rue d'Ulm, 75005 Paris, and *Laboratoire d'Immunologie Cellulaire et Tissulaire, Groupe Hospitalier Pitié-Salpêtrière, 75013 Paris, France.

Stimulation of a human T cell clone (P28D) by antigen presenting cells (APC, i.e. murine fibroblasts transfected with appropriate MHC class II molecules and presenting a diptheria toxoid antigen) results in marked changes of the T cell shape.

These changes, observed within a few tens of seconds after the beginning of the interaction between the two cell types, are characterized by a flattenning, and the appearance of long protrusions which allow T cells to interact with several APC at the same time. T cells deformations take place before any detectable increase in intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$ (as measured with fura-2 on the same imaging system) can be observed. When $[Ca^{2+}]_i$ increases, T lymphocytes stay flat, retract their protrusions but remain in contact with at least one APC.

Protein kinase C (PKC) is probably involved in these shape changes. Indeed, PKC activators like phorbols esters also provoked shape changes in T cells (even though less dramatic than those elicited by APC). More importantly, pretreatment of T cells with a PKC inhibitor (calphostin C) prevented the appearance of the morphological changes elicited by APC. The large increase in $[Ca^{2+}]_i$ triggered by APC plays a key role in the retraction of cell protrusions. T cells which fail to give a $[Ca^{2+}]_i$ response stay deformed. Application of ionomycin or thapsigargin to these cells provoked not only a $[Ca^{2+}]_i$ response but also a retraction of elongated pseudopods.

Thus, shape changes represent early and transient events in T cells responding to antigen presentation. Their role in the activation of T lymphocytes remains to be determined.

V 164 FUNCTIONAL EXPRESSION OF THE COSTIMULATORY MOLECULE B7 ON ACCESSORY CELLS FOR MHC CLASS I-RESTRICTED IMMUNE RESPONSES, Adelheid Elbe, *Hans Reiser, Dirk Strunk and Georg Stingl, Dept. of Dermatol., DIAID, VIRCC, Univ. of Vienna Med. School, Vienna, Austria and * Div. of Lymph. Biology, Dana-Farber Cancer Inst., Boston, MA

We have generated a cell line (80/1) from murine fetal skin displaying the $CD45^+$/MHC Class I^+/MHC Class II^- /FcγRII$^+$/$CD18^+$/$CD54^+$/$CD3^-$ phenotype. We have previously shown that this cell line is capable of activating naive, allogeneic $CD8^+$ T cells in a MHC Class I-restricted fashion. This finding was surprising in that other MHC Class I^+/II^- cell types (P388D1, L929, BW5147, PAM212, peritoneal exudate cells) were substantially less effective. Antigen-specific T cell stimulation requires the delivery of both an antigenic and a costimulatory signal e.g. cytokines and/or adhesion molecules. In this regard, particular attention has focused on B7/BB1 molecules which are strongly expressed on cells of the Langerhans cell/dendritic cell family. Using a murine anti-B7 mAb, we found that 80/1 cells diplayed anti-B7 reactivity similar in magnitude to that seen with cultured Langerhans cells. In contrast, P388D1, L929, BW5147 and PAM212 were negative. Importantly, the continuous presence of anti-B7 mAb in cocultures of 80/1 cells and allogeneic $CD8^+$ lymph node T cells resulted in a 90 - 94% inhibition of the MLR. Moreover, pretreatment of 80/1 cells with the anti-B7 mAb led to a 57% inhibition of the allogeneic MLR, whereas pretreatment of responder $CD8^+$ T cells with anti-B7 mAb had no effect.

In summary, we have demonstrated that presence of the costimulatory molecule B7 on the cell line 80/1 is required for optimal activation of Class I disparate allogeneic $CD8^+$ T cells, suggesting that the addition of costimulatory molecules may render non-immunogenic MHC Class I-bearing cells capable of eliciting primary productive immune responses. These findings have important clinical implications concerning the development of vaccination strategies against endogenous pathogens.

V 165 SIALYLATION OF PROTEIN ANTIGEN PRESENTED BY ADHERENT PERITONEAL EXUDATE CELLS (PEC) REDUCES ITS CAPACITY TO STIMULATE T-HYBRIDOMA CELLS. O.Förster, M.Köller, W.Krugluger, G.Boltz-Nitulescu. Inst. of Exptl.Pathol., Univ. Vienna, Austria.

Macrophages (MΦ) may express lectin-like surface structures with binding specificity for sialic acid, called sialic acid receptor (SAR) in rats (1) and sialoadhesin in mice (2). SAR^+ MΦ were found to suppress T-cell proliferation (3). Sialic acid is considered to function as an "immunological mask" (4), reducing the immune response. We have, studied the role of sialylation of protein antigen (ag) for its capacity to stimulate T-cells. $I\text{-}A^k$-positive, adherent PEC containing about 70% SAR^+ MΦ were pulsed with hen eggwhite lysozyme (HEL), HEL coupled to sialyl lactose (sialo-HEL) or lactose (lacto-HEL). These ag presenting cells (APC) were used to stimulate $I\text{-}A^k$ restricted HEL-specific 3A9 T-hybridoma cells. IL-2 production was estimated in a biological assay on IL-2 dependent CTLL cells. The experiments showed clearly that APC pulsed with sialo-HEL were less stimulatory for 3A9 cells than APC pulsed with native HEL. No difference to native HEL was found with lacto-HEL. Furthermore, no difference in immunogenicity between the 3 different ag glycoforms was found when the SAR-negative lymphoblastoid cell line LK35.2 was used as APC. We would like to raise the hypothesis, that sialylated protein antigens bind preferentially to SAR^+ MΦ which suppress T-cell stimulation.

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V 166 ANALYSIS OF HLA-DQ EXPRESSION BY TRANSFECTION AND TRANSGENESIS.
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HLA-DQ, *-DR* and *-DP* genes are found within the major histocompatibility complex in man. The homologous genes in mice are *H2-A* and *H2-E* respectively, *HLA-DP* having no expressed murine counterpart. HLA-DQ is of particular interest because of it's association with a number of autoimmune diseases, eg. IDDM, coeliac diease and multiple sclerosis. However this situation represents rather a paradox as this molecule generally plays a marginal role in antigen presentation in the periphery. It has been suggested that variation in the level of expression of DQ on thymic cells compared to peripheral cells, as well as differences in the distribution of polymorphic residues in the peptide binding groove of DQ and DR molecules, may imply a different mode of operation for DQ in the immune response. Since *DQ* and *DR* differ with respect to both the regulatory and coding sequences a comparative study of both these regions is being carried out using transfectants and transgenic mice.

In vitro analysis of the variation between isotype promoters has involved the sub-cloning of various upstream segments (160, 650 and 1000bp in length) of *DQ*, *DR*, *H2-A* and *H2-E* to a reporter gene and subsequent transfection into a panel of mouse and human cell lines. These represent examples of thymic, macrophage/monocyte and B cell lineages. It is hoped that these experiments will help to determine whether the high level of DQ found in the thymus relative to antigen presenting cells in the periphery, can be attributed to tissue specific promoter elements upstream of the gene.

In vivo studies involve the further examination of the level of expression and tissue distribution of DQ in HLA-DQw3 transgenic mice, compared to mice in which the endogenous *DQ* promoter has been replaced by that of *H2-E*. To this end we have generated FVB/N mice containing multiple copies of *DQB1*0302* cDNA linked to a 2.2Kb segment of the *H-2Ea* promoter. Analysis of these mice will be described.

V 167 ANTIGEN PRESENTING PROPERTIES OF A MURINE MYOBLAST CELL LINE, Michael J. Garlepp[1], Hyacinth Tabarias[1], Weisan Chen[2] and James McCluskey[2], Australian Neuromuscular Research Institute[1], University of Western Australia, Nedlands, Western Australia, 6009 and Dept of Clinical Immunology[2], Flinders Medical Centre, S A, Australia.

Although the most efficient processing and presentation of antigen is carried out by professional antigen presenting cells (eg macrophages, dendritic cells, B cells) evidence has accumulated that other, but not all, cell types are capable of performing these functions. We have examined the capacity of a murine myoblast cell line (C2C12) to process and present antigen to class I restricted T cell hybridomas. Transfectant C2C12 clones constitutively expressing H-2K^b were produced by lipofection and limiting dilution. Two K^b expressing clones, Kb-5 and Kb-17, when exposed to the ovalbumin (OVA) peptides OVA 55-62 and OVA 257-264 stimulated IL-2 secretion by the Kb-restricted T cell hybridomas IG8 (OVA 55-62) and GA4.2 (OVA 257-264). To analyse antigen processing capacity, native OVA was introduced into the cytoplasm of Kb-17 by electroporation and then co-cultured with IG8 or GA4.2. Both T cells were activated to produce IL-2. The untransfected parent cell line was inactive in both assays.

We have also demonstrated by FACS analysis that the C2C12 cell line constitutively expresses low levels of the co-stimulatory molecule B7 and that its expression can be up-regulated by 24 hour exposure to the cyclic AMP analogue dibutyryl cyclic AMP (dcAMP).

Thus this murine myoblast cell line can present antigenic peptides to, and activate, antigen-specific MHC restricted T cells. The stimulation of both T cell hybridomas after introduction of native OVA into the myoblast cell line indicates that processing of OVA by myoblasts generates peptides similar to those produced during processing by professional antigen presenting cells. Furthermore the expression of B7 on this cell line can be upregulated via a cAMP driven pathway which is activated after interaction of T cell receptors with MHC molecules. These data suggest that under the appropriate conditions skeletal muscle myoblasts may act as antigen presenting cells, consistent with the possibility that skeletal muscle cells can contribute to T cell activation in autoimmune inflammatory muscle diseases.

V 168 HOST BONE MARROW DERIVED CELLS, NOT TUMOR CELLS, PRESENT MHC CLASS I RESTRICTED TUMOR ANTIGENS IN PRIMING OF ANTITUMOR IMMUNE RESPONSE, Alex Y.C. Huang, Paul Golumbek, Elizabeth M. Jaffee, Mojgan Ahmadzadeh, Drew M. Pardoll and Hyam Levitsky, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Many tumors have now been shown to express tumor specific antigens capable of being presented to CD8+ T cells by major histocompatibility complex (MHC) class I molecules. Current antigen presentation dogma predicts that the tumor cell itself should present its own MHC class I-restricted antigens to T cells. However, earlier cross-priming experiments have demonstrated that at least some MHC class I-restricted antigens may also be presented by bystander cells. Here, we show that there is no detectable presentation of MHC class I restricted tumor antigens by the tumor itself during priming of tumor-specific responses. Rather, the tumor antigens are exclusively presented by host bone marrow derived cells. We demonstrated that mice primed with a MHC class I negative tumor produced a CD8$^+$ T cell dependent antitumor immune response. To further investigate this phenomenon, we have constructed bone marrow chimera mice, C57BL6 (H-2^b) -> CB6F1 (H-2^{b}xH-2^d), which were immunized with a Balb/C derived murine colonic epithelial tumor, CT26, that has been transduced with a Moloney-based defective recombinant retrovirus containing the nucleoprotein (NP) gene from the PR8 influenza strain. The tumor-restricted (H-2^d) NP epitope, NP147-155, showed no detectable recognition by the CTLs generated from the immunized chimeric mice in an *in vitro* CTL assay. In contrast, the H-2^b restricted NP epitope, NP366-374, was strongly recognized by the CTLs. T cells from the chimeric mice primed with splenocytes produced a strong CTL response against minor histocompatibility antigens in the context of either H-2^d or H-2^b molecules, indicating that the failure of generating CTL against H-2^d restricted NP epitope was not due to the selection bias during TCR maturation in the chimeric mice. These results imply that an efficient mechanism exists *in vivo* for transfer of MHC I-restricted antigens to bone marrow derived antigen presenting cells (APCs). They also suggest that HLA matching may not be critical in the clinical application of allogeneic tumor vaccines.

V 169 DIFFERENT ACTIVATION LEVELS ARE PROVIDED BY HUMAN MELANOMA CELLS, EXPRESSING THE APPROPRIATE MHC-PEPTIDE COMPLEX,TO TUMOR-SPECIFIC T CELL CLONES Francine Jotereau Yannick Guilloux, Nadine Gervois, Christophe Viret, Marie-Christine Pandolfino and Elisabeth Diez, Unité 211 INSERM. Institut de Biologie, 9 quai Moncousu - 44 035 Nantes Cedex 01 - France.

Human melanomas are frequently infiltrated by tumor-reactiveT lymphocytes. The ability of these cells to elicit a specific anti-tumor response *in vivo* remains however to be established. Because lymphokine production is critical for both proliferative and effector functions of T lymphocytes, we have analyzed the ability of CD4+ and CD8+ melanoma-specific TIL clones to produce major lymphokines: interleukin-2 (IL-2), interferon-γ (IFN-γ) and interleukine-4 (IL-4), as well as tumor necrosis factor (TNF), in response to autologous and allogeneic tumor cells. We report here that all the TIL clones did produce TNF upon stimulation by autologous and by some MHC-sharing melanoma cells. Since this production was not observed on allogeneic melanoma lines not sharing a HLA molecule, and neither in response to autologous lymphoblasts, this established that tumor-restricted antigens were effectively recognized by these clones on melanoma cells. Melanoma cell recognition was however unable to induce the production of measurable IL-2, IL-4 or IFNγ amounts by all the CD8+ clones from 2 patients (out of 4 analyzed) as well as by the majority of the CD4+ clones from the 4 patients under study. Only the CD8+ clones from 2 patients and some CD4+ clones from one of these 2 patients, did produce IL-2 and IFNγ under these conditions. Nonetheless all TIL clones did produce normal lymphokine patterns upon stimulation by the phorbol myristate acetate-calcium ionophore combination (PMA-CaI), showing the existence of at least three lymphokine secretion profiles among these clones, which could also be activated by anti-CD3 mAb. Differential capability of melanoma cells, expressing the specific MHC-peptide complex, to activate lymphokine secretion was then established by showing that CD8 and CD4 IL-2 producer clones failed to produce IL-2, in response to some allogeneic melanoma cells which induced as efficient lysis and/or TNF production by these clones as the autologous tumor cells. Defective lymphokine production in response to either autologous or allogeneic melanoma cells, suggests that antigen presentation by some, but not all, these cells is suboptimal or/and inducing anergy .This likely explains the failure of melanoma-reactive TIL to control tumor development at least in some melanoma patients.

V 170 EXPRESSION AND ANALYSIS OF H-2O AND H-2M

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The murine MHC class II region encodes two well characterized heterodimers, H-2A and H-2E. In addition, the region contains two other loci encoding potential class II-like proteins. We have biochemically characterized the heterodimer encoded by one of these, H-2O. The other locus, H-2M, has not yet been shown to give rise to any translated proteins. Analysis of cDNA sequences indicate that H-2M is present in common laboratory strains of mice and that it, like H-2O, is virtually non-polymorphic.

We have made soluble variants of the H-2O and H-2M molecules by expressing truncated cDNA constructs in Schneider-2 Drosophila cells. These constructs encode the extracellular domains of the α and β chains, either directly, or as fusion proteins with immonoglobulin Fc domains. Protein yield of 0.5-1mg per liter of culture medium has allowed us to purify both H-2O and H-2M to homogeneity. The purified proteins have allowed us to raise antibody reagents against the two molecules and these are now being used to study the expression and tissue distribution of these class II molecules. Purified molecules are also being used to study peptide binding and to determine binding affinities for the invariant chain and for CD4.

V 171 DEFICIENT EXPRESSION OF COSTIMULATORY MOLECULES BY MACROPHAGES INFECTED WITH *LEISHMANIA DONOVANI*, Paul M. Kaye, Nicola J. Rogers, Julia C. Scott, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, WC1E 7HT.
Visceral leishmaniasis (VL) is characterised by a lack of recall Th1-type responses *in vitro*. In mouse models of VL, a disease spectrum ranging from early cure to non-cure can be observed, the latter displaying some, though not all, of the characteristics of human VL. Initial studies had demonstrated that strains representative of these different disease patterns elicited an early Th1-type response, but that this waned with time in non-curing mice. Importantly, no evidence was found for a reciprocal T cell response involving IL-4 or IL-5 (Kaye et.al. *J.Immunol.***146** 2763-2770 1991). As antigen presenting cells isolated from these mice, or infected *in vitro* with *L. donovani*, are deficient in their ability to support anti-CD3 mediated proliferation of $CD4^+$ T cells, we examined the effect of infection on the expression of costimulatory molecules. Bone marrow macrophages, grown in CSF-1, express low levels of both B7 and HSA. This can be enhanced by 24-48h exposure to LPS, heat killed *Listeria* and to a lesser extent by IFN-γ. In contrast to bacterial stimuli, infection with *L. donovani* amastigotes fails to up-regulate expression of these molecules and indeed abrogates the basal levels observed in untreated macrophages. Furthermore, infection with this parasite inhibits, in a dose dependent way, the subsequent response to bacterial and cytokine inducers of B7 and HSA. Immunohistochemical analysis in the liver of non-cure mice demonstrates that HSA and B7 are either absent or expressed at very low levels by infected Kupffer cells and epitheliod cells. These data are the first to support the contention that deficient expression of costimulatory molecules may underlie some chronic parasitic infections.

V 172 PEYER'S PATCH DENDRITIC CELLS: PHENOTYPIC CHARACTERIZATION BY FACS ANALYSIS AND *IN VITRO* PRIMARY AND SECONDARY T-CELL RESPONSES USING A TCR-TRANSGENIC MOUSE MODEL, Brian L. Kelsall, Rolf O. Ehrhardt, and Warren Strober, Laboratory for Clinical Investigation, National Institutes of Health, Bethesda, MD 20892
Oral immunization with protein antigens often results in antigen-specific systemic suppression as well as the induction of local secretory IgA. This unique regulation may be due to antigen processing and presentation by peyer's patch (PP) dendritic cells (DC). We isolated DCs from the PPs of B10.A mice by transient plastic adherence and analyzed their surface phenotype by FACS analysis, and their ability to stimulate both primary and secondary T-cell responses *in vitro* using T-cells from mice transgenic for a TCR (Va11/Vb3) specific for the 88-104 COOH-terminal peptide of pigeon cytochrome C (CyC) and IE^k. After one day in culture, as compared with spleen DCs, PP DCs had the same surface expression of B7/BB1 and antigens recognized by the DC-specific antibodies 33D1, and N418. A 5-10 fold higher level of MHC class II molecules was found on PP DCs. In addition, only PP DCs expressed a novel antigen recognized by monoclonal antibody G8.8. Primary stimulation of peripheral lymph node (LN) T-cells with CyC pulsed spleen and PP DCs resulted in equal proliferation and IL-2 production, but significant levels of Interferon-gamma (IFN-g) only with the PP DCs (121 U/ml for $5x10^5$ T-cells/ml vs. 9 U/ml for spleen DCs, p=0.006). Both FACS sorted CD4+ and T-cells depleated of CD8+ cells with immunomagnetic beads produced IFN-g (155U/ml and 138U/ml, respectively), as did FACS sorted CD8+ T-cells when supplemented with 50U/ml IL-2 (80U/ml IFN-g) and stimulated with CyC pulsed PP DCs. Primary IFN-g production was blocked with antibodies to murine IL-12 (10 U IFN-g/ml). Secondary stimulation of LN or spleen T-cells with CyC pulsed DCs resulted in a similar increased production of IFN-g with PPDCs (mean 10 fold higher than with spleen DCs in 3 experiments). The predominant IFN-g production induced by PPDCs may have implications for understanding tolerance and priming of the systemic immune responses by antigens presented to the intestinal mucosa.

V 173 HALF-LIVES OF PEPTIDE/MHC CLASS II COMPLEXES *IN VIVO* VARY BETWEEN DIFFERENT ORGAN MICROENVIRONMENTS, Bruno A. Kyewski and Klaus-Peter Müller, Tumor Immunology Programme, German Cancer Research Center, D 69 120 Heidelberg, F.R. Germany.
We have determined the half-life *in vivo* of antigen/MHC class II complexes in different organ microenvironments. Mice were pulsed with myoglobin intravenously and MHC class II-positive antigen presenting cell populations from different organs were isolated after various time intervals. Specific antigen/MHC complexes were quantitated by co-cultivation of the APC subsets with myoglobin-specific T-T hybridoma cells *in vitro*. Half-lives of antigen/MHC complexes differed both between organs and between compartments of the same organ. Half-lives in peripheral organs (spleen, bone marrow, mesenteric lymph node, and skin) ranged between 2 and 8 h, whereas in the thymus half-lives between 13 h (cortical epithelial cells) and 22 h (medullary dendritic cells) were observed. Half-lives *in vivo* were independent of antigen processing, since intact protein or antigenic peptides yielded similar values. The regulation of half-lives *in situ* by biosynthetic MHC class II turn-over and tissue kinetics of the different APC subsets will be discussed. The considerably longer half-life of peptide/MHC complexes in the thymus as compared to peripheral organs may reflect the distinct role which antigen presentation plays in both organs, i. e. induction of tolerance versus induction of immunity.

V 174 **IS A DEFECT IN ANTIGEN PRESENTATION THE REASON FOR THE LOSS OF PROTECTIVE IMMUNITY TO *PLASMODIUM FALCIPARUM*?** Wolfgang W. Leitner* and Urszula Krzych#, Departments of *Biology, The Catholic University of America, Washington, DC 20064, and #,Immunology, Walter Reed Army Institute of Research, Washington, DC 20307
Although sporozoites (SPZ)-specific T cell responses, as partial effectors of protective immunity, and parasitized red blood cells (pRBC)-specific T cell responses have both been induced by exposure to radiation-attenuated *Plasmodium* sporozoites (SPZ), the cellular and antigenic requirements for the maintenance of the immune protective status have not been investigated. The availability of PBL from protected as well as SPZ-exposed but no longer protected human volunteers prompted us to address this process. Since a repeated *in vitro* exposure of macrophages to pRBC results in an eventual loss of phagocytic activity, we hypothesized that an intact macrophage function may be one of the key factors in the maintenance of protective immunity by providing steady availability of recognizable antigens required for the expression of memory T cells. Accordingly, our investigations include analysis of macrophage APC function and its interphasing relationship with the pRBC-responding T cells. Preliminary results demonstrate that macrophages pulsed with pRBC lysates induced proliferative T-cell responses of SPZ-immune PBL, whereas such responses were suppressed in the presence of macrophages pulsed with intact pRBC. Furthermore, T cells from protected volunteers secreted IL-2, IL-4 and IFN-γ in response to intact or lysed pRBC, while only IL-2 was induced in non-protected volunteers. The IL-2 receptor was upregulated only in cell cultures from malaria-protected volunteers. By analyzing the course and mechanism of lymphocyte activation and monitoring the expression of various cell surface markers we hope to gain more insight into the interaction between the parasite and cells of the immune system.

V 175 **RETINAL PIGMENT EPITHELIAL CELLS SUPPRESS THE PROLIFERATION OF ACTIVATED T CELLS.**
Janet Liversidge, *Peter Grabowski, and John V. Forrester, Departments of Ophthalmology and * Medicine and Therapeutics, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD, Scotland UK.
Local presentation of retinal antigen by organ resident antigen presenting cells has been suggested as an early event in autoimmune uveoretinitis, leading to activation of autoreactive T cells and enabling their migration across the blood-retina barrier. We have shown that the retinal pigment epithelium (RPE), which forms part of this barrier, profoundly suppresses the proliferation of lymphocytes to specific antigen, mitogen or IL-2, even in the presence of exogenous antigen presenting cells. Analysis of supernatants from these cultures revealed very high levels of PGE_2 (90ng/ml) and nitrite (35μM),the stable end product of nitric oxide synthesis. A cDNA probe for the inducible form of nitric oxide synthase (iNOS) was generated by RT/PCR of total RNA from cytokine stimulated RPE cells which on sequencing showed identity to iNOS cloned from rat hepatocytes. Using this probe, Northern analysis of total RNA extracted from cytokine stimulated RPE cells showed induction of a 4.5 kbp mRNA transcript upon cytokine stimulation. Treatment of cultures with indomethacin (1μg/ml) reduced PGE_2 levels to background and restored proliferation to control levels despite continued generation of elevated nitrite levels (upto 127.5 μM) in the supernatants. L-NMMA, a competitive inhibitor of l-arginine metabolism reduced both nitrite and PGE_2 levels and partially restored proliferation. These results support the theory that nitric oxide enhances cyclooxygenase activity and may act as an inducible mediator of immunosuppressive mechanisms within the microenvironment of the eye at the site of lymphocytre activation.

V 176 HETEROGENEITY AMONG CELL LINEAGES TO PROCESS TRANSFERRIN-ANTIGEN CONJUGATES, Kathleen L. McCoy, M. Shayne Page, Brian J. Merkel, John K. Inman, and Robert Stutzman, Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298
The antigen, pigeon cytochrome c, was coupled to human ferric transferrin by a heteroligation technique to target antigen into the endosomal transport pathway via transferrin receptors. The ability of various types of presenting cells that do or do not express transferrin receptors to process exogenous antigen in their endosomes was investigated by the stimulation of antigen-specific $CD4^+$ T cells with the transferrin-antigen conjugate in a serum-free assay. When three B lymphoma cells were the source of presenting cells, the conjugate was significantly more potent than native antigen in activating the T cells. The conjugate and antigen were similarly presented by splenic B cells that lack transferrin receptors. Treatment of the presenting cells with chloroquine or paraformaldehyde interfered with the T cell response to the conjugate, indicating that the conjugate still required processing to activate T cells. The conjugate followed an intracellular pathway similar to transferrin, remaining in low density vesicles. Within 5 minutes after internalization, degraded conjugate was detected in isolated early endosomes and did not accumulate in these organelles, which is consistent with an early processing compartment. However, both a macrophage hybridoma and a MHC class II-L cell transfectant hardly elicited a T cell response to the conjugate, although stimulation with native antigen was readily observed. These findings could not be attributed to an absence of transferrin receptors or receptor-mediated internalization of the conjugate, nor to differential expression of MHC class II molecules or Ii chain by non-B presenting cells. The poor presentation of the conjugate by the L cell transfectant was associated with diminished catabolism of the conjugate, however the macrophage hybridoma rapidly degraded the conjugate to a comparable extent as a B lymphoma cell. Peritoneal macrophages, which lack transferrin receptors, and the macrophage hybridoma induced a response to the conjugate only at concentrations that allowed internalization by fluid-phase pinocytosis, suggesting that the processing of the conjugate by these cells may require entry into the lysosomes. The lower potency of the conjugate compared to native antigen with non-B presenting cells indicates that these cell types process the conjugate by a different mechanism than used by B cells. Differences in the mechanism of antigen processing utilized by presenting cells of distinct cell lineages may influence immune responsiveness.

V 177 **MACROPHAGES BUT NOT EPIDERMAL LANGERHANS CELLS PRESENT TYPE II COLLAGEN TO T-CELL HYBRIDOMAS,** **Erik Michaëlsson, Meirav Holmdahl, Catharina Johansson*, Annika Scheynius* and Rikard Holmdahl, Department of Medical and Physiological Chemistry, Uppsala University, Uppsala , Sweden and *Department of Clinical Immunology, Karolinska Institute, Stockholm, Sweden**

Dendritic cells have been reported to be very competent antigen-presenting cells for various soluble protein antigens. Langerhans cells (LC) are dendritic cells that reside in the epidermal skin. It is generally believed that LC, after they have acquired antigen in the skin, migrate to the draining lymph node and prime antigen-specific T-cells. In H-2q mice, an intradermal injection with rat type II collagen (CII) in adjuvant results in a strong proliferative T-cell response towards CII and eventually also development of arthritis. To investigate whether LC could be the initiating APCs for the activation of CII-specific T-cells, we have analyzed the capacity of freshly isolated LC, compared to spleen cells (SC) and peritoneal macrophages (PEC), to stimulate CII-specific T-cell hybridomas.

It was found that LC were unable to present two distinct T-cell determinants on CII, unless the antigen was administered as a synthetic peptide. Both of these T-cell determinants were, however, well presented by both PEC and SC. In contrast, three other protein antigens, i.e. myelin basic protein, ovalbumin and pepsin, were efficiently presented by LC. Furthermore, both ovalbumin and pepsin were also presented by PEC and SC, whereas myelin basic protein was presented by SC, but not by PEC.

These findings suggests that macrophages, rather than LC, are the initiating APCs in the immune response towards CII. Alternatively, macrophages degrade CII into peptides capable of binding to the MHC class II molecule on the cell surface of the LC, which then could serve as the APC interacting with the T-cells in the draining lymph node.

V 178 LYMPH NODE DENDRITIC CELLS IN LEISHMANIA MAJOR-IMMUNE MICE SERVE AS LONG-TERM HOST CELLS THAT INDUCE AN ANTIGEN-SPECIFIC T-CELL RESPONSE, Heidrun Moll*+ and Martin Röllinghoff*, *Institute of Clinical Microbiology, University of Erlangen-Nürnberg, 91054 Erlangen, +Infectious Diseases Research Center, 97070 Würzburg, Germany.
Upon infection with **Leishmania major**, a cause of human cutaneous leishmaniasis, mice of resistant strains are able to control the disease with lesions resolving spontaneously. A long-lasting cell-mediated immunity protects the host from subsequent infections. Nevertheless, significant numbers of viable parasites persist in the lymph nodes of those mice. We have recently documented that epidermal Langerhans cells can ingest **L.major** and have the unique ability to transport viable parasites from the infected skin to the draining lymph node for presentation to antigen-specific T cells and initiation of the cellular immune response. During migration, Langerhans cells develop into dendritic cells. In the present study, we analyzed whether parasite persistence in immune hosts is associated with dendritic cells. Immunohistological studies showed that in the lymph nodes of mice that have recovered from infection with **L.major**, both macrophages and dendritic cells harbor parasites. However, only dendritic cells were able to induce a vigorous T-cell immune response to **L.major** in vitro in the absence of exogenous antigen. In vivo tracking experiments suggested that the infected dendritic cells are derived from Langerhans cells that have emigrated from the skin. The data indicate that **L.major**-infected lymph node dendritic cells in immune animals represent long-term host cells that may be required for maintenance of a population of antigen-specific memory T cells protecting the mice from reinfection.

V 179 GENERATION OF IMMORTALIZED FUNCTIONAL DENDRITIC CELL LINES, Ricciardi-Castagnoli P., Paglia P. and Granucci F., CNR Center of Cytopharmacology, University of Milan, Milan 20129, Italy

Dendritic cells are extremely efficient in the presentation of MHC associated peptides and the most potent activators of naive T lymphocytes but the mechanisms responsible for these properties are mostly unknown. Previous studies from our laboratory have shown that a recombinant retrovirus transducing a *myc*^{MH2}-*env*^{AKR} fusion gene was able to immortalize cells of the monocytic-macrophage lineage. We employed this vector to generate dendritic cell (DC) lines. One of these, named D2SC/1, constitutively expressed B7/BB1 and the HSA molecules as well as an array of β_2 integrins such as the CD11c (N418) which are known to be good markers of mouse DC. In addition, D2SC/1 cells also express CD2 and CD44 without prior activation. We also tested the ability of D2SC/1 cells to present class II restricted antigens. For this purpose, D2SC/1 cells were pulsed *in vitro* with various amounts of soluble native hen egg lysozyme (HEL). HEL was presented very efficiently by IFNγ-treated D2SC/1 cells to HEL-specific T cell hybridoma 1H11.3. Surprisingly, IFNγ treatment did not affect the level of class II expression although IFNγ was necessary to generate APC function. The identification of the IFNγ-induced molecules which regulate antigen presentation in DC is in progress but neither cell surface expression of MHC molecules nor the expression of adhesion or co-stimulatory molecules seems to be affected by IFNγ.

V 180 ANTIGEN PRESENTATION BY B CELLS AND NOT BY MACROPHAGES FAVORS IL-4 SYNTHESIS IN HUMAN MEMORY CD4+ T CELLS. Heather Secrist, Jason D. Marshall, Carl J. Chelen, Rosemarie H. DeKruyff and Dale T. Umetsu. Department of Pediatrics, Stanford University, Stanford, CA 94305

We have previously demonstrated that CD4+ T cells from allergic individuals are predisposed to produce IL-4 in response to allergens, and that allergen immunotherapy greatly reduces IL-4 production in an allergen specific fashion (J.Exp.Med. in press). The mechanism that results in the reduction of IL-4 synthesis in treated individuals is not known, but may be related to the route of antigen administration (subcutaneously versus naturally inhaled) and/or the antigen dose (μg injected vs. pg levels inhaled).

In order to test the effect of antigen dose and antigen-presenting cell (APC) type on cytokine production, CD4+ T cells from allergic donors were cultured with various concentrations of antigen and different APC, including purified irradiated autologous macrophages (Mac) or mitomycin C-treated E rosette negative (E-) cells (containing B lymphocytes and Mac). We demonstrated that low concentrations of allergen (0.003 μg/ml) favor IL-4 production whereas high concentrations of allergen (3-30 μg/ml) favor IFN-γ production. This effect was observed both with allergen and with the nonallergen tetanus toxoid, in both allergic donors and nonallergic donors who had been recently boosted with TT. Proliferation of the antigen specific CD4+ T cells was inversely related to IL-4 production, in that maximal proliferation of CD4+ T cells occurred at high concentrations of antigen, when IL-4 production was minimal and IFN-γ production maximal. Addition of anti-IFN-γ mAb to cultures did not reverse the suppressive effect of high concentrations of antigen on IL-4 production, indicating that enhanced levels of IFN-γ were not responsible for the observed decrease in IL-4 production. Since at low concentrations of antigen, B cells efficiently present antigen for which they express surface receptors, we tested the effect of APC type on cytokine production in response to allergen by CD4+ T cells from allergic donors, and demonstrated that mitomycin C-treated E- cells induced significant IL-4 synthesis whereas irradiated Mac induced minimal IL-4 production.

These results demonstrate that cytokine production in memory CD4+ T cells can be modulated by the antigen dose and APC type, and suggest that immunotherapy operates through such mechanisms. Since previous studies have shown that the cytokine profiles of memory CD4+ T cells are difficult to alter with anti-cytokine antibodies, soluble cytokine receptors or cytokines themselves, manipulation of the APC type may be an alternative method to modify the cytokine profiles of such CD4+ T cells.

V 181 INVARIANT-CHAIN PROTEOLYSIS DIFFERS AMONG ANTIGEN PRESENTING CELLS, Susan J. Stull, Maja Marić, Austin Lampert, and Janice S. Blum. Immunology and Diabetes Programs, Virginia Mason Research Center and the University of Washington, Seattle, WA 98101.

Dissociation of the Invariant (I) chain from MHC class II molecules is required for peptide binding to mature class II dimers. The I-chain is cleaved from class II molecules by proteases residing in an acidic intracellular compartment. Subsequently class II/peptide complexes are expressed on the surface of antigen presenting cells (APC). Macrophages and B-cells both function as APC, however, they differ in their capacity to present antigens. Preliminary studies indicate these two cell types also differ in I-chain processing. In human B-lymphoblastoid cell lines (B-LCL) a 21 kilodalton fragment of the invariant chain, termed LIP, along with a series of 11-14 kilodalton fragments, termed SLIPs accumulate following treatment with a cysteine protease inhibitor. By comparison only the SLIP fragments accumulate in IFNγ-induced human monocytes and macrophages. Biochemical analysis indicates that there are differences between SLIPs from B-LCL and macrophages. Potential explanations for these differences are being investigated through studies to: 1) identify and compare the proteases involved in I-chain processing, 2) define post-translational modifications of the I-chain in each cell type, and 3) localize the site(s) of I-chain proteolysis in B-LCL and macrophages. As surface expression and the generation of functional class II complexes is dependent on I-chain release, understanding the molecular events which regulate I-chain processing is critical.

V 182 GENERATION OF AN ANTI-TUMOR IMMUNE RESPONSE BY IN VITRO PRIMING OF DENDRITIC CELLS, Caryn D. Tong, Josh Lauring, Drew M. Pardoll, and Elizabeth M. Jaffee, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Recent studies have provided strong evidence that the failure of the immune system to eliminate tumors that arise *de novo* is due to an inability to respond to tumor-specific antigens. We have recently shown that vaccination with irradiated B16 melanoma cells transduced with retroviral vectors carrying the GM-CSF gene results in generation of potent, specific, and long-lasting anti-tumor immunity. The anti-tumor response was found to be dependent on both CD4+ and CD8+ T cells. In addition, examination of the vaccination site revealed an extensive local influx of activated macrophage-like cells as well as a moderate number of granulocytes. Since several studies have suggested that GM-CSF plays an important role in the maturation and/or function of specialized antigen presenting cells, we are examining the possibility that localized expression of GM-CSF by vaccinating tumor cells might specifically enhance tumor-antigen presentation by host dendritic cells.

Using the method of Steinman, we have isolated dendritic cell precursors from murine bone marrow and stimulated their maturation with GM-CSF. These cells were characterized using a combination of flow cytometry and viral antigen presentation assays. When immature day 5 or day 6 dendritic cells were pulsed overnight with irradiated wild type B16 melanoma *in vitro* and subsequently injected into C57BL/6 mice, tumor-specific CD8+ T cells were generated *in vivo* as demonstrated by *in vitro* CTL assays. This *in vitro* MHC-restricted antitumor activity also correlated with rejection of challenge tumor by mice previously vaccinated with the *in vitro* primed dendritic cells.

V 183 ABNORMAL THYMIC IgG2b EXPRESSION CO-SEGREGATES WITH T-CELL LYMPHOPENIA IN CROSSES INVOLVING THE SPONTANEOUS DIABETIC BB RAT, S. Tullin, L. Hornum, M. Jackerott and H. Markholst, Hagedorn Research Institute, Gentofte, Denmark

One genetic component of spontaneous autoimmune diabetes in BB rats is linked or identical to the recessive T cell lymphopenia trait on chromosome 4. The trait is characterized by severe reduction of peripheral T cells, absence of regulatory TR6 +T cells, and poor response to T cell mitogens. The remaining T cells are enriched for autoreactive capacities. The underlying genetic defect is expressed either in thymocytes or in the thymic APC's of bone marrow origin. Differential screening of a cDNA library from MHC class II purified thymic cells from a normal rat with total cDNA probes from lymphopenic and non-lymphopenic rats, respectively, resulted in identification of 10 different cDNA's detectable with the latter probe only. The sequence of one of these showed 100% homology to the $\gamma 2\beta$ heavy chain of immunoglobulin. Northern blotting experiments with size separated thymic RNA from lymphopenic and non-lymphopenic rats showed that the Igγ2b transcript was present only in non-lymphopenic rats. However, FACS analysis showed similar numbers of B lymphocytes present in thymi of lymphopenic and non-lymphopenic rats. Since the Igγ2b locus is not on chromosome 4, the observed Igγ2b mRNA level difference appears to be a secondary phenomenon. It remains to be shown whether or not the T-cell lymphopenia of the BB rat is caused by deficient thymic B-lymphocytes.

V 184 TOLERANCE INDUCTION TO A PROTEIN ANTIGEN USING B CELLS FROM A TRANSGENIC MOUSE

Victoria N. Yuschenkoff and David C. Parker, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01655

To test the hypothesis that B cells are tolerizing antigen presenting cells in acquired immunologic tolerance we have been examining the role of B cells as APC in vivo. We transfuse spleen cells from transgenic mice expressing the membrane-bound form of human IgM on their B cells into syngeneic, non-transgenic Fvb mice and then challenge the mice with human IgM (Fc fragment) and measure serum antibody responses. We find long-lasting and antigen-specific tolerance in mice transfused with transgenic spleen cells as compared to non-transfused controls or mice receiving normal cells. Adoptive transfer experiments indicate that tolerance exists in both the B cell and T cell compartments of transgenic cell recipients. When mice are transfused with transgenic cells 2 weeks post-challenge priming to human IgM can be blocked and mice transfused 8 weeks post-challenge display reduced anti-human IgM antibody titers. Spleen cell proliferative responses in these mice are also reduced as compared to controls. Experiments in progress include determining where and how long the transfused transgenic cells persist in the recipient mice, examining the capabilities of resting vs. activated transgenic B cells in inducing tolerance to human IgM and determining how the transfused transgenic B cells are able to interfere with priming to human IgM.

Autoimmune Diseases and Models

V 185 TOLERANCE AND AUTOIMMUNITY IN THYROID-HEL TRANSGENIC MICE, Srinivas Akkaraju, Karen Canaan, Bill Ho, and Chris Goodnow, HHMI, Stanford University, Stanford, CA. 94305.

Central to the process of organ-specific autoimmunity is the question of the immune system's reaction to peripherally-expressed antigen. Although deletion or anergy seems to act upon self-reactive cells specific for ubiquitous antigens, these processes may not account for tolerance to tissue-specific antigens. To investigate tolerance mechanisms evoked toward proteins expressed specifically on the thyroid epithelium, we have developed a transgenic mouse model using membrane-bound Hen Egg Lysozyme [HEL] under the control of the rat thyroglobulin promoter [TLK-1 and TLK-2 lines]. Both lines of mice do not display spontaneous infiltration, and in the low-responder b haplotype, immunization with HEL or HEL peptide does not induce pathology. Double-transgenic mice have been made by breeding TLK-2 with HEL-specific Ig or TCR transgenic mice. B cell numbers, phenotype, and function in (Ig x TLK) mice are equivalent to those from Ig single transgenics, indicating antigen expression in the thyroid has little effect on B cell tolerance. Despite the presence of anti-HEL Ig in the serum, the thyroids in these animals do not display immune infiltration. T cells from HEL-specific TCR transgenics uniformly express the transgenic Vβ at high levels, but show a range of expression of the transgenic Vα. (TCR x TLK) mice show a reduction in the numbers of both immature and mature CD4+ T cells expressing high levels of idiotype TCR. Despite this reduction, these mice spontaneously develop marked thyroid infiltration. Double-transgenic animals, nonetheless, are of normal size and weight, and can reproduce. These results suggest that tolerance to some peripheral antigens is incomplete, and that autoimmune pathology can be induced by increasing the frequency of specific effectors.

V 186 HLA-DR RESTRICTED T CELL LINES AND CLONES FROM NEWLY DIAGNOSED TYPE I DIABETIC PATIENTS SPECIFIC FOR INSULINOMA AND NORMAL ISLET MICROSOMAL MEMBRANE PROTEINS, J. Paul Banga, Guo-Cai Huang, Jennifer Tremble, Elaine Bailyes, Susan D. Arden, Tony Kaye, Werner A, Scherbaum and Alan M. McGregor, Department of Medicine, King's College School of Medicine, South Thames Blood Transfusion Service, London, UK; University of Cambridge, Cambridge, UK and Department of Medicine, Universitat Leipsig, Germany

T cells reactive with pancreatic islet beta cell proteins play a pivotal role in the pathogenesis of Type 1 diabetes in experimental animal models and man although the islet cell autoantigens recognised have not been characterised. We have previously demonstrated that disease-related antigens residing in the subcellular fractions of rat insulinoma RIN cell line are recognised by T cells in the diabetic NOD mice but not in the non-diabetogenic NON.NOD-H-2g^7 mice (Diabetologia, 36; 385, 1993). Antigen specific, CD4+ T cell lines and twenty T cell clones have been propagated from the peripheral blood lymphocytes (PBL) of four newly diagnosed Type 1 diabetic patients (patient MC, DR4, 7, DR54, DQ2, 3; patient HS, DR1, 7, DR53, DQ1; patient MR, DR3, 4, DR52, 53, DQ2, 7; patient KC, DR3, DR52, DQ2) with RIN microsomal membrane proteins and IL-2 using autologous, irradiated PBL as an antigen presenting cells (APC). These T cells also proliferate specifically to stimulation with normal rat islet microsomal proteins demonstrating that the RIN antigenic specificities were present in the normal islets. Using Class II, allele specific monoclonal antibodies in inhibition of proliferation studies in two patients identified DR-restricted, antigen specific T cells (patients HS and MR). Studies using microsomal preparations from several rat tissues demonstrated that rat brain cortex, cerebellum and cerebrum proteins specifically stimulated the human diabetic T cell lines and clones, in addition to normal islet proteins. Using electroeluted proteins of RIN microsomal membrane preparations fractionated by SDS-polyacrylamide gel electrophoresis, showed that the T cell lines (HS and MR) recognised a number of different molecular weight polypeptides which varied in the two cell lines. The fact that some of the antigens recognised by the diabetic patient derived T cell lines are common between islets and brain suggests that either glutamic acid decarboxylase (GAD), peripherin or some other unknown antigen(s) may be recognised. Present studies are aimed at using recombinant islet proteins and differential screening of an islet cDNA library to begin to define some of the autoantigens recognised by these T cell lines and clones.

V 187 INHIBITION OF HUMAN IL-10 PRODUCTION BY GLUCOCORTICOIDS DEPENDS ON STIMULUS,
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Proliferation of peripheral blood mononuclear cells (PBMC) in response to anti-CD3 antibody or Staphylococcal enterotoxin A (SEA) is only partially inhibited by glucocorticoids (GC) at optimum concentrations. GC-resistant proliferation driven by anti-CD3 is dependent on IL-2, while that driven by SEA is not. Since Interleukin-10 (IL-10) has been demonstrated to be both immunostimulatory and inhibitory, we have examined the role of IL-10 in GC-resistant proliferation. Dexamethasone (Dex) had no effect on IL-10 production in SEA stimulated cultures. In anti-CD3-stimulated cultures, IL-10 production was inhibited 50% by 10^{-6} M Dex. Steady-state levels of IL-10 mRNA were not correspondingly decreased, indicating an acceleration of message decay or a post-transcriptional inhibition. Anti-IL-10 antibodies failed to reduce proliferation to either stimulus in cultures containing GC, indicating that IL-10 produced during a proliferative response (peak on day 1-2) did not support GC-resistant proliferation. When exogenous IL-10 (2-20 ng/ml) was added at the beginning of culture, IL-2 produced in response to either anti-CD3 or SEA was inhibited 83-89% and proliferation in response to anti-CD3 was inhibited 79% (See also *J. Immunol.* **150**:4754, 1993). Proliferation in response to SEA was inhibited only 21%, and this inhibition was increased only slightly, to 32%, upon inclusion of neutralizing antibody to IL-2. These data indicated that GC-treated cultures remained sensitive to inhibition by IL-10. In addition, these data also suggest that proliferation to superantigen may be supported by an IL-2 independent mechanism which is insensitive to both IL-10 and GC inhibition.

V 188 THE CLONING OF CD70 AND IT'S IDENTIFICATION AS THE LIGAND FOR CD27,
Michael R. Bowman, Mary A. V. Crimmins, Joanne Yetz-Aldape, Ron Kriz, Kerry Kelleher, and Steven H. Herrmann, Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140

CD70 is a surface antigen found on activated but not resting T and B lymphocytes. The biological activity of this antibody defined cell surface molecule on lymphocytes has not been established. Therefore, toward understanding the function of the CD70 protein, a mAb defining the CD70 antigen was used to isolate by expression cloning cDNA responsible for the CD70 molecule. The predicted protein product is a type II transmembrane protein. Bioassays demonstrated that the CD70 cDNA clone expressed in COS cells would induce the proliferation of PHA costimulated T cells. Comparison with known sequences indicates identity with the CD27 ligand. Therefore, the molecule defining the CD70 antigen is identical with the recently defined ligand for CD27.

V 189 THE INDUCTION OF AUTOIMMUNE ARTHRITIS BY BOVINE αI(II)-CB11 IS MEDIATED BY A SINGLE IMMUNODOMINANT T CELL DETERMINANT IN H-2^q MICE,
David D. Brand, Linda K. Myers, Kuniaki Terato, Karen B. Whittington, John M. Stuart, Andrew H. Kang, and Edward F. Rosloniec, Department of Medicine and the Department of Pediatrics, University of Tennessee, and the Veterans Affairs Medical Center, Memphis TN 38104

Collagen induced arthritis (CIA) is an experimental autoimmune disease elicited in genetically susceptible strains of mice by immunization with heterologous type II collagen. This experimental disease is mediated by the immune response of both T cells and B cells and susceptibility is restricted by the class II molecules of the MHC . In order to study the T cell determinants of bovine type II collagen (CII) that mediate the autoimmune response in H-2^q mice, we have identified a cyanogen bromide fragment of bovine CII, CII(124-402), that induces arthritis in DBA/1 mice. Using an overlapping set of mimotope peptides to map the T cell response to CII(124-402), we have determined that the I-A^q-restricted T cell response to this collagen fragment is mediated by a single immunodominant antigenic determinant. Consequently, this determinant plays a central role in promoting the production of the collagen specific antibodies and the induction of CIA in H-2^q mice. Characterization of this immunodominant determinant revealed that the core residues required for T cell stimulation consists of only 8 amino acids and is located at amino acids 260 through 267 of bovine CII. In order to identify which of these amino acids are critical for I-A^q-restricted antigen presentation, alanine-substituted analog peptides were tested for their ability to be recognized by CII(124-402)-primed T cells. Alanine substitutions at positions 260 (I), 262 (G), 264 (K), and 266 (E) all reduced the ability of the analog peptides to stimulate the proliferation of CII(124-402)-primed T cells with respect to wild type peptide, indicating that these amino acid residues are critical to I-A^q-restricted T cell stimulation. Alanine substitutions at the other four positions did not significantly alter their stimulatory capacities. This systematic analysis of the contribution of each of these amino acids, in conjunction with sequences of other peptides known to bind I-A^q, have allowed us to propose a peptide binding motif for the collagen arthritis susceptibility allele, I-A^q.

V 190 INSULIN-SPECIFIC T CELLS ARE A PREDOMINANT COMPONENT OF NOMINALLY ISLET-SPECIFIC T CELLS ISOLATED FROM ISLET INFILTRATES OF PRE-DIABETIC NOD MICE.
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The non-obese diabetic (NOD) mouse is a widely studied animal model of type I diabetes. Investigations of the pathogenesis of diabetes in NOD mice have provided evidence that autoreactive T cells participate in the process of beta cell destruction. It is thus of interest to identify antigens that target beta cells to this T cell mediated destruction. In an analysis of islet-specific T cell lines established from islet infiltrates obtained from pre-diabetic NOD mice, we observed a potent proliferative response to insulin. An analysis of nominally islet cell-specific T cell clones derived from these lines revealed that a high frequency of these clones were insulin-specific. T cell hybrids derived from the islet-specific T cell lines also exhibited insulin specificity. The prevalence of insulin-specific T cells within populations of islet infiltrating cells and the availability of antigen within the beta cells may combine to produce extensive beta cell damage.

V 191 ANTI-CD8 mAB THERAPY PREVENTS THE ACCUMULATION OF B220+ DN T CELLS BUT NOT AUTOANTIBODY PRODUCTION IN C3H-*lpr/lpr* AND C3H-*gld/gld* MICE

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Mice homozygous for *lpr* or *gld* develop autoimmunity and progressive lymphoproliferative disease characterized by the accumulation of an unusual population of functionally impaired B220+,TCR α/ß+,CD4-,CD8- DN T cells. Although these cells are thymus-derived and appear to have undergone thymic negative selection, the identity of their immediate precursors and the mechanisms leading to their accumulation are poorly understood. Here we investigated the role of CD8+ T cells in the development of lymphoproliferative disease and autoantibody production. We showed that treatment of C3H-*lpr* or C3H-*gld* mice with anti-CD8 mAb beginning at 3 wk of age, and continuing to 15 wk of age caused a dramatic reduction in lymphadenopathy. The change in lymph node size resulted predominantly from a very significant decrease in both the proportions and the total numbers of B220+ DN T cells. The proportions of these cells were reduced up to 20-fold and the total numbers per LN up to 400-fold. Chronic treatment with anti-CD8 mAb also decreased the numbers of CD4+ T cells, CD4+B220+ T cells and B cells in *lpr* and *gld* LN up to 5-fold. In contrast to its impact on lymphoproliferative disease, anti-CD8 mAb therapy had no significant effect on B cell hyperactivity, hypergammaglobulinemia or the levels of circulating autoantibodies.These results suggest that in *lpr* and *gld* mice, CD8+ T cells play a crucial role in the accumulation of B220+ DN T cells and also may contribute to the characteristic increase in the numbers of B cells and CD4+ T cells in these mice, but have no significant effect on B cell hyperactivity or autoantibody production.

V 192 DIFFERENCES IN THE REACTIVITY OF AUTOIMMUNE ANTI-DNA ANTIBODIES TO DNA FROM DIFFERENT SPECIES, Dharmesh D. Desai, Melissa A. Stuart and Tony N. Marion, Department of Microbiology and Immunology, University of Tennessee, Memphis, Memphis ,TN 38163.

Results from the structural analyses of autoimmune antibodies to DNA have indicated that autoimmunity to DNA develops as a selective, antigen-specific immune response to DNA. The recurrent and preferential expression of both germline and somatically-derived variable region structures among autoimmune anti-DNA antibodies suggests that DNA or, more likely, DNA-protein complexes may provide the immunogenic stimulus necessary for the development of autoimmunity to DNA. More recent results from similar analyses of immune antibodies to DNA have confirmed the previous results. Normal, non autoimmune mice immunized with calf thymus DNA-peptide complexes produce anti-DNA antibody with serological and structural characteristics similar to those of autoimmune anti-DNA antibodies. Under appropriate conditions, DNA can be immunogenic even in mice that are not predisposed to autoimmunity.

When analyzed for their ability to bind to DNA from different species, both serum and monoclonal antibodies from both autoimmune (NZB x NZW) F_1 mice and calf thymus DNA-peptide immune, normal mice bind to DNA from different organisms with different relative avidities. Antibodies from both immune and autoimmune mice bound to viral DNA poorly. Both IgM and IgG monoclonal antibodies bound better to mammalian DNA than to bacterial DNA. Autoimmune IgM monoclonal antibodies bound calf thymus DNA with higher avidity than mouse DNA. Autoimmune IgG antibodies generally bind equally well to calf thymus and mouse DNA. These results suggest that tolerance to DNA may not be complete in normal, non autoimmune mice. Immunization with heterologous DNA can eventually induce antibody with specificity for homologous DNA. It is not yet clear whether this possibility can also apply to autoimmune responses to DNA. Further analyses of both immune and autoimmune antibodies should provide more information about how specificity for self DNA develops in autoimmunity to DNA and about the nature and specificity of immunological tolerance to DNA.

V 193 INFLAMMATORY RESPONSE IN TRANSGENIC MICE EXPRESSING INCREASED IFN-γ IN THE LENS OF THE EYE. Rita M. Egan, W. David Martin, Julia Stevens and Jerold G. Woodward, Department of Microbiology and Immunology, University of Kentucky, Lexington, Ky 40536

The anterior chamber (AC) of the eye is known to be an immune privileged site, due in part to the lack of MHC antigen expression, the lack of lymphatic drainage and the presence of repressive factors such as transforming growth factor-β (TGF-β). Injection of IFN-γ into the AC is known to overcome the suppression of antigen-specific DTH responses normally seen in the eye. Transgenic mice expressing increased IFN-γ in the lens under the αA-crystallin promoter were produced to determine whether the proinflammatory effects of IFN-γ could abolish immune privilege. In non-immune privileged tissue such as the pancreas, expressing increased IFN-γ under the insulin promoter led to lymphocytic infiltration of islets and loss of tolerance to self-islet proteins. By two weeks of age, both of the αC\IFN-γ transgenic lines produced demonstrate a marked acute and chronic inflammatory cell exudate in the vitreous and AC. By 6 weeks of age the cellular infiltrate has resolved and anterior and posterior synechiae and corneal fibrosis are seen, consistent with the previous inflammation. MHC class I, which is known to be upregulated by IFN-γ, was found by immunohistolgic staining to be strongly expressed in the lens, ciliary body and iris. These results suggest that increased IFN-γ expression in the AC results in the loss of immune privilege, possibly by overcoming the suppressive effects if TGF-β. We are in the process of determining whether tolerance is lost to self ocular antigens in these mice and whether DTH responses are no longer suppressed.

V 194 PERIPHERAL TOLERANCE INDUCTION IN αβ T CELL RECEPTOR TRANSGENIC MICE. **Eidelman, F.J. Oehen, S.U. and Hedrick, S.M. Department of Biology and Cancer Center, University of California, San Diego, La Jolla, CA**

Recent work has shown that the immune response to a variety of antigens can be manipulated *in vivo* so as to diminish the T cell immune response. In experimental allergic encephalomyelitis, successful downregulation of the immune response has been accomplished by administration of antibodies specific for the β chain of the predominantly used T cell receptor (TCR) or by injection of T cell clones, antigenic peptide analogs, or TCR peptides. Despite these successes, the mechanism of the downmodulation remains obscure, largely because it is difficult to follow a small population of T cells with a specific TCR *in vivo*. In order to explore the mechanisms involved in immunomodulation we have used αβ TCR transgenic(TG) mice, in which > 90 % of the T cells are $CD4^+$ and bear the same cytochrome *c*-specific antigen receptor, and thus have a large population of T cells which can be followed *in vivo* . *In vivo* responses of the mice depend on the route of administration and the vehicle. Subcutaneous injection of PCC in CFA leads to sustained activation whereas intraperitoneal injection in IFA gives rise to initial activation followed by a state of relative anergy. Examination of cell surface markers in both cases reveals the T lymphocytes isolated from the animals demonstrate a $CD4^+CD69^{hi}CD45RB^{lo}$ phenotype. In neither case do cells develop a cell surface phenotype which has been associated with T cell memory. Initial attempts at manipulating this model system have shown that monoclonal antibodies specific for the β chain lead to elimination of antigen specific cells and to a downmodulation of the response. TCR peptide injection has led to decrease in T cell activation as measured by CD69 levels. We are currently evaluating the mechanism of this phenomenon. The results of these studies will prove useful in optimizing ways to manipulate the T cell immune response in autoimmune disease.

V 195 GENERATION OF CD4+ TCR TRANSGENIC MICE SPECIFIC FOR THE SV40 T ANTIGEN: A NEW TOOL TO STUDY INDUCTION OF TOLERANCE VERSUS AUTOIMMUNITY IN A TRANSGENIC MOUSE MODEL, *Irmgard Förster, #Ryo Hirose, #Jeff Arbeit and #Douglas Hanahan, *Institut für Genetik, Universität Köln, 50931 Köln, Germany, and #Hormone Research Institute, UCSF, San Francisco, CA 94143

Transgenic mice expressing the SV40 large T antigen (Tag) under the control of the rat insulin promoter (RIP) have been shown to be either tolerant toward Tag or develop an autoimmune reaction against their pancreatic ß cells depending on the onset and level of Tag-expression in ontogeny. We have isolated CD4+ and CD8+ Tag-specific T cell lines from infiltrated pancreatic islets of an autoimmune RIP1-Tag5 mouse and have cloned the T cell receptor (TCR) variable region genes expressed in one of the CD4+ T cell clones. The genomic Vß8.3-Dß2.1-Jß2.4 and Vα2-JαMT1-27 V gene rearrangements encoding an I-A^k restricted Tag-specific TCR were inserted into cosmid vectors already containing the TCR α and ß constant region genes (provided by D. Loh) and used to generate TCR transgenic mice. Two lines of transgenic mice were obtained in which either 5-10% (Tag-TCR1) or 70-80% (Tag-TCR2) of peripheral T cells express the transgenic T cell receptor as identified by a clonotype specific antibody. Naive T cells from both transgenic lines proliferate vigorously in response to Tag-stimulation in vitro. The Tag-TCR mice are presently being bred to the RIP-Tag transgenic lines. Since the transgenic TCR has been cloned from a T cell known to be involved in the Tag-specific autoimmune reaction it will be interesting to follow the fate of the transgenic T cells during their development in either the tolerant or autoimmune Tag-transgenic backgrounds.

V 196 REQUIREMENTS FOR INDUCTION OF AUTOIMMUNE DISEASE MEDIATED BY CD8+ T CELLS. William R. Heath and Jacques F.A.P. Miller, Thymus Biology Unit, The Walter and Eliza Hall Institute for Medical Research, Melbourne, Victoria 3050, Australia.

To investigate the requirements for induction of autoimmunity mediated by CD8+ T cells, we have generated transgenic mice that express the class I molecule K^b in the ß cells of the pancreas under the control of the rat insulin promoter (RIP-K^b mice), and these mice were crossed to TCR transgenic mice that expressed a K^b-specific TCR. Autoreactive CD8+ T cells were detected in the periphery of these mice, although those cells expressing the highest density of the K^b-specific TCR (presumably the highest avidity cells) appeared to be deleted in the thymus. Under normal conditions, the lower avidity CD8+ cells present in TCR x RIP-K^b mice did not cause autoimmunity, even after priming. They were, however, able to cause disease when supplied with IL-2, suggesting that, in the presence of help, lower avidity cells may be autoaggressive. To examine whether higher avidity CD8+ cells could cause autoimmunity in the absence of exogenous help, RIP-K^b mice were manipulated so as to enable the maturation of K^b-specific T cells expressing the highest density of TCR. These cells normally ignored islet K^b antigens, but in contrast to lower avidity cells, were able to cause transient autoimmunity after priming. These data support a model where higher avidity CD8+ cells, in contrast to lower avidity cells, can cause autoimmune disease in the absence of exogenously-supplied "help".

V 197 Fc GAMMA RECEPTOR PRODUCTION BY CON-A ACTIVATED T-SUPPRESSOR LYMPHOCYTES IN INSULIN-DEPENDENT * DIABETICS. T.S.Hephzibah Rani, Mohd Ishaq. N. Sudhakar Rao, Dept. of Genetics, Osmania University, Hyderabad-7, A.P. India. * Prof. & Head, Dept. of Endocrinology, Gandhi General Hospital, Sec. A.P. India.

T - cells bearing Fc receptors for Fc portions of immunoglobulin (Ig) heavy chains have been reported to play a regulatory role in Ig isotype production. T-cell Fc gamma receptor, a factor produced by activated T-suppressor cells during immune response plays an important role in the termination of IgG type of antibody response. Any derangement in the levels of the production of these receptors genetically or otherwise, can result in the continued production of antibodies beyond the required levels. A substantial autoimmune damage to B-cell of pancreas in type I diabetics is supported by autoantibodies to pancreatic islet cells and to insulin. An indication of elevated immunoglobulin levels as hyper B cells function is also been related to altered helper to suppressor ratio; latter being reported decreased in type I diabetes. We estimated T-cell Fc gamma receptor levels in IDDM patients and compared with the controls. The method employed was to obtain lymphocytes from whole blood and subjected to Con-A mediated activation for the expression of these receptors. The activated T-cells (ATC) release these receptors when suspended in serum free medium. The mean levels of T-cell Fc gamma receptor in control subjects (12.5 ± 2.90), IDDM patients (3.3 ± 0.72). Our observation suggest that the significant reduction in the receptor levels appears to be due to reduced number of T-suppressor cells. Significance of the results will be discussed.

V 198 ESTROGEN INFLUENCES LYMPHOCYTE ACTIVATION IN AUTOIMMUNE ARTHRITIS IN MICE. Liselotte Jansson and Rikard Holmdahl, Department of Medical and Physiological Chemistry, Uppsala University, Uppsala, Sweden.

Type II collagen induced arthritis (CIA) in mice is a T cell dependent, MHC class II associated disease with similarities to human rheumatoid arthritis (RA). In mice, estrogen treatment has been shown to suppress the disease both prophylactically and therapeutically. Both sexes of mice, castrated or normal, are modulated by estrogen treatment. To try to found out the mechanism through which estrogen perform its suppressive effect in CIA we have investigated the effects on lymphocyte activation. The T cell dependent anti CII autoantibody response are decreased but the B cells in the spleen are polyclonally stimulated by estrogen. Analyses of anti CII T cells *in vivo* by the delayed type of hypersensitivity test, DTH, showed a suppressed response after estrogen treatment.

We now report a reduction of gIFN producing T cells in draining lymphnods 10 days after immunization with CII after estrogen treatment, measured by *ex vivo* ELISPOT assay. A correlation to a suppressed proliferation of anti CII specific T cells, measured with ^{3}H-thymidine incorporation, was found. Further analyses of the cytokine profile of T cells during estrogen treatment are ongoing, using bioassays with cytokine dependent cell lines, mice with disrupted Il-4 gene and semiquantitative PCR techniques.

V 199 ROLE OF MRL BACKGROUND GENES IN THE INDUCTION OF ANTI-Sm ANTIBODY RESPONSE IN LPR MICE, V. N. Kakkanaiah, Eric S. Sobel, Philip L. Cohen and Robert A. Eisenberg, Departments of Medicine and Microbiology / Immunology, University of North Carolina, Chapel Hill, NC 27599.

Anti-Sm antibodies are specific markers of human SLE and of murine models of this disease. In humans, the anti-Sm Abs are mostly IgG1 and in MRL/lpr mice, IgG2a; both are T-dependent isotypes. Other lpr strains, such as B6/lpr, do not produce anti-Sm Ab spontaneously. The present study is aimed at identifying the cellular expression of background genes responsible for generation of the anti-Sm Ab response in MRL/lpr mice. We used double chimeras made by transferring MRL/lpr and B6/lpr-H-2^k bone marrow into irradiated allotype heterozygous F1 mice. We used two different set of F1 host mice, viz., (MRL/lprxMRL/lpr-Igh^b)F1 and (MRL/lprxB6/lpr-H-2^k)F1 mice to examine the influence of non-lymphoid genes on auto Ab production. FACS analysis after 5 months of reconstitution revealed that both MRL/lpr and B6/lpr-H-2^k cells co-existed in roughly equal numbers. Antibody produced by each donor could be distinguished by allotype-specific assays. IgG2a anti-Sm was made only by MRL-derived B cells despite the presence of T cells which might potentially provide help to the B6/lpr B cells. The frequency of anti-Sm Ab producing individuals was similar to that of unmanipulated MRL/lpr mice (about 25%). IgG2a anti-chromatin and total IgG2a was mostly dominated by the MRL-derived B cells in both sets of mice. B6-derived B cells produced more rheumatoid factor (RF) against their own anti-IgG2b^b, while RF against anti-IgG2a was dominated by MRL-derived B cells. Also, the antibody profiles in both sets of host F1 mice were identical. This suggests that the commitment to produce anti-Sm and other auto Abs is determined by MRL- or B6-background genes which are expressed in (?pre) B cells and not in T cells or non-lymphoid cells.

V 200 MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENE DISRUPTION PREVENTS EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS$^+$. Rashmi Kaul*, Mohan Shenoy, Elzbieta Goluszko and Premkumar Christadoss. Department of Microbiology and Immunology. University of Texas Medical Branch, Galveston, TX. 77555.

To analyze the impact of lack of MHC class II gene expression, and to demonstrate the direct genetic evidence for the involvement of the MHC class II gene product in the development of experimental autoimmune myasthenia gravis (EAMG), MHC class II gene disrupted C57BL6 mutant (-/-) and EAMG-susceptible MHC class II wild-type C57BL6 mice (+/+) were evaluated for the clinical and immunopathological manifestations of EAMG. Eight of 15 (53%) wild type (+/+) mice developed muscle weakness, while none of the 15 (0%) MHC class II mutant (-/-) mice displayed muscle weakness. The deficiency of MHC class II, and therefore, CD4$^+$ T cells, completely prevented the C57BL6 MHC class II mutant (-/-) mice from mounting both the cellular and humoral autoimmune response to the nicotinic acetylcholine receptor (AChR). Further, the mutant (-/-) mice failed to show any immunopathological (muscle AChR loss) manifestation of EAMG. The data unequivocally provide direct genetic evidence for the essential role of MHC class II molecule in the induction of EAMG, and rules out any pathogenic effector role for MHC class I restricted CD8$^+$ T cells, $\gamma\delta$ TCR bearing cells, and NK cells, which are intact in the MHC class II mutant mice. The lack of pathogenic effector role for MHC class I restricted CD8$^+$ T cells is further demonstrated in the β2 microglobulin deficient (MHC class I and CD8$^+$ T cell deficient) mice, which had high incidence of EAMG. Therefore, induction of EAMG is solely mediated by MHC class II restricted CD4$^+$ T cells which help B cells to produce pathogenic anti-AChR antibodies. *McLaughlin Fellow. $^+$Supported by MDA and Sealy Smith Endowment.

V 201 SIMILARITY BETWEEN THE POTENTIAL ANTI-DNA ANTIBODY REPERTOIRES IN AUTOIMMUNE AND NORMAL MICE, Krishnan, M.R. and Tony N. Marion, Department of Microbiology and Immunology,University of Tennessee,Memphis,TN 38163

Structural analyses of anti-DNA antibody variable regions by us and others have demonstrated that both IgM and IgG anti-DNA antibodies in autoimmune (NZB x NZW)F_1 mice have the characteristics of secondary immune antibodies. These characteristics include recurrent and preferential expression of particular V_H and V_L genes and somatically derived structures that promote antibody binding to DNA. The most striking of these somatically derived structures is the expression of one or more arginines in VH-CDR3. Moreover, the avidity of anti-DNA antibodies for binding mammalian duplex, B-form DNA can be correlated to the relative positions of arginines in VH-CDR3. Arginines in VH-CDR3 of anti-DNA antibodies appear to be generated by junctional diversity during V-D-J recombination. There is a much higher frequency of appearance of arginines in VH-CDR3 of autoimmune anti-DNA antibodies than for antibodies in general. The purpose of this study was to determine whether antibodies from autoimmune mice have a higher frequency of arginines in VH-CDR3 in general when compared to antibodies from non-autoimmune mice. To answer this question we have compared the heavy-chain variable region sequences for monoclonal antibodies derived from LPS-stimulated B cells from pre autoimmune (NZB x NZW) F1 mice with those derived from LPS stimulated B cells from, non-autoimmune BALB/c mice. Both DNA-binding and non-DNA binding monoclonal antibodies from both types of mice were analyzed.

Our results indicate that variable region structures of anti-DNA mAbs from the normal BALB/c and the (NZB x NZW) F1 mouse respectively are similar in all respects including the frequency and position of arginine residues in VH-CDR3. Therefore the recurrent expression of somatically derived variable-region structures among autoimmune anti-DNA antibodies, particularly arginines in VH-CDR3, is due to clonal selection and not to inherent differences in antibody formation and expression in autoimmune mice. Since the selected structures common to anti-DNA antibodies promote protein binding to DNA, DNA or DNA containing complexes are likely to be the antigen(s) that stimulate and drive the anti-DNA antibody response in autoimmune mice.

V 202 THE ROLE OF SELF VERSUS NONSELF IN T CELL RECOGNITION OF A TYPE II COLLAGEN PEPTIDE, Laurie Lambert and Jennifer Berling, Marion Merrell Dow Research Institute, Cincinnati, OH, 45215

T lymphocytes play a critical role in the development of murine collagen-induced arthritis (CIA). As in rheumatoid arthritis, susceptibility to CIA is associated with the expression of particular MHC Class II molecules. In susceptible mouse strains, immunization with heterologous type II collagen (cII) induces an autoimmune disease with progressive joint destruction. To analyze T cell recognition of cII we have isolated T cell hybridomas that recognize a chick cII peptide fragment, cII(255-270) (TGELGIAGFKGEQGPK). We examined the response of these T cell hybrids to both denatured chick cII and mouse cII and found that chick cII was recognized, but mouse cII was not. We then assessed the role of particular amino acid differences between chick and mouse cII. Three residues in cII(255-270) differ in these two species: residue 255 (T in chick to A in mouse), residue 258 (L to P) and residue 266 (E to D). Truncation analysis indicated that loss of residue 255 resulted in a 30 fold drop in activity. The peptide 258-270 was 100 fold more potent than 260-270. Replacement of residue 266 with alanine, or removal of this residue, resulted in loss of detectable peptide activity. These results suggested that all three positions that differed between chick and mouse were important in determining the stimulatory capacity of this peptide. Finally, we examined the activity of chick cII(255-270) bearing individual mouse substitutions, as well as the mouse cII(255-270) peptide. The mouse substitutions at positions 255 and 258 had no effect on peptide activity. In contrast, substitution of the mouse residue (Asp) for the chick residue (Glu) at position 266 resulted in a 3000 fold reduction in the stimulatory capacity of the peptide. The activity of the peptide bearing this single substitution was identical to mouse cII(255-270). These results indicate that a single, very conservative difference between self and nonself can dramatically effect T cell peptide recognition.

V 203 CLONAL DIVERSION: A NEW MECHANISM OF TOLERANCE INDUCTION FOR CD4+ T-CELLS, Roland S. Liblau, Bernadette Scott, Sylvie Degermann, Lori Anne Marconi, Lynn Ogata, Andrew Caton, Hugh O. McDevitt and David Lo, Deparment of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305 and Deparment of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Immunological tolerance to tissue specific autoantigens can be achieved by deletion or inactivation of autoreactive T cells, or by "ignorance", although other mechanisms might be involved. We have generated a T-cell receptor transgenic line (HNT-TCR) in which most CD4 T-cells express an I-A^d restricted, influenza hemagglutinin (HA) specific TCR. These mice were crossed with Ins-HA transgenic mice expressing HA in the pancreatic islet cells. Double transgenic mice showed no evidence of thymic or peripheral clonal deletion or clonal inactivation. The peripheral CD4 T-cells from double transgenic animals responded as well as cells from HNT-TCR mice to HA in calcium flux and proliferation experiments. However, two distinct phenotypes were observed in double transgenic mice depending on the strain on which the transgenes were backcrossed. While mice backcrossed on the BALB/c background did not develop diabetes and rarely showed some low grade insulitis, double transgenic mice on the B10.D2 background developed early spontaneous autoimmune diabetes with a penetrance rate of more than 70% in some litters. The islet infiltrates were extensive, consisting mostly of CD4 and CD8 T-cells.
The genetic effect noted here bears a striking resemblance to the genetic influence over immune responses to *Leishmania major* infection in which BALB/c mice develop a predominant Th2 response while B10.D2 mice generate a Th1 inflammatory response. Preliminary results suggest that a similar mechanism operates in our system. The presence of antigen reactive, but non-pathogenic CD4 T-cells in BALB/c mice suggests a new mechanism of tolerance induction in which autoreactive CD4 T cells are induced to differentiate away from a pathogenic phenotype. This might be classified as "clonal diversion".

V 204 SINGLE T CELL PEPTIDE OF A SELF PROTEIN ELICITS AUTOANTIBODIES TO THE PROTEIN ANTIGEN: IMPLICATION FOR SPECIFICITY AND PATHOGENETIC ROLE OF ANTIBODY IN AUTOIMMUNITY, Ya-Huan Lou, M. Fairley McElveen and Kenneth S.K. Tung, Department of Pathology, University of Virginia Health Sciences Center, Box 214, Charlottesville, Virginia 22908

The zona pellucida is an extracellular structure that surrounds the ovarian and ovulated oocytes. It has three major glycoproteins, ZP1, ZP2 and ZP3. ZP3 functions in fertilization as the sperm receptor. A 13-mer peptide from mouse ZP3 has a T cell epitope that induces autoimmune oophoritis, and a B cell epitope that reacts with antibody to murine ZP3. When the B epitope was partially truncated, the ZP3 peptide no longer induced antibody to the B epitope, but unexpectedly they elicited an antibody of the IgG class to the zona pellucida. That an exclusive T cell peptide of murine ZP3, without co-injection of the whole ZP3 protein, elicited autoantibodies whole ZP3, was confirmed as follows. First, the ZP3 T cell peptide did not contain additional B cell epitopes that cross-reacted with native ZP3. Second, a T cell peptide from ZP2 was able to elicit the antibody which reacted with ZP3 protein. Interestingly, induction of autoantibody to the zona pellucida did not follow the ovarian disease process since the antibody response proceeds ovarian pathology, and ovariectomy 2 days following immunization did not affect induction of antibody response. These results suggest that ovarian self antigens normally drain from the ovaries and, in the extra-ovarian sit, stimulate B cell response in the presence of activated T cells. This autoantibody amplification phenomenon demonstrates conclusively that 1) self reactive B cells for ovarian autoantigens respond to endogenous ovarian antigen in vivo following activation of ZP3(or ZP2)-specific helper T cells, and 2) serum antibody in an autoimmune disease need not mirror the immunogen that initiates the disease process. Nonetheless, the autoantibodies bound to the zona pellucida in vivo and are potentially important in disease pathogenesis.

V 205 SUSCEPTIBILITY TO THE INDUCTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMIYELITIS (EAE) DEPENDING ON EFFECTOR CELL ACTIVATION AT THE LEVEL OF THE TARGET TISSUE. Miodrag L. Lukic, Immunology Unit, Department of Medical Microbiology, UAE University, Faculty of Medicine, P O Box: 17666, Al Ain, UAE.

EAE is an autoimmune disorder of the central nervous system (CNS) mediated by $CD4^+$ T lymphocytes specific for myelin basic protein (MBP). MBP-specific T lymphocytes can be isolated from healthy individuals, suggesting that the auto-reactivity to MBP is normally controlled, either in the induction phase and/or in the effector phase of the autoimmune response. In the present report we have examined whether regulatory mechanisms controlling the effects of MBP-specific autoagressive T cells could exert their activity at the level of the target tissue. In order to by-pass the inductive phase of the immune response, we have generated a MBP-specific T cell line derived from F1 hybrids between EAE-susceptible (DA) and EAE-resistant (AO) strains of rats. A myelin basic protein (MBP)-specific T cell line derived from F1 hybrids between EAE-susceptible DA strain and EAE-resistant AO strain was capable of inducing clinical EAE in F1 hybrids and DA, but not in AO rats. In vitro restimulation with MBP presented by AO antigen-presenting cells (APC) resulted in the generation of a MBP-specific subline restricted by $RT1^u$ MHC products which induced clinical EAE in F1 hybrids but not in the AO parental strain. Deletion of hosts' leukocytes using sublethal irradiation and cytotoxic drugs did not abrogate the resistance of AO rats, which argues against the involvement of hosts' lymphoid cells in the regulation of autoagression. However the preliminary results indicate that the transforming growth factor-β (TGF-β) affect the induction of EAE in this model. When susceptible DA host were treated with Salmonella typhi murium aroA mutant producing TGF-β the clinical signs of the disease did not develop. We postulate that TGF-β may differentially affect the functions of resident cells in the CNS of susceptible and resistant strain and the *in situ* activity of the inflamatory cytokines during EAE development.

V 206 T CELL RECOGNITION OF TYPE II COLLAGEN IN CARTILAGE **Vivianne Malmström[1], Eero Vuorio[2], Harald Burkhardt[3] and Rikard Holmdahl[1]. 1. Dept of Medical & Physiological Chemistry, Uppsala University, Sweden. 2. Dept of Medical Biochemistry, Turku University, Finland.3.Max-Planck-Gesellschaft, Klinische Arbeitsgruppen für Rheumatologie, University of Erlangen-Nürnberg, Germany.**

Collagen type II Induced Arthritis (CIA) is an experimental model for rheumatoid arthritis. A dominant, heterologous T-cell epitope in CIA was introduced into a genomic clone of mouse type II collagen (CII) by changing one nucleotide which lead to an amino acid shift from D to E. The mutated gene was micro injected into fertilized C3HQ eggs (a H-2q mouse strain susceptible to CIA) and two transgenic lines were founded; MMC-1 and MMC-2.
The same epitope was also introduced into a cassette construct of collagen type I (which is a systemically available protein) and microinjected in C3HQ. Again 2 founders were identified and given the name TSC-1 and TSC-2.
LNC from MMC-transgenes respond both to heterologous CII and a synthetic peptide of the T cell epitope, but the proliferation is reduced. LNC from TSC-transgenes have no proliferative response to the peptide.This supports the belief that CII is not negatively selected for in the thymus in contrast to systemically available collagen. Rather the T cells appear to be tolerized in the periphery. The MMC-1 mice do not spontanously develop arthritis but they are susceptible to CIA with a lower incidence compared to normal C3HQ mice (40 and 100 % respectivily). This system will be further investigated to enable us to follow the precise regulation and maturation of T-cells towards a specified auto antigen not present in the thymus.

V 207 CLONAL IGNORANCE IN TRANSGENIC MICE EXPRESSING ALLO-MHC EXCLUSIVELY IN THE LENS, W. David Martin, Rita Egan, Julia Stevens and Jerold G. Woodward, Departments of Microbiology and Immunology, and Ophthalmology, University of Kentucky Medical Center, Lexington KY 40536.

Previous transgenic mouse experiments have demonstrated that the expression of foreign or allo-antigens in extra-thymic sites results in the establishment of peripheral tolerance to that antigen. In these cases, the transgene was expressed in tissue freely surveyed by lymphocytes. We have asked whether tolerance would also be invoked to transgenic antigens expressed in "immunologic privileged sites". Using the lens specific α-A crystallin promoter, we have generated transgenic mice expressing the H-2D^d alloantigen exclusively in the lens of mice of H-2(b x k) background. Seven transgenic lines were established and all show strict lens-specific H-2D^d expression at both the RNA and protein levels. Two of the lines with the highest copy number and D^d mRNA levels have non-immunological developmental ocular defects including microphthalmia and cataracts. Most of these mice also have ruptured lens capsules by six weeks of age. Inflammatory cells were consistently observed in mice with ruptured lens capsules. When crossed to BALB/c, these mice showed lens capsule ruptures but no inflammatory cells demonstrating that the inflammation was D^d specific. In lower copy lines which have much less severely affected eyes and intact lens capsules, no inflammation was observed in spite of clearly detectable D^d expression. Low copy number mice were capable of generating D^d specific CTL's in vitro at levels equivalent to normal B6 mice. Thus, our results indicate that in mice whose lenses have intact capsules, there is no immunologic recognition of lens proteins, and as a consequence, no tolerance. This lack of tolerance allowed for the development of an inflammatory response in those mice with ruptured lens capsules. These results demonstrate that, for at least some tissues, lack of self reactivity is maintained by "clonal ignorance" rather than clonal anergy.

V 208 Qualitative difference between encephalitogenic and non-encephalitogenic PLP-specific T cells. William J. Morrison, Cain Hoy, Halina Offner, Arthur A. Vandenbark and Ruth Whitham, Neuroimmunology, VA Med.Center, Portland, OR 97201.

This study compares encephalitogenic spinal cord-derived T cells (SC8) that exhibit lower activity towards PLP(139-151) in vitro with non-encephalitogenic lymphnode-derived T cells (P14) that show high PLP-reactivity. Both lines showed predominant utilization of Vβ2 in their T cell receptors; however, SC8 demonstrated a PLP-stimulation index (SI, [^{3}H]-thymidine uptake) of 54 and transferred (10^7 cells/mouse) severe EAE in all (28/28) SJL mice tested. In comparison, P14 showed a SI of 260 but failed to cause EAE in any (0/31) mice after passive transfer of $2x10^7$ cells. This was reflected by total incorporation of ^{35}S-cys/met and [^{3}H]-glycerol into cellular proteins and lipids, respectively. Compared to P14, SC8 showed 53% less ^{35}S-protein and 28% less [^{3}H]-lipid labeling after PLP-stimulations. However, fractionation of cellular protein and lipid classes revealed qualitative differences. Gel electrophoresed proteins showed that SC8 cells had significantly higher labeling of proteins weighing 40,000. In contrast, P14 showed greater labeling of all other proteins with prominent labeling of proteins near 70,000. Lipids separated by TLC showed that P14 had incorporated more [^{3}H]-glycerol into phosphatidic acid (PA) and produced low levels of [^{3}H]-diacylgycerol (DG). Although PA can act as a precursor for DG, it represents a common intermediate for phospholipid biosynthesis. SC8 produced high levels of [^{3}H]-DG, a protein kinase C activator, rather then channeling lipid metabolism into the biosynthetic intermediate PA. These results demonstrate that encephalitogenicity does not always directly correlate to PLP-stimulated proliferation or total protein and lipid production. Fractionation of protein and lipid classes demonstrate quality differences in the types of proteins and active lipid metabolites generated during PLP-stimulation that may explain why less reactive SC8 cells are encephalitogenic and highly PLP-reactive P14 cells are not.

V 209 REGULATION OF ANTI-DNA B CELLS IN NON-AUTOIMMUNE TRANSGENIC MICE: FUNCTIONAL AND BIOCHEMICAL ANALYSES OF SELF-TOLERANCE, Kim-Anh Nguyen, Joshua Kavaler, and Jan Erikson. The Wistar Institute, Philadelphia, PA

Anti-DNA antibodies are associated with the autoimmune disease systemic lupus erythematosus (SLE). We have evaluated the mechanism by which anti-DNA B cells are regulated in a non-autoimmune background by generating Balb/c mice whose B cells bear the V_H3H9 heavy chain and the V_k8 light chain transgenes (Tg's). These transgenes encode an immunoglobulin (Ig) specific for single-stranded DNA (ssDNA). Earlier work had shown that although these anti-ssDNA B cells dominate the peripheral B cell pool and can be recovered as hybridomas, anti-DNA antibodies are not elevated in the serum of V_H3H9/V_k8 mice. In this study, we have attempted to define these anti-ssDNA B cells phenotypically and functionally to determine if they are inactivated (anergized). We have found that splenic V_H3H9/V_k8 Tg B cells consistently express reduced surface Ig when compared to their non-Tg counterparts. In response to the B cell mitogen LPS, V_H3H9/V_k8 Tg B cells proliferate as well, or nearly as well, as non-Tg B cells. In contrast, when stimulated in an Ig-specific manner via Goat anti-Mouse IgM F(ab)'$_2$ fragments, Tg B cells proliferate much less than non-Tg B cells. Finally, we have assessed V_H3H9/V_k8 Tg B cell function in cognate T-B interactions. Non-Tg B cells exposed to Rabbit anti-Mouse IgM F(ab)'2 fragments internalize, process, and present the F(ab)'2 fragments to T cell clones specific for rabbit Ig, thereby activating the T cells. The B cells themselves are also induced to proliferate and secrete Ab with the cognate T cell help. V_H3H9/V_k8 Tg B cells function equally well as antigen presenting cells in this system, and they also proliferate well. Despite their proliferative ability, however, V_H3H9/V_k8 Tg B cells could not be be stimulated under any of the above conditions to produce significant amounts of antibody relative to the non-Tg controls. We conclude, therefore, that these anti-ssDNA Tg B cells are anergized, and that this functional inactivation involves a block in their differentiation into antigen secreting cells upon stimulation. We are in the process of determining whether this block is mediated by differences in early signalling events, such as tyrosine phosphorylation of activation-associated proteins.

V 210 MYELIN PROTEOLIPID PROTEIN (PLP) REACTIVITY IN MULTIPLE SCLEROSIS. C. Pelfrey, J. Trotter, L. Tranquill, H. McFarland. NIH, NINDS, Bethesda, MD 20892 and Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Research into the pathogenesis of multiple sclerosis (MS) has focused on myelin antigens as potential targets of autoimmune attack. Proteolipid protein (PLP), which makes up more than 50% of central nervous system myelin, is a hydrophobic membrane protein with many properties that historically have made it difficult to study. The use of synthetic peptides based on the PLP sequence provides an alternative method for studying the immunologic properties of PLP. Using PBL from MS patients, long term TCL established in the presence of PLP reacted weakly to PLP in proliferation assays, however, these same lines were much more reactive to synthetic peptides of PLP. Thus, we established T cell lines (TCL) from the peripheral blood lymphocytes (PBL) of MS patients in the presence of 5 separate synthetic PLP peptides. In 6/7 MS patients, proliferative responses were elicited most often to PLP 40-60 compared to four other PLP peptides (PLP 89-106, 103-120, 125-143, and 139-154), however, in 5/7 MS patients the response to PLP 89-106 gave the highest stimulation indices. Characterization of PLP 40-60-responsive TCL from a single MS patient, MS1, indicated that 6/7 TCL proliferating to the peptide also lysed PLP 40-60 pulsed autologous targets. All cytolytic PLP 40-60 TCL were CD4$^+$ and MHC class II restricted and further analysis of MS1 TCL showed that the PLP 40-60 TCL were restricted by DR4 whereas the MBP TCL from MS1 were restricted by DR6. These results suggest that MS PBL recognize several PLP peptides, with the predominant response to PLP 40-60. Since these cells phenotypically resemble T cells known to mediate experimental autoimmune encephalomyelitis, it is possible that they may play a role in the pathogenesis of MS. To determine whether PLP peptide reactivity is associated with MS, we are currently examining PBL reactivity to various peptides of PLP and MBP in MS patients and normal HLA DR matched controls. (Supported in part by National Multiple Sclerosis Society grant # FG 946-A-1).

V 211 MODIFICATION OF CYTOKINE PRODUCTION BY T CELLS ACTIVATED IN THE PRESENCE OF RETINOIC ACID, Michael K. Racke, Dale E. McFarlin, and *Dorothy E. Scott, Neuroimmunology Branch, NIH and *FDA, Bethesda, MD 20892

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease characterized by central nervous system inflammation and demyelination. Retinoids are important regulators of cell diffferentiation and growth. The effects of retinoids on chronic relapsing EAE produced by the transfer of myelin basic protein (MBP)-specific T cell lines was studied. All-*trans*-retinoic acid (tRA) inhibited the proliferation of MBP-specific lymph node cells (LNC) in vitro. However, the capacity of these cells to transfer EAE was markdly reduced with concentrations of tRA which only mildly inhibited T cell proliferation. The presence of tRA during in vitro MBP-specific LNC activation resulted in a marked increase in the production of IL-4 mRNA and protein, while mRNA for IL-2, TNF-α, and IFN-γ was decreased. However, the presence of a neutralizing IL-4 antibody during the in vitro activation did not reverse the inhibition of encephalitogenicity. These findings suggest that T cell activation in the presence of tRA results in the development of T cells of the Th2 phenotype and a decrease in the encephalitogenicity of MBP-specific T cells. These findings may have potential relevance for human demyelinating diseases such as multiple sclerosis.

V 212 MOLECULAR MODELING OF HLA-DR2 MOLECULES SHOW NO DIFFERENCES IN SURFACE PHYSICOCHEMICAL PROPERTIES BETWEEN SUSCEPTIBLE AND RESISTANCE MOLECULES IN INSULIN-DEPENDENT DIABETES. C.B.Sanjeevi, T.P. Lybrand and Å. Lernmark. Department of Endocrinology, Karolinska Hospital Stockholm, Sweden and Molecular Bioengineering Program, University of Washington, Seattle, WA 98195, USA.

HLA-DR2 is negatively associated with insulin-dependent diabetes mellitus (IDDM). The aim of the present study was to determine, by molecular modeling, whether differences in the surface physicochemical properties between subtypes of DR2 molecules explain the mechanisms of DR2 mediated protection. We have previously observed a strong correlation between surface electrostatic potentials (ESP) and diabetes susceptibility and resistance in HLA-DQ. In our earlier report, analysis of HLA-DRB1, DQA1 and DQB1 genes in 425 consecutively diagnosed unrelated IDDM patients and 367 matched controls showed eleven patients and 97 controls to be DR2 positive. Among the five subtypes of DR2-DRB1, DRB1*1601 was positively associated (8/10 patients and 5/97 controls, OR 73.6, Pc<0.001) while DRB1*1501 was negatively associated (2/10 patients and 92/97 controls, OR 0.01, Pc<0.001). Three dimentional models of the peptide binding and T cell recognition areas were constructed for the five DR2 molecules, by substitution of amino acids at polymorphic positions in the DR1 molecules (from the coordinates kindly provided by D. Wiley). Amino acid side chains were adjusted and the side chain packing interactions were evaluated to insure that final model structure posses native-like packing densities. Model building and structural analysis were performed using programs Midas Plus and PSShow. ESP were computed over solvent accesible surfaces (at 298K) using a simple coulomb model with charges from united atom potential function of Weiner et al. (values are given in kT/e, where k is Boltzman's constant and e is standard electron charge).

DR molecule	Mean	Standard Deviation
DRB1*1501	-201.1	108.6
DRB1*1502	-207.9	120.5
DRB1*1503	-247.1	134.6
DRB1*1601	-207.7	109.6
DRB1*1602	-207.6	110.0

No difference in the ESP was observed between the negatively associated DRB1*1501 and positively associated DRB1*1601. It is suggested that DQ molecules rather than the DR molecules may be responsible for protection from IDDM. In 2/10 patients with DRB1*1501, DQA1*0102, DQB1*0602, second haplotype was DR4, DQA1*0301, DQB1*0302. Since DQA1*0102-DQB1*0302, DQA1*0301-DQB1*0602 trans combinations do not form stable heterodimers diabetes in these 2 patients may be due to DR and DQ transcombinations.

V 213 ROLE OF T-CELL FC-GAMMA RECEPTORS IN THE IMMUNOREGULATORY ABNORMALITIES IN RHEUMATOID ARTHRITIS

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Soluble T-cell Fc-gamma receptors are a class of immunoregulatory molecules which bind to the Fc-region of homologous Ig-G. These receptors (also known as immunoglobulin binding factor-IBF) play an important role in the regulation of Ig G synthesis to antigens. The levels of T-cell Fc-gamma receptors are under genetic control; decreased levels resulting in abnormally high levels of IgG isotype. Decreased levels of this immunoregulatory molecule may lead to genetic predisposition to autoimmune diseases like Rheumatoid Arthritis. With this objective semi-quantitative estimations of T-cell Fc-gamma receptors (obtained from Con-A Activated cultured T-cells) were performed in 25 clinically proven cases of Rheumatoid Arthritis by haemagglutination assay employing Ig G coated human 'O' erythrocytes. An equal number of age and sex-matched healthy normal individuals served as controls. Mean titres of the receptors were found to be significantly decreased in the test group compared to healthy subjects. A negative correlation was observed between levels of receptors and Ig G istotype. These observations indicate the role of T-cell Fc-gamma receptors in the causation of Rheumatoid Arthritis.

V 214 MHC CLASS I RESTRICTED CD8$^+$ T CELLS ARE NOT THE EFFECTORS DURING THE INDUCTION OF AUTOIMMUNE MYASTHENIA GRAVIS IN C57BL10 MICE$^+$.

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The acetylcholine receptor (AChR) deficit in the neuromuscular junctions of myasthenia gravis (MG) patient is predominantly mediated by antibody and complement. However, MHC class II restricted CD4$^+$ Th cells play a very critical role in the production of pathogenic anti-AChR antibody. Serum anti-AChR antibodies are absent in 10% of patients with MG. Therefore, a cell mediated attack of the neuromuscular junction cannot be ruled out. To study the involvement of MHC class I restricted CD8$^+$ T cells (cytotoxic T cells ?) in MG pathogenesis C57BL10 (B10) mice deficient in β2 microglobulin ($\beta 2m^{-/-}$) and MG susceptible heterozygous ($\beta 2m^{+/-}$) B10 mice were immunized thrice with *Torpedo* AChR in complete Freund's adjuvant, and assessed for the clinical and immunopathological manifestations of the disease. The cumulative data from two experiments revealed that 15/22 (68%) $\beta m^{-/-}$ mice developed myasthenic muscle weakness in contrast to 8/20 (40%) $\beta 2m^{+/-}$ mice. This finding analyzed with our recent observation on the class II gene disrupted B6 mice which failed to develop MG rules out any pathogenic effector role for class I restricted CD8$^+$ T cells. Further, the current finding suggests a possible regulator/suppressor role for class I restricted CD8$^+$ T cells during the development of MG. $^+$Supported by the MDA and Sealy Smith Endowment Fund. *J.W. McLaughlin Fellow.

V 215 IL-4 REGULATES THE AUTOIMMUNE RESPONSE TO CARDIAC MYOSIN, Stacy C. Smith, Manfred Kopf, and Paul M. Allen,Departments Medicine and Pathology, Washington University ,St. Louis, MO, and Max Planck Institute for Immunobiology, Freiburg, Germany.

Autoimmune myocarditis is induced in susceptible mouse strains by the injection of purified mouse cardiac myosin emulsified in Complete Freund's Adjuvant. We have previously demonstrated that this autoimmune myocarditis is induced by cardiac myosin-specific CD4+ T cells, and that antibodies are not involved in the induction of disease.

We were interested in the role of T helper subsets in the inductive phase of this disease, and postulated that ineffective regulation of the immune response by Th1 and Th2 cells is responsible for the breakdown of tolerance to the self antigen, cardiac myosin. IL-4 is critical for the differentiation of Th2 cells. Because a Th1-mediated response against cardiac myosin is most likely to be essential for the induction of disease, we studied the role of IL-4 in controlling disease induction. First, we found that a non-responder strain, C57BL/6, can be converted to a responder strain by systemic neutralization of IL-4 with the monoclonal antibody 11B11. 4 of 5 11B11-treated mice got severe myocarditis, where 4 of 5 rat IgG-treated controls had no myocarditis. To further define the role of IL-4 in disease induction, we immunized mice which are deficient in Th2-derived cytokines due to disruption of the IL-4 gene (Nature 362: 245, 1993). The IL-4 knockout mice immunized with myosin got severe myocarditis (5 of 5), whereas the immunized normal control mice did not get disease (6 of 7). These data show that IL-4 is critical in the regulation of the immune response to the self protein cardiac myosin. Whether this regulatory function is mediated directly by IL-4, or in conjuction with Th2 cells is not currently known. The mechanism of IL-4 mediated regulation of this autoimmune response is actively being investigated.

V 216 **Effects of Polymorphic Class II Residues on MHC-Restricted T cell Responses to a Myelin Basic Protein Peptide** by Keri Tate, Christopher Lee*, Stacy Edelman, Irena Conboy and Patricia Jones. Department of Biological Sciences, Department of Cell Biology*, Stanford University, Stanford, CA 94305.

Both A^u and A^k murine class II alleles present the immunodominant N-terminal Ac1-11 peptide of myelin basic protein (MBP). In *u*-haplotype mice immunization with MBP Ac1-11 induces experimental allergic encephalomyelitis (EAE), whereas A^k-expressing strains are less susceptible. We have been investigating how differences in the A^u vs. A^k presenting molecule and in the TCR repertoire may give quantitatively and qualitatively different responses to this self peptide. A^k and A^u differ by 16 residues, 6 in A_α and 10 in A_β; the A_β differences appear to be responsible for T cell restriction. Transfectants have been generated expressing chimeric $A^{k/u}$ molecules with single or multiple residues from the other allele; effects of these substitutions on T cell responses have been examined with panels of MBP Ac1-11-specific A^k- and A^u-restricted T cell hybridomas. Peptide-binding studies indicate that the peptide binds approximately 6-fold better to A^u than A^k and that this may be due to the origin of residue 26 in the A_β β-sheet. Evidence from substitutions at polymorphic residues 38 and 61 suggests that these residues may affect peptide conformation in an allele-specific way. The side chains of these residues are in van der Waals contact. Substituting the *k* or *u* residue at either or both of these positions dramatically reduces the T cell responses without corresponding reductions in peptide binding, suggestion effects of these substitutions on peptide conformation. Functional assays with the transfectants and computer modeling studies suggest that the positioning of the side chains of 38 and 61 depends on the combination of residues at these positions and also on the origins of other nearby polymorphic A_β residue(s).

V 217 ANTIGEN MIMICRY IN AUTOIMMUNE DISEASE:SHARING OF AMINO ACID RESIDUES CRITICAL FOR PATHOGENIC T CELL ACTIVATION, Kenneth S.K. Tung, An-Ming Luo, Kristine M. Garza, Department of Pathology, University of Virginia Health Sciences Center, Box 214, Charlottesville, Virginia 22908

This study investigates antigen mimicry in autoimmune disease induction at the level of the T cell peptide. A nonamer peptide from murine nicotinic acetylcholine receptor δ chain (ACRδ), which shared four amino acid residues with a nonamer peptide of murine ovarian zona pellucida glycoprotein ZP3, induced murine autoimmune oophoritis and IgG autoantibody to the zona pellucida. Crossreaction between the ACRδ and ZP3 peptides was established by the response of a ZP3 peptide-specific, oophoritogenic T cell clone to both peptides in association with $IA(\alpha^k\beta^b)$.

By substituting the ZP3 peptides by single alanine, four amino acids within the ZP3 peptide were found to be important for ovarian autoimmune disease, autoantibody response, and stimulation of the ZP3-specific T cell clone. Substitution with conservative amino acid of three residues also ablated activity, whereas the fourth, a phenylalanine, was replaceable by tyrosine without loss of activity. Of the four critical amino acids, three were shared between the ZP3 peptide and the ACRδ peptide. Moreover, polyalanine peptides with the four critical ZP3 amino acids or the four amino acids common to the ZP3 and ACRδ peptides, induced immune response to ZP3 and elicited severe ovarian autoimmune disease. Thus, organ-specific autoimmune disease can occur through immune response against unrelated self (or foreign) peptides that share with a self peptide sufficient common amino acid residues critical for activation of pathogenic, autoreactive T cells.

V 218 CDR3 REGION HETEROGENEITY IN T CELLS SPECIFIC FOR MYELIN BASIC PROTEIN INCREASES WITH THE SEVERITY OF MULTIPLE SCLEROSIS, Ursula Utz, Janet A. Brooks, and William E. Biddison, Neuroimmunology Branch, NINDS, NIH, Bethesda, MD 20892.

Genes of the HLA and T cell receptor (TCR) complex seem to contribute to the susceptibility of a number of autoimmune diseases. Nonetheless, they seem not to be sufficient for disease induction. To eliminate the influence of the genetic background we decided to study monozygotic twin pairs that were dis- or condordant for multiple sclerosis (MS). A general skewing of the TCR repertoire in affected individuals, visible only after stimulation with antigens was detected.[1] In vitro stimulation of PBL from MS patients with myelin basic protein (MBP), a suspected target antigen in MS, resulted most often in an overrepresentation of Vα8-positive T cells.[1] The sequence analysis of those T cells showed extensive CDR3 region heterogeneity for all individuals with severe MS. Normal control twins, the healthy individual of a discordant twin set and a twin set with a very mild form of MS showed restriction in their CDR3 regions. Tetanus toxoid (TT)-specific Vα8-positive T cells that had been generated from the same individuals displayed very limited heterogeneity regardless of disease. The CDR3 regions of MBP- and TT-specific T cells differed from each other. Common motifs within MBP-specific CDR3 regions were identified.

[1] Utz et al. (1993), Nature 364:243.

V 219 SEARCHING FOR ANTIGENS POTENTIALLY INVOLVED IN RHEUMATOID ARTHRITIS (RA), Gijs F. Verheijden, Ron J.M. Schöningh, Catherina J. van Staveren, Annemieke M.H. Boots, Anton W.M. Rijnders, Department of Immunology, Organon International BV, P.O. BOX 20, 5340 BH Oss, The Netherlands.

Rheumatoid arthritis (RA) is a chronic articular inflammatory disease that afflicts about 1% of the population world-wide. Increased risk for RA appears to be associated with certain HLA-DR haplotypes, such as DRB1*0101 (DR1) and DRB1*0401 (DR4Dw4), suggesting that these HLA-DR molecules are involved in presentation of antigens to the arthritogenic T-cells in the inflamed joints of RA patients. Thus far, the nature of the antigens responsible for initiation of RA pathogenesis or for sustaining the chronic inflammation in RA are unknown. We have searched for peptide sequences in cartilage proteins which encompass a DR4Dw4 peptide binding motif and synthesized 30 peptides thus selected. Using a direct, semi-quantitative *in vitro* binding assay (Joosten et al. submitted) we determined the relative affinity of these peptides for HLA-DR4Dw4. Subsequently, we investigated whether these peptides have the potential to induce T-cell activation in peripheral blood mononuclear cells from RA patients. The results of these studies will be presented and discussed in light of the inflammatory response.

V 220 MHC CLASS II GENES INFLUENCE THE CYTOKINE PROFILE AND THE DEVELOPMENT OF EAE IN THE RAT AFTER IMMUNIZATION WITH A PEPTIDE OF MYELIN BASIC PROTEIN, Carina Vingsbo[1], Maha Mustafa[2], Tomas Olsson[2], Rikard Holmdahl[1], 1 Dept of Medical and Physiological Chemistry Uppsala University Sweden, 2 Dept of Neurology Hudding University Hospital Karolinska Insitut, Sweden

The genetic influence on autoimmune diseases is exerted mainly by the MHC genes. We have here analyzed the development of experimental autoimmune encephalomyelitis (EAE) in MHC-congenic Lewis rats.

Immunization with the immunodominant peptide of Myelin Basic Protein, MBP 63-88, showed that the $RT1^a$, $RT1^c$, $RT1^l$ haplotypes were susceptible to clinical EAE, whereas the $RT1^d$, $RT1^f$, $RT1^n$ and $RT1^u$ were resistant. Further investigation of the immune response, with ELISPOT and in-situ hybridization, revealed that lymphocytes from susceptible strains responded to MBP with proliferation and a γ-IFN response. Of the resistant strains the$RT1^u$ rats developed a weak γ-IFN response but no proliferation was detected. Interestingly, IL-4 and TGF-ß mRNA was detected early and at a significant level in the $RT1^u$ strain but not in the EAE suseptible strains. Furthermore, F1 hybrids between the suseptible LEW.1A ($RT1^a$) and the resistant Lew.1W ($RT1^u$) behave identical to the parental strain LEW.1W indicating that $RT1^u$ genes allow a suppressive immune response

To connect the EAE permissive effects of the T cells to the MHC class II genes we analyzed two intra-MHC recombinant Lewis strains. The strains are recombinants between the $RT1^a$ and $RT1^u$ haplotypes, both exhibiting class II genes of the a haplotype but are $RT1^u$ on respective sides of the class II region. These strains respond to MBP and develop EAE like LEW.1A and we therefore suggest the MHC class II genes for being responsible for these disease mediating effects.

V 221 IMPLICATION OF THE THYMUS AND CD4 CELLS DURING DEVELOPMENT OF AUTOIMMUNE DIABETES IN A TRANSGENIC MODEL.

Matthias G. von Herrath, Janel Dockter and Michael B.A. Oldstone, The Scripps Research Institute, La Jolla, CA 92037

Transgenic (tg) mice expressing nucleoprotein (NP) or glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) under control of the rat insulin promotor (RIP) in beta-cells of the pancreas develop autoimmune insulin dependent diabetes mellitus (IDDM) after LCMV challenge. Several tg lines exhibited rapid or slow onset of IDDM. Two lines, RIP-GP 34-20 (IDDM in 10 days) and RIP-NP 25-3 (IDDM in 5 months), were studied. PCR technique indicated that RIP-NP 25-3 mice expressed the NP transgene in both the pancreatic beta-cells and the thymus, whereas, by contrast, the RIP-GP 34-20 mice expressed the tg (LCMV GP) only in the pancreas. LCMV-specific CTL responses in RIP-GP 34-20 mice show the same activity as nontransgenic littermates, but RIP-NP 25-3 mice have a partial defect in the generation of LCMV-specific (anti self) NP CTL. NUDE mice grafted with newborn thymi from RIP-NP 25-3 mice and challenged 6-8 weeks later with LCMV made a low but definite CTL response (3 times less than non-tg controls), whereas NUDE mice receiving a thymic graft from non-tg RIP-NP 25-3 littermates or tg RIP-GP 34-20 mice made normal LCMV CTL responses. In both RIP-NP 25-3 and RIP-GP 34-20 tgs, LCMV-specific CD8 CTL are necessary for IDDM. However, RIP-NP 25-3 mice also require CD4 cells in addition to CD8 lymphocytes to develop IDDM. Thus, thymic involvement plays a critical role in the delay of the virus-induced autoimmune IDDM. Further, the negative selection by the thymus is not absolute, and T cells (presumably of low affinity) escape to the periphery, where they require added participation of CD4 lymphocytes to cause disease.

V 222 INHIBITION OF EXPERIMENTAL AUTOIMMUNE DISEASES BY COMPETITOR-MODULATOR PEPTIDES

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Blocking of the antigen presentation function of MHC molecules by competitor peptides with high MHC binding affinity has been proposed as a potential immunotherapy for MHC-associated autoimmune diseases. To study the mechanism of disease inhibition by competitor peptides, we selected the MBP72-85 induced EAE, and the Mycobacteria induced AA model in Lewis rats. After *in vitro* definition of an EAE- and AA associated competitor peptide it appeared that, in MBP72-85 induced EAE the efficacy to inhibit disease development correlated with the MHC binding affinity of the competitor peptides. Furthermore, by increasing the MHC binding affinity of the EAE inducing peptide the capacity to inhibit disease induction by addition of competitor peptides decreased. However, In the AA model, although the EAE analogue had a superior MHC binding affinity, the AA analogue was a better inhibitor of the disease. Remarkably, in contrast to what was seen in the EAE model and what was expected of a MHC competitor peptide, the AA analogue induced T cell responses cross-reactive with the original disease associated epitope. Therefore, it is concluded that besides MHC blockade an antigen specific mechanism was involved in AA inhibition by the AA analogue. Whether this antigen specific effect is due to a direct effect of the analogue on the arthritogenic T cells or to T cells activated by the analogue inducing protective regulatory T cell responses is currently under investigation.

V 223 ARBITRARY PRIMED PCR: A NEW TECHNIQUE TO DETECT CD4+ T CELL SPECIFIC AUTOIMMUNE GENE PRODUCTS, Weinberg, A.D., Lemon, M., Sullivan, T., Jones, R., Bourdette, D., Offner, H., and Vandenbark, A. VA Medical Center, Immunology 151-D, Portland, OR 97207.

T helper cells specific for myelin basic protein (MBP) will induce experimental autoimmune encephalomyelitis (EAE) upon MBP activation and adoptive transfer into naive recipients. We have crossed Lewis and Buffalo rat strains to generate F1 progeny that express both forms of the allelic marker RT7.1 (Lewis) and RT7.2 (Buffalo). F1 progeny were immunized with MBP and an MBP specific F1 line was derived that expressed both RT7 1 and 7.2. The F1 line was Ag activated and transferred into irradiated Lewis recipients so that the transferred cells could be detected and purified with the RT7.2 Ab. On the first day of EAE onset RT7.2 positive cells were sorted from the inflamed tissue where MBP is present (spinal cord) and the spleen where no Ag is present. RNA was isolated from the two populations and made into cDNA. Previously we had shown (by PCR) that MBP specific cells isolated from the spinal cord preferentially produced IL-2, IL-3, IFN-γ, and the OX-40 Ag when compared to the same population isolated from the spleen. More recently, we have used Arbitrary Primed PCR (Nuc. Acids Res. 1992. 20(19):4965) to find new gene products that were associated with RNA from MBP specific cells isolated from the spinal cord vs. RNA from MBP cells isolated from the spleen. We feel the cDNA clones isolated from this type of analysis will provide new insights into the T cell genes involved with the pathogenic process leading to autoimmune paralysis. This work is supported by MS society grant RG 2521-A-1, by NIH grants NS23444, NS23221, and by the Dept. of Veterans Affairs.

V 224 HEAT SHOCK PROTEIN REACTIVE T CELLS ARE NEPHRITOGENIC AND CYTOTOXIC TO STRESSED RENAL TUBULAR CELLS. R.A. Weiss, C.J. Kelly, and M.P. Madaio, U. of Penn., Phila., PA and USCD, La Jolla, CA.

HSP70 reactive T cells have been isolated from inflammatory sites in several models of autoimmune diseases. Toxins that induce HSP70 expression (i.e. lead, cadmium, aminoglycosides) are known causes of interstitial nephritis, often associated with T cell infiltration. Our studies were designed to investigate whether locally induced HSP and HSP-reactive T cells participate in chronic inflammation in experimentally induced tubulointerstitial nephritis.

Immunoreactive HSP70 was identified in kidneys of cadmium-treated mice after 5-6 weeks,whereas interstitial infiltrates were seen after 8-10 weeks. Western blots of total cellular protein from cultured syngeneic renal tubular cells (MCT) probed with an Ab to inducible HSP70 revealed no detectable expression in unmanipulated cells, but induced expression with heat shock (42°, 4h). Dose dependent increases in HSP expression were observed in cells treated with $CdCl_2$ $3.4x10^{-6}$M at 12 and 16 h but not with lower cadmium doses. To investigate the role that HSP70 recognition plays in the development of interstitial nephritis, a T cell line (CdCL) was established by eluting T cells from the nephritic kidneys of SJL mice treated with $CdCl_2$ for 13 weeks. In parallel, a T cell line (HSP-1) was produced by immunizing SJL mice with an immunodominant synthetic peptide fragment of M. bovis HSP65; an antigen-specific control T cell line (HEL) was used for comparison. All T cell lines were $CD4^+$, $CD8^-$, α/β TCR^+ and caused a DTH response to the appropriate antigen, but not to irrelevant peptides. HSP-1 and CdCl T cells were cytotoxic to heat shocked and $CdCl_2$ pretreated cultured tubular cells in a dose dependent manner, whereas cytotoxicity was not observed with HEL cells. None of the T cell lines were cytotoxic to unmanipulated tubular cells. Moreover CdCl and HSP-1 T cells, but not HEL T cells, induced inflammatory renal parenchymal infiltrates on subcapsular transfer into cadmium treated mice prior to the development of $CdCl_2$ infiltrates, but at a time when immunoreactive HSP70 was detectable in the kidney.

These results demonstrate that T cells reactive to an endogenous neoantigen are activated and participate in the inflammatory response initiated by an exogenous toxin. We believe that this paradigm is common and that interruption of these events could ameliorate progressive interstitial nephritis, irrespective of the initial insult.

V 225 IMMUNOGLOBULIN SYNTHESIS & GENERALISED AUTO-IMMUNITY IN MICE CONGENITALLY LACKING αβ(+) T CELLS Li Wen, Scott Roberts, Joanne Viney, Susan Wong, Caroline Mallick, Joseph Craft, Michael Owen and Adrian Hayday, Departments of Biology, Immunobiology & Rheumatology, Yale University, New Haven, CT 06511.

B cells accumulate in the spleens of mice lacking αβ T cells. We tested serum Ig isotypes in 16 TCRα-/- and 14 matched TCRα+/- heterozygotes. Surprisingly, the homozygotes showed higher levels of IgG (mean 11.9g/l) due to an increase of IgG, while the heterozygotes showed consistently lower levels of IgG (mean 3.4 g/l). Co-culturing B and γδ T cells from the TCRα-/- mice showed that γδ T cells augment B cell expansion and IgG1 production. To examine the 'non-αβ T cell' -mediated help, T cells from TCRα-/- and control Balb/c mice were cultured with either Con A or PPD. As expected high levels of IL2 & IL4 were secreted by T cells from the control Balb/c mice in response to Con A but not to PPD. In contrast, IL4 was detected in the supernatants of cultures derived from TCRα-/- mice in response to both Con A & PPD. Hypergammaglobulinemia and autoantibodies are hallmarks of some autoimmune diseases. We found significantly higher levels of anti DNA autoantibodies in TCRα-/- mice compared with TCRα+/- mice, more apparent for anti dsDNA ($p= 0.0002$) than for anti ssDNA ($p= 0.01$). Likewise, positive results were obtained with the TCRα-/- sera tested by Western blot of Hela cell lysates & by immunoprecipitation of labeled Hela cell lysates. These results suggest that a cause of generalised auto-immunity may be an absence of αβ T cell regulation.

V 226 EXPRESSION OF THE COSTIMULATOR B7 ACCELERATES DIABETES IN THE NOD MOUSE, Susan Wong, Sylvie Guerder, Karl E. Swenson, Irene Visintin, Richard A. Flavell and Charles A. Janeway Jr., Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

The NOD (Non-Obese Diabetic) mouse is a model for human type I diabetes. Lymphocyte infiltration into the islets of Langerhans occurs and later, destruction of islet cells leads to diabetes on a unique genetic background which includes I-A g^7. In our colony, diabetes usually occurs between 12 and 24 weeks of age with over 90% incidence of diabetes in the female animals. The aim of the study was to examine the role of the addition of the costimulatory molecule B7 onto islet cells in relation to a genetic background that predisposes to diabetes. NOD mice were crossed with transgenic C57BL/6 mice expressing the human B7 molecule specifically on the islets of Langerhans. By 10 weeks of age, intense insulitis is seen in the (NODxB7) F1 mice and diabetes has developed in one mouse at this age. When the F1 generation were backcrossed to NOD, 4 out of 21 mice, which were B7 transgene positive and homozygous for NOD MHC, developed diabetes at 4 weeks of age and one mouse at 6 weeks. Histology has shown that, at 4 weeks, there is an intense lymphocytic infiltrate in the remaining islets of those mice developing early diabetes compared with littermates which are transgene positive but heterozygous for NOD MHC. The lymphocytic infiltrate comprises B cells, CD8 positive and CD4 positive cells. These results indicate that the increased costimulatory capacity of islet cells allows the activation of lymphocytes which have the potential to cause diabetes in F1 mice which have a reduced genetic susceptibility and would not normally develop the disease. This increased level of activation also causes accelerated diabetes in mice that have a genetic background that predisposes to spontaneous diabetes.

V 227 POSSIBLE REGULATOR/SUPPRESSOR ROLE OF TCR Vß6+ CELLS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. Bo Wu, Mohan Shenoy, Elzbieta Goluszko, Rashmi Kaul and Premkumar Christadoss. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555

Genomic deletion of Vß5.2, 8.3, 5.1, 8.2, 5.3, 8.1, 13, 12, 11, 9, 6, 15 and 17 genes in RIII/SJ (H-2^r; Vß^c) and B10.TCR^c (H-2^b; Vß^c) mice augmented experimental autoimmune myasthenia gravis (EAMG) susceptibility, when compared with B10.RIII (H-2^r; Vß^b) and B10 (H-2^b; Vß^b) mice with intact Vß genome. However, deletion of Vß5.2, 8.3, 5.1, 8.2, 5.3, 8.1, 13, 12, 11 and 9 in B10.TCR^a congenic mice (H-2^b; Vß^a) failed to augment EAMG. Therefore, the increased susceptibility observed in the Vß^c haplotype mice might be due to, the overexpressed non-deleted Vß genes, and/or the deleted Vß6/Vß15 or the linked genes. To study the contribution of Vß6 gene in EAMG pathogenesis, C57L mice (H-2^b; Vß^a) were depleted of Vß6+ cells with anti-Vß6 monoclonal antibody before each immunization with AChR in CFA. FACS analysis of PBLs showed <1% Vß6+ cells in the antibody-treated group against 11.8% in the PBS-treated group. Functional depletion of Vß6-positive cells was confirmed by TCR-Vß cross-linking assay. Five of nine (56%) Vß6 cell depleted mice developed muscle weakness characteristic of myasthenia gravis(MG) in contrast to two of eight (25%) PBS-treated mice. This preliminary finding suggests that Vß6+ cells might play an important role in the suppression/regulation of an autoimmune response to AChR and EAMG pathogenesis. The augmented EAMG susceptibility in TCR-Vß^c mice might be due to the deletion of the regulatory Vß6 gene. Similar TCR-specific regulatory mechanism might operate during the onset, remissions and relapses associated with MG or other autoimmune diseases. Supported by Sealy Smith Endowment, MDA, McLaughlin Foundation.

V 228 THE CRITICAL ROLE OF TNF-a IN LYMPHOCYTE DEVELOPMENT AND IN THE PATHOGENESIS OF AUTOIMMUNE DIABETES, Xiao-dong Yang, R. Tisch, S.M. Singer, Z. Cao, R. Schreiber, S. Nagata and H.O. McDevitt, Department of Microbiology and Immunology,Stanford University School of Medicine, Stanford, CA94305; Department of Pathology, Washington University School of Medicine, St. Louis, MI 63110, USA; Osaka Bioscience Institute, Osaka, Japan

Tumor necrosis factor-a (TNF-a) is a pro-inflammatory cytokine that has pleiotropic effects on a wide variety of tissues. It has been shown that TNF-a is produced very early during thymic development and that administration of anti-TNF-a antibody can block normal thymic development in mice. Our recent study has demonstrated that administration of TNF-a in newborn NOD mice, in contrast to the results obtained with adult mice, accelerates disease onset, while anti-TNF-a treatment inhibits insulitis and completely prevents diabetes. The T cell response in TNF-a treated mice to a panel of pancreatic beta-cell autoantigens including murine GAD65 and 67, peripherin, CPH and HSP65 was enhanced, but was almost absent in anti-TNF-a treated animals. These data suggest that the acceleration of IDDM by TNF-a is possibly via up-regulation of an autoimmune response to the target organ while prevention of the disease by anti-TNF-a treatment may be mediated by down-regulation of these responses. To further determine the mechanism by which these treatments elicit their effects, we have investigated the regulatory effect of TNF-a on *bcl-2* and *Fas* proto-oncogene expressions in lymphocytes during early development, and on thymocyte activation. Preliminary data has shown that TNF-a does not appear to modify *bcl-2* expresssion in thymocytes, but does reduce *Fas* expression in $CD4^-CD8^-$ cells, whereas Fas expression is up-regulated in $CD4^+CD8^-$ subsets. Moreover, TNF-a inhibits thymocyte activation as measured by antibody specific for CD69, an early T cell activation marker. These TNF-a mediated effects on thymocytes may correlate with positive or negative selection and could therefore contribute to the enhanced T cell autoimmune response and the acceleration of IDDM in the young NOD mice treated with TNF-a.

V 229 THE AUTOANTIGEN MYELIN BASIC PROTEIN CAUSES ENCEPHALOMYELITIS IN MICE THAT DEVELOP SPONTANEOUS SYSTEMIC "LUPUS-LIKE" ILLNESS. Scott S. Zamvil*, Ahmad Al-Sabbagh#, Patricia A. Nelson#, Depak Kaul*, Howard L. Weiner*, and Vijay K. Kuchroo*, *Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115, and #AutoImmune Inc, Lexington, MA

The multideterminant protein myelin basic protein (MBP) is the autoantigen in experimental allergic encephalomyelitis (EAE), a model for antigen specific T cell mediated autoimmune disease. For mice that express the H-2^u haplotype, MBP Ac1-11 is the dominant encephalitogenic determinant. It is known that most T cell clones specific for Ac1-11 express T cell receptor (TCR) V_β8.2, and of all clones analyzed, regardless of TCR V_β, all utilize $D\beta2$ and $J\beta2$ elements. The NZW mouse strain (H-2^z), which contributes to the spontaneous "lupus-like" illness in (NZB x NZW)F_1 mice has deleted $D_{\beta2}$ and $J_{\beta2}$ gene segments. NZW express class II (I-A and I-E) gene sequences with H-2^u class II. We investigated whether these strains are susceptible to EAE induced with intact MBP and MBP peptides. In vitro analysis demonstrated that NZW antigen presenting cells (APC) can present MBP and MBP peptides to encephalitogenic T cell clones derived from H-2^u mice, confirming the functional identity of NZW I-A and I-E molecules. In vivo results demonstrated that NZW and (NZB x NZW)F_1 mice are susceptible to EAE induced with intact MBP and Ac1-11. MBP p35-47, a potent subdominant encephalitogenic determinant for H-2^u mice, caused EAE in (NZB x NZW)F_1 mice containing both the NZW TCR β deletion and the normal TCR β gene locus. In contrast, p35-47 did not cause clinical and histologic EAE in homozygous NZW mice, suggesting that the encephalitogenic T cell repertoire in mice containing only the mutant TCR had been altered. These results emphasize the importance of both MHC class II and additional host genes in shaping the autoimmune T cell repertoire.

T Cells with Gamma/Delta Receptors

V 250 EXPRESSION OF KERATINOCYTE GROWTH FACTOR BY DENDRITIC EPIDERMAL T CELLS, **Richard Boismenu and Wendy L. Havran, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.**

Dendritic epidermal T cells (DETC) reside in the epidermal layer of skin where they establish intimate contact with keratinocytes. The strict tissue localization and absence of clonal diversity in the TCR of DETC led to suggestions that the immunological role of these cells may be distinct from that of peripheral α and β T cells expressing diverse TCR. We previously established that freshly isolated as well as cloned DETC release IL-2 and proliferate in the presence of stressed keratinocytes. This response was specific to DETC and judged by several criteria appeared to involve a signal transduced through the invariant Vγ3/Vδ1 TCR. However the functional consequence of this interaction remained to be characterized. We now demonstrate that activated DETC secrete at least one growth factor with specificity for keratinocytes. The mRNA coding for this cytokine was amplified following reverse transcription and cloned. Analysis of the DNA sequence indicated that the mRNA coded for a protein identical to mouse keratinocyte growth factor (KGF). To our knowledge this represents the first report of KGF synthesis by a cell type other than stromal fibroblasts. We have failed to detect expression of KGF by T cells other than DETC consistent with the notion that DETC have a specialized function which involves KGF. Thus our current view is that, expression of KGF by DETC follows recognition of stressed or injured keratinocytes with the functional consequence of maintaining the integrity of the skin by allowing for timely and localized proliferation of keratinocytes.

V 251 IMMUNOREGULATORY EFFECTS OF AN HLA CLASS II PEPTIDE, Michelle L. Boytim, Jodi E. Goldberg, Shu-Chen Lyu, and Carol Clayberger, Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA 94305-5247

Donor specific tolerance mediated by blood transfusions is a well established phenomenon. Class I MHC molecules have been shown to be critical for this effect, and peptides obtained from the structure of class I MHC were capable of mediating similar immunoregulatory effects in a non-allele specific manner. To investigate whether MHC class II was also capable of this effect, peptides were synthesized corresponding to the conserved alpha helical domains of several human MHC molecules. One 25 amino acid peptide, derived from the alpha helix of DQ A 03011, was found to inhibit in vitro proliferation, CTL precursor formation, and lysis of target cells by established CTL. This inhibition was not reversible with the addition of IL-2. The peptide was also found to be effective in vitro on rat lymphocytes. A series of 15 amino acid peptides that encompassed the 25 amino acid peptide and a series with single amino acid substitutions were synthesized. The activity of this peptide was defined to the 15 amino acid peptide from residues 62 to 76 of the primary sequence. Amino acid 69 was found to be critical for the peptide's effect. Additionally, data on the mechanism of effect of this peptide will be presented.

V 252 $\gamma\delta$CTL PROLIFERATE IN RESPONSE TO VIRUS-INFECTED CELLS AND MEDIATE THEIR DESTRUCTION Jack F. Bukowski, Craig T. Morita, and Michael B. Brenner. Department of Rheumatology/Immunology, Harvard Medical School, Boston, MA 02115.

PBMC from herpes simplex virus (HSV) seropositive individuals were stimulated with autologous HSV-infected PHA blasts. There was a 3- to 20-fold expansion of $\gamma\delta$CTL which were >95% Vγ2Vδ2. In contrast, stimulation with live virus or irradiated virus-infected PBMC resulted in T cell proliferation but no expansion of $\gamma\delta$CTL. In addition, there was no T cell proliferation or expansion of $\gamma\delta$CTL when PBMC were from seronegative individuals, or when mock-infected PHA blasts were used as stimulators. PBMC from these cultures lysed HSV-infected, but not mock-infected targets. Surprisingly, they also lysed targets infected with vaccinia virus (VV), an unrelated virus, equally well. Their cytotoxic activity was not restricted by classical HLA class I or class II molecules, and could be blocked with monoclonal antibodies to CD3 and the $\gamma\delta$TCR. The cytotoxicity was contained entirely within the CD3$^+$ subset; in addition, depletion of $\alpha\beta$CTL enriched the cytotoxic activity whereas depletion of $\gamma\delta$CTL abrogated it. Cloned Vγ2Vδ2 CTL lines exhibited the same cytotoxic spectrum as polyclonal CTL. These data show that Vγ2Vδ2 CTL proliferate in response to virus-infected cells and that they may mediate a newly described antiviral effector function which is most likely TCR-dependent and directed against a cellular ligand induced or modified by acute infection with unrelated viruses.

V 253 CD8 CYTOTOXIC T LYMPHOCYTE (CTL) RESPONSE IS UNDER THE NEGATIVE REGULATION OF $\gamma\delta$ T CELL WHICH CAN BE ACTIVATED BY EXOGENOUS VIRAL SUPERANTIGEN MLS-1^a, Chow Kai-ping N., Department of Microbiology and Immunology, Chang-Gung College of Medicine and Technology, Kwei-Shan, Taoyuan, Taiwan, Republic of China.

The regulatory function of $\gamma\delta$ T cells in CD8 CTL responses has been exploited in exogenous minor lymphocyte stimulating (Mls) antigen-1^a-injected mice. Adult mice of Mls-1^b intravenously given 2000R-irradiated Mls-1^a bearing cells were found unable to mount hapten trinitrophenol (TNP)-self specific CTL response if Mls is co-injected in the *in vivo* CTL immunization . Previously a CD5$^+$CD8$^-$ T cell from Mls-treated mice was shown to mediate the CTL tolerance by adoptive transfer experiments. Here we further identify the CD5 T cell to be not of Vβ 2, 6, 7, 8 or 11 subtypes. In stead, it is a CD3$^+\alpha\beta^-\gamma\delta^+$ T cell. Thus, a $\gamma\delta$ T cell can be activated in a viral infection (for instance, by Mls-1^a as in this report) and it down-regulates the CTL responses non-specifically in the individuals.

V 254 *IN VITRO* ACTIVATION OF T CELLS WITH *PLASMODIUM FALCIPARUM* ANTIGEN IS PREFERENTIAL FOR THE γδ T CELL SUBSET OVER THE αβ T CELL SUBSET AND CORRELATES WITH PARASITE GROWTH INHIBITORY ACTIVITY, M. Merle Elloso, Henri C. van der Heyde, and William P. Weidanz, Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI 53706.

During malaria, γδ T cells increase in number in the peripheral blood of humans and in the spleens of both humans and mice. Cells of this subset also proliferate in response to parasite antigen *in vitro*. To further characterize this response, we obtained peripheral blood mononuclear cells from malaria-naive donors and examined the γδ T cell population after stimulation for 5-7 days with lysate prepared from schizont-infected erythrocytes. We observed that γδ T cells are preferentially activated over αβ T cells, with higher percentages of blast cells and cycling cells among the γδ T cell population. Furthermore, this response was greater than that obtained with cultures stimulated with either uninfected erythrocytes or medium alone, but was comparable to that obtained by stimulation with the Daudi Burkitt's lymphoma cell line. Previously, we have shown that cloned human γδ T cells can inhibit the growth of *P. falciparum in vitro*. In the present study, we show that increases in the proportion of activated γδ T cells correlates with parasite growth inhibitory activity, regardless of the stimulus. This suggests that once activated, either by direct antigenic stimulation or by products elaborated by other cell types during infection, γδ T cells may exert similar growth inhibitory activity *in vivo*.

V 255 THE CAPACITY OF Vγ1.1Cγ4 T CELLS TO INFLUENCE THE DEVELOPMENT OF A FUNTIONAL T CELL REPERTOIRE IN *SCID* MICE.

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The *scid* genetic mutation in mice produces a severe deficiency of functional lymphocytes. Associated with this defect is abnormal organization of the microenvironment in lymphoid organs. Mice can be cured of this lymphoid deficiency by grafts of bone marrow from immunocompetent mice. Mice transgenic for the Vγ1.1Cγ4 TcR chain demonstrate a marked skewing of the γδ repertoire towards expression of the Vγ1.1Cγ4 TcR chain throughout development. These mice are characterized by alterations of lymphoid stromal elements and an early appearance of T cell reactivity. Herein, we have examined the effects of reconstituting *scid* mice with bone marrow cells from mice transgenic for the Vγ1.1Cγ4 TcR chain and non transgenic littermates. Both *scid* and reconstituted *scid* mice were analysed using multicolor flow cytometery and extensive immunohistological staining. The *scid* thymic microenvironment showed a rudimentary medulla, relatively disorganized and collapsed cortex, with T cell maturation arrested at the CD3-4-8- stage. The spleen and lymph nodes of these mice show no delineation between T & B cell areas. Following reconstitution with bone marrow from the transgenic mice the isolated thymic medullary elements were observed to undergo massive expansion, contrasting normal development, organizing into a morphologically and phenotypically delineated cortex and medulla. In addition, peripheral lymphoid organs in these mice showed distended PALS, extensive T cell areas and B cell follicles. These observations indicate that T cells expressing the transgenic Vγ1.1Cγ4 TcR chain may play a developmental role in the formation of a functional thymic microenvironment and therein a repertoire of immunocompetent T cells.

V 256 THE ABILITY OF γδ T CELLS TO INFLUENCE THE OUTCOME OF T_h1 AND T_h2 RESPONSES TO PARASITES, David A. Ferrick, Thera Mulvania and Beryl Hsieh, Department of Pathology, Microbiology & Immunology, University of California School of Veterinary Medicine, Davis, CA 95616

It has been postulated, based on several investigations in mouse and human that γδ T cells are activated in response to cellular stress induced by trauma or infection. Their functional response to these activation signals and how this contributes to host immunity is poorly defined. It has been demonstrated that γδ T cells can perform effector functions similar to αβ T cells, most notably cell cytotoxicity and cytokine production. It is the latter function that we have investigated in Vγ1.1 and Vγ2 transgenic mice infected with parasites that produce either T_h1 or T_h2 responses. Mice (C57BL/6) that usually develop protective T_h1 responses to infection with *Listeria monocytogenes* or *Leishmania major*, but are transgenic for the Vγ1.1 TCR chain, are less protected than their corresponding nontransgenic littermates. This was measured by following the magnitude of the localized inflammatory response, enumerating the infectious organisms and quantifying cytokine production. In contrast, in preliminary experiments with mice infected with *Nippostrongylus brasiliensis*, a parasite that is repelled by a T_h2 response, the Vγ1.1 transgenic mice generated a more aggressive response to the parasite compared to nontransgenic littermates. Experiments with infected Vγ2 transgenic mice are ongoing and the results will be presented at the meeting. These data suggest that at least a subpopulation of γδ T cells may function at sites of trauma and injury to recognize stressed cells and, as a result, initiate and/or influence the type of immune response generated.

V 257 ANALYSIS OF THE HUMAN T-CELL RECEPTOR Vγ REPERTOIRE BY MEANS OF A NOVEL ANTI-Vγ2/3/4 ANTIBODY, Dieter Kabelitz and Susanne Schondelmaier Department of Immunology, Paul Ehrlich Institute, P.O. Box 1740, D-63207 Langen, Germany

The majority (50 - 90%) of γδ T cells in the peripheral blood of adult individuals expresses a T cell receptor (TcR) which uses Vγ9 and Vδ2 as variable elements. Little is known about the distribution of other Vγ gene elements in the remaining 10 - 50% of γδ T cells. We have generated a monoclonal antibody (mAb) termed 23D12 which recognizes 3 different Vγ elements, i.e. Vγ2, Vγ3, and Vγ4. The specificity of mAb 23D12 was defined by flow cytometry (FCM) analysis of a large panel of T cell clones and transfectants expressing molecularly well-defined γδ TCR elements. Here we have studied the Vγ gene expression in peripheral blood γδ T cells by three color FCM analysis applying established mAb directed against Vγ9 and Vγ4, as well as mAb 23D12 directed against Vγ2, Vγ3 and Vγ4. On average, 79.9% of γδ T cells expressed Vγ9, 11.9% Vγ2/Vγ3, 4.4% Vγ4, and 7.5% one of the remaining Vγ5, Vγ8, Vγ10 or Vγ11 elements. There were remarkable variations in the γδ subset composition between individual donors. The majority (69.8%) of Vγ2/Vγ3/Vγ4-bearing cells coexpressed Vδ1. This is in contrast to Vγ9-bearing γδ T cells, of which 83.1% used Vδ2 and only 12.7% Vδ1. Taken together, this data identifies Vγ2/Vγ3 as the second most frequently used set of Vγ elements in human peripheral blood γδ T cells.

V 258 OLIGOCLONALITY OF THE Vδ1 T CELL RECEPTOR REPERTOIRE IN HUMAN SMALL INTESTINE AND COLON, Martin F. Kagnoff, Ewa Morzycka-Wroblewska, Wolfgang Holtmeier, Julia Harwood and Yehuda Chowers, Laboratory of Mucosal Immunology, Department of Medicine, University of California, San Diego, La Jolla, CA 92093

Vδ1 bearing T cells represent a major fraction of the γ/δ T cell population in the human intestinal tract. To assess human intestinal γ/δ T cell diversity, we have cloned and sequenced the variable region junctions of Vδ1 T cell receptor transcripts in the human small intestine and colon. Intestinal mucosal biopsies were obtained from defined regions of small intestine and colon via fiberoptic endoscopy. After extraction and reverse transcription of biopsy RNA, Vδ1 specific transcripts were amplified by PCR and then cloned and sequenced. RESULTS: One Vδ1 transcript predominated within each intestinal biopsy, although a broader population of sequences was represented at a very low frequency. Moreover, individual biopsies obtained within a several centimeter region of either the small intestine or colon contained identical dominant transcripts. The Vδ1 transcripts that were dominant in the small intestinal biopsies differed from those that predominated in the colon biopsies of the same individual. Moreover, the dominant transcripts present in the small intestine and colon biopsies differed among different individuals. The majority of Vδ1 transcripts in the small intestine and colon were in frame. These data indicate there is marked regional oligoclonality of the Vδ1 bearing γ/δ T cell population in the human small intestine and colon, and favor the notion that positive selection plays a major role in shaping the Vδ1 repertoire in the intestinal tract. This work was supported by NIH grant DK35108.

V 259 **RECONSTITUTION OF *SCID* MICE WITH HAEMATOPOEITIC PRECURSORS - A DETAILED ANALYSIS OF γδ T CELL RECONSTITUTION**

Katherine A. Kelly, Rebecca O'Brien and Willi Born. Department of Medicine, National Jewish Center for Immunology & Respiratory Medicine. Denver CO 80206

Several unique features distinguish γδ T cells from the more frequent αβ T cells. γδ cells are, for instance, more abundant at epithelial sites such as the gut, skin, liver and female reproductive tract, while αβ cells dominate in the lymph nodes and spleen. In addition, γδ cells are produced in distinct waves during ontogeny with cells expressing TCR Vγ5 being produced earliest and seeding the epidermis. These are followed by $V\gamma6^+$ cells, then other γδ T cell types. In addition, it has previously been shown that while the precursors of $V\gamma5^+$ cells are to be found in fetal liver they are not detectable in adult bone marrow. Furthermore, a fetal thymic microenvironment is required. Specific circumstances may be required for the generation of other γδ cell types. We have reconstituted unmanipulated *scid* mice with 3 sources of haematopoeitic precursor cells - fetal liver, neonatal spleen and adult bone marrow. Recipients were examined 8 weeks post-transfer for reconstitution of lymphoid cells in the thymus, lymph nodes, spleen, liver, gastrointestinal epithelium, epidermis and uterine epithelium. Both the T and B cell compartments were reconstituted, with fetal liver giving closest to normal reconstitution (normal cell numbers were observed in some organs) and adult bone marrow gave the poorest reconstitution. This difference could not be fully overcome by increasing the bone marrow inoculum. Regardless of the source of precursors, the T cell compartment was more extensively reconstituted than the B cell compartment. Within the T cell compartment γδ cells generally were not reconstituted as well as αβ cells. While some γδ subsets appeared in normal numbers others, such as the $V\gamma2^+$ subset, were present in significantly lower numbers than normal. These results may well reflect the pattern of T cell development in ontogeny with subsets that normally develop prenatally and around birth relatively diminished in number compared with normal, and later developing subsets present as normal.

V 260 γ,δ T CELL RECOGNITION OF TUMOR IMMUNOGLOBULIN PEPTIDE, Alan M. Krensky, Hubert T Kim, Edward L. Nelson, and Carol Clayberger, Departments of Pediatrics and Cardiovascular Medicine, Stanford University, Stanford, CA 94305

Although γ,δ T cells have been postulated to act as a surveillance mechanism which eliminates transformed or otherwise damaged cells, little is known about tumor recognition by γ,δ T cells, including the antigens that are recognized and the molecules that present them. Previously, we described human γ,δ cytotoxic T lymphocytes (CTL) which recognize autologous B cell lymphoma (J. Exp. Med. 169:1557, 1989). We now show that these γ,δ T cells lyse heterologous cells transfected with tumor immunoglobulin light chain gene. Furthermore, the light chain is recognized as processed peptide in an idiotype specific manner. T cell recognition does not involve classical MHC antigens, but could be blocked by antibodies directed against the heat shock protein grp75. These findings show that γ,δ T cells can recognize highly polymorphic antigens such as tumor idiotype, and implicate heat shock protein as the antigen presentation molecule.

V 261 THE TL ANTIGEN, A NONCLASSICAL CLASS I MOLECULE, SELECTS γδ T CELLS, Mitchell Kronenberg, Hilde Cheroutre, Hilda Holcombe, Beate Sydora, Pirooz Eghtesady and Michael Teitell, Department of Microbiology & Immunology, UCLA, Los Angeles, CA 90024.

The Thymus Leukemia (TL) antigen is a nonclassical class I molecule encoded by the *T3* and *T18* genes in the mouse MHC. It is expressed on epithelial cells in the mouse intestine and in other sites. The TL antigen shares a number of structural features with classical class I molecules. In addition, we have shown that it requires β2m for surface expression and it is capable of interacting with CD8. However, transfection of both RMA-S and *D. melanogaster* cells demonstrates that unlike classical class I molecules, stable surface expression of TL antigen is completely independent of TAP (peptide antigen transporter) function.

We have shown previously that γδ intestinal epithelial cells (IEL) become activated and release granules containing serine esterase in response to short term culture with intestinal epithelial cells. The IEL responsible for this are mostly Vγ5+, CD8+ T cells. Studies of epithelial cells from β2m deficient mice demonstrates that class I molecules are required this response, and it can be blocked by anti-TL mAbs, suggesting that the TL antigen is recognized by Vγ5+ IEL.

We have now studied γδ T cells in transgenic mice that overexpress TL antigen under the control of the H-2D gene promoter. The total number of γδ T cells or Vγ5+ cells is not increased in the spleen or circulation of these mice. However, among the IEL, there is a 2-3 fold decrease in Vγ5+ lymphocytes. These data suggest that overexpression of TL antigen leads to negative selection of γδ IEL, and they are consistent with the proposed recognition of TLa by many Vγ5+ IEL. Results from bone marrow chimeras will determine the cell type responsible for this apparent negative selection.

V 262 THE LIGAND FOR CD40 ACTS AS A COSTIMULATORY SIGNAL FOR THYMIC γδ T CELLS. Fred Ramsdell, Michael Seaman, Ky Clifford and William C. Fanslow. Department of Immunobiology, Immunex Research and Development Corp., Seattle, WA 98101

The stimulatory requirements for T cells bearing γδ T cell receptors are distinct from those of αβ T cells. We have analyzed the ability of the CD40 ligand (CD40L) to activate neonatal thymic γδ T cells. CD40L is expressed on activated T cells and has been shown to induce B cell proliferation and immunoglobulin secretion as well as monocyte activation. We now demonstrate that, in the presence of an anti-TCRγδ antibody, CD40L is able to induce the proliferation of neonatal thymic γδ cells. The presence of CD40L also leads to enhanced expression of a variety of activation associated antigens including CD25, CD69, CD44 and Ly6C. In addition to proliferation, CD40L induces non-specific cytolytic activity in thymic γδ T cells as well as the production of interferonγ (IFNγ) and TNFα. We were unable to detect IL-2 or IL-4 production in response to CD40L, and antibody blocking studies indicate that the mechanism of activation appears to involve IL-1 but is independent of IL-2, IL-4 and IL-7. These results suggest that, in addition to its effects on B cells and monocytes, CD40L can co-stimulate the activation of thymic γδ T cells.

V 263 A MOLECULAR CHARACTERIZATION OF LIVER γδ T CELLS IN THE HOST RESPONSE TO *LISTERIA MONOCYTOGENES*, Christina Ellis Roark, Michaelann Vollmer, Willi K. Born, and Rebecca L. O'Brien, Department of Medicine, National Jewish Center for Immunological and Respiratory Medicine, Denver, CO 80206.

The role of γδ cells in bacterial infections is currently being investigated. In *Listeria monocytogenes*, γδ cell percentages have been reported to increase in the peritoneum and the liver during the course of the infection. Our initial studies in C57BL/10 mice have shown that γδ cells percentages are not notably increased in the livers of Listeria-infected mice but do show surface changes during infection, including a change in light scatter (size change). Around day 5, the Vδ6.3$^+$ γδ cell percentage begins to decrease as compared to total γδ$^+$ cells; these cells encompass the HSP-60 reactive subset. At the same time, hybridoma analysis indicates a preferential expansion of a different subset of liver γδ cells, the Vγ6/Vδ1 cells previously reported to reside in the epithelium of the female reproductive tract and of the tongue. This increase suggests this subset is activated in the response to listeria. Functional studies on this subset are in progress.

V 264 PREFERENTIAL USAGE OF FcRγ CHAIN IN THE TCR COMPLEX BY γ/δ T CELLS LOCALIZED IN EPITHELIA, Takashi Saito, Hiroshi Ohno, and Satoru Ono, Division of Molecular Genetics, Center for Biomedical Science, School of Medicine, Chiba University, Chiba, Japan.

ζ and η chains of the T cell antigen receptor (TCR) complex and the γ chain of Fc receptors (FcRγ) constitute a family of proteins important for the expression of, and signal transduction through, these receptors in hematopoietic cells. In order to analyze the *in vivo* function of these molecules for developement and function of T cells, we have generated mice lacking the expression of ζ chain by gene targeting and characterized T cell developement in these mice. Differentiation of thymotytes was impaired in double positive and single positive populations in ζ-deficient mice. TCR expression was reduced in most T cells from thymus, spleen, lympho node and peripheral blood. By contrast, CD8αα$^+$ TCRγδ$^+$ intestinal intraepithelial lymphocytes (iIEL) in these mice expressed a normal level of TCR. Biochemical analysis of the TCR complex in these cells from ζ-deficient as well as normal mice revealed the predominant usage of FcRγ in the TCR complex. Furthermore, γδ$^+$ T cells in epithelia of the skin and female reproductive organs from ζ-deficient mice also showed relatively high TCR expression, indicating the usage of FcRγ. These observations demonstrate the preferential usage of FcRγ by γδ$^+$ T cells localized in epithelia of normal mice. Considering that these cells are thought to play an role in the surveillance of body surfaces that are exposed to environment, the cells may exhibit distinct signaling capability and function.

V 265 DEVELOPMENTAL PATHWAYS OF γδ T CELLS IN THE MURINE THYMUS. Roland Scollay and Michelle Zorbas. Walter and Eliza Hall Institute of Medical Research, PO RMH, VIC 3050, and Centenary Institute of Cancer Medicine and Cell Biology, Locked Bag #6, Newtown, NSW 2042, Australia.

We have attempted to learn more about the development of T cells expressing the γδ-TCR by analysing the biology of the subsets of γδ expressing cells among the CD4$^-$CD8$^-$ cells of the mouse thymus. Three such subsets can be defined using the surface molecules HSA and Thy-1 as markers, these being HSA$^+$Thy-1$^+$, HSA$^-$ Thy-1$^+$ and HSA$^-$Thy-1$^-$. The proliferation rate, population turnover, survival in culture, lymphokine profiles, TCR V-gene usage and appearance during ontogeny have been analysed for these three populations. The data show that essentially only one population (HSA$^+$Thy-1$^+$) has many cells in division or is turning over at a significant rate. Since γδ thymic emigrants express the same HSA$^+$Thy-1$^+$ phenotype, it seems likely that this thymic population is the one that gives rise to exported γδ cells. However the production rate by the thymic population (~10^5 cells per day) exceeds the number previously estimated to be exported per day to the spleen and lymph nodes (~10^4 cells per day, Kelly et al, Int Immunol 5, 331, 1993), suggesting that some form of selection may be occurring. This is supported by differences in the Vγ usage between the thymic population and the exported cells. There are also differences in Vγ usage between the three thymic populations, with more Vγ2$^+$ cells found in the HSA$^+$Thy-1$^+$ group. Vγ3 and Vγ4 are detectable but rare in all populations. The origin and function of the two HSA$^-$ populations remains mysterious, but their low turnover makes it unlikely that they are precursor or product cells of the HSA$^+$Thy-1$^+$ population. They appear to be long lived and stable, and this is supported by their survival in culture in the absence of cytokines, while the population which is turning over *in vivo*, requires several cytokines for optimal growth *in vitro*. Data on the ontogeny of the three populations will be presented.

V 266 TCR-$\gamma\delta$ CELLS WITH $V\gamma2$-Vα10 RECEPTORS ARE SELECTIVELY EXPANDED IN C57BL/6 MICE.
Anne I. Sperling, Donna C. Decker, Anthony K. Shum, Darryl A. Stern, and Jeffrey A. Bluestone. The Ben May Institute and the Department of Pathology, University of Chicago, Chicago, IL.

The goal of the present studies have focused on determining the basis for TCR-$\gamma\delta$ cell activation and repertoire development. We have found that TCR$\gamma\delta$ cells require two signals for activation. The first signal is transduced through the CD3/TCR-$\gamma\delta$ complex. However, autocrine IL-2 production and proliferation are dependent on costimulation through CD28 ligation.

In addition, we have previously shown that TCR-$\gamma\delta$ cells expressing certain V region elements are selectively expanded *in vivo*. The expansion of $V\gamma2^+$ cells in C57BL/6 mice was found to be genetically linked to TCRδ allotype. The percentage of $V\gamma2^+$ cells in the adult is four-fold greater than in the neonate, thus expansion of $V\gamma2^+$ cells occurs after birth. The junctional diversity of the $V\gamma2$-$C\gamma$ rearrangements after birth also supports a selective expression of a subset of $V\gamma2^+$ cells. Finally, B6 mice housed in germfree conditions were found to express similar levels of $V\gamma2^+$ $\gamma\delta$ T cells as their normally housed counterparts. Thus, expansion and diversification of $V\gamma2^+$ cells is a postnatal event that does not require microbial antigenic exposure.

B6 $V\gamma2^+$ hybridomas were produced in order to examined Vδ usage. A total of 64 hybridomas were produced in three independent fusions. Strikingly, 81% of the hybrids used V$\alpha10^+$ δ-chain. In contrast, no $V\gamma2^+$ hybridomas (0/11) from a non-positively selecting strain, DBA/2, were V$\alpha10^+$. Analysis of the Vα10-Jδ rearrangements in the B6 $V\gamma2^+$ hybridomas found that two junctional lengths predominate. Further characterization of the nature of the predominate junctional length is presently being examined by sequencing the Vα10 gene the relevant T cell hybridomas. Thus, TCR-$\gamma\delta$ cells that utilize $V\gamma2$-Vα10 receptors are selectively expanded in B6 mice. In addition, these rearranged receptors may express a restricted CDR3 region.

V 267 EXPRESSION OF THE PIM-1 PROTO-ONCOGENE: DIFFERENTIAL INDUCIBILITY BETWEEN α/β- AND γ/δ-T CELLS AND B CELLS,
Denise Wingett, Diane Stone, William C. Davis and Nancy S. Magnuson, Department of Microbiology, Washington State Univ., Pullman, WA 99164-4233
The *pim-1* gene encodes a serine/threonine protein kinase and is thought to play a role in the signal transduction events associated with lymphocyte activation. Stimulation of PBMCs with phorbol ester and calcium ionophore results in a rapid but transient increase in *pim-1* mRNA levels with the peak expression occurring 4 hours post-stimulation. Treatment of PBMCs with phorbol ester alone was found to induce *pim-1* mRNA expression suggesting that the activation of protein kinase C is one pathway involved in the accumulation of this transcript. In contrast, treatment with ionomycin alone caused only a minimal increase in *pim-1* mRNA expression. *Pim-1* expression was also examined in lymphocyte subpopulations and found to be constitutively expressed in resting T cells and B cells. Furthermore, the basal level of *pim-1* mRNA could be increased by PMA/ionomycin stimulation of α/β- T cells (~5-fold) and γ/δ-T cells (~7-fold). In contrast, *pim-1* expression was not inducible in normal B cells. Examination of *pim-1* mRNA turnover in resting and PMA/ionomycin treated PBMCs revealed that *pim-1* mRNA stability was increased after stimulation. *Pim-1* mRNA stability was also compared between transformed cell lines and resting primary lymphocyte cultures. The results of these studies suggest that the increased stability of *pim-1* transcripts observed in transformed cell lines and mitogen stimulated primary lymphocytes may be linked to the growth rate of the cell. (Funded in part by USDA grant 91-37206-6867 and NIH grant T32-A107025).

Antigen Processing and Presentation

V 268 PEPTIDE SELECTIVITY OF THE TRANSPORTERS ASSOCIATED WITH ANTIGEN PROCESSING (TAP MOLECULES),
Matthew J. Androlewicz and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510
We and others have shown that TAP molecules can translocate antigenic peptides across the endoplasmic reticulum membrane in an ATP-dependent fashion. These results are consistent with TAP being a member of the ATP-binding cassette family of transporters. A major question remaining is the peptide specificity of TAP. Our initial report (PNAS 90, 9130) suggested that MHC class I binding peptides of 8-10 amino acids in length were the optimum substrate for TAP, with the exception of one 9-mer peptide derived from a signal sequence. Furthermore, longer peptides (>20 amino acids) were not good substrates. Here, we have further defined the length and sequence requirements for peptide translocation through TAP. Competition analysis was performed using a ^{125}I-labeled peptide which contains an N-linked glycosylation acceptor site. Peptide translocation into the ER was measured in permeabilized cells (Swei) by the extent of glycosylation of the labeled peptide. Peptides of length 7 amino acids are very poor substrates, while peptides of length 11 or 12 amino acids are still good substrates. However, the efficiency of transport is significantly reduced for peptides > 15 amino acids. Sequence specificity was also addressed through the substitution of specific amino acid residues within the peptides with alanine. Furthermore, a photoactive peptide analogue was synthesized which can photolabel TAP molecules. This allowed us to directly demonstrate that the competition analysis measures the specificity of TAP-dependent translocation and not other steps in the peptide transport pathway.

V 269 TRANSPORT PROPERTIES OF HUMAN INVARIANT CHAIN ISOFORMS AND THEIR ASSOCIATION WITH CALNEXIN,
Balasubramanian Arunachalam and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510
Intracellular association of invariant chain with MHC class II prevents class II from prematurely binding peptides in the endoplasmic reticulum (ER) and targets and retains class II in endocytic compartments. Human invariant chain exists in four forms, p33, p35, p41, and p43, generated by a combination of alternative initiation of translation and alternative splicing. Here we have compared all the four forms for their transport characteristics in the absence of class II and their ability to associate with molecular chaperone calnexin. Hetero oligomers containing p35 or p43 remain in the ER, whereas homo oligomers containing p33 or p41 exit the ER and are sorted to endosomes. The sorting of invariant chain to endosomes takes place via the Golgi apparatus and their accumulation leads to enlargement of the endosomes. p43/41 forms are more stable than p35/33 forms, both in the ER and following transport through the Golgi apparatus. Experiments conducted using dithiothreitol (DTT)-treated cells suggest the possible role of ER-redox potential in the transport of invariant chain forms. All the four forms of invariant chain associate with calnexin. The roles of glycosylation and transmembrane sequences of the invariant chain forms in their association with calnexin will be discussed.

V 270 αβCLIP: AN INTERMEDIATE IN MHC CLASS II PROCESSING, Ravi R. Avva and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

A complex composed of the αβ dimer of HLA-DR and a peptide derived from the Invariant chain (CLIP) has been extensively characterized in the MHC Class II antigen processing mutant T2. Pulse-chase analyses in wild type cells suggests that this complex may be an intermediate in MHC Class II processing. Dissociation kinetics and peptide displacement studies indicate that CLIP may bind to a polymorphic region of HLA-DR. Further experiments suggest that the transition of αβCLIP to a mature αβ complex capable of binding a potential antigenic peptide is catalyzed by low pH and a second agent containing an intermediate length aliphatic carbon chain. Experiments designed to elucidate the physiological mechanism for this transition will be presented.

V 271 ENDOGENOUS ANTIGEN PROCESSING IN CELL MEDIATED CYTOLYSIS OF CHLAMYDIA TRACHOMATIS INFECTED L CELLS, P. Robert Beatty and Richard S. Stephens, Program in Infectious Disease, University of California at Berkeley, Berkeley, CA 94720

CD8 T cell mediated cytotoxicity has been well documented for bacteria that infect and replicate inside macrophages. *Chlamydia trachomatis* primarily infects non-phagocytic cells, grows within an endosomal vacuole, and evades degradation by avoiding lysosomal fusion. Important questions for bacteria that do not enter the cytoplasm is whether cytotoxic CD8 T cells recognize infected cells and what antigen processing pathway might be utilized inside non-phagocytic host cells. We used L cells infected with *C. trachomatis* to examine in vitro cytolysis of target cells by immune spleen cells. The cytotoxicity was CD8 T cell mediated and dependent on the increased expression of ICAM-1 on the target cells. To examine mechanisms of antigen processing, we added brefeldin A to infected target cells which eliminated the cytolytic response. Cycloheximide treatment of infected cells reduced lysis to background levels. The brefeldin A results demonstrate the need for intact golgi apparatus, and the effect with cycloheximide establishes the need for host protein synthesis. In contrast, inhibitors of exogenous antigen processing mechanisms did not alter chlamydia specific lysis. Chloroquine and ammonium chloride when added to the assay had no effect on cytolysis and indicate that exogenously obtained antigens are not required for immune lysis. Inactivated chlamydia, still capable of attachment and uptake, did not elicit killing in our cytotoxicity assay indicating that active chlamydia replication is necessary and that production of nascent protein antigens is required. Our results implicate the endogenous processing of chlamydial antigens to achieve specific lysis of infected target cells in vitro. It will be important to identify in which endosomal vesicles chlamydia proteins encounter class I MHC molecules because no antigens have been detected outside the chlamydia vacuole.

V 272 CYTOTOXIC T CELL INDUCTION TO PROMISCUOUS CLASS I BINDING PEPTIDES, Cornelia Bergmann, Lili Tong, Racquel Cua, John Sensintaffar and Stephen Stohlman. Dept. of Neurology and Microbiology, USC School of Medicine, Los Angeles, CA 90033.

Major histocompatibility (MHC) class I restricted cytotoxic T lymphocyte (CTL) responses are governed by intracellular antigen processing, affinity of peptides to class I molecules and the T cell repertoire. In BALB/c mice (H-2^d) the CTL response to native gp160 of HIV-1 strain IIIB is specific for a 10-mer peptide (p18-10) comprising residues 318-327. This response is exclusively D^d restricted, although this epitope contains a 9 amino acid sequence (residues 319-327) which comprises an L^d specific binding motif. To evaluate the factors contributing to T cell repertoire selection, the potential to induce dually restricted CTL to this epitope was analyzed in the H-2^d haplotype.

Peptide sensitization of D^d target cells demonstrated that the 10-mer was recognized more efficiently than the 9-mer by gp160 specific CTL. However, the 9-mer was more potent than the 10-mer in inhibiting lysis of L^d target cells by CTL specific for an L^d-restricted peptide derived from mouse hepatitis virus, demonstrating that the 9-mer bound efficiently to both class I molecules *in vitro*. To enhance promiscuous class I presentation *in vivo* 'preprocessed' forms of the gp160 epitope were expressed using recombinant vaccinia viruses (vac). Immunization with recombinant vac expressing either the 10-mer, or 9-mer including an initiation Methionine (M318-327 and M319-327), induced D^d-restricted CTL specific for native gp160. By contrast, recombinant vac expressing 8 gp160 residues (M320-327), generated L^d-restricted CTL specific for peptide, but not native gp160. The lack of a L^d restricted response to native gp160 may therefore not be attributed to a limited T cell repertoire, but to inefficient processing of gp160 for presentation on L^d. The switch in class I restriction, controlled by a single amino acid within one epitope, demonstrates that non anchor residues may have a profound effect on differential MHC restriction and CTL induction.

V 273 CLASS II-RESTRICTED PRESENTATION OF AN ENDOGENOUS ANTIGEN BY THE MUTANT ANTIGEN PRESENTING CELL, T2, *Janice Blum, *Susan Kovats, *Carol Fang, *Gerald Nepom, Jeffrey Hermes, Dennis Zaller, Alka Bansal, Daniel Freed, and Phyllis E. Whiteley, * Immunology and Diabetes Programs, Virginia Mason Research Center and the University of Washington, Seattle, WA 98101 and Department of Autoimmune Diseases Research, Merck Research Laboratories, Rahway, NJ 07065.

A series of mutant human APC have been identified which are unable to present exogenous antigens in the context of class II histocompatibility antigens. One such mutant APC, T2 expressing DR4, is defective in the presentation of exogenous protein antigens such as influenza hemagglutinin (HA) and tetanus toxoid. However, T2 cells can stimulate T cells when provided with preprocessed peptides derived these antigens, such as the HA peptide 307-319. The defect in T2 cells results in the association of a large proportion of the class II molecules with endogenous peptides derived from the invariant chain, a chaperone protein associated with class II antigens. We examined T2 cells to determine whether other endogenous antigens were processed and bound to cell surface class II proteins. T2 cells were found to contain peptide/DR4 complexes derived from the processing of endogenous human immunoglobulin, as detected using antigen-specific T cell hybridomas. Two distinct peptide epitopes derived from the kappa light chain are expressed bound to DR4 on the surface of T2 cells. These same peptides are generated by normal antigen presenting cells (APC) and each bind with a high affinity to DR4. Studies using another mutant APC, defective in the presentation of exogenous antigens, yielded similar results with endogenous kappa light chain peptides being displayed by DR4. Both of the mutant APC were shown to synthesize endogenous kappa light chains. Mechanistic analysis of the T2 defect that allows presentation of endogenous but not exogenous antigens is underway.

V 274 INVARIANT CHAIN LIMITS THE DIVERSITY OF ENDOGENOUSLY SYNTHESIZED PROTEIN EPITOPES PRESENTED BY MHC CLASS II MOLECULES, H. Bodmer*, S. Viville, C. Benoist and D. Mathis, LGME/U-184 INSERM, Institut de Chimie Biologique, 11 rue Humann, Strasbourg, France. * Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford. Oxford.

A central role of major histocompatibility complex (MHC) class II molecules is to present antigenic peptides to CD4+ T cells[1]. Primarily, they display peptides derived from exogenous proteins, but can sometimes present endogenously synthesized protein derivatives[1]. MHC class II molecules are closely associated with the invariant chain (Ii) during assembly and intracellular transport. Ii is thought to have several functions[1-3], including: direction of class II molecules to the endosomes, where they can encounter degraded, endocytosed proteins[4-7]; as well as blockage of the peptide-binding groove so that it is not available until the Ii/class II complex dissociates in the endosomes[8-10]. Both functions suggest that the presentation of endogenous proteins might be altered with antigen presenting cells (APCs) lacking the invariant chain. We have tested this by mating a "knock-out" mouse line devoid of the invariant chain[2] with transgenic lines expressing a fragment of myelin basic protein (MBP84-105) in MHC class II positive cells. By screening a panel of MBP reactive T cell hybridomas, with differing fine specificities within this complex epitope, we find that Ii^{0} splenic APCs present a broader range of endogenously synthesized epitopes from the MBP fragment, than APCs from Ii^{+} mice. Moreover, these additional epitopes are represented among specificities of T cells derived from Ii^{+}, transgenic mice, expressing the MBP fragment in the thymus. This apparent lack of self-tolerance has interesting implications for autoimmunity.

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V 275 THE REGULATORY COMPLEX OF HLA CLASS I PROMOTERS EXHIBITS LOCUS-SPECIFIC CONSERVATION WITH LIMITED ALLELIC VARIATION, Nezih Cereb and Soo Young Yang, Department of Pediatrics and Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

The extensive genetic polymorphism of the classical MHC class I molecules provides an important distinguishing feature of the host's immune system, influencing the selection and function of effector cells against tumor and virally-infected cells. In these targets, class I molecules are often found to be selectively suppressed. This suppression is locus-specific and, in certain cases, allele-specific. We examined the molecular basis for this phenomenon by analysis of HLA class I promoter sequences, which include class I regulatory complex, CRC. A total of 41 well-characterized HLA homozygous B-LCL were analyzed, using SSOPs complementary to the overlapping HLA-A, -B, and -C CRC elements. These include κB_1, κB_2, the IRS, the NRE, and the HLA counterpart of the H-2RII region that contains the RxRβ-binding motif. The CRC of HLA promoters displayed locus-specific conservation; however, limited allelic variation was also observed in each of the cis elements. In some, variations were apparently generated by gene conversion. The palindromic κB_1 site, which has an active role in enhancing promoter activity, was found to be conserved in almost all HLA-A and -B alleles, but not in HLA-C. The core DNA-binding motif for RXRβ was absent within the CRC region, in all HLA alleles examined. Sequence analysis of promoters from HLA-A31, -B13, and -Cw1 genomic clones, as well as pairwise inter- and intra-allelic comparison with those of published alleles, showed that locus-specific conservation extended throughout the promoter sequences. Locus-specificity and the allelic variation seen in the CRC regions may provide a structural basis for the selective modulation of HLA class I genes.

V 276 **Identification and isolation of a novel MHC class II containing compartment from B-lymphocytes and its involvement in class II peptide loading,** James R. Drake, Sebastian Amigorena, Paul Webster and Ira Mellman, Department of Cell Biology, Yale University School of Medicine. 333 Cedar St., PO Box 208002, New Haven, Connecticut 06520-8002, USA.

A novel subcellular compartment containing the majority of the intracellular MHC class II in murine B-lymphocytes has been identified and isolated using density gradient centrifugation and free flow electrophoresis. These class II-containing vesicles (CIIV) are biochemically, morphologically and functionally distinct from conventional endosomes, lysosomes and biosynthetic organelles. The class II molecules in the CIIV are largely free of bound invariant chain and are capable of efficiently presenting peptide antigen to T-cells *in vitro*. Newly synthesized MHC class II molecules, no longer associated with intact Ii chain, are found to transiently accumulate in the CIIV before transport to the plasma membrane. Moreover, MHC class II-peptide complexes, detected by increased stability to dissociation by SDS, are also transiently detected in the CIIV, suggesting that this may be the location were complexes of processed antigen and MHC class II form. Thus, the CIIV are likely to represent a population of B cell-specific endosome-derived vesicles, specialized for antigen processing and the transport of MHC class II-peptide complexes to the cell surface.

V 277 ALTERNATIVE PROCESSING EVENTS IN PROTEINS EXPRESSED INTRACELLULARLY AND PRESENTED IN THE MHC CLASS II PATHWAY, Elizabeth E. Eynon and Ada M. Kruisbeek Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Myelin Basic protein (MBP) is an intracellular protein associated with the myelin sheath in the central nervous system. Tolerance to this cytoplasmic protein in $H\text{-}2^{s}$ strains of mice has been hypothesized to be due to immunological ignorance, that is, the lack of availability of the antigen to the immune system. However, there is some evidence that some form of myelin basic protein is present in the thymus in neonatal animals, but the fact remains that there are MBP reactive T cells present in adult mice of the $H\text{-}2^{s}$ haplotype. We are considering another alternative explanation for the presence of MBP reactive T cells in the periphery of unprimed mice and that is alternative processing of cytoplasmic proteins. We are investigating whether MBP present intracellularly is processed into different epitopes from exogenous MBP for T cell recognition.

Sequences 2.0 kb 5' of the MHC classII promoter have been shown to confer tissue specific expression for the MHC class II gene product. We have inserted this 2.0 kb sequence upstream of an MBP minigene construct and transfected it into a B cell lymphoma LS102.9. This minigene has also been placed under the control of an immunoglobulin enhancer/promoter from an Ig/myc translocation. An additional construct is being made providing targeting to the ER and endosomal vesicles by adding sequences encoding the cytoplasmic tail and transmembrane domain of the MHC class II associated invariant chain, which has been shown to target to endosomal vesicles. Cells transfected with these constructs are being tested with a T cell hybridoma which recognizes the major epitope produced by challenge with exogenous MBP. In addition, these cells will be tested against bulk T cells generated by injection of exogenous MBP or injection of the transfected cells into $H\text{-}2^{s}$ mice.

V 278 COMPLEMENTARY MUTANTS IN MHC CLASS II/PEPTIDE COMPLEX ASSEMBLY Steven P. Fling and Donald A. Pious, Department of Pediatrics, University of Washington, Seattle, WA 98195.

Our lab has previously mapped to the MHC a gene involved in class II antigen processing and presentation, using a set of mutant B-LCL derived by mutagenesis and immunoselection of B-LCL 8.1.6; 8.1.6 contains an 800 kb hemizygous deletion within the class II region of the HLA. The lesion in these mutants results in a failure to form normal intracellular class II/peptide complexes which is manifested by: 1) conformationally altered, unstable class II dimers; 2) association of Ii chain derived peptides with DR; and 3) an inability to present whole exogenous antigens. Available evidence suggests that the affected gene maps to a 230 kb interval within the MHC hemizygous deletion of 8.1.6.

We describe here the isolation of a set of mutants which manifest global alterations in class II conformation and stability similar to those seen in the 8.1.6 derived mutants. The new mutants, which were derived by mutagenesis and immunoselection of a B-LCL which contains a different hemizygous deletion of chromosome 6p, are complementary with the 8.1.6 derived mutants in somatic cell hybrids. These results suggest the possibility of an additional locus whose product is involved in the assembly of class II/peptide complexes. Alternatively, complementation in these mutants could occur by intragenic means, perhaps by molecular complementation in a homomultimeric protein..

V 279 GENERAL AND UNIQUE PEPTIDE-SPECIFICITIES EXHIBITED BY TRANSPORTERS ASSOCIATED WITH ANTIGEN PROCESSING (TAP) FROM MOUSE, RAT, AND HUMAN. Lars Franksson, Elisabeth Wolpert and Klas Kärre, Microbiology and Tumorbiology Center, Laboratory of Tumor Biology, Karolinska Institutet, S-17177 Stockholm, Sweden.

Transporters associated with antigen processing , TAP, are considered to transport short peptide fragments from the cytosol to the lumen of the endoplasmic reticulum, where they are loaded on MHC class I molecules. In vivo the lack of functional TAP2 molecules allow tumor cells to escape from T cell mediated immune surveillance. In vitro the TAP molecules have been shown to influence the rate and spectrum of peptides being transported over the ER membrane. Here we address the questions how different TAP molecules i) affect the tumorigenicity of tumor cells in vivo, and ii) affect the peptide repertoire presented by MHC class I molecules at the cell surface. Ad i), three independent TAP2 transfectants (mouse, rat, and human) of the antigen presentation defect mouse lymphoma RMA-S were inoculated s.c. over a minor histocompatibility barrier, MiHa, and monitored for outgrowth. All three TAP2 transfectants grew out and were subsequentially rejected equally well as the wild type line RMA, whereas the non-transfected RMA-S grew out and killed the A.BY mice. Ad ii), naturally processed peptides were eluted under acid conditions from either intact cells or immuno-affinity purified MHC class I molecules of the three RMA-S TAP2 transfectants. The eluates were analysed on reverse phase HPLC and capillary electrophoresis, CE. In addition to this a functional identification of epitopes was performed utilising cytotoxic T lymphocytes specific for either three MiHa, or a tumor antigen. The HPLC- and CE-profiles differed between the three TAP2 transfectants. However, the three MiHa and the tumor antigen previously characterised on the wild type line RMA were identified by the CTL in each peptide-eluate. The data suggests a general capacity of the TAP molecules to transport certain peptides, among which the CTL epitopes screened for here are found. In addition to this each TAP molecule appear to have a unique fine specificity for peptides as indicated by the differences in the HPLC-and CE-profiles, though further chracterisation of the material remains to be done. Finally, the restored rejectability by the TAP2 transfections pinpoints the decisive role that one single gene can have on T cell mediated immune surveillance.

V 280 INTERFERON-γ INDUCIBLE PROTEASOME SUBUNITS REPLACE CONSTITUTIVELY EXPRESSED SUBUNITS Klaus Früh, Per A. Peterson and Young Yang
The Scripps Research Institute, Dept. of Immunology, 10666 North Torrey Pines Road, La Jolla, CA 92037

The proteasome is thought to be the cytoplasmic protease responsible for the generation of peptides presented by MHC class I molecules. Two subunits, LMP2 and LMP7, of the proteasome are encoded in the MHC class II region of the genome and the expression of LMP2 and LMP7 can strongly be induced by IFN-γ. We have observed previously that this induction not only leads to the enhanced presence of LMP2 and LMP7 in the proteasomal complex, but also to the disappearence of certain other subunits. We have now further characterized the events taking place during the incorporation of LMP2 and LMP7 into the proteasome. Our data indicate that both LMP2 and LMP7 replace a disinct other subunit. The respective subunits are constitutively expressed and highly homologous to the MHC-encoded ones. The results will be discussed with regard to proteasome function and antigen processing.

V 281 PATHWAYS INVOLVED IN ANTIGEN PROCESSING FOR MHC CLASS II-MEDIATED ANTIGEN PRESENTATION, Anand M. Gautam and Hugh O. McDevitt#, Human Genetics Group, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia and #Department of Microbiology and Immunology, Stanford University, California 94305, USA.

Generally, major histocompatibility complex (MHC) molecules present processed foreign peptides to specific T cells. The recently described genes, LMP2, LMP7 (subunits of proteosome complex) and TAP1, TAP2 (ATP-dependent transporters) have been shown to regulate antigen processing for MHC class I molecules. However, such genes have not been defined for MHC class II molecules. We have generated antigen processing mutants for MHC class II-mediated antigen presentation. These mutants fail to present whole antigens but retain the ability to present peptides to antigen-specific and MHC class II-restricted T cell clones. This indicates that these cells have certain defects in antigen processing. Our aim is to utilise these mutants to study pathways and genes which may control MHC class II-mediated antigen processing. The putative gene (s) involved in antigen processing for class II molecules have been localised in the MHC class II region. Invariant chain (Ii) prevents binding of endogenous peptide to MHC class II molecules in the endoplasmic reticulum and hence dissects MHC class II from MHC class I pathway of antigen presentation. MHC class II molecules in processing mutants are predominantly occupied with an Ii peptide, called CLIP (class II-associated Ii peptide). We have also developed an experimental system to determine binding characteristics of Ii to MHC class II molecules by utilizing the CLIP.

V 282 ISOLATION OF CANDIDATE GENES FOR THE PROCESSING AND PRESENTATION OF ANTIGENS BY MHC CLASS I USING PROMOTER TRAP RETROVIRUSES. Alison E George, Keith Gould, Bin Goto, Justin P Newton, Catherine Hubbard*, Wen Chang†, H Earl Ruley†, George Brownlee and D Jasper G Rees, The Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, United Kingdom, *Genzyme Corp., Cambridge, MA 02139, †Department of Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232.

Antigen processing and presentation to T cells is observable only in intact cells, and a genetic approach to the identification of the proteins involved in this pathway is therefore attractive. We are using a defective "promoter-trap" retrovirus as an insertional mutagen in order to isolate new antigen presentation mutants. We have used Chinese hamster ovary (CHO) cells, which are functionally haploid, as our antigen presenting cell, which have been transfected with the murine ecotropic retroviral receptor to make them susceptible to infection by the promoter-trap retrovirus U3-Hygro (Chang et al, 1993, Virology, **193**, 737). The U3Hygro retrovirus has a promoterless hygromycin^R gene 30 nucleotides from the 5' end of the provirus LTR, such that it is only expressed following integration downstream of an active cellular promoter. A CHO line transfected with murine MHC Class I K^k, and a cytoplasmic influenza virus hemaglutinin (HA) was previously used for U3Hygro mutagenesis, but was found to have high background in the experiment due to loss of transfected markers. Therefore this cell line has been further transfected with a second Class I K^k, and an HA-puromycin^R fusion protein construct. A cell line (Y10) that is highly susceptible to CTL lysis and extremely stable against loss of transfected markers has been isolated and characterised. 3 x 10^7 cells of line Y10 were infected with the U3-hygro virus selected with HA specific, K^k restricted CTL and hygromycin to obtain approximately 100 potential antigen presentation mutants. We are characterising these cell lines to identify the sites of retroviral insertion using 5' RACE PCR, and will use the criterion of multiple independent insertions to identify candidate genes for further investigation.

V 283 LISTERIOLYSIN O (LLO) IS A TARGET FOR H-2K^b RESTRICTED CD8 T CELLS. John T. Harty*, Marc A. Gavin and Michael J. Bevan, *Department of Microbiology, University of Iowa, Iowa City, IA 52242 and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195.

The LLO molecule is a virulence factor secreted from *Listeria monocytogenes* which is required for the organism to escape the phagocytic vesicle and replicate in the cytoplasm of the infected cell. Previous work has shown that amino acids 91-99 are presented to protective CD8 T cells in an H-2K^d restricted fashion.

We have recently determined that the LLO molecule is a target for Listeria specific H-2^b restricted CD8 T cells. *Listeria* specific polyclonal CD8 T cells recognize EL-4 (H-2^b) cells after transfection with the coding region of the LLO gene. LLO specific CD8 T cell lines recognize two peaks of biological activity in HPLC separated natural peptide extracts of *Listeria* infected spleens from H-2^b mice. Both fractions 29 and 32 are presented by the H-2K^b molecule. Interestingly, the targeting activity present in fraction 32 can be immunoprecipitated with both H-2K^b and H-2D^b specific mAbs suggesting that the peptide contains residues permitting binding to these two class I molecules. In contrast, detection of fraction 29 does not require surface expression of either the H-2K^b or H-2D^b molecules as it is found after HPLC separation of natural peptide extracts from *Listeria* infected H-2^k, H-2^d , and β2-/- strains of mice. Fraction 32 is not detectable in non-H-2^b extracts. These data suggest the possibility that fraction 29 is a processing intermediate that does not require protection in a class I groove for detection. Experiments to identify the targeting peptide(s) are in progress.

V 284 DIFFERENT PEPTIDES ARE TRANSLOCATED BY ALLELIC VARIANTS OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PRESENTATION (TAP). Marie-Thérèse Heemels*, Ton N.M. Schumacher*, Kurt Wonigeit• and Hidde L. Ploegh* Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA • Medizinische Hochschule Hannover, Klinik fur Abdominal- und Transplantations Chirurgie, 30625 Hannover, Germany

MHC class I molecules associate with peptides that are delivered from the cytosol to the ER lumen by the Transporter associated with Antigen Presentation (TAP). We have exploited TAP polymorphism to investigate the specificity of the rat peptide translocator. Liver microsomes of SHR and Lewis rats, each expressing a distinct allelic variant of TAP (*cim*^{b} and *cim*^{a} respectively), accumulate different sets of peptides. The use of MHC congenic rats allowed unequivocal assignment of this difference in translocation capacity to the MHC, independent of the class I products expressed. The COOH-terminal residue of the peptide seems of overriding importance for substrate recognition, although other sequence elements of the peptide also contribute to the specificity of the translocation process. Both the *cim*^{a} and *cim*^{b} TAP complexes translocate peptides with a hydrophobic COOH-terminus. However, the ability to translocate peptides with a COOH-terminal His, Lys or Arg residue is unique to the *cim*^{a} transporter. Thus, the specificity of the TAP peptide translocator imposes restrictions on the availability of peptides for antigen presentation by class I molecules.

V 285 INTERFERENCE WITH TRANSPORT OF HLA CLASS I MOLECULES BY HERPES SIMPLEX VIRUS. Ann B. Hill*, Barbara Barnett†, Duncan McGeoch† and Andrew McMichael*. *Institute of Molecular Medicine, J.R.H., Headington, Oxford. OX39DU, U.K. †Institue of Virology, Church St, Glasgow, U.K.

Antigen presentation by class I MHC moleules, enabling recognition by cytotoxic T lymphocytes, plays a crucial role in host defence against most viruses. We have investigated the assembly and transport of class I MHC in cells infected with herpes simplex virus (HSV). We used mutant viruses with the UL41 gene which shuts off host protein synthesis deleted. Here we find that class I is synthesized normally, but fails to become sialated. In contrast, transferrin receptor is sialated normally, indicating a selective retention of MHC in a sialyl-transferase negative compartment, probably the endoplasmic reticulum. Failure of sialation was seen with both HSV-1 and HSV-2. Drug treatment of cells indicated that an early gene or genes are responsible for the effect. A pulse chase study showed that whereas some retained class I allelic products were stably associated with ß2microglobulin, others were retained as free heavy chain, suggesting that HSV interferes with assembly as well as transport of class I. No HSV protein was found to co-precipiate with class I MHC. The effect of HSV may be similar to that recently described for murine and human cytomegaloviruses (CMV), which are distantly related to HSV in evolutionary terms. However, in contrast to hCMV, class I heavy chains in HSV-infected cells are not degraded.

V 286 EVIDENCE FOR QS-21 AFFECTING ANTIGEN PROCESSING AND PRESENTATION, Kristy Kikly and Mark Moore, Department of Cell Genetics, Genentech, Inc., South San Francisco, CA 94080

QS-21 is a purified saponin from the bark of the *Quillaja saponaria* Molina tree from South America. When QS-21 is used *in vivo* as an adjuvant with soluble proteins there is a 10 to 1000 fold higher antibody response compared to antigen alone but most interestingly, a CTL response is generated. We are using an *in vitro* model system of antigen processing and presentation to study how QS-21 is able to stimulate a Class I restricted CTL response. The CTL clone B3 recognizes an ovalbumin (OVA) peptide in the context of H-2K^b. When intact OVA is mixed with EL-4, H-2^b target cells, the OVA cannot enter the cytosol of the cell and therefore is not processed and presented by H-2^b on the cell surface. However, when QS-21 is mixed with the OVA and EL-4, the OVA is able to enter the Class I processing pathway and sensitize the EL-4 for lysis by B3. This does not appear to occur simply because the QS-21 has permeablized the cell membrane and OVA has entered the cytosol. Other saponins and surfactants do not allow OVA to enter the cytosol of the cell, and the target cells can be labeled with sodium chromate to the same extent as the controls. EL-4 can be pulsed with OVA, washed, and then exposed to QS-21 and there is still enhanced OVA presentation. Additionally, EL-4 can be pretreated with QS-21 for 10 minutes and rested for up to four hours before the addition of OVA and still show enhanced antigen processing and presentation. Since QS-21 can be added before, during, or after OVA, these data suggest that QS-21 may be affecting the cellular mechanism of antigen processing resulting in enhanced antigen presentation on the cell surface.

V 287 THE PRESENTATION OF EXOGENOUS CLASS II-RESTRICTED ANTIGENS IS DEFECTIVE IN A BARE LYMPHOCYTE SYNDROME CELL. Susan Kovats, Janice Blum and Gerald Nepom, Immunology and Diabetes Programs, Virginia Mason Research Center and the University of Washington, Seattle, WA 98101.

Bare Lymphocyte Syndrome (BLS) is an inherited immune deficiency in which HLA class II structural genes are not expressed due to mutations in trans-acting transcription factors encoded outside the MHC. Fusion of different BLS cell lines restores class II expression, indicating that defects in class II transcription differ among BLS cell lines and defining four genetic complementation groups. BLS cells transfected with HLA-DR were used to study antigen presentation and found to display a mutant APC phenotype similar to the APCs 721.174 and T2. The mutation in 721.174 and T2 cells maps in the class II region of the HLA gene complex, and the gene involved is thought to control the ability of cells to process and present exogenous class II-restricted antigens. BLS cells that express DR molecules stimulate influenza hemagglutinin (HA) restricted T cell clones after incubation with the preprocessed peptide HA307-319, but are defective in the presentation of the exogenous HA protein. The DR molecules on the surface of BLS cells do not display epitopes recognized by conformationally sensitive antibodies that bind peptide-class II complexes, suggesting that DR molecules in BLS cells are not stably associated with peptides. These data suggest that the mutated gene which controls class II structural gene transcription also regulates the expression of a gene controlling antigen processing. This transcription factor does not regulate the synthesis of MHC Class I or TAP genes. Cell fusions using BLS lines from different complementation groups resulted in the surface expression of wild-type class II molecules. Thus, HLA class II structural genes and a gene required for class II-restricted antigen presentation are transcriptionally coregulated.

V 288 INCREASED ANTIGEN PRESENTATION BY PEPTIDES BINDING TO THE SAME MHC. Ming-Zong Lai and Ming-Hsien Lin Feng, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan, R. O. C.

We have recently identified a weak I-A^d-binding peptide PB1 capable of enhancing the reactivity of other I-A^d-restricted T cells. The augmentative effect was not due to the cross-reactivity of PB1 peptide with antigens. The strict I-A^d specificity suggests that PB1 enhanced the recognition of antigen-I-A^d complex by TCR. The association of PB1 with I-A^d was a priori to its additive effect, because late addition of PB1 had little effect after antigen-MHC complex was immobilized. Many of I-A^d-binding peptides displayed a similar additive effect even though such enhancing effect could only be detected under very restricted condition. PB1 was distinct for its prominently augmentative effect on all the I-A^d-restricted T cells analyzed. A similarly enhancing activity was demonstrated on a synthetic transferrin receptor peptide with minimum affinity for I-A^d. In contrast, no augmentative activity was detected on another weak I-A^d-binding peptide hen egg lysozyme 46-61. It was found that the binding of PB1 and transferrin peptide, but not hen egg lysozyme peptide, stabilized I-A^d expression. It is postulated that for a peptide to enhance the presentation of other antigens, two criteria must be met: (1) the binding of peptide should increase the biochemically stable compact form of MHC molecule, (2) the affinity for MHC should be relatively low to allow the displacement of other peptide. This is supported by that the binding of specific peptides to I-A^d was increased in the presence of PB1. The inclusion of PB1 in the immunization mixture also enhanced T cell responses *in vivo*, suggesting the possibility of using low-affinity peptide to promote specific immunity.

V 289 DETECTION OF HLA-DQA2 PROTEIN EXPRESSION Andrew M. Lew and George B. Rudy Walter & Eliza Hall Institute, P.O. RMH, Parkville, Vic. 3050, Australia. Ph: 61-3-345 2555; Fx: 61-3-347 0852

The more centromeric paired MHC Class II DQ loci, DQA2 and DQB2 (formerly DXA and DXB), have not previously been shown to be translated, despite apparently normal gene architecture. Transcription of DQA2, but not DQB2, has been reported. We have raised polyclonal antisera in rabbits to synthetic immunogens representing the C-termini of DQA1, DQB1, and the putative products of DQA2 and DQB2. Western blotting of cell lysates from a panel of homozygous typing cells (EBV-transformed lymphoblastoid lines) shows that the antisera to DQA1 and DQA2 both detect 33K proteins (consistent with Class II α chains). Competition experiments with the peptides used for immunization demonstrate the specificity of both these reagents. Immunoprecipitation of biotin-labelled cell surface proteins indicates that DQA2 is expressed on the surface of at least one cell line, although at a much lower level than DQA1. Preliminary results suggest that surface-expressed DQA2 in this line is associated with a 28K protein (consistent with a Class II β chain).

V 290 PROTEOLYTIC PROCESSING OF ANTIGENIC PEPTIDES IN THE ENDOPLASMIC RETICULUM. Heidi Link, Jack Bennink and Jonathan Yewdell, Laboratory of Viral Diseases, NIAID, NIH. Bethesda, MD 20892.

MHC class I molecules bind peptides of 8-10 residues derived by proteolytic events in the cytosol. The translocation of antigenic peptides into the endoplasmic reticulum (ER) is mediated by TAP, MHC encoded membrane transporters. Although the transport of peptides into the ER has been extensively studied, the contribution of events in the ER to the production of class I binding peptides is uncertain. It remains unknown whether all proteolysis occurs in the cytosol and correctly sized peptides are transported or whether a variety of potential class I substrates are transported and further modified in the ER. To characterize the contribution of this intracellular compartment to antigen presentation, recombinant vaccinia viruses (rVV) were constructed to contain two MHC class I determinants in tandem: peptide 147-155 of influenza virus nucleoprotein (NP), and peptide 52-59 of vesticular somatitis virus N-protein. Translocation of the 17-mer peptides into the ER was facilitated by the hydrophobic signal sequence of E3/19K glycoprotein of adenovirus, and experiments were conducted in T2 cells, eliminating TAP dependent transport of peptides. Both $N_{(52-59)}$ and $NP_{(147-155)}$ were efficiently presented in T2 cells when located at the C-terminal position of the tandem peptides. A control construct without the signal sequence did not present either peptide in T2 cells, demonstrating that presentation was dependent upon signal sequence translocation into the ER. To assess the extent of ER processing, rVV expressing full length NP, containing the α-interferon signal sequence to facilitate translocation to the ER, was examined. None of the three defined class I determinants of NP (50-57, K^k; 147-155 K^d; 366-374 D^b) were presented in T2 cells, demonstrating that the observed processing was limited to peptides. The results demonstrate that that peptides longer than the minimal residues can be modified in the ER/secretory pathway to bind MHC class I molecules, provided that MHC class I-binding C-terminal residues are available. Although the nature of ER peptidase activity is unknown, the results are consistant with an N-terminal peptidase activity involved in peptide trimming.

V 291 AN HLA-B7 LEADER PEPTIDE IS RECOGNIZED BY AN HLA-B7-REACTIVE CTL CLONE, Charles T. Lutz, Kelly D. Smith, Eric L. Huczko, and Victor H. Engelhard, Depts of Pathology and Microbiology, U. of Iowa, Iowa City, IA 52242 and the Beirne Carter Center for Immunology Research and the Dept of Microbiology, U. of Virginia, Charlottesville, VA 22901.

To investigate peptide determinants recognized by alloreactive CTL, we tested 12 anti-HLA-B7 CTL clones with HLA-B7-binding synthetic peptides. All CTL clones specifically killed HLA-B7-positive JY cells and HLA-B7-transfected 721.221 cells, but not HLA-B7-transfected T2 cells (T2-B7). These 12 CTL clones recognize multiple HLA-B7-bound peptides: 1. Seven CTL clones reacted with T2-B7 cells incubated with different HPLC fractions from HLA-B7-positive cell extracts. 2. Each CTL clone was sensitive to HLA-B7 peptide binding groove mutations. 3. Each clone recognized a unique set of HLA-B7 variants. The CTL clones were tested with 12 synthetic peptides incubated with T2-B7 cells and β–2 microglobulin . The synthetic peptides represented cellular peptides bound in moderate to large quantities to HLA-B7 on JY cells (Huczko et al, J Immunol 151: 2572, 1993). Only one CTL clone, KID-8, responded to a synthetic peptide. This implies that prevalent MHC-bound peptides are not necessarily common targets for alloreactive CTL. The synthetic peptide recognized by KID-8, L V M A P R T V L, matches the HLA-B7 leader sequence. KID-8 does not kill T2-B7 cells without synthetic peptide, implying that the HLA-B7 leader peptide was not efficiently processed for MHC association in TAP1 and TAP2 deficient T2 cells. The HLA-B7 leader peptide is unusual because the A P R motif at positions P4-6 is found at positions P1-3 in many HLA-B7-binding peptides. KID-8 CTL did not recognize the closely related synthetic peptide, R V M A P R A X X (where X = leucine or isoleucine), suggesting that residues 1, 7, or 8 are critical CTL recognition epitopes.

V 292 TNP-MODIFIED PEPTIDES INDUCE CTL REACTIONS TO THE UNMODIFIED CARRIER PEPTIDES. Stefan Martin and Hans Ulrich Weltzien, Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-79108 Freiburg, Germany.

We have previously shown that H-$2K^b$ restricted, trinitrophenyl (TNP)-specific CTL reactive to the hapten in position 7 of octameric K^b-binding peptides are highly dependent in their antigen specificity on the sequence of the carrier peptide (J. Immunol. 151:678 (1993)). Primary *in vitro* induction of CTL with different H-$2K^b$-binding peptides modified on the ε-amino group of lysine in position 7 revealed that such CTL are capable of specifically recognizing the unmodified carrier structure, although with lower efficiency. Using glycine designer peptides we could demonstrate that the TCR of these T cells contained two independent contact sites for a) TNP-lysine in position 7 and b) a second "subepitope" made up of amino acid side chains in positions 3 and 4 of the K^b-associated octamer. In the case of chemically altered self peptides this specificity might be expected to result in the induction of self reactive CTL. To test this hypothesis we used a K^b-binding self peptide from C57BL/6 mice (Falk, K., and O. Rötzschke. 1993. Sem. Immunol. 5:81). This peptide was TNP-modified on its lysine residue in position 7 and used for primary *in vitro* induction of CTL. A CTL line was generated which was not only reactive to the TNP-modified self peptide but also to the unmodified self peptide. These findings lead us to propose the following hypothesis concerning the induction of autoimmune reactions by chemicals: self reactive TCR may possibly not be eliminated by thymic negative selection if they react to only one of two possible contact points on MHC-associated self peptides. Haptenization of such "monoreactive" non-immunogenic self peptides could lead to the generation of a second "subepitope" and hence to an immunogenic structure capable of primary induction of resting T cells. Once activated some of these T cells might react to the hapten-free self peptide resulting in autoimmune reactions. Studies to evaluate the *in vivo* relevance of our results are underway.

V 293 A PROCESSING DEFECT LIMITED TO ANTIGENS WITH DISULFIDE BONDS, Brian J. Merkel and Kathleen L. McCoy, Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298

A Chinese hamster ovary fibroblast, transfected with murine MHC class II genes, inefficiently stimulated $CD4^+$ T cells specific for antigens containing disulfide bonds including ovalbumin, hen egg lysozyme, and pork insulin. By contrast, the fibroblasts efficiently presented synthetic peptides indicating that the cells express a processing defect. In agreement with our previous findings with cytochrome c and Staphylococcal nuclease, the cells effectively activated a λ-repressor-specific T cell, which recognizes an antigen lacking disulfide bonds. The poor ability of the fibroblasts to process antigens was associated with the presence of disulfide bonds within the antigens. Diminished aspartic acid-mediated proteolysis of the antigen could not account for the phenotype, because cell lysates from the fibroblast possessed significantly more acidic aspartyl proteolytic activity than lysates from a B lymphoma cell. A somatic cell hybrid, generated by fusing the hamster fibroblast with a murine L cell fibroblast, very efficiently processed ovalbumin and hen egg lysozyme, suggesting that impaired processing was genetically complemented and was a recessive trait. The hamster fibroblasts were capable of processing two distinct denatured forms of ovalbumin and a carboxymethylated preparation of hen egg lysozyme either as effectively or more efficiently than a B lymphoma cell. Thus, the Chinese hamster ovary cells are defective in processing antigen with disulfide bonds, which involve an impaired ability to unfold these antigens.

V 294 ENHANCEMENT OF HUMAN T CELL RESPONSES BY THE CHONDROITIN SULFATE FORM OF INVARIANT CHAIN.

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We have previously shown that expression of murine invariant chain-chondroitin sulfate (Ii-CS) can enhance stimulation of primary murine T cells stimulated by alloantigens or by superantigens such as Staphylococcal enterotoxin B (SEB). This enhancement is mediated by interactions between Ii-CS and CD44 (Cell 74:257). Using EL4 cells transfected with murine MHC class II (I-A^d) in the absence and presence of mutant forms of murine Ii, we showed that expression of Ii-CS was required for enhanced T cell responses. The ability of CD44 to bind Ii-CS was demonstrated by precipitation of murine Ii-CS with a soluble form of human CD44, CD44Rg . Given that human soluble CD44 could bind to murine Ii-CS, we tested whether the panel of EL4 transfectants, expressing murine class II and Ii, could be used to stimulate human T cells. For this purpose, we tested proliferative responses of human T cells to superantigens presented by murine class II I-A^d expressed on the transfectants. We found that SEB stimulated human $CD4^+$ T cells when presented by murine class II, and SEB was required for stimulation because human T cells did not proliferate to I-A^d alone. Using this system, we found that the proliferative response of human T cells to SEB was significantly enhanced when murine Ii was co-expressed with I-A^d. This enhancement was dependent on the expression of Ii-CS, because transfectants expressing a mutant form of Ii lacking chondroitin sulfate did not enhance the proliferative response as well as wild-type Ii. Similar results were found using SEA as antigen. These data suggest that, as with murine T cells, the proliferative response of human T cells can be enhanced by Ii-CS. We are exploring whether Ii-CS provides accessory function for unique subsets of T cells, though preliminary data indicated that responses of negatively-selected naive (CD45RA+) and memory (CD45RO+) T cells were both enhanced by Ii-CS. Other subset differences will be explored. Furthermore, because CS addition is a post-translational modification, the relative expression of Ii-CS may differ between cell types or under different conditions. These studies are in progress.

V 295 ASSOCIATION OF MHC CLASS I MOLECULES AND TRANSPORTERS ASSOCIATED WITH ANTIGEN PROCESSING

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One of the most important defense mechanisms of the mammalian imune system against virus infected cells, tumor cells, and allografts involves cytotoxic T-lymphocytes. These MHC restricted cells recognize antigenic peptides presented by MHC class I molecules on the surface of their target cells. Peptides bound to these class I molecules are derived from endogenous, mainly cytoplasmic proteins. Most probably they are generated by the action of a cytoplasmic multicatalytic proteinase complex, the so called proteasome. As shown by three independent groups, transport of these peptides into the endoplasmic reticulum (ER), where binding to the class I molecule occurs, is facilitated by a heterodimeric transmembrane protein complex formed by the transporters associated with antigen processing (TAP 1 and TAP 2). In the ER class I heavy chains and to some extent class I-β_2-microglobulin dimers are bound to the ER resident chaperone calnexin (IP90 in human, p88 in mouse). Little is known about the relationship of the peptide transporters and assembling class I heavy chain-β_2-microglobulin-peptide complexes. Here we provide evidence that TAP 1 is associated with the chaperone calnexin and that class I molecules are retained in the ER in association with TAP proteins.

V 296 TWO PROCESSING PATHWAYS FOR THE CLASS II MHC-RESTRICTED PRESENTATION OF EXOGENOUS ANTIGENS,

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Presentation of exogenous antigens to CD4+ T cells involves delivery to acidic compartments where antigen is degraded into peptides that can bind to MHC class II molecules. There has been much debate about which endocytic compartment is involved in antigen processing and whether the invariant chain is necessary for class II-restricted presentation. Here we show that two distinct processing pathways can function in the class II-restricted presentation of exogenous antigens. The natural antigen influenza virus A was used to test the requirements for the HLA-DR1-restricted presentation of the immunodominant epitopes 18-29 in the matrix protein (M1) and 307-318 in the hemagglutinin protein (H3) to specific CD4+ T cells. The processing and presentation of M1 followed the "classical pathway" in that it required (i) Ii expression, (ii) protein synthesis, and (iii) a function encoded in the MHC class II region. In contrast, processing of H3 could follow an alternative pathway in that it was independent of (i) Ii expression, (ii) protein synthesis, and (iii) other functions encoded in the MHC class II region. Thus, mature class II $\alpha\beta$ molecules have the ability to acquire immunogenic peptides derived from intact antigens for presentation to CD4+ T cells.

V 297 TARGETING OF MHC CLASS II $\alpha\beta$Ii COMPLEXES TO ENDOSOMES,

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MHC class II α- and β-chains associate immediately after translocation in the ER with a third polypeptide known as the invariant chain (Ii). Ii association inhibits peptide binding to immature class II $\alpha\beta$Ii complexes and plays an important role in the delivery of newly synthesized class II MHC glycoproteins to endosomes. However, the intracellular transport pathway(s) for the targeting of these molecules to endosomes has not been fully elucidated. We have previously demonstrated that class II $\alpha\beta$Ii complexes could be targeted to the endosomal pathway by rapid internalization from the cell surface. We now show that, following internalization, Ii dissociates from the $\alpha\beta$Ii complexes with a $t_{1/2}$ of approximately 1 hr. In addition, we demonstrate that dissociation of Ii from the surface $\alpha\beta$Ii complex is ATP dependent and requires the activity of endosomal proteases. Additional data will be presented demonstrating that the internalization of surface $\alpha\beta$Ii complexes is an important mechanism for the delivery of class II molecules to endosomes.

V 298 INTRACELLULAR ASSEMBLY OF CLASS I HLA COMPLEXES IS BLOCKED BY A PHOSPHATASE INHIBITOR, Russell D. Salter, Matthew Tector, and Qing Zhang, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Stable complexes are formed when class I MHC heavy chains associate with β_2m and peptides in an early biosynthetic compartment within the cell. Although the kinetics of class I assembly have been examined in detail, it is not clear exactly where peptides bind to class I molecules, and what role chaperonins such as calnexin play in the process. To address these questions, we examined the assembly of class I molecules in CIR cells treated with drugs which cause retention of proteins in the ER. Okadaic acid, a membrane permeable phosphatase inhibitor, blocked both the assembly and transport of HLA-B7 from the ER. In contrast, Brefeldin A blocked transport, but not assembly of HLA-B7 molecules. Combined treatment with both drugs resulted in intermediate levels of assembled HLA-B7. These data suggest that endogenous phosphatases are required for assembly of HLA-B7 complexes. One potential explanation for these results is that okadaic acid blocks the transport of class I molecules at a site before loading with peptides occurs, thereby sequestering class I molecules from a peptide loading compartment, whereas treatment with Brefeldin A, which induces retrograde transport of Golgi components to the ER, does not. A second possibility is that okadaic acid blocks peptide loading by interfering directly with molecules involved in class I biosynthesis such as calnexin. Current experiments are directed towards distinguishing between these possibilities.

V 299 *XENOPUS LAEVIS MHC*-LINKED GENES: POLYMORPHISM AND EXPRESSION, Luisa Salter-Cid and Martin F. Flajnik, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101.

Xenopus is the only ectothermic vertebrate in which a true *MHC* has been identified at the functional, biochemical and genetic levels. Moreover, *X. laevis* is a pseudotetraploid, whose *MHC* genes seem to have been diploidized by a yet unknown silencing mechanism(s). Our work shows that *Xenopus* class I molecules are differently expressed during ontogeny: while tadpoles have no detectable mRNA, class I transcripts begin to appear at the time of metamorphosis. This expression gradually increases until it reaches adult levels. *Xenopus* is, therefore, a suitable model to begin to understand the developmental regulation and evolution of *MHC*-linked genes. The *Hsp 70* gene family, being very conserved, can help to elucidate the mechanism of silencing of *MHC* loci in polyploid animals. Polymorphism studies demonstrate that two to three *Xenopus Hsp 70* genes segregate with known *MHC* haplotypes. Another *Hsp 70* gene was detected which, as in mammals, does not map to the *MHC*. We conclude that the physical association between *MHC* class I, class II and *Hsp 70* genes is an ancient one. A large number of non classical Class I genes have also been identified and they appear all to be linked on a chromosome distinct from the one containing the *MHC* proper. Data relating to the expression of these genes during development will also be discussed.

V 300 **CONVERSION OF HLA-DR4Dw4 POLYMORPHIC RESIDUES TO ALA: EFFECTS ON ANTIGEN PRESENTATION** K. Signorelli, L. Watts and L. Lambert, Department of Immunology, Marion Merrell Dow Research Institute, Cincinnati, OH 45215

Specific alleles of the human HLA-DR locus are associated with increased risk for the development of rheumatoid arthritis (RA). Examination of the amino acid sequence of the DR β chain (the α chain being monomorphic for DR) has revealed that risk for RA correlates with a cluster of polymorphic residues located between a.a. 67-86. This is exemplified by the DR4Dw4 and DR4Dw10 alleles which differ at only 4 a.a.'s: positions 67,70,71, and 86. DR4Dw4 has the highest relative risk factor for RA among DR alleles while the Dw10 subtype has one of the lowest. Based on the recently solved Class II DR1 structure, these residues are located in the alpha-helical region of the molecule which is involved in peptide binding and T cell receptor contact. Previous studies have utilized site-directed mutagenesis to examine the role of these residues in T cell stimulation. These have typically involved conversion of amino acids in a starting "backbone" DR molecule (usually DR7 or DR1) into that found in another allele, usually DR4Dw4 or Dw10. Such inter-allele conversions frequently result in a change in the charge of the amino acid side chain which might be expected to have drastic effects on T cell stimulation. Furthermore, the effect of such changes on peptide binding has not been measured directly. In an effort to examine the fundamental role of these residues in antigen presentation, we have converted a.a.'s 70, 71, and 86 of the DR4Dw4 β chain into alanine residues, either singly or as a double 70+71 mutant by site-directed mutagenesis. Wild-type or mutated DR $\alpha\beta$ heterodimers were expressed in CHO cells as a GPI-linked surface protein. This allows for isolation of soluble molecules for use in direct binding assays. The effects of these alterations on antigen presentation function will be presented.

V 301 ENGINEERING A NOVEL INTRACELLULAR PATHWAY FOR MHC CLASS II PRESENTATION OF ANTIGEN. Staveley-O'Carroll, K.F., Guarnieri, F.G., Levitsky, H.I., Golumbek, P., August, J.T., and Pardoll, D.M. Johns Hopkins University School of Medicine, Baltimore, MD, 21205

CD4+ T cell activation is central to the generation of an active immune response. These cells recognize peptides derived from antigenic proteins that have been processed and presented on the MHC class II molecules of specialized antigen presenting cells (APCs). Antigen processing is thought to occur in a compartment related to late endosomes or lysosomes. Normally, antigens enter this compartment via endocytosis. We have utilized the targeting signal of the lysosomal membrane LAMP-1 protein to target a model antigen directly into the endosomal/lysosomal compartment. This unique intracellular pathway concentrates the antigen in this compartment, resulting in marked enhancement of presentation to MHC class II restricted T cells. The data directly implicate LAMP-1 containing compartments as the site for antigen processing and association with MHC class II molecules and suggest a novel strategy to enhance the potency of recombinant vaccines.

V 302 AVAILABILITY OF ENDOGENOUS PEPTIDES LIMITS EXPRESSION OF THE $M3^a$-L^d MHC CLASS I CHIMERA, Jatin M. Vyas, John R. Rodgers, Dewey D. Howell, Said M. Shawar, and Robert R. Rich, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030.

Evidence suggests oligomorphic class I molecules bind a stenotopic (narrow) range of peptides and are expressed at low levels at the cell surface. The MHC class I-b molecule $M3^a$ is specific for N-formyl peptides and presents endogenous mitochondrial peptides from subunit 1 of NADH dehydrogenase (ND1). Taking advantage of our understanding of this specificity, we sought to investigate the molecular basis for poor surface representation of $M3^a$. We constructed a chimeric molecule with the α_1 and α_2 domains of $M3^a$ and the α_3 domain of L^d, allowing use of available mAb against a conformation-independent epitope of L^d to quantify surface expression and measure peptide binding using a cell-free approach. Transfected, but not control, B10.CAS2 (H-$2M3^b$) cells were lysed readily by $M3^a$-restricted mCTL. Thus, the chimera bound, trafficked and presented endogenous ND1 peptides. However, despite high levels of $M3^a$-L^d mRNA, transfectants were negative by surface staining. This finding was consistent with inefficient trafficking to the cell surface. Incubation at 26°C resulted in detectable cell surface expression of $M3^a$-L^d. Incubation with exogenous specific peptides at 26°C (but not at 37°C) greatly enhanced expression of $M3^a$-L^d in a dose-dependent manner, suggesting stabilization of unoccupied molecules. β_2-microglobulin (β_2m) associated stably with the chimeric heavy chain in labelled cell lysates only in the presence of exogenous specific peptide, indicating $M3^a$-L^d heavy chains were in excess of endogenous peptide availability. We used the β_2m-association assay to determine the optimal length of peptides binding to $M3^a$-L^d. Peptides of eight or twelve amino acids bound effectively. Our data show that surface expression of $M3^a$-L^d is limited largely by the steady-state availability of endogenous N-formyl peptides. Moreover, the antigen binding cleft of $M3^a$ may accept a range of peptide lengths longer than do other class I molecules. This broader range may be an adaptation to temper the N-formyl requirement, thus expanding somewhat the range of bacterial peptides restricted by $M3^a$. This MHC molecule may normally be expressed at higher levels only during infection by intracellular bacteria.

V 303 BIOSYNTHETIC RATE AND TRANSPORT OF MHC CLASS II MOLECULES, Petra A. M. Warmerdam, Paul A. Roche, and Eric O. Long, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852.

MHC class II αβ heterodimers assemble shortly after synthesis in the endoplasmic reticulum (ER). The αβIi complexes are subsequently transported to an endosomal compartment. It is not yet clear whether the transport route goes directly to endosomes from the trans-Golgi network, or whether newly synthesized class II molecules are targeted to endosomes by rapid internalization from the cell surface. To address this question we have quantitated the biosynthetic rate of class II molecules and the rate of internalization of surface αβIi complexes. In addition we have determined that some of the internalized αβIi complexes recycle back to the cell surface. Altogether these data suggest that an important fraction of newly synthesized class II molecules are transported to endosomes via the cell surface.

V 304 REQUIREMENT FOR T CELL ACTIVATION BY INTACT *PLASMODIUM BERGHEI* SPOROZOITES VERSUS CIRCUMSPOROZOITE PROTEIN PEPTIDES. Katherine L. White*, Heidi T. Link@, and Urszula Krzych#, Departments of Biology *The Catholic University of America, Washington, D.C., 20064, @Viral Diseases NIAID, NIH, Bethesda MD, 20892, and #Immunology Walter Reed Army Institute of Research, Washington D.C., 20307

Immunization with irradiated *P. berghei* SPZ induces protective immunity characterized in part by CD4+ Th1 and CD8+ T cell responses. The Th1 cells proliferate to SPZ, and to recombinant circumsporozoite (rCS) protein, but not to CS protein peptides. Alternative priming with CS protein peptides induces T cells recognizing CS protein peptides but not intact SPZ, and hence, these were considered cryptic specificities. Among the cryptic determinants, three peptides mapping to a.a. 51-70, 71-90, and 271-290 correspond to the conserved regions of the Plasmodia CS protein and the fourth to the central repeat region. T cell lines and clones obtained from the cryptic peptide priming acquired anti-SPZ proliferative reactivity following *in vitro* expansion without loosing peptide specificity. This unexpected finding led us to investigate the usefulness of these clones in *in vivo* anti-SPZ protective immunity. Furthermore, the availability of T cell clones with anti-SPZ and peptide reactivities allowed us to investigate the mechanisms of recognition of SPZ vs. CS protein peptides. Preliminary data based on the requirements for SPZ and peptide presentation to T cells and lymphokine production suggest that activation by these two forms of the malaria antigen is distinct. Currently, we are in the process of evaluating specific mechanisms of T cell activation by CS peptides and SPZ by examining Vβ usage, signal transduction, fine epitope mapping and costimulatory molecules. These results will enhance our understanding of the immune response to malaria parasites. Supported in part by WHO/World Bank TDR.

V 305 THE MOLECULAR BASIS OF DOMINANT AND SUBDOMINANT RESPONSES IN HUMAN CLASS II TRANSGENIC MICE. Phyllis E. Whiteley, Daniel Freed, Patricia Cameron, Dennis Zaller, Jeffrey Hermes, Alison Pressey, and Linda S. Wicker, Department of Autoimmune Diseases Research, Merck Research Laboratories, Rahway, N.J. 07065.

Immune responses to Human-derived proteins were studied in mice that are transgenic for the Human MHC-class II protein, DR4Dw4. The DR4 in the transgenic mice is chimeric such that the antigen-binding portion of the molecule (α1β1 domains) is retained, whereas the rest of the molecule corresponds to the murine I-E protein. The transgenic mice are fully functional and have normal DR4-restricted immune responses. Endogenous peptides were sequenced from DR4 that was purified from the Human B cell line, Priess. Two of the peptides identified were from the constant region of the Kappa protein of Human Immunoglobulin (Ig), residues 145-159 and 188-203. T cell hybridomas specific for both kappa peptides were generated from transgenic mice injected with Human Ig. The T cell hybridomas respond to Priess cells in the absence of exogenous antigen and are peptide-specific and MHC-restricted (both chimeric and native DR4). In addition, the T cell hybridomas secrete lymphokines in response to immobilized kappa/DR4 complexes and both hybridomas respond to similar numbers of relevant peptide/DR4 complexes. The T cell hybridomas were used to quantitate the number of kappa/DR4 complexes in Priess cell lysates. Approximately 17% of the Priess-derived DR4 is bound with the kappa 188-203 epitope; whereas only 1% of the DR4 is occupied with the 145-159 epitope. The data is consistent with the HPLC analysis of endogenous peptides bound to Priess-derived DR4 in that peptide 188-203 is found in higher abundance than peptide 145-159. Transgenic spleen cells, in the presence of Human Ig, also elicit responses from the T cell hybridomas. However, much less Human Ig is required to stimulate the T cell hybridoma specific for 188-203, suggesting that the kappa 188-203 peptide is the dominant epitope of the kappa protein. Thus, Human Ig is processed similarly whether the antigen is endogenous or exogenous to the antigen presenting cell. Additional studies have demonstrated that the two kappa peptides bind with similar affinities to purified DR4 over a wide pH range, suggesting that affinity alone does not dictate the pattern of kappa epitope dominance. A role for intracellular processing in epitope selection is suggested by two observations: 1) the dominant epitope contains an internal cysteine residue that is involved in a conserved intramolecular disulfide bond and 2) the subdominant epitope contains a cathepsin B site.

V 306 PARAMETERS EFFECTING THE EFFICIENCY OF PROCESSING ANTIGENIC EPITOPES EXPRESSED IN *SALMONELLA TYPHIMURIUM*, Mary Jo Wick[1], Clifford V. Harding[2], Staffan J. Normark[3], and John D. Pfeifer[2], The Immunology Unit[1], Lund University, Lund, Sweden, and Department of Pathology[2] and Molecular Microbiology[3], Washington University School of Medicine, St. Louis, MO 63110.

Using an *in vitro* model system where *Salmonella typhimurium* expresses a defined T cell epitope, we investigated the parameters that effect the efficiency of processing antigenic epitopes expressed in this bacterium for presentation to T lymphocytes. The HEL(52-61) epitope, which binds the murine major histocompatibility complex class II (MHC-II) molecule, I-A^k, was expressed as various soluble fusion proteins in *S. typhimurium* such that the epitope was expressed in different bacterial compartments (i.e. surface-exposed, facing the periplasmic space, or in the cytoplasm). Murine peritoneal macrophages mediated phagocytic processing of viable *S. typhimurium* expressing the HEL(45-62) epitope for presentation via I-A^k regardless of which bacterial compartment the epitope was contained within. Minor differences in processing efficiency, which may be due to bacterial compartmentalization or local protein context of the epitope, could be overcome by increasing the relative amount of epitope per bacterium, indicating that epitope abundance is an important factor for efficient processing of epitopes from bacteria. This processing pathway required phagocytosis of bacteria followed by passage through an acidic compartment, suggesting a pathway involving phagolysosomal degradation of the bacteria to liberate epitopes that bind MHC-II for presentation for T cells. When low levels of HEL(52-61) was constitutively expressed, the epitope was processed more efficiently from rough lipopolysaccharide (LPS) *S. typhimurium* strains compared to their isogenic smooth LPS counterparts. In addition, expressing HEL(52-61) in *S. typhimurium* strains containing a mutation in *phoP*, a locus involved in delaying macrophage phagosome acidification, resulted in more efficient processing and presentation compared to the same epitope expressed in strains with a wild type *phoP* allele. These data suggest that factors associated with virulent strains of *S. typhimurium*, such as smooth LPS and *phoP* expression, may have a role in effecting the efficiency of phagocytic processing of epitopes expressed in *S. typhimurium* for presentation via MHC-II.

V 307 TRANSGENIC MICE EXPRESSING FUNCTIONAL HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II MOLECULES.

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Transgenic mice expressing human MHC class II molecules would provide a valuable model system for studying human immunology. However, attempts to obtain human class II-restricted T-cell responses in such transgenic mice have had only limited success, possibly due to an inability of mouse CD4 to interact efficiently with human MHC class II molecules. To circumvent this problem, we have created transgenic mice using recombinant MHC class II genes in which the peptide-binding domain was derived from human sequences while the CD4-binding domain was derived from mouse sequences. These recombinant Class II molecules fold properly, bind specific peptides, and can present antigen to human T-cell clones. Transgenic mice expressing these chimeric Class II molecules were fully capable of mounting human MHC class II-restricted immune responses. In addition, these transgenic mice could be used to generate antigen-specific mouse T-cells that interact with human antigen presenting cells. Human self antigen-specific, class II-restricted mouse T-cell hybridomas could respond to human antigen presenting cells without the addition of exogenous antigen.

Receptors, Co-Receptors and Others

V 308 **IgM ASSEMBLY IN B LYMPHOCYTES**, Klaus D. Bornemann, Gabriele B. Beck-Engeser, Joseph W. Breuer*, Ronald B. Corley*, Ingrid G. Haas° and Hans-Martin Jäck, Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL 60153; *Department of Microbiology and Immunology, Duke Medical Center, Durham, NC 27710, °Institut für Biochemie I, Universität Heidelberg, 6900 Heidelberg, Germany

Immunoglobulin M (IgM) can be secreted by plasma cells as a pentameric and hexameric polymer. Pentameric IgM consists of five μ_2L_2 monomeric subunits that are disulfide bonded in a circular array and are held together by one J chain molecule. The hexameric form of IgM consists of an additional monomeric subunit and lacks J chain. J chain, a 15-kDa glycoprotein, is associated via disulfide bonds with the terminal cysteines of two μ chains of a pentameric molecule. The formation of pentameric IgM is favored in plasma cells that express J chain, whereas in absence of J chain the hexameric form dominates. There are no clear data showing in which subcellular compartment the assembly of IgM occurs. Therefore, the goal of this work is to determine where in the cell and in what order IgM is assembled. We found that in a μ only expressing murine hybridoma cell line μ assembles together with J chain into oligomeric forms. Since these polymers can be precipitated with BiP, an ER-resident protein, we conclude that the polymerization process occurs in the ER. In addition, we showed that in a μ,κ producing hybridoma line the assembly of pentameric IgM occurs by combining μL-subunits. Since almost all of the oligomeric intermediates could be precipitated with BiP, we conclude that pentameric and hexameric IgM is assembled in the ER of a plasma cell.

V 309 TRANSFECTED HUMAN T CELL RECEPTOR GENES RECONSTITUTE HLA RESTRICTED ANTIGEN RECOGNITION IN A RECEPTOR NEGATIVE HUMAN T CELL LINE, James V. Brawley and Patrick Concannon, Virginia Mason Research Center, 1000 Seneca St., Seattle, WA 98109 and Department of Immunology, University of Washington, Seattle, WA 98195

We have been studying human T cell clones and lines which recognize the influenza hemagglutinin peptide 307-319 (HA 307-319) in the context of different class II DR molecules. Two T cell clones,3BC6.6 and BC8, recognize HA 307-319 in the context of DR4. Clone JS515.11 recognizes HA 307-319 only in the context of DR7. We have sequenced cDNAs coding for the T cell receptors expressed by all of these clones. 3BC6.6 and JS515.11 each use Vα1 and Vβ3 gene segments yet interact with different DR molecules. 3BC6.6 and BC8 have identical HLA and peptide specificities yet use different Vα and Vβ T cell receptor gene segments. In order to better understand the molecular basis for these results, we have set up an *in vitro* system to study recognition of major histocompatibility complex plus peptide by transfected cognate or mutated T cell receptor chains. A reporter gene coding for ß-galactosidase under the control of an interleukin 2 promoter element has been introduced into a human T cell receptor negative Jurkat cell (Glaichenhaus *et al*, 1991, J. Immunol. 146, 2095-2101). α and ß T cell receptor cDNAs were transfected into the recipient cell line using various expression vectors. ß-galactosidase activity was observed as a result of activation by the appropriate peptide plus MHC molecule. ß-galactosidase activity was peptide dose dependent and could be observed as early as four hours after presentation. Chimeric T cell receptors constructed from the receptor cDNAs derived from clones 3BC6.6, BC8, and JS515.11 have also been constructed and expressed in this system. Analysis of the reactivity of cells expressing these constructs and other site directed mutants should help to map important TCR and HLA contact residues.

V 310 REGULATION OF IgM SECRETION AT THE LEVEL OF POLYMER ASSEMBLY, Joseph W. Brewer, Troy D. Randall, and Ronald B. Corley, Department of Immunology, Duke University Medical Center, Durham, NC 27710

Classically, IgM has been described as a pentameric molecule consisting of five μ_2L_2 monomeric subunits and one joining (J) chain. Recently, it has been shown that a variety of B cell lines make IgM hexamers as well pentamers. Hexamers contain an additional μ_2L_2 subunit and lack J chain. Increases in the level of J chain in responding B cells as a result of stimulation by lymphokines such as IL-2, IL-5, and IL-6 favor pentamer formation at the expense of hexamers. Hexamers mediate complement fixation 15-20 fold more efficiently than pentamers. Therefore, the manner in which B cells are stimulated may influence the biological activity of the resulting secreted IgM by regulating its polymeric structure. While a role for J chain in the regulation of IgM polymer structure is established, the mechanism by which it mediates its effect is poorly understood. To understand the mechanisms that regulate the type of polymeric IgM secreted by responding B cells, we are studying the process of IgM assembly and secretion. We have identified the intermediates of assembling IgM polymers and found that assembly proceeds by stepwise addition of μL half-monomer subunits and possibly also by μ_2L_2 addition. IgM pentamers and hexamers are assembled with similar kinetics. Analysis of the maturation of N-linked oligosaccharides on both the μ and J chain proteins demonstrates that complete assembly, including J chain addition, occurs prior to the mid-Golgi. Complete assembly of both pentamers and hexamers is not inhibited by brefeldin A which blocks protein transport from the endoplasmic reticulum (ER). We further localize the subcellular compartment of assembly by demonstrating complete assembly of pentamers and hexamers under conditions of low temperature which block transport from the ER. J chain is observed to be associated with at least some of each assembly intermediate, including a small fraction of μL and μ_2L_2. We have proposed and are currently testing a model whereby J chain is added to assembling polymers in a stochastic manner, perhaps as part of a μL-J or μ_2L_2-J complex. These findings are important to our understanding of how different modes of B cell stimulation may influence the resulting IgM response.

(Supported by NIH grant AI31209)

V 311 THE INTERACTION OF CD4 AND CLASS II MHC PROTEINS Jennifer Brogdon and Carolyn Doyle, Department of Immunology, Duke University Medical Center, Durham, NC 27710.

We have generated a panel of approximately fifty mutant class II proteins, each containing a single amino acid substitution in either the β1 or β2 domain of the HLA-DR1 protein. The altered gene products have been expressed in a class II-negative human B lymphoblastoid cell line that has been co-transfected with the HLA-DRα cDNA. Transfected cells have been analysed for CD4 binding using a cell adhesion assay that measures a direct CD4-class II interaction. Mutations in an exposed loop encompassing amino acids 41-55 of the β1 domain of HLA-DR1 appear to effect the CD4 interaction. Crystallographic analyses suggest that this region may be involved in heterodimer formation of the DR1 protein. Implications of this model will be discussed.

The mutant class II proteins have also been analysed using alloreactive and antigen-specific CD4-dependent DR1-restricted T cell clones. Results obtained in these studies may reflect a subtle difference in the requirement for peptide involvement in allorecognition as compared to T cell receptor recognition of nominal antigen. Additional mutations in the β2 domain also appear to alter CD4 binding and T cell recognition. These results are consistent with previous studies of CD4-dependent T cell recognition of the murine class II molecule I-A^d.

V 312 IDENTIFICATION OF CONSERVED T CELL RECEPTOR CDR3 RESIDUES CONTACTING KNOWN EXPOSED PEPTIDE SIDECHAINS FROM AN MHC CLASS I-BOUND DETERMINANT By F. R. Carbone, S. J. Sterry and J. M. Kelly, Department of Pathology and Immunology, Monash Medical School, Prahran, Victoria 3181. 03-276-2744

We have analysed the T cell receptor (TCR) repertoire found in the MHC class I-restricted cytotoxic T lymphocyte (CTL) response to the protein ovalbumin (OVA). These CTL exhibit a high degree of diversity in V(D)J usage in both TCR α- and ß-chains. Closer examination shows that the majority of these sequences encode negative and positive charged residues at their respective TCR α- and ß-chain VJ or VDJ junctions. These junctions form the third complementarity determining regions (CDR3) of the TCR polypeptides involved in direct interaction with the class I-bound peptide. Crystallographic analyses of K^b-peptide complexes predict that the major determinant from OVA, peptide $OVA_{257-264}$ (SIINFEKL), contains two exposed charged sidechains which can contact the TCR. These are the negatively charged glutamic acid at determinant position 6 (P6) and the positively charged lysine at P7. To examine whether the TCR α-chain makes contact with P7 lysine, we have established a single chain TCR transgenic C57BL/6 mouse line where all T cells express a TCR ß-chain derived from the OVA-specific CTL clone B3. Stimulation of naive TCR ß-chain transgenic T cells with a P7 substitution peptide analogue induces CTL that no longer recognise the wildtype $OVA_{257-264}$ determinant, suggesting that the TCR α-chain from the T cell clone B3 can determine the specificity for this residue. Consequently, these results identify CDR3 residues contacting known exposed peptide sidechains from a structurally defined class I-bound antigen.

V 313 T-CELL RECEPTOR ANALYSIS IN A SYSTEMIC GRANULOMATOUS DISEASE OF UNKNOWN ETIOLOGY: EVIDENCE FOR ACTIVATION OF ANTIGEN-SPECIFIC T-CELLS WITH A COMMON V(D)J JUNCTIONAL REGION MOTIF. Jeffrey D. Forman, Brian Greenlee, David R. Moller. Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Analysis of the T-cell receptor (TCR) repertoire has been used to identify specific T-cells which may play a critical role in the pathogenesis of certain autoimmune and infectious diseases. We have been using this approach in our investigations of sarcoidosis, a multiorgan granulomatous disease of unknown etiology that frequently affects the lungs, lymph nodes, skin and eyes. T-cells at sites of disease are predominantly CD4+ and show signs of recent activation via the TCR receptor pathway. We hypothesized that this activation would result in preferential expansion of a limited number of TCR specific T-cells. TCR Vß expression was analyzed in bronchoalveolar lavage lung and blood T-cells of sarcoid patients and for comparison, normal individuals using anti-TCR antibodies and a quantitative polymerase chain reaction. Compared to normal lung and blood, subgroups of sarcoid patients demonstrated biased expression of one or more Vß genes with Vß5, 8, 15, 16 and 18 utilized most frequently in a biased fashion. Furthermore, dramatic skewing of the TCR repertoire was apparent when sarcoid lung and blood T-cells were expanded by short-term culture with IL2. Sequence analysis demonstrated this bias was the result of oligoclonal expression of TCR Vß genes and was usually dominated by a single Vß-gene, consistent with an antigenic response. Interestingly, the V(D)J junctional region of several of the dominant Vß sequences contained a common RGR or RGGR sequence motif. Such homology in the hypervariable CDR3 region of the TCR suggests recognition of a similar or identical antigenic determinant. Together, these observations suggest it is possible to identify critical antigen-specific T-cells in a granulomatous disease of unknown etiology. Isolation of these T-cells would allow testing of their antigenic specificity in hopes of identifying the underlying stimulus causing this disorder.

V 314 A CIS-ACTING DNA ELEMENT AND CELL TYPE-SPECIFIC NUCLEAR PROTEINS MAY PLAY A ROLE IN REGULATION OF MOUSE *CD8α* (*Lyt-2*) GENE TRANSCRIPTION, P.D. Gottlieb, W.-H. Lee, M. Banan, J.V. Harris, and H.J. Youn, Department of Microbiology, University of Texas at Austin, Austin TX 78712

Fusion of mouse $CD8^+$ class I MHC-restricted T cells with the BW5147 thymoma invariably yields hybridomas in which transcription of the *CD8α* (*Lyt-2*) gene has been shut off. To determine whether cis-acting DNA sequences are involved in this apparent negative regulation, BW5147 cells were stably transfected with the *Lyt-2* gene containing from 1 to 11,000 nt of 5' flanking DNA and surface expression of Lyt-2 was monitored by FMF. Results suggest the presence of a negative element between 1,400 and 5,000 nt upstream of the site of transcription initiation. Further studies suggest the presence of a regulatory element within a 269 nt *AccI/SstI* fragment which lies approximately 4,500 nt upstream of the transcription initiation site which could be shown in EMSA assays to bind nuclear proteins from both $CD8^+$ and $CD8^-$ cell lines. EMSA studies performed using nuclear extracts from a variety of cell lines and tissues indicate that unique retarded complexes correlate with expression and non-expression of Lyt-2. EMSA analysis of proteins fractionated from nuclear extracts of the CD8+ VL3 T lymphoma cell line suggest a model by which three proteins (called L2a-P1, L2a-P2 and L2a-P3) could play a role in regulation of *Lyt-2* gene transcription.

V 315 CHARACTERIZATION OF A NOVEL HUMAN T CELL RECEPTOR β CHAIN VARIABLE REGION FAMILY BY TRANSPECIES HYBRIDISATION, Margaret A. Hall, Emma E.M. Jaeger, Ronald E. Bontrop[2], Jerry S. Lanchbury, Molecular Immunogenetics Laboratory, Division of Medicine, UMDS, London SE1 9RT, UK. 2. ITRI-TNO Primate Centre, 2280 HV Rijswijk, The Netherlands

T cell receptor (Tcr) α and β chain heterodimers are responsible for the antigen specificity of 90% of T lymphocytes while the remainder carry heterodimers composed of γ and δ chains. Human Tcr β variable genes have been classified into 24 families with a family defined by members which share greater than 75% nucleotide sequence homology. It has recently been estimated that the 24 human Vβ families characterized encompass some 51 transcriptionally active gene segments. Surveys of several hundred Tcrβ cDNA clones have failed to establish further Vβ families which presumably, if they exist, must be poorly represented in the peripheral blood repertoire. It is likely, therefore, that the majority of human Vβ regions which are rearranged conventionally and are transcriptionally active have already been described.

In order to investigate whether additional Vβ familes characterize the human T cell receptor repertoire we have examined Vβ sequences from an inverse PCR Tcrβ cDNA library of the chimpanzee, man's closest living relative. One clone which could not be assigned to any human Vβ family cross-hybridized with human Tcr β chain cDNA. This new family has been called Vβ25. Chimpanzee and human Vβ25 are closely related. Comparison using the Lipman-Pearson method gives a similarity index of 94.6% over a consensus length of 112 amino acids. On the evidence of southern blotting with cDNA probes, Vβ25 segments in human and chimpanzee appear to represent single member gene families and no restriction fragment length polymorphism was evident with the restriction enzymes used. Equalisation of TCRβ cDNA followed family-specific quantitative PCR showed that the gene is expressed at similar levels in the chimpanzee and the human. The actual level of expression in the human is less that 0.5% of the total Tcrβ repertoire. Transpecies DNA hybridization using novel sequences from closely related species may be a powerful tool for establishing the full extent of T cell receptor α and β repertoires.

V 316 A CRUCIFORM STRUCTURE FORMED BY REPETITIVE ALU-ELEMENTS REGULATES A T-CELL-SPECIFIC ENHANCER IN THE HUMAN CD8α GENE. Jeffrey H. Hanke, John E. Hambor, Lynda J. Kieffer and Paula Kavathas. Department of Molecular Genetics and Protein Chemistry, Pfizer Central Research, Groton, CT 06340, and Departments of Laboratory Medicine, Genetics and Immunobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

Expression of the human CD8α gene is restricted to cytotoxic/suppressor T cells and a subset of NK and $TcR_{\delta\gamma}$ cells of the lymphoid lineage. Furthermore, its expression is developmentally regulated during thymic development. In order to better understand the transcriptional mechanisms that control thymic development of CD8+ cells, hypersensitivity mapping was used to map two hypersensitive sites (HS) in both cell lines and fresh human peripheral T cells. A minimal 691bp T cell-specific enhancer was identified in proximity to a prominent T cell-restricted HS in the last intron of the CD8α gene. This enhancer was composed of a cluster of binding sites for T cell-specific factors as well as a 5'-*Alu* element which contained functional GATA-3, bHLH and LyF-1 binding sites. This minimal enhancer was located just downstream of a half *Alu*-element which when included in reporter constructs significantly reduced enhancer activity. Interestingly, the repetitive *Alu*-sequences appear to form a cruciform structure as demonstrated by P1-nuclease mapping, chloroacetaldehyde mapping of unpaired bases, and deletion/site-directed mutation analysis of DNA secondary structure.

V 317 **The CDR domains of the T Cell Receptor Beta Chain Affect the Fine Specificity of MHC Recognition**

Soon-cheol Hong, Sangwook Tim Yoon, Charles A. Janeway Jr. HHMI and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

We have analyzed the interaction of T cell antigen receptor (TCR) and its ligands by mutational and transfectional analysis. The D10 T cell clone originally isolated from AKR mice by immunizing chicken conalbumin responds to this peptide presented by A^k molecule. This clone is also alloreactive to $A^{b,v,p,q, \text{ and } d}$. The TCRß chain used by this clone is Vß8.2 and therefore reactive to SEB but not to Mls-1^a. Several amino acids in the CDR1, CDR2 and CDR3 domain of this TCRß chain have been mutagenized and transfectants expressing these mutations have been generated. Several interesting observations have been made by these transfectants. All the transfectants expressing amino acid substitution in the CDR1 or CDR2 domain of this TCR ß-chain abolish the recognition of non-self MHC molecule with little effect on the recognition of antigenic peptide presented by self-MHC molecule. This suggests the possible modification of the interactions of the CDR1 or CDR2 of the TCR ß chain with the α-helical region of the MHC molecule without affecting the interaction of the antigenic peptide. Some of this lack of alloreactivity can be restored by the mutation in the α–helical region of the MHC Aβ chain residue facing TCR, implying the direct amino acid interaction between these residues. None of the CDR1 and CDR2 TCR ß-chain mutations affect the recognition of SEB confirming the involvement of the CDR4 region for superantigen recognition. Single amino acid substitution at residue 22 of TCR ß chain with Asparagine (N) to Histidine (H) restore the reactivity of the retroviral superantigen Mls-1^a confirming the ealier studies, but this reactivity is abolished by the additional mutations in the CDR1 domain showing the MHC involvement in superantigen recognition by TCR. A possible model of TCR:Ag:MHC:sAg interaction is proposed.

V 318 MHC, EPITOPE AND TCR INTERACTION IN THE RECOGNITION OF ACETYLCHOLINE RECEPTOR IN A MOUSE MODEL OF MYASTHENIA GRAVIS, Anthony J. Infante[1], Jessica Pierce[2], Pat Currier[1], Kim Zborowski[3], Ellen Kraig[3], Ji-Yang Hu[4], Katherine Wall[4], Scott Southwood[5], and Alessandro Sette[5], Depts of [1]Pediatrics, [2]Biochemistry, and [3]Cellular and Structural Biology, Univ of Texas Health Science Center, San Antonio, TX 78284; [4]Dept of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH 43606; [5]Cytel, Inc., San Diego, CA 92121

Experimental autoimmune myasthenia gravis (EAMG) is inducible in C57BL/6 (B6;I-A^b) mice by immunization with *Torpedo californica* acetylcholine receptor (TAChR). Congenic B6.C-H-2^{bm12} mice with discrete substitutions in the MHC class II peptide binding site are relatively resistant to EAMG. As a consequence of MHC structure bm12 mice fail to recognize an epitope which is immuno-dominant in B6 mice, peptide 146-162 of the TAChR α subunit. This peptide has been shown by others to be capable of inducing neonatal tolerance to EAMG induction. Binding of p146-162 to I-A^b is mediated by side chain interactions with Y151, V156 and to a lesser degree S157; these residues define a binding motif which is conserved among three other AChR epitopes. The strength of MHC binding correlates with the pattern of epitope dominance. Residues D152 and K155 are dominant T cell contacts. S157 and I158 are also important; substitutions of these positions yield TCR antagonist peptides. Interestingly, although individual T cell clones could be inhibited by one or more antagonists, no single antagonist inhibited all clones. Since recognition of p146-162 was previously shown to preferentially involve Vβ6, V-D-J sequences of p146-162 reactive clones, hybridomas and lymph node T cells were determined. Highly conserved β chain junctional sequences were found to contain glutamic acid (E) and to a lesser extent aspartic acid (D). A minor population of p146-162 reactive T cells utilized Vβ8.2, the Vβ gene with highest homology to Vβ6, associated with V-D-J sequences containing E or D. These studies indicate considerable structural constraints on TCR recognition of the important p146-162/I-A^b ligand and several potential targets for immune intervention in EAMG.

V 319 MOLECULAR BASIS FOR INTERACTION OF CD8α/α WITH ITS LIGAND MHC CLASS I

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The CD8 dimer interacts with the α3 domain of its ligand, MHC class I, through two Ig variable-like domains. A crystal structure informed mutational analysis has been performed to identify amino acids in the CD8α/α homodimer that are likely to be involved in binding to class I. Several key residues are situated on the top face of the dimer within loops analogous to the complementarity determining regions (CDR) of immunoglobulin. In addition, other important amino acids are located in the A and B β strands on the sides of the dimer. We find that the positive surface potential of CD8 appears to be important in class I binding thus complementing previous work demonstrating the importance of a negatively charged loop on the α3 domain of class I for CD8α/α-class I interaction. The potential involvement of amino acids on both the top and the side faces of the molecule is consistent with a bivalent model for the interaction between a single CD8α/α homodimer and two class I molecules and may have important implications for signal transduction in class I expressing cells. A model was generated showing how two molecules of HLA calss I can interact symmetrically with one CD8 dimer in an orientation analogous to the orientation found in crystal structures for dimers of HLA class II molecules. This model predicts that CD8α/α interacts not only with the α3 domain of class I but also the α2 domain.

V 320 AFFINITY MEASUREMENT BETWEEN TCR AND MHC WITH PEPTIDES OR WITH SUPERANTIGENS, Haruo Kozono, Janice White, Janice Clements, Philippa C. Marrack and John W. Kappler, Department of Medicine, National Jewish Center for Respiratory Medicine and Immunology, Howard Hughes Medical Institutes, Denver, CO 80206

T cell receptors (TCRs) recognize peptide antigens bound to MHC molecules. According to the affinity hypothesis, TCRs should have a lower affinity for the peptide/MHC complexes which positively selected them than for the peptide/MHC complexes which could negatively select them. To test this, soluble TCR and MHC protein were made using the baculovirus expression system. Two TCRs were chosen for this study, 5KC(Vβ3) specific for a moth cytochrome C (MCC) peptide in the context of I-E^k and DO-11.10(Vβ8.2) specific for an ovalbumin (OVA) peptide bound to I-A^d. Soluble I-E^k made in moth cells was functional since it could present peptide antigens as well as SEB and SEA to T-cell hybridomas. Likewise soluble TCR molecules made in moth cells were also functional since they bound to MHC with SEB or specific peptide and inhibited the antigen presentation to T-cell hybridomas specifically. Affinities were measured using a BIAcore™ (Pharmacia Biosenser). I-E^k plus MCC peptide had significantly higher affinity compared to I-E^k plus non-relevant peptide for the 5KC TCR. Currently we are testing the affinity of the 5KC TCR for I-E^k plus several peptides that were isolated from thymus I-E molecules. The hierarchy of affinity may suggest candidate peptides for positive selection of the TCR *in vivo*.

V 321 ANALYSIS OF THE STRUCTURE, DIVERSITY AND EVOLUTION OF T CELL RECEPTOR β-CHAINS IN PRIMATES, Jerry S. Lanchbury, Emma E. M. Jaeger, and Ronald E. Bontrop* Molecular Immunogenetics Unit, Division of Medicine, UMDS, London, SE1 9RT. UK. *TNO Medical Biological Laboratory, PO Box 5815, 2280 HV, Rijswijk, The Netherlands.

The non-human primates *Pan troglodytes* and *Macaca mulatta* have been used as animal disease models for the investigation of AIDS and collagen-induced arthritis, due to their close phylogenetic relationship with humans. MHC, immunoglobulin and T cell receptor molecules are components of specific immune responses. The MHC class I and class II molecules of *Pan troglodytes* and *Macaca mulatta* have been extensively investigated and studies have revealed that most primate MHC allelic lineages are stable structures that predate speciation. To investigate the adaptive immune response in these primates further, we have cloned and sequenced TCRβ cDNA that is derived from the peripheral blood of a healthy chimpanzee and rhesus monkey after inverse PCR. We have obtained the sequences of 58 chimpanzee and 56 rhesus monkey TCR β chains, and in both species Vβ genes are rearranged to D and J segments as in humans. Alignment of variable region sequences shows that key residues are conserved relative to other TCR β chains and immunoglobulins and are probably responsible for the conserved domain framework. Vβ genes from both chimpanzee and rhesus monkey can be assigned to equivalent human Vβ families, and are usually identified with particular family members. We have sequenced representatives from 15 chimpanzee and 18 rhesus monkey Vβ families, and in general the chimpanzee/human relationship is closer than the human/rhesus relationship as expected from phylogeny. Phylogenetic trees created using Vβ amino acid sequences from all three species show that Vβ family members from different species cluster together rather than by species, indicating relative stability of these genes through primate evolution. This indicates that T cell receptor variable regions are inherited in a trans-species manner analogous to MHC alleles. Lack of Vβ divergence between species, the absence of novel Vβ's and relative conservation of Vβ mRNA levels suggests that the ancestral primate of human, chimpanzee and rhesus macaque which existed approximately 30 million years ago, had reached an equilibrium between germline TCR variability resulting from tandemly repeated Vβ genes and junctional variability which is mostly somatically generated.

V 322 TCR-LIGAND INTERACTIONS ON CTL CLONES RECOGNIZING A H2-K^d RESTRICTED, PHOTOREACTIVE PEPTIDE DERIVATIVE, Immanuel F. Luescher, Manuel Peitsch*, Fabienne Anjuère, Jean-Charles Cerottini and Pedro Romero, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland and *Institute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland.

The K^d restricted Plasmodium berghei circumsporozoite peptide PbCS 253-260 (YIPSAEK)I was N-terminally conjugated with photoreactive iodo, 4-azidosalicylic acid (IASA) and at the TCR contact residue Lys 259 with 4-azidobenzoic acid (ABA), to make IASA-YPSAEKI. Seven independent CTL clones were derived from mice immunized with this conjugate. All clones recognized the peptide derivative as well as YIPSAEK(ABA)I in a K^d restricted manner, but failed to recognize IASA-YIPSAEKI or the parental PbCS peptide. Selective photoactivation of the IASA group allowed the covalent attachment of the peptide derivative to K^d molecules. Incubation of covalent P815 cell-associated or soluble K^d peptide derivative complexes with the cloned CTL and photoactivation of the ABA group resulted in TCR photoaffinity labeling. The TCR of two clones were selectively labeled at the β chain, while the TCR of the other clones were labeled to various relative extents at both chains. The TCR of the latter clones expressed Vβ1 encoded β chains that were paired with α chains containing the Jα TA28 segment. These TCR gene elements were not utilized by PbCS specific CTL clones. The TCR of the two other clones expressed different gene elements. Molecular modeling of one of the Vβ1, Jα TA28 expressing TCR showed a prominent hydrophobic pocket formed in part by the CDR3 equivalent region of the α chain and the CDR2 region of the β chain. In the bound state the Lys (ABA) side chain of the ligand intruded into this pocket, with the ABA group being stacked between the Jα TA28 encoded Trp and Tyr 48 of the Vβ1 segment. The photoaffinity labeled site on the α chain was localized by peptide mapping in the CDR3 region. It is concluded that TCR expressing these particular gene elements can avidly bind this photoreactive ligand, which favoured their preferential positive selection in vivo.

V 323 ABNORMAL SIGNAL TRANSDUCTION THROUGH CD4 LEADS TO ALTERED TYROSINE PHOSPHORYLATION IN T CELLS DERIVED FROM LUPUS PRONE MICE, Michael. P. Madaio & Ji Ming Duan University of Pennsylvania, Philadelphia, PA 19104-6144

CD4+ T cells play a crucial role in the development of lupus in MRL-*lpr/lpr* mice: i) incomplete deletion/ silencing of CD4+ T cells results in both polyclonal B cell activation and infiltration of T cells within the spleen, lymph nodes, kidney and other organs; and ii) anti-CD4 antibody therapy ameliorates disease and prolongs survival. Because CD4 is normally involved in both tolerance induction and T cell activation, we questioned whether abnormal signal transduction through CD4 influenced activaton of autoreactive T cells in this strain. For this purpose, signal transduction in CD4+ T cells derived from MRL-*lpr/lpr* and normal mice were compared, using both CD4+T cell clones and bulk CD4+T cell populations, isolated from mice of varying ages.

Cross-linking of surface CD4 resulted in deficient tyrosine phosphorylation of cellular proteins in MRL T cells. By comparison, tyrosine phosphorylation was similar among MRL and normal CD4+ T cells after cross-linking with either anti-TcR antibody or anti-CD3 antibody, and following co-culture with Con A. *lck* protein expression in MRL, CD4+ T cells was lower than normal. However, following stimulation with Con A, *lck* enzyme activity was comparable in MRL and normal T cells. The observed differences could not be explained by variation in surface CD4 expression, and they were present in an autoreactive MRL T cell clone as well as in T cells isolated from pre-diseased and diseased mice.

These results raise the possibility that abnormal signaling through CD4 may contribute to the impaired tolerance and the expansion of autoreactive T cells exhibited in MRL-*lpr/lpr* mice.

V 324 ORGANISATION AND STRUCTURE OF T CELL RECEPTOR Vα GENE SEGMENTS OF THE MOUSE.
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The number of Vα gene segments varies from mice to mice and is estimated between 75 to 100. On the basis of their structure, Vα are grouped into about 20 subfamilies. Southern blot analysis revealed that most of the Vα are organized in clusters composed of a copy of Vα belonging to different subfamilies. To seek further the organisation of the mouse TCR α locus, we analyzed in detail a Vα subfamily and search for new Vα.

From a serie of genomic clones derived from B10.A strain encompassing these clusters, the structure of all the Vα2 was determined. Comparisons indicate that the Vα2 can be organized in 3 structural subgroups composed of 3 members for Vα2I, 6 for Vα2II and 3 for Vα2III. Several Vα2 are identical indicating recent duplications, the most divergent Vα2 display 7.4% of nucleotide differences leading to 5.2% of amino acid replacements. All the crucial residues of the folding in Ig like domain are conserved in the Vα2, this allowed us to use an Ig model to predicted the localization of the variable positions. The great majority of the differences are in regions that are predicted to be exposed to the solvent, including the loops corresponding to the 4 CDR.

By the study of T cell lines, hierto undescribed Vα were found, one of them corresponds to a new subfamily composed of two members localized to each extremity of the TCR Vα locus, in association with one member of the Vα13 and VαBMB subfamilies.

Together, these data highlight the successive duplication units involved in the process of expansion and contraction of the Vα gene subfamilies.

V 325 SINGLE C-TERMINUS TRUNCATIONS OF AN IMMUNOGENIC PEPTIDE ANTIGEN ALTER THE SDS-CONFORMATION OF THE PEPTIDE-MHC COMPLEX AND SWITCH AGONIST/ANTAGONIST EFFECTS ON CD4 T-CELL FUNCTIONS, Joseph S. Murray, Isabel Ferrandis-Edwards, Curtis J. Wolfe, Nelsen Petersen, and Tony Schountz, Center for Basic Cancer Research, Division of Biology, Kansas State University, Manhattan, Kansas 66506.

CD4 T-cells recognize antigen as peptide fragments bound to the groove of class II major histocompatibility complex (MHC) molecules through the α/β T cell antigen receptor (TCR). Peptide-MHC complexes are presented on the surface of various accessory cells, and with appropriate co-stimulation, activate the T cell through the TCR. One theory of T cell activation states that TCR occupancy drives the activation of specific T cell functions in a manner quantitatively dependent upon the presentation-density of the peptide-MHC complex. Another view is that qualitative differences between T cell ligands can independently control the activation of specific T cell response genes. We now demonstrate that single C-truncations of a peptide antigen are associated with a structural change in the extracted I-A^s-peptide complex as revealed by SDS-PAGE. Furthermore, we show that these single C-truncations alter the presentation density of the peptide on live APC. C-truncated antagonist peptide doses were increased to effect peptide presentation-density equal with the peptide agonist, and part of the CD4 T cell clone's proliferative response was restored. By contrast, a dose approximately ten-fold in excess of this level of the antagonist peptide was required for interferon-gamma release. These data therefore support the role of qualitative differences in ligand structure in determining the functional outcome of CD4 T-cell recognition; and, suggest that peptide antagonism may be partially overcome by increasing the presentation-density of a particular ligand conformation.

V 326 DECREASED SIGNALING COMPETENCE AS A RESULT OF RECEPTOR OVEREXPRESSION: OVEREXPRESSION OF CD4 REDUCES ITS ABILITY TO ACTIVATE $p56^{lck}$ AND TO REGULATE TCR EXPRESSION IN IMMATURE $CD4^+CD8^+$ THYMOCYTES

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Thymic selection of the developing T cell repertoire in immature $CD4^+CD8^+$ thymocytes, with the fate of individual thymocytes determined by the specificity of TCR they express. However, TCR expression in immature $CD4^+CD8^+$ thymocytes actively downregulated in $CD4^+CD8^+$ thymocytes by CD4-mediated tyrosine kinase signals that are generated in the thymus as a result of CD4 engagement by intrathymic ligands. In the present study we have examined the effect of CD4 overexpression in $CD4^+CD8^+$ thymocytes on activation of CD4-associated $p56^{lck}$ tyrosine kinase and regulation of TCR expression. Augmented CD4 expression in $CD4^+CD8^+$ thymocytes did not result in commensurate increases in associated $p56^{lck}$ molecules, so that CD4 expression was quantitatively disproportionate to that of its associated signaling molecule $p56^{lck}$. Interestingly, we found that CD4 overexpression significantly interfered with the ability of CD4 crosslinking to activate associated $p56^{lck}$ molecules and significantly interfered with the ability of CD4 to regulate TCR expression. Thus, this study provides an example in which receptor overexpression leads to decreased receptor signaling competence.

V 327 RECOGNITION OF MULTIPLE PEPTIDE CORES BY A SINGLE T CELL RECEPTOR,

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A single T cell clone raised against a peptide of sperm whale myoglobin (SWM) (110-121) can recognize at least four other essentially "unique" peptides, each with its distinct core structure, in the context of the same major histocompatibility complex (MHC) molecule. We truncated this determinant so as to abrogate T cell recognition but retain MHC binding, and then examined 180 mutants of the truncated peptide for their potential to activate the TCR. Further analysis of the core structures of 3 of 6 mutant stimulatory peptides revealed that four other peptides in this region of SWM are able to stimulate the TCR of the SWM 110-121-specific T hybrid. The 5 stimulatory peptides have a different distribution of charge and hydrophobicity. Our results suggest that the T cell receptor (i) has multiple sets of contact residues for alternative peptide-MHC ligands, the binding to any one of which can trigger the cell and/or (ii) may be able to attach to the peptide-MHC complex in more than one orientation. In this sense, the TCR is a multi-subsite structure capable of being stimulated by a variety of peptide ligands associated with the same MHC molecules. (Supp. by grants from NIH and ACS)

V 328 SPECIES SPECIFIC T CELL RECOGNITION OF BOTH THE α2 AND α3 DOMAINS OF MHC CLASS I AND THE ROLE OF CD8,

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Murine T cell responses to human MHC class I molecules are 40 to 80 fold lower than responses to murine class I molecules. Reports from different laboratories have implicated either the α1+α2 domains or the α3 domain of the MHC class I molecule in determining the strength of the response by $CD8^+$ T cells. In this study, primary murine T cell responses were raised against HLA-A2.1, $H\text{-}2D^d$, and all six hybrid class I molecules containing the α1, α2 and α3 domains of either the human or murine molecule. Responses to a hybrid containing human α1 and murine α2+α3 were as strong as those to intact $H\text{-}2D^d$. Conversely, responses to a hybrid containing murine α1 and human α2+α3 were as weak as those to the intact HLA-A2.1. Responses to hybrids in which the α2 and α3 domains were from different species, however, were intermediate or lower in strength. Neither a murine α2 nor a murine α3 alone could elicit a T cell response as strong as that to $H\text{-}2D^d$. Thus, the weakness of the murine T cell response to human class I molecules is due to regions in both the α2 and α3 domains. Previous studies have shown that the CD8 molecule interacts with the α3 domain of MHC class I molecules, and that murine CD8 does not interact optimally with human class I. The possibility that CD8 interacts with the α2 domain, as well as α3, is consistent with the crystal structures obtained for CD8 and class I and the effect of CD8 point mutations on class I binding. A cell-cell binding assay is being used to investigate the influence of the species of origin of the α1, α2 and α3 domains of hybrid class I molecules on murine CD8 binding. The extent to which weak responses to class I molecules containing human α2 or α3 domains is due to species specific interactions with murine CD8 will be discussed.

V 329 PREFERENTIAL ASSOCIATION OF LOW MOLECULAR WEIGHT ISOFORMS OF CD45 WITH THE TCR/CD3 COMPLEX AND THE CD4 CO-RECEPTOR.

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A cell line, $BW(T200^-)$, which lacks expression of TCR/CD3, CD4 and CD45 on its surface was stably transfected with cDNAs encoding TCR α and β chains of known antigen specificity (conalbumin + H-2k). These $TCR/CD3^+$ lines secreted IL2 in response to immobilized anti-TCR MAbs, but they failed to respond when challenged with Ag+APC or with allo-stimulators (H-2b). Transfection of these cells with cDNAs encoding the ABC, BC, C or null isoforms of CD45 increased the sensitivity of the cells to low concentrations of anti-TCR MAbs, but did not increase the maximal amount of IL2 secreted. These $TCR/CD45^+$ lines also failed to respond to Ag+APC and alloantigen. The restoration of CD4 expression by a further round of transfection resulted in $TCR/CD45/CD4^+$ cell lines which could respond to both Ag+APC and alloantigen. Efficient allo-responses were limited to cells expressing the C and Null isoforms of CD45. Antibody-induced co-capping experiments showed that the C and Null isoforms of CD45 could form a complex with CD4 on the cell surface while the ABC and BC isoforms could not. All isoforms were capable of co-capping with the TCR/CD3 complex. These results suggest that responses to low avidity ligands such as alloantigen require close approximation of the TCR with CD4 and CD45 co-receptors, and that only low molecular weight isoforms of CD45 can participate.

V 330 ASSOCIATION OF IgG1 ANTIGEN RECEPTOR WITH THREE NOVEL PHOSPHOPROTEINS IS MEDIATED BY THE HEAVY CHAIN CYTOPLASMIC DOMAIN, Sarah L. Pogue* and Christopher C. Goodnow*!, Department of Microbiology and Immunology* and Howard Hughes Medical Institute!, Stanford University, Stanford, CA 94305.

The B cell response to antigen depends, in part, on the developmental stage of the lymphocyte. Exposure of immature B cells in the bone marrow to antigen results in B cell tolerance, whereas, exposure of mature B cells results in activation and differentiation. The memory B cell response to antigen is characterized by the rapid production of high affinity antibodies. At each of these developmental stages the isotype of the antigen receptor differs: immature B cells express IgM, mature B cells co-express IgM and IgD and memory B cells typically express IgG, IgA or IgE antigen receptors. To determine if the structural or isotypic differences of the antigen receptors are responsible for differences in B cell responses to antigen we constructed chimeric IgM antigen receptors which contain the transmembrane and cytoplasmic regions of IgG1. Wild-type and chimeric receptors have been expressed in a B lymphoma line, M12. Analysis of the antigen receptor complex expressed by the transfectants revealed three novel phosphoproteins which immunoprecipitate with the wild-type IgG1 antigen receptor, as well as, with the IgM/IgG1 chimeric receptors but not IgM or IgD receptors. Characterization of these isotype specific proteins is presented.

V 331 EXTRACELLULAR DOMAINS OF MEMBRANE IGM MEDIATE ITS ASSOCIATION WITH MB-1 AND B29 GENE PRODUCTS, Allen Rosenspire, Qiao Li and Ronald Santini, Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

Recent work in a number of laboratories has shown that membrane immunoglobulin (mIg) is associated with mb-1 and B29 gene products, and several other proteins including tyrosine kinases, $p95^{vav}$, and possibly CD 45 on the surfaces of B lymphocytes within a structure now referred to as the B cell receptor complex. Experiments utilizing site directed mutagenesis of mIg constructs has suggested that the association of the mb-1 and B29 gene products with mIg is mediated through interactions involving the transmembrane domains of these proteins. However mIgM as well as the mb-1 and B29 gene products all express large glycosylated exodomains. While the Fc domain of secreted immunoglobulins is known to participate in several recognition phenomena, the function of the Fc domain of mIg, as well as the function of the mb-1 and B29 exodomains are unknown. To investigate the possibility that these domains are important for mb-1 and B29 gene products to bind to membrane IgM, we examined mb-1 and B29 gene product binding to secreted IgM. Secreted IgM is similar to the extracellular domains of membrane IgM, but lacks the transmembrane and cytoplasmic domains. In our experiments, mb-1 and B29 gene products were demonstrated to bind to 7S secreted IgM, and to a lesser extent to 19S secreted IgM. The binding was prevented by treating secreted IgM with N-glycanase or neuraminidase. These observations imply that mb-1 and B29 gene products can bind to membrane IgM through an interaction solely involving extracellular domains, and that this interaction is mediated by carbohydrate. Supported in part by NSF grant MCB 9220169.

V 332 THE ROLE OF CD45 ISOFORMS IN T CELL ACTIVATION: EXPRESSION OF INDIVIDUAL CD45 ISOFORMS IN AN ANTISENSE TRANSFECTED CD45-NEGATIVE HUMAN T CELL LINE. David M. Rothstein, Linda Gorman, and Daniel W. McKenney. Section of Nephrology, Yale University School of Medicine, New Haven, CT 06510.

CD45 is a family of transmembrane protein tyrosine phosphatases (PTP'ases) shown to play a key role in T cell activation presumably by regulating the tyrosine phosphorylation of protein tyrosine kinases (PTKs) or their substrates. In man, regulated alternative splicing generates 5 CD45 isoforms that differ only in their extracellular domains. These isoforms are differentially distributed on T cells having distinct activation requirements and in vitro functions. However, the role of the different CD45 isoforms is unknown and has been difficult to study because individual T cells simultaneously express multiple isoforms. Furthermore, re-expression of different individual CD45 isoforms into CD45-negative mutant cell lines has proved difficult to accomplish. We have now developed a unique CD45-negative human CD4+ T cell line (J-AS-CD45) by stably transfecting Jurkat cells with an antisense gene that specifically targets endogenous CD45 expression. These cells lack both cell surface and cytoplasmic CD45 as determined by immunofluorescence and immunoblotting of whole cell lysates. J-AS-CD45 demonstrates defective activation signaling through both the CD3 and CD2 pathways, as evidenced by markedly decreased IL-2 production and minimal calcium flux compared to wild-type controls. We have recently transfected J-AS-CD45 with cDNAs encoding the smallest (180 kDa) and largest (220 kDa) CD45 isoforms, generating J-AS-CD45[0] and J-AS-CD45[ABC] cell lines, respectively. Each of these stable cell lines expresses the appropriate single isoform as detected by immunofluorescence and western blotting. Preliminary studies reveal that expression of either of these isoforms can restore activation-induced IL-2 production and calcium flux. Studies comparing $p56^{lck}$ and $p59^{fyn}$ activity, activation-induced tyrosine phosphorylation of intracellular substrates, and preferential activation requirements between these cells, are currently underway. Results of these studies will be discussed.

V 333 CD4 MEDIATED T LYMPHOCYTE CHEMOTAXIS REQUIRES THE PRESENCE BUT NOT THE ENZYMATIC ACTIVITY OF THE TYROSINE KINASE, $p56^{lck}$. §Thomas C. Ryan, §William W. Cruikshank, †Steven J. Burakoff, †Tassie L. Collins, †Barry P. Sleckmann, §David M. Center. §Boston University School of Medicine, Boston, MA 02118, †Dana Farber Cancer Institute, Boston, MA 02115.

CD4, is a 55 kDa surface glycoprotein which, in addition to acting as a co-receptor providing modulatory signals for antigen receptor-driven T lymphocyte activation, also participates in the motility of CD4+ lymphocytes and myelocytes following exposure of cells to a number of factors. The signal transduction mechanism which is induced during T cell motility has, however, yet to be defined. A signaling component that may be directly involved in CD4 mediated chemotaxis is the T-cell specific protein-tyrosine kinase, $p56^{lck}$, a member of the src kinase family, which associates with the cytoplasmic domain of a number of molecules including CD4. Because previous studies have demonstrated that the chemoattractants HIVgp120 and CD4 monoclonal antibodies (Mab) when crosslinked on the cell surface are capable of inducing $p56^{lck}$ enzymatic activity, we investigated the possible requirement for $p56^{lck}$ in CD4-mediated motile responses induced by CD4 Mab. We examined antibody induced lymphocyte chemotaxis in a series of murine T-cell hybridoma lines which have been transfected to express human CD4 and mutants of CD4 which have modifications in their cytoplasmic domains which result in the disruption of the association of $p56^{lck}$ with CD4. Our experiments demonstrate that CD4 antibodies induced dose dependent motile responses in cells expressing normal human CD4, however, no migration occurred in cells with CD4 mutations incapable of binding $p56^{lck}$. Anti-T cell receptor antibody induced chemotaxis in all the murine cell clones under all conditions. An examination of the enzymatic activity of the kinase in response to maximal chemotactic concentrations of CD4 antibodies, however, demonstrated no increase in CD4 associated $p56^{lck}$ activity. In addition, the tyrosine-kinase specific inhibitor, Herbimycin A, at doses and exposure times which inhibited all CD4 associated kinase activity, had no effect on lymphocyte migration. Our data suggests that the physical association of $p56^{lck}$ with CD4, and not the enzymatic activity of the kinase, is the essential component for lymphocyte motile responses. We propose that $p56^{lck}$ is acting, under these conditions, as an "adapter", linking CD4 with other signaling or structural molecules.

V 334 T CELL ACTIVATION CAN BE INDUCED VIA CROSS-LINKING CHIMERIC RECEPTORS CONTAINING TAM'S FROM THE HIGH AFFINITY IgE RECEPTOR OF MAST CELLS AND BASOPHILS, Patricia A. Schimke, Mary Godfrey, Daniel J. Price, Ernie Kawasaki and Benjamin Rivnay, Procept, Inc., Cambridge, MA 02139

Activation of T cells via TCR results in the phosphorylation of tyrosines in the three tyrosine activation motifs (TAMs) of the ζ subunit of CD3, which is believed to involve the tyrosine kinase Zap-70. Activation of mast cells and basophils via the high affinity IgE receptor results in the phosphorylation of tyrosines in the single TAM of both the γ and β subunits, which appears to involve the tyrosine kinase, syk. We investigated the specificity of the TAMs in the γ and β subunits of the high affinity IgE receptor, to determine if these were capable of activating T cells. Chimeric receptors with the extracellular domain of CD8α, the transmembrane domain of either CD8α, γ, or β, and the cytoplasmic domains of γ or β were constructed, transfected into TCR- Jurkat 31-13 cells, and the ability of these receptors to activate after cross-linking with anti-CD8 antibodies was determined. We observed that chimeric receptors with cytoplasmic domains from either γ or β subunits induced both early, (phosphorylation and Ca+2 mobilization) and late (IL-2 secretion) events in cellular activation. Chimeras with transmembrane and cytoplasmic γ domains could not be expressed on the cell surface, while those with transmembrane and cytoplasmic β domains were expressed on the cell surface, but were non-functional. The role of the transmembrane domains of γ and β and TAMs that interact with syk rather than Zap-70 in the activation of T cells which contain Zap-70 will be discussed.

V 335 MUTATIONAL ANALYSIS OF T CELL RECEPTOR ALPHA CHAIN AMINO ACID RESIDUES POSTULATED TO CONTACT SOLVENT BUT NOT THE CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULE AND ITS ASSOCIATED PEPTIDE, Jeffrey L. Seibel, John W. Kappler and Philippa Marrack, Howard Hughes Medical Institute, Denver, CO 80206

To understand the role that the T cell receptor (TCR) alpha chain plays during T cell activation, other than recognition of the class II major histocompatibility complex molecule (MHC) and its associated peptide, we have subjected it to mutational analysis. In order to identify sites that are involved in TCR signal transduction and/or interaction of the TCR with other surface molecules, specific mutations were made in amino acid residues postulated to lie on the face of the alpha chain that contacts solvent but not Class II MHC and peptide. The ability of such mutant receptors to interact with antigen on presenting cells was assessed *in vitro*.

V 336 CO-RECEPTOR AND CO-LIGAND FUNCTION OF CD4 INVOLVE DIFFERENT SITES ON CD4 AND ON MHC CLASS II MOLECULES. Rafick-P. Sékaly*, Bei Huang*, Sylvain Fleury*, Abdelkader Yachou*, H.E. Aronson†, Wayne Hendrickson† and Jacques Thibodeau*, *Laboratoire d'Immunologie, IRCM, 110 ouest ave des Pins, Montréal, (Québec), Canada. H2W 1R7; †Department of Biochemistry and Molecular Biophysics, Columbia University, New York, N.Y. 10032, USA.

The CD4 molecule interacts with a monomorphic domain of MHC class II molecules leading to enhanced T cell activation. CD4 has been proposed to act either as a co-receptor by interacting with the same MHC class II molecule which presents the peptide to the T cell receptor. Alternatively, *in vitro* systems have demonstrated that CD4 can enhance T cell activation by interacting with an MHC molecule which is not involved in peptide presentation to the T cell receptor. Three different *in vitro* systems have been set up in the lab which address the two functions of CD4. Using a battery of CD4 mutants spanning the four extracellular domains of CD4, we have shown that the co-receptor function of this molecule involves residues which are situated on the gp120 binding domain and others which are located on the other face of CD4. In contrast, the co-ligand function of CD4 involves the face of CD4 which does not encompass the gp120 binding site. Recent experiments have enabled us to show on the crystal structure of class II the presence of two sites implicated in an interaction with CD4. The implication of these sites in monomeric and dimeric forms of class II will be discussed.

V 337 STRONG PREDOMINANCE OF Vβ8 OR 14 TCR USAGE BY CYTOTOXIC T CELLS CROSSREACTIVE TO TWO HIV-1 EPITOPES AND DEGENERATE IN PRESENTATION TO CLASS I MHC MOLECULES, Mutsunori Shirai*, Melanie S. Vacchio#, Richard J. Hodes#, Jay A. Berzofsky* *Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, and #Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The MHC class I molecule D^d as well as H-$2^{u,p,\text{ and }q}$, were found to present peptides P18 and HP53, two determinants of HIV-1 gp160, to $CD8^+$ murine cytotoxic T lymphocytes (CTL). The usage of Vβ in T cells showing an unexpected crossreaction between these two peptides was remarkably conserved (primarily Vβ8 family, with some use of Vβ14) despite the extensive TCR Vβ diversity of the non-crossreactive CTL, which did not use Vβ8 or 14. This correlation of Vβ usage with fine specificity was consistent in H-$2^{d,u,\text{and }p}$ ($P<0.01$), but not in H-2^q. The correlation of Vβ use with peptide fine specificity independent of MHC restriction is all the more surprising in view of the finding that mice bearing a genomic deletion of Vβ8 can still produce T cells with cross-reactive phenotype, implying that other Vβ can still produce this specificity. We therefore asked whether the MHC restriction of these T cells was different, and found that H-$2^{d,p,\text{and }u}$ cells mutually cross-present the peptides P18 and HP53 to allogeneic CTL lines and individual clones of each of the other haplotypes, whereas none of these cross-present to H-2^q CTL, nor do H-2^q targets present to CTL of the other halotypes. This degeneracy of MHC restriction is novel for class I molecules. Moreover, the observed restriction in Vβ usage occurs only in the unique set of CTL that exhibit both peptide-crossreactive fine specificity and MHC allogeneic cross-presentation. The observation that a strain of mice in which the Vβ8 family is genomically deleted can still make CTL of this phenotype using another Vβ demonstrates the plasticity of the class I MHC-restricted repertoire when the dominating receptor is not available.

V 338 AFFINITY AND KINETICS OF REACTIONS BETWEEN AN ANTIGEN-SPECIFIC T-CELL RECEPTOR AND PEPTIDE-MHC CLASS I COMPLEXES, Yuri Sykulev*, Anders Brunmark+, Michael Jackson+, Richard J. Cohen#, Per A. Peterson+ and Herman N. Eisen*, *Center for Cancer Research and Department of Biology, and #Harvard-M.I.T. Health Science and Technology Program, Massachusetts Institute of Technology, Cambridge, MA 02139, and +Department of Immunology, IMM 8, The Scripps Research Institute, La Jolla, CA 92037

It is widely assumed that differences in antigen-specific T-cell receptor (TcR) affinities for various peptide-MHC (pepMHC) complexes exercise a major influence on T cell development and function. Since T cells interact transiently with target cells or APC, only a limited time is available for specific reactions to occur between the TcR on T cells and pepMHC complexes on target cells or APC. Therefore, it is important to determine how rapidly this reaction can approach equilibrium and thus whether TcR affinity values have physiological relevance. We found that the TcR on a clone of $CD8^+$ cytotoxic T cells reacts rapidly with its principal naturally occurring pepMHC ligand (equilibrium was approached in 1-2 min), and that the affinities of this TcR for a set of closely related soluble pepMHC complexes varied over a 1000-fold range, with values for individual complexes correlating well with the ability of their peptide moieties to sensitize target cells for lysis by the cytotoxic T lymphocytes. Even with an intrinsic affinity constant as low as 3×10^3 M^{-1} for a particular pepMHC ligand, target cells that presented this complex could stimulate a specific T cell response.

V 339 STUDIES ON T-CELL RECEPTOR REPERTOIRE OF TUMOR INFILTRATING LYMPHOCYTES IN BREAST AND OVARIAN CANCER. Sohel Talib, Bharati Sanjanwala, Lydia Kilinski, Katharine Fredericks, Dewey Moody, Ramila Philip, Jane Lebkowski, and Thomas B. Okarma, Applied Immune Sciences, Santa Clara, CA 95054.

Tumor infiltrating lymphocytes (TIL) are often associated with human tumors and are believed to be enriched for tumor-specific cytotoxic T-cells. TIL activated and expanded *in vitro* by interleukin-2 (IL-2) are currently used in cell therapy of human cancer under protocols sponsored by AIS. Molecular characterization of the variable region of the T-cell receptor (TCR) in the infiltrating lymphocytes may provide insight into the mechanism of anti-tumor immunity. We have studied the T-cell repertoire expressed in TIL from patients with breast and ovarian cancer using the anchor polymerase chain reaction (PCR) and the multiprobe RNase protection assay. This analysis demonstrated limited heterogenicity of the Vß repertoire in 6 breast cancer patients, Vß3 and Vß15 being the predominant repertoires. The levels of clonal restriction, as measured by the usage of Jß gene rearrangements within Vß3 and Vß15 families, was analyzed by southern blotting of PCR amplified products. VDJ sequence analysis of restricted repertoires further confirmed the clonal restriction of T-cells in breast cancer patients. To study the effect of long term *ex vivo* culture of TIL on their TCR repertoire, TIL from 2 patients were cultured in the presence of IL-2 for 23 and 46 days, respectively. In both cases, there was an increase in the total number of TCR repertoires expressed in long term cultured cells (from 5/22 at day 0 to 18/22 at day 23). These results indicate that in breast cancer, the TCR repertoire may be restricted and long term culture of TIL in the presence of IL-2 may result in polyclonal activation of T-cells. In the case of ovarian cancer, TIL from 3 patients were cultured in the presence of IL-2 or in the presence of irradiated tumor, and TCR from these samples was analyzed by RNase protection assay. Selective usage of the Vß6.3, Vß8.1 and Vß13.3 T-cell receptor gene products was found. Moreover, amplification of restricted repertoires was relatively enhanced in TIL cultured in the presence of irradiated tumor, potentially due to antigen-specific stimulation provided by irradiated tumor. These findings will guide in the development of antigen-specific cellular therapies for breast and ovarian cancer.

V 340 **A ubiquitous system for expressing functional α–β heterodimeric T cell receptors,** Luc Teyton, Brunmark A., Scott C., Jackson M., Boismenu R., and P.A.Peterson.
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The study of major histocompatibility complex (MHC) molecules - T cell receptor (TCR) interactions have been limited by the difficulties to produce large amounts of soluble molecules. The production of functional empty MHC molecules has been reported recently in a Drosophila expression system. We have taken advantage of this same system to engineer and produce soluble TCRs. The transmembrane domain of each α and β TCR cDNAs has been deleted by PCR to connect the extracytoplasmic region directly to the cytoplasmic tail. A six histidine tail was added at the N-terminal part of each construct to allow an efficient first purification step. Both cDNAs were subcloned in the metallothionein-driven Drosophila expression vector pRmHa-3 and transfected along with a neomycin-resistance gene into SC2 *Drosophila melanogaster* cells. After G-418 selection the expression was tested after 3 days of copper sulfate induction by immunofluorescence and purification by nickel-agarose chromatography. Transfected cells produced a 65 kD heterodimer made of one α and one β chain associated by a disulfide bridge which were recognized by anti-idiotype antibodies. Functionality of the purified molecules was shown by blocking specific T cell responses. The following series of 5 different receptors and their corresponding MHC ligands has now been produced : 2C (Ld), HY (Db), HA ($I\text{-}A^d$), OVA ($I\text{-}A^b$), OVA (Kb).
The affinities of TCR 2C for Ld+p2CL peptide complexes and TCR HY for Db+M80 peptide complexes have been measured using plasmon resonance technique. They both were found to be in the micromolar range and to correlate with the functional blocking experiments.

V 341 T-CELL RECEPTOR α CHAIN REPERTOIRE IN THE NON-HUMAN PRIMATES PAN TROGLODYTES AND MACACA MULATTA, Cornelia Thiel, Ronald E. Bontrop* and Jerry S. Lanchbury. Dept. of Medicine, UMDS, Guy's Hospital, London SE1 9RT,UK. *TNO Medical Biological Laboratory, 2288 GJ, Rijswijk, The Netherlands.

Chimpanzees and rhesus monkeys are suitable for the production of animal models for human autoimmune and immune-mediated diseases due to their close evolutionary relationship with humans. As a first step towards investigating the contribution of specific T-cell receptors (TCR) to disease, we analysed the α chain T-cell receptor repertoire in a healthy animal of each species. RNA was extracted from peripheral blood mononuclear cells of one chimpanzee and one rhesus monkey. Using human Cα amplification primers for linear PCR we cloned and sequenced ten Cα clones of the rhesus monkey and five of the chimpanzee. Nucleotide sequences (amino acid sequences) of the human and chimpanzee C α genes showed a similarity of 97.1% (95.6%). Cα nucleotide sequences (protein sequences) of the more distantly related rhesus monkey were 93.4% (86.7%) similar to humans. Conservation at both the nucleotide and the amino acid level is evident from a comparison of the Cα region in eight different mammalian species. RFLP's were generated with multiple restriction enzymes on human, chimpanzee and rhesus monkey genomic DNA using Cα probes. The results indicate the existence of a single Cα gene in the two non-human primates with evidence of polymorphism. We have designed primers for the inverse PCR on the basis of the Cα sequence of the rhesus monkey. Twenty Vα and Jα regions have been characterized. Further investigation of the α chain repertoires of non-human primates is in progress.

V 342 THE ROLE OF THE TRANSMEMBRANE AND CYTOPLASMIC DOMAINS OF THE TCR ß CHAIN IN TCR-MEDIATED SIGNALLING, Georg Tiefenthaler and Ed Palmer, Basel Institute for Immunology, CH-4005 Basel, Switzerland.

The antigen specific T cell receptor (TCR) is composed of the α/β or γ/δ TCR heterodimers which are non covalently associated with a group of invariant polypeptides collectively referred to as CD3. While the antigen specificity of the TCR is determined by the variable domains of the α/β or γ/δ chains, the components of the CD3 complex are necessary for the assembly of, and signal transduction through, the TCR complex.
We were interested in the role of the constant domain of the TCR ß chain in connecting antigen recognition with triggering T cell functions. To this end we truncated the TCR ß chain within the transmembrane and cytoplasmic domains and introduced these mutant TCR ß chains into a TCR ß-negative T cell hybridoma.
Preliminary results suggest that we have identified a region within the transmembrane and cytoplasmic domains that does not affect TCR surface expression but is absolutely required for the induction of T cell functions in response to TCR stimulation. Since the region does not display any homology to a known signalling motif, it is unclear at present whether this domain directly interacts with other protein (s) or is involved in a conformational change of the TCR ß chain during TCR-mediated signal transduction.

V 343 TARGETED MUTATION OF THE B CELL ANTIGEN RECEPTOR-ASSOCIATED MOLECULE, mb-1. Raul M. Torres, Heinrich Flaswinkel, Michael Reth, and Klaus Rajewsky, Institute for Genetics, Cologne and Max Planck Institute for Immunobiology, Freiburg.

The B cell antigen receptor complex (BCR) on B lymphocytes is composed of the clonotypic immunoglobulin molecule together with the gene products of mb-1 and B29, Ig-α and Ig-β. These Ig-associated proteins function as the signal-transducing moiety each harboring a cytoplasmic tyrosine-based activation motif (TAM) also found in the signal-transducing proteins associated with the T cell receptor, FcγRIII, and FcεRIβ/γ. Several studies using chimeric proteins transfected into cell lines have previously identified this motif as a critical element in the signal transduction via these surface receptors. Within the context of a plasmacytoma cell line, reconstitution of a BCR with an Ig-α, mutated at both TAM tyrosines, results in defective antigen receptor signalling as assayed by increased tyrosine phosphorylation. Nevertheless, the in vivo significance of this motif within mb-1 and B29, as well as the molecules per se, has not been addressed. To approach these questions, we have generated embryonic stem (ES) cell clones in which both tyrosines of the Ig-α TAM have been mutated to phenylalanine. In addition, using these clones, mice will be generated which lack Ig-α, either throughout or conditionally in development. We wish to discuss the phenotype of this null mutant upon reconstitution of the B cell lineage in RAG-2-deficient mice with ES clones homozygous for the deleted locus.

V 344 DISCRETE USAGE OF T-CELL RECEPTOR Vβ GENE REGIONS IN THE LUNG OF PATIENTS WITH INTERSTITIAL LUNG DISEASES. L. Trentin, R. Zambello, A. Cerutti, C. Enthammer, R. Sancetta, M. Facco, A. Cipriani, C. Agostini, G. Semenzato. Padua University School of Medicine, Departments of Clinical Medicine and Pneumology, Padova, Italy.

The T-cell repertoire was evaluated in lung T cells and peripheral blood lymphocytes (PBL) from 20 patients with sarcoidosis, 18 patients with hypersensitivity pneumonitis (HP) and 19 subjects with HIV-1 infection. TCR β-chain variable-region (Vβ) gene usage was determined using flow cytometry analysis with mAbs recognizing TCR Vβ gene families (Vβ2, Vβ3, Vβ5, Vβ6, Vβ8, Vβ12, Vβ13, Vβ17, Vβ19) and, in 15 patients, by quantitative PCR analysis with 22 primers specific for 20 TCR Vβ gene families and a 3' constant β primer. The TCR-α constant region was co-amplified as a standard for the calculation of the percentage of each TCR-Vβ gene expressed. In 10 patients BAL lymphocytes and PBL were cultured in vitro with staphylococcal enterotoxins (SEA, SEB, SEC1, SEC2, SED, SEE). Moreover, the role of IL-2 receptors in enterotoxin induced proliferation was evaluated.

TCR Vβ region analysis demonstrated an overexpression of discrete TCR Vβ region (Vβ2, Vβ5, Vβ6) on bronchoalveolar lavage (BAL) T cells with respect to PBL of some sarcoid patients. In particular, an increased percentage (>8%) of BAL Vβ2+ cells has been observed in 15 of 25 sarcoid patients, while the percentage of PBL Vβ2+ lymphocytes was usually lower than 8%. Similarly Vβ5 and Vβ6 were overrepresented in the lung with respect to PBL in 6 and 7 patients, respectively. In HP group, the highly expressed Vβ on BAL T cells were Vβ2 (4 patients, values ranging from 10 to 34%), Vβ5 (4 subjects, values ranging from 8 to 12%), Vβ6 (4 cases, values ranging from 8 to 12.5%) and Vβ8 (4 patients, values from 8 to 11%). The percentage of HP PBL expressing the above reported Vβ was usually lower than 6%. HIV-1 infected patients showed an overexpression of BAL Vβ2 (4 patients) and Vβ3 (3 patients) with respect to PBL. When BAL T cells and PBL were cultured in the presence of staphylococcal enterotoxins, a proliferative index varied according to the different toxins used. Furthermore, when BAL T cells or PBL were cultured in the presence of different enterotoxins and mAbs recognizing the p55 and p75 IL-2 receptors we observed a marked inhibitory effect (ranging from 75 to 85%) by these antibodies on the in vitro enterotoxin induced proliferation. These data demonstrate that the TCR repertoire of lung lymphocytes is not determined by random sampling of peripheral blood mononuclear cells but is likely to be the consequence of the interaction with specific disease related antigens.

V 345 FUNCTIONAL INTERACTION BETWEEN THE D3/D4 DOMAINS OF CD4 AND THE T CELL RECEPTOR:CD3 COMPLEX. Dario A.A. Vignali, Department of Immunology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38101.

During antigen presentation, a close association between CD4 and the T cell receptor (TCR) occurs due to both molecules interacting with the same major histocompatibility complex (MHC) class II molecule. The potential consequences of such an intimate interaction on TCR specificity were recently addressed using CD4 loss variants of murine T cell hybridomas specific for the hen egg lysozyme (HEL) peptide 52-61[1-3]. In the presence of CD4, T cells responded strongly to any peptide containing the core sequence, HEL 52-61, regardless of N- and C-terminal composition[2]. However, in the absence of CD4, most T cells could only respond to peptides containing the C-terminal tryptophans, Trp62 and Trp63. Thus, T cells require CD4 in order to tolerate changes in the composition of the peptide termini. In order, to investigated which portion of the molecule regulates this phenotype, a series of CD4 mutants were transfected into a CD4- hybridoma[3]. While cells which lack the cytoplasmic tail of CD4 responded comparably to the wild-type cell, any alteration of the D3/D4 domains of CD4 abrogated their ability to respond to peptides which lack Trp62 and Trp63. Furthermore, using fluoresence resonance energy transfer, a direct interaction between the D3/D4 domains of CD4 and the TCR/CD3 complex was demonstrated. These observations are currently being investigated further using CD4 domain-shuffled and site-directed mutants.

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V 346 HS1 AS A NOVEL SIGNAL TRANSMITTER FROM B CELL ANTIGEN RECEPTOR, Takeshi Watanabe, Daisuke Kitamura, Ichiro Taniuchi, Takahiro Fukuda, Yoichi Maekawa,Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University. 3-1-1 Maidashi, Higashi-ku, Fukuoka 812 Japan.

Cross-linking of membrane-bound immunoglobulin (mIg) receptors on B cells causes proliferation and differentiation of B cells or the inhibition (apoptosis) of their growth. It has been reported that the mIg receptor-mediated signaling induces rapid activation of Src-like kinases and tyrosine phosphorylation of cellular proteins in B cells. Various kinds of Src-like kinases such as $p53/56^{lyn}$, $p55^{blk}$, $p59^{fyn}$ and $p72^{syk}$ have been shown to associate with B cell antigen receptor complex and to be activated after cross-linking of receptors. HS1 gene is specifically expressed in hematopoietic cells and encodes a 75 kD protein. The N-terminal half of HS1 protein is rich in basic and acidic residues and has three and half copies of a 37 amino acid repeating motif, each of which contains a helix-turn-helix motif. The C-terminal motif is rich in acidic residuces and has potential a-helix containing an amphipathic region and an SH3 motif (Kitamura et al, 1989). HS1 protein is identified both in cytoplasm and nucleus. We showed that $p75^{HS1}$ is a major substrate of protein-tyrosine kinase, $p53/56^{lyn}$, activated by cross-linking of B cell antigen receptors. Our recent study indicates that HS1 protein plays a crucial role in regulating or transducing signals from B cell antigen receptors via protein tyrosine kinases directly to the nucleus. Currently we are analyzing the mice devoid of HS1 protein due to the targeted gene disruption. Preliminary results indicate that HS1 protein may be involved in signaling for cell proliferation as well as induction of apoptotic cell death of the lymphocytes.

V 347 CARRIER-DEPENDENT TNP-SPECIFIC T-CELLS DERIVE FROM A HIGHLY PRE-SELECTED PRECURSOR POOL.
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Previous data of our laboratory demonstrated that the H-$2K^b$ class I MHC-restricted T cell response to trinitrophenylated "self" is directed against K^b-associated, TNP-conjugated peptides. The overwhelming proportion of these TNP-specific cytotoxic T cells (CTL) was found reactive to TNP-lysine in the central position 4 of K^b-bound octapeptides. A large part of these CTL did not differentiate between "TNP-4" peptides of differing amino acid composition, i.e. they reacted in a carrier-independent fashion. Despite apparent restrictions in the statistical variability of their antigen specific receptors (TCR), T cells of greatly varying primary TCR structures were found to react to a given "TNP-4" peptide/K^b combination. More recently, we have produced a second, highly carrier-dependent type of TNP-specific CTL by primary in vitro stimulation with K^b-binding octapeptides carrying TNP-lysine in the peripheral position 7. The TCR of these CTL were shown to contact not only the hapten determinant in position 7 but also an amino acid epitope of side chains in positions 3 and 4 of the haptenated peptides. The structural repertoire of these carrier plus TNP specific TCR revealed itself as extraordinarily limited: CTL induction with different "TNP-7" peptides, using unprimed T cells of different individual mice, selected for α,β-TCR of defined fine-specificities resulting from rearrangements of identical $V\alpha/J\alpha$ and $V\beta/J\beta$ combinations. Besides contributing valuable information concerning the TCR contact sites for hapten and peptide determinants, these findings imply that carrier-specific in contrast to carrier-unspecific anti-hapten CTL are recruited from an extremely preselected pool of precursor T cells. Thus, the hapten/carrier-specific TCR repertoire appears to be particularly narrowed by negative thymic selection. The few "successfull" T cells of this type may, therefore, be enriched for "errors", i.e. for cells which in their effector phase or in recall reactions may also react to the respective un-haptenized peptides. In the case of *bona fide* self peptides, this would result in drug-induced autoimmune reactions.

V 348 ANTIGEN RECEPTOR-MEDIATED SIGNALING IN B CELLS MAY BE REGULATED BY CD45 IN A MATURATIONAL STAGE-SPECIFIC MANNER. Hidetaka Yakura, Mami Ogimoto, Tatsuo Katagiri, Kiminori Hasegawa and Kazuya Mizuno. *Department of Microbiology and Immunology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183, Japan*

Cross-linking of membrane IgM receptor on B cells by anti-IgM antibody leads to activation and growth in mature B cells but induces growth arrest and eventually cell death by apoptosis in immature B cells. To study the dichotomy of antigen receptor-mediated signaling at different stages of B cell differentiation, we chose to focus on the regulatory role of CD45, a membrane-bound protein tyrosine phosphatase (PTP). To this end, CD45-negative clones were isolated from immature B cell line WEHI-231 ($IgM^{hi}IgD^{lo}$) and mature B cell line BAL-17 ($IgM^{hi}IgD^{hi}$) by mutagenesis, complement-mediated cytotoxicity and limiting dilution. The CD45-negative clones were then compared to the parental line with respect to anti-IgM antibody-induced biochemical changes.

In immature WEHI-231 cells, tyrosine phosphorylation was constitutively induced in CD45-negative clones and was not enhanced by anti-IgM antibody stimulation. Ca^{2+} flux induced by anti-IgM antibody stimulation was slightly delayed but significantly prolonged when compared with the parental line. Further, CD45-negative clones showed stronger growth arrest and DNA fragmentation than the parental line and revertant clones, indicating that IgM receptor-mediated growth arrest and apoptosis in immature B cells are negatively regulated by CD45. In contrast, CD45-negative clones derived from mature BAL-17 cells had distinctly different characteristics in protein tyrosine phosphorylation, Ca^{2+} flux, and cell growth induced by anti-IgM antibody.

One interpretation is that CD45 may have differential effects on antigen receptor-initiated signaling events in immature and mature B cells. It may be that the content of protein tyrosine kinases or the substrate of CD45 is different in these B cells. These factors may ultimately affect the bifurcation of signals leading to cellular activation and tolerance.

V 349 RESIDUE 51 OF THE T CELL RECEPTOR α-CHAIN MAPS TO A SINGLE AMINO-ACID RESIDUE OF THE ANTIGENIC PEPTIDE

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T cell receptor (TCR) specificity for Ag:MHC ligand is determined by the complementarity determining regions of the TCR αβ chains. The importance of the CDR3's has been addressed in previous studies. Here, the influence on specificity by non-CDR3 of the TCR α-chain is studied by genetic mutational analysis. A TCR mutant with changes in the amino-terminal TCR α-chain can still recognize the antigenic peptide. However the specificity for the MHC ligand is dramatically different, allowing recognition of antigenic peptide only within the context of certain MHC's. By using MHC mutants and peptide antigen, an interaction between TCR α-chain and MHC HVR2 is mapped. Interestingly, a change in residue 51 (CDR2) of TCR α-chain causes a change in peptide specificity while leaving the MHC specificity largely unchanged. Thus, α-chain TCR amino-terminal region is involved in both MHC and peptide interaction. Furthermore, the identification of an interaction between a single TCR amino-acid residue and a single antigenic-peptide residue provides powerful information in determining the rotational orientation of the TCR on Ag:MHC.

V 350 T CELL ACTIVATION RESULTS IN PHYSICAL MODIFICATION OF THE MOUSE CD8β CHAIN.

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The T lymphocyte glycoprotein, CD8, is an essential component of the response of class I Major Histocompatibility Complex (MHC) restricted T cells to antigen. CD8 is expressed on the surface of class I restricted T cells as disulphide-bonded heterodimers and higher multimers of two distantly related polypeptides, α and β. The CD8α polypeptide, expressed in transfection studies as homodimers, is able to reproduce both the adhesive and stimulatory properties of CD8, leaving the function of the CD8β polypeptide unresolved. We have shown that there are reversible alterations in the physical nature of the CD8β chain which occur during T cell maturation and upon T cell activation. These changes are a specific decrease in the sialic acid content of the O-linked sugars of the β chain. The O-linked sugars are likely to be located in the connecting peptide region of CD8β - the area between the Ig-like domain and the transmembrane. In CD8β this area is about 10 amino acids shorter than the corresponding region of the CD8α chain which is very homologous and contains O-linked sugars , but does not vary with activation. In order to approach the size of CD4 and presumably traverse the length of the TCR, it has been predicted that the connecting peptide region of CD8 will have to adopt an extended structure. Therefore, altering the charge in this region of the molecule may have a profound influence on either the conformation of the CD8 heterodimer itself, or on the way in which CD8 can interact with other molecules on the T cell surface. These changes occur specifically on β and not CD8α, indicating that the primary role of the CD8β chain may be regulatory, influencing the physical structure of the CD8 complex.

T Cell subsets

V 351 ICAM-R INTERACTION WITH LFA-1 COSTIMULATES CD45RA$^+$ AND CD45RO$^+$ T-LYMPHOCYTES.

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T-lymphocyte activation in response to T-cell Receptor (CD3) engagement is supported by interaction with costimulatory molecules including ICAM-1, ICAM-2, B7/BB-1, LFA-3, and VCAM-1. To identify whether ICAM-R costimulates T-cells we measured T-cell proliferation, IL-2 receptor (CD25) and transferrin receptor (CD71) expression in response to recombinant ICAM-R coimmobilized with a suboptimal concentration of the CD3 specific antibody OKT3. We report that ICAM-R induced upregulated expression of CD25 and CD71, as well as dose-dependent proliferation of human peripheral blood T-cells when coimmobilized with suboptimal OKT3. Furthermore, costimulation with either ICAM-R or ICAM-1 induced comparable proliferation at identical concentrations. Anti-CD18 (TS1/18) or anti-CD11a (TS1/22) inhibited ICAM-R mediated costimulation, demonstrating at least a partial dependence on interaction of ICAM-3 with LFA-1. ICAM-R, but not ICAM-1 is expressed on "resting" (CD45RA$^+$) as well as "memory" (CD45RO$^+$) T-cells. To identify whether ICAM-R and ICAM-1 costimulate different T-cell subsets we tested the relative ability of each molecule to costimulate proliferation of CD45RA$^+$ or CD45RO$^+$ T-cells. Our studies indicate that either ICAM-R or ICAM-1 is able to costimulate proliferation of CD45RA$^+$ or CD45RO$^+$ T-cells. These results demonstrate that ICAM-R interacts with LFA-1 to costimulate both "resting" and "memory" T-cells.

V 352 CHARACTERIZATION OF *P. FALCIPARUM* BLOOD STAGE ANTIGEN-REACTIVE T-CELLS: USAGE OF THE TCR VARIABLE REGIONS AND MECHANISM(S) OF T CELL ACTIVATION, Elke S. Bergmann* and Urszula Krzych#, Departments of *Biology, The Catholic University of America, Washington, DC 20064 and # Immunology, Walter Reed Army Institute of Research, Washington, DC 20307

Exposure to irradiated *P. falciparum* sporozoites (SPZ) induces protective immunity in human volunteers and the arising T-cells respond to SPZ and blood stage antigens (pRBC). The analysis of pRBC-specific, CD4/CD8- and CD45RO/RA-bearing T-cells revealed striking differences between protected and non-protected individuals. pRBC-specific CD45RO$^+$ (memory) T-cells were evident only in protected volunteers. Furthermore, the majority of pRBC-reactive T-cells from protected and naive persons were CD4$^+$, whereas induction of CD8$^+$T-cells correlated with the loss of protective immunity. Current data suggest that the CD8$^+$T-cells have suppressive function leading to down-regulation of IL-4 and IFN-γ. The analysis of the TCR variable (V) chain usage among the pRBC-reactive T-cells showed preferential induction of Vβ5, Vγ4, and Vδ2 sub-populations and that depended on the stage-specificity of malarial antigens used for the *in vitro* expansion of T cells. We are currently evaluating the phenotype and the function of the induced subpopulations, including cytokine-patterns, and are analyzing mechanisms of T-cell activation by pRBC-stage antigens by examining the cell surface molecules that might be involved in the signal transduction and the subsequent activation events in response to pRBC antigens of *P. falciparum*. The outcome of this study will clarify mechanisms and cellular interactions that lead to immunity against malaria or that are responsible for the loss of protection. Supported in part by Deutsche Forschungsgemeinschaft (Be 1553/1-1).

V 353 SM D PEPTIDES IN THE INDUCTION OF LUPUS AUTOIMMUNITY, Linda K. Bockenstedt and Mark J. Mamula. Department of Internal Medicine, Yale University School of Medicine. New Haven, CT 06510

Autoantibodies specific for the Sm B and D proteins of uridine rich small nuclear ribonucleoprotein particles are a serologic hallmark of systemic lupus erythematosus (SLE) and constitute one diagnostic criterion for the disease. Indirect evidence suggests that the production of Sm autoantibodies is T cell dependent and antigen driven. How T cell tolerance to the Sm proteins is abrogated in SLE is unknown. We have recently identified the presence of T cells reactive with a model autoantigen, cytochrome c (cyt c) in the T cell repertoire of normal mice. These T cells are specific for "cryptic" cyt c peptides not normally produced during processing of the intact cyt c by antigen presenting cells (APCs). Such T cells therefore escape known mechanisms of thymic tolerance and remain functionally quiescent. We propose that T cell responses to cryptic epitopes on the Sm D protein can initiate and perpetuate autoimmune responses characteristic of SLE. In order to determine whether cryptic T cell epitopes exist on the Sm D protein, nonautoimmune strains of mice were immunized with synthetic murine Sm D peptides emulsified in complete Freund's adjuvant. One peptide spanning Sm D amino acids 46-60 consistently elicited strong autoreactive T cell proliferative responses from Balb/c (H2-d) mice. In contrast, immunization of mice with native Sm D failed to induce proliferative responses from murine lymph node cells, nor did T cell hybridomas specific for peptide 46-60 respond to native Sm D, indicating that peptide 46-60 constitutes a cryptic T cell epitope. Immunization of B10.BR (H2-k) mice with Sm D peptides revealed T cells reactive with peptides 26-40 and 56-70, demonstrating that cryptic epitopes are determined, in part, by the MHC background of the mice. T cells from MRL/lpr (H2-k) mice immunized with Sm D peptides responded to a broader array of peptides (11-40, 56-80) than those from B10.BR mice. Because the lpr gene results in loss of thymic deletion of self-reactive T cells, these findings indicate that T cells specific for peptides which normally would be deleted in mice with H2-k haplotype, are permitted to exist in MRL/lpr mice. Taken together, these results suggest two important mechanisms by which lupus-related T cell responses can occur: 1) T cells specific for cryptic Sm D epitopes exist as part of the normal T cell repertoire, but remain quiescent until the appropriate cryptics are presented by APCs, as might occur during aberrant processing of self proteins; and 2) the genetic background may allow autoreactive T cells to escape thymic deletion and contribute to the spontaneous development of autoimmune disease.

V 354 PREDOMINANCE OF MHC CLASS II-RESTRICTED CD4$^+$ CYTOTOXIC T CELLS AGAINST MHV-A59 MIGHT BE DUE TO THE REDUCTION OF MHC CLASS I SURFACE EXPRESSION ON VIRUS INFECTED CELLS, Claire J.P. Boog, Mirjam H.M. Heemskerk, Henriette Schoenmaker, Willy J.M. Spaan*, and , Dept. of Immunology, Fac. of Veterinary Medicine, University of Utrecht, and *Dept. of Virology, Fac. of Medicine, University of Leiden, The Netherlands.

Mouse hepatitis virus (MHV-A59), coronavirus causes a variety of acute and chronic infections in mice and rats, ranging from acute hepatitis and encephalomyelitis to chronic demyelination. We have analyzed the role of cytotoxic T cells (CTL) in MHV-A59 infection. Surprisingly, we detected only a very clear virus-specific MHC class II-restricted cytotoxicity in mice infected with MHV-A59. We found no evidence for the activation of the classical CD8$^+$MHC class I-restricted CTL. The virus-specific CD4$^+$ CTL derived from two different mouse strains having different MHC haplotypes recognize the same immunodominant epitope. This epitope comprizing the amino acids 329-343 of the viral S-glycoprotein, was recognized both at the polyclonal level and by virus-specific cytotoxic T cell clones. Facs analysis showed that infection with MHV-A59 clearly reduces the cell surface expression of MHC class I molecules. Our data suggest that this decrease in class I MHC expression could prevent recognition and lysis of virus infected cells by MHV-A59-specific class I-restricted CD8$^+$CTL. However, this inability of the host to generate CD8$^+$CTL can probably be compensated by the expansion of MHV-specific class II -restricted CD4$^+$CTL

V 355 DOUBLE LABEL *IN SITU* HYBRIDIZATION ANALYSIS OF ANTIGEN ACTIVATED T CELL CLONES SHOWS HETEROGENEITY OF CYTOKINE EXPRESSION AMONG SINGLE CELLS. R. Pat Bucy, Laurel Karr, Guo qaing Huang, Angela Panoskaltsis, Jimin Li, Kenneth M. Murphy*, and Casey T. Weaver. University of Alabama at Birmingham. Birmingham, AL 35294 and Washington University*, St. Louis, MO 63110.

Th1, Th2, and Th0 T cell clones derived from an ovalbumin peptide/I-A^d specific TCR transgenic mouse by selective exposure to IL-4 during primary antigenic stimulation *in vitro* were analyzed for cytokine mRNA expression by a double label *in situ* hybridization protocol utilizing both ^{35}S and digoxigenin labeled riboprobes. The frequency of cells expressing cytokine mRNA correlates well with the protein detected in the supernatant by sandwich ELISA and bioassay. Activation of these T cell clones with peptide antigen/APC demonstrates significant heterogeneity among individual T cells in the population. Stimulation of these clones with graded antigen doses demonstrates that the frequency of mRNA positive cells is increased with increasing antigen dose, without significantly changing the brightness of individual cells as detected by either the Digoxigenin or ^{35}S labeled riboprobes. Stimulation with peptide presented by fibroblasts co-transfected with I-A^d and murine B7, compared to I-A^d single transfectants, demonstrates a profound effect of B7 costimulation on the expression of IL-2 and IFNγ by the Th1 line and on IL-10 by the Th2 line, but relatively little effect on IL-4 expression by the Th2 line. The grain density per cell for the IL-2 probe on cells activated with B7+ APC was significantly increased, but for IFNγ and IL-4 the grain density per cell was not significantly affected by B7 costimulation. Double label analysis demonstrates that the IL-2 mRNA expressing cells are a subset of the IFNγ mRNA expressing cells in the Th1 clone, while the IL-5 and IL-10 mRNA expressing cells are subsets of the IL-4 mRNA expressing cells in the Th2 clone. Analysis of the cloned Th0 population that produces all of these cytokines shows that very few individual cells co-express IFNγ and IL-4, indicating that a "Th0 clone" may have an unstable cytokine expression phenotype rather than a mixed phenotype. These data emphasize that T cell activation is a fundamentally heterogeneous process both within cloned populations and between different cytokine genes as indices of activation. Supported by AR03555 and DK44240.

V 356 RELATIONSHIP OF HELPER-DEPENDENT AND HELPER-INDEPENDENT CD8+ CELLS, Zeling Cai and Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

The relationship of helper-dependent (HD) and helper-independent (HI) CD8+ cells was addressed with the aid of the 2C TCR TG mouse. 2C cells recognize three class I alloantigens: K^{bm3}, K^{bm11} and L^d. Purified resting 2C CD8+ cells proliferate strongly to Ld (B10.D2) in the absence of exogenous IL-2 and produce large amounts of IL-2 and show high expression of IL-2R. By contrast, 2C cells do not proliferate to bm11 unless the cells are supplemented with IL-2. Without IL-2, the cells synthesize IL-2R but not IL-2. These findings indicate that the response of 2C cells to Ld is HI, whereas the response to bm11 is HD. Since HI and HD responses can be generated by the same population of resting 2C cells, the data rule out the possibility that HI and HD CD8+ cells are derived from different lineages or represent different development stages. The data favor the idea that HD and HI responses of CD8+ cells are a reflection of a difference in the avidity of T/APC interaction: HI responses depend on high-avidity T/APC interaction whereas HD responses reflect weak T/APC interaction. We have found that CD8 molecules play an essential role in HI responses. Thus addition of anti-CD8 mAb converts the response of 2C to B10.D2 from an HI to an HD response. Conversely, the HD response of 2C to bm11 changes to an HI response when the 5% of 2C cells expressing the highest density of CD8 were tested. At the effector stage, CD8+ CTL cause strong lysis of B10.D2 target cells and intermediate lysis of bm11 target cells. By contrast, purified CD8-2C CTL kill only B10.D2 and not bm11 target cells. The finding that CD8+ and CD8- 2C cells cause equivalent lysis of B10.D2 target cells implies that lysis of these target cells is CD8 independent. Interestingly, however, CD8- 2C CTL are 30-fold more sensitive to inhibition with anti-TCR antibody (1B2 Fab) than CD8+ CTL. The implication of this finding will be discussed.

V 357 T CELL ACTIVATION IN RESPONSE TO *T.ANNULATA*

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The protozoan parasite *Theileria annulata* causes the lymphoproliferative disease tropical theileriosis, which threatens millions of cattle worldwide. The disease is particularly severe, and often fatal, in "exotic" cattle imported into endemic areas such as Africa, India and Asia. Although the role of the immune response in fatal cases is poorly understood, vaccinated or drug treated animals produce anti theilerial antibodies and theileria specific cytotoxic T cells. Recent work in this department has demonstrated that *T.annulata* infected cells induce strong proliferation of both CD4 and CD8 T cells from both naive and immune animals *in vitro*. However, it has not been possible to establish theileria specific $CD4^+$ lines and clones from the periphery of immune animals. This finding has important implications for the generation of immunity in naive animals. Currently we are investigating the activation of T cells within lymph nodes draining the sites of parasite inoculation in naive animals. IL $2R^+$ cells are seen reacting to areas of parasite induced lymphoproliferation early in the infection. In addition, IL $2R^-$ cells expressing a T cell activation marker are observed after IL $2R^+$ cells have left the node, and many of these cells are apoptotic. These findings are discussed in relation to our *in vitro* observations.

V 358 THE REQUIREMENT FOR SELF-RECOGNITION IN THE RESPONSE TO A BACTERIAL POLYSACCHARIDE

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Although >90% of B cells from M167 (μ, κ) immunoglobulin transgenic (Tg) mice express surface Ig that binds phosphorylcholine (PC), we find that these mice are unresponsive to immunization with pneumococcal cell wall polysaccharide (PnC), a type II thymus independent antigen that contains PC. However, when the PnC antigen is presented as a complex with TEPC-15 or McPC-603 antibodies (which are specific for PnC), a vigorous immune response occurs in which the TG mice produce 10 - 50 fold more anti-PnC antibody than when immunized with antigen alone. Interestingly, MOPC-167, which expresses the VH and VL regions used to encode the transgene antibody, was found to be a relatively poor "carrier" for PnC eliciting a weak anti-PnC antibody response in M167 (μ, κ) TG mice. In vivo administration of anti-CD4 antibody dramatically reduced the response to TEPC-15/PnC complexes suggesting that the response is mediated by immunoglobulin (idiotype)-dependent helper T cells. Furthermore, we find that M167 Tg mice primed with TEPC-15/PnC complexes and subsequently challenged with MOPC-167/PnC complexes produced fivefold less anti-PnC antibody than mice primed and challenged with TEPC-15/PnC complexes. The results indicate that unresponsiveness to PnC is due not to tolerance of the transgenic B cells but rather to the lack of T cell help resulting from T cell tolerance to the transgene-encoded idiotype. The requirement for self-recognition in the TG mouse response to PnC was also demonstrated by showing that the unresponsiveness to PnC could be overcome by immunizing mice with PnC coupled to self erythrocytes; Tg mice respond to PnC-MRBC as vigorously as to PnC coupled to foreign (sheep) erythrocytes. These results demonstrate that "autoimmunity" may be required to promote beneficial immune responses.

V 359 HUMAN PRIMARY CYTOTOXIC T CELLS RECOGNIZING ENDOGENOUSLY SYNTHESIZED ANTIGEN CAN BE INDUCED IN VITRO

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Synthetic peptides containing HLA class I binding motifs have been utilized as a tool to define the molecular targets of the cytotoxic T cell (CTL) response. We have used this approach for the in vitro expansion of in vivo sensitized CTL in patients with hepatitis B (HBV) and hepatitis C virus (HCV) infection. This has led to the definition of multiple HLA-A2 restricted CTL epitopes for both viruses. In an attempt to define the experimental conditions for successful in vitro priming we have developed two CTL induction strategies both based on the use of synthetic virus derived peptides and peripheral blood monunuclear cell (PBMC) isolated from normal uninfected donors. First, *repetitive long-term stimulation:* cultures containing 4 $x10^6$ PBMC set up in the presence of peptide and IL-2 and restimulated weekly with autologous antigen presenting cells and peptides for up to six weeks. Second, *bulk cultures:* cultures containing up to $25x10^6$ PBMC set up in the presence of peptide and IL-2 and restimulated weekly with autologous antigen presenting cells and peptides for up to four weeks. Both techniques were found to successfully induce HLA-A2 restricted CTL lines and clones specific for HBV core $_{18-27}$, HBV env $_{183-191}$, HBV env $_{251-260}$, HCV core $_{131-140}$, HCV core $_{178-187}$, HCV NS3 $_{1073-1081}$ and HCV NS3 $_{1406-1415}$. Some of these CTL lines and clones specific for HBV env $_{183-191}$ and HCV NS3 $_{1406-1415}$ were capable of recognizing endogenously sythesized antigen presented by target cells infected with recombinant Vaccinia virus containing HBV or HCV sequences. The system described may serve as a tool to dissect the cellular and molecular requirements for the induction of naive human cytotoxic T cells.

V 360 CD4-DEPENDENT AND -INDEPENDENT PRIMING OF ANTI-VIRAL CTLS IN VIVO.

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We have analyzed vesicular stomatitis virus (VSV)-specific CTL responses to determine whether VSV precursor CTLs (pCTLs) can be primed *in vivo* in the absence of $CD4^+$ cells. Our studies demonstrated that secondary anti-VSV CTL responses *in vitro* were markedly reduced by CD4-depletion prior to priming *in vivo* with VSV. Limiting dilution analysis indicated that the vast majority (>90%) of VSV pCTLs failed to become primed when exposed to VSV in the absence of CD4+ cells. A second minor population (<5-10%) of pCTLs was identified that was reproducibly primed in CD4-depleted mice. VSV-infection of CD4-deficient mice was associated with the appearance with time of a splenic $CD5^+CD8^+\alpha\beta$-TCR^- population that was amplified relative to intact, VSV-infected mice. Three-color flow cytometry indicated that the majority of the $CD5^+CD8^+$ cells were $\tau\delta$-T cells. Thus, despite the presence of $\tau\delta$-T cells, a population known to secrete cytokines, most VSV pCTLs still required classical CD4+ Th cells to achieve efficient priming *in vivo*. In contrast to CD4-depleted mice infected with free, infectious virus, CD4-deficient mice primed with VSV-infected, activated B cells mounted normal secondary anti-VSV CTL responses *in vitro*. Precursor estimates indicated that virtually all VSV pCTLs became primed using this cellular immunogen. CD4-independent priming could not be achieved using VSV-infected, activated T cells, another permissive cell type for VSV replication. Thus, most of VSV pCTLs require inductive signals from classical CD4+ Th cells for priming and that this requirement may be regulated by the antigen presenting cell *in vivo*.

V 361 ENDOGENOUS IL-2 AS A KEY REGULATOR FOR DEVELOPMENT OF TH1 OR TH2 EFFECTOR CELLS FROM C57BL/10 AND BALB/c MICE. Elaine K. Codias, Oliver A. Perez, and Thomas R. Malek. Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL, 33101.

After an identical antigenic challenge, e.g. *Leishmania major*, BALB/c mice develop a strong, but unprotective, Th2 response while C57BL/6 elicit a protective Th1 response. The basis for this phenomenon is not known. In the current study we developed an *in vitro* model that permitted us to preferentially elicit Th1 or Th2 cytokine profiles from BALB/c and C57BL/10 mice. Briefly, purified CD4 cells were primed *in vitro* by two rounds of polyclonal stimulation, and the resulting CD4 effector cells were restimulated with anti-CD3 to assess their lymphokine-producing potential. For both strains of mice, exogenous IL-2 and IL-4 added during both priming cultures resulted in effector cells producing high levels of IL-4 upon final restimulation. These data demonstrate that CD4 cells from both mouse strains have the capacity to produce high levels of IL-4. In contrast, when only exogenous IL-2 was added to the first culture, and no exogenous cytokines were added to the second culture, BALB/c mice generally developed IL-4-producing effector cells while C57BL/10 mice did not. Although IL-2 was added to the first culture in all cases, the level of IL-2 during the second priming culture was a critical regulatory element. Thus, when anti-IL-2 mAb was added to the second culture of cells from BALB/c, IL-4-producing effector cells were converted to a C57BL/10-like pattern of high IL-2 and low IL-4 production. Further, when IL-2 alone was added to the second prime of cells from C57BL/10 mice, the resulting effector cells produced substantial levels of IL-4 and little IL-2, similar to the results seen in BALB/c. Therefore, the higher endogenous level of IL-2 in BALB/c mice as compared to C57BL/10 mice was an important factor regulating the preferential development of IL-4-producing cells in BALB/c mice. These data suggest IL-2 plays a complex role in Th cell regulation, i.e. the levels of IL-2 at different times during activation may be critical to the development of appropriate immune responses.

V 362 ANTIGEN DOSE CONTROLS THE FUNCTIONAL DIFFERENTIATION OF NAIVE CD4$^+$ T CELLS, Stephanie Constant, Christiane Pfeiffer, Theresa Pasqualini and Kim Bottomly, Immunobiology Department, Yale University School of Medicine and HHMI, New Haven, CT 06510

Naive CD4$^+$ T cells can differentiate into cells predominantly involved in humoral immunity, known as Th2 cells, or cells involved in cell-mediated immunity known as Th1 cells. Dominance of one or the other effector cell type has been largely attributed to cytokines present in the priming environment, with high doses of exogenous IFNγ and/or IL-12 leading to Th1-like cells, and IL-4 to Th2-like cells. These findings leave open the mechanism by which these cytokine environments may be generated in the in vivo setting. We have previously predicted that antigen dose might be one critical parameter in determining the differential activation of naive CD4$^+$ T cells. Here, we show that the differentiation of naive CD4$^+$ T cells into either Th1 or Th2-like cells can indeed be controlled by varying the dose of priming antigen. To contol the specificity of the TCR we used CD4$^+$ T cells from TCR-transgenic mice. The cells were stimulated in vitro with various doses of antigen plus IL-2 for 4 days, 'rested' in the absence of antigen for 2 days, and then restimulated with a fixed dose of antigen for a further 2 days. Cytokines were then measured in supernatant from each culture. We found that priming in vitro with high doses of antigen generated Th1-like cells producing abundant IFNγ, whereas low doses of the same antigen induced cells to differentiate into Th2-like cells producing abundant IL-4. Importantly, this dichotomy in differentiation was observed only when using resting naive CD4$^+$ T cells as the starting population. The presence of previously activated cells in the primary cultures resulted in cytokines being produced which overcame the observed dichotomy in differentiation generated with antigen dose alone. We are investigating further the potential interplay between antigen dose, cytokine environment and antigen presenting cells on the CD4$^+$ T cell differentiation process.

V 363 NAIVE CD4 CELLS CAN PRODUCE IL-4, HELP B CELLS, AND DRIVE THEMSELVES TOWARDS A TH2-TYPE PHENOTYPE. Michael Croft and Susan L. Swain, Dept. of Biology, University of California San Diego, La Jolla, CA 92093

Naive CD4 T cells cannot help resting B cells for Ig secretion. Using T cells from TCR transgenic mice, able to react to a defined peptide Ag (pigeon cytochrome c fragment 88-104) presented on IE^k, we show that lack of help is due to the fact that naive T cells are not activated on resting B cells, with activation requiring a high level of costimulation. Provision of a costimulus (anti-CD28, or Ag-presenting dendritic cells, activated B cells, fibroblasts expressing B7 alone or B7 and ICAM-1) induced naive cells to help Ag-specific resting B cells immediately in co-culture experiments, and as little as a 1 day preactivation period also promoted help for IgM secretion. FACS, Ab blocking, and mRNA analysis showed that helper activity correlated with T cell CD40L expression and IL-4 synthesis. Initial naive T cell activation on costimulatory APC resulted in IL-2 secretion alone, and no CD40L expression, suggesting that IL-4 and CD40L were only induced after a second stimulation event following re-encounter with Ag.

IL-4 is the dominant factor in driving naive Th to secrete Th2-type cytokines, although the source of IL-4 in vivo is in doubt. Because we found naive Th could produce some IL-4 early (24-36 hr), but only after encountering Ag/APC more than once, we reasoned that multiple restimulation events might induce enough endogenous IL-4 to allow naive Th to drive themselves towards the Th2 phenotype. Preliminary experiments using highly costimulatory APC (B7 and ICAM expressing fibroblasts) support this hypothesis, and show that 3 or 4 restimulations during a 12-day culture can produce Th effectors capable of secreting large quantities of IL-4 and IL-5, as well as some IFN-γ and IL-2, and able to induce good IgM, IgG1 and IgE secretion from B cells. Secretion of Th2-type cytokines correlated with increasing amounts of endogenous IL-4 accumulated during the culture period. A single stimulation resulted in effectors producing only IL-2 and IFN-γ. Interestingly, multiple stimulations on resting B cells did not induce significant IL-4 or IL-5 secretion, suggesting that autocrine production of IL-4 may require a high level of costimulation during multiple Ag recognition events. These studies promote the hypothesis that naive Th may be capable of directing their own differentiation toward the Th2 phenotype and that the type of cell presenting Ag and the frequency of Ag/APC encounters may be critical to this process.

V 364 IL-13, AN IL-4 LIKE CYTOKINE THAT HAS NO T CELL STIMULATING ACTIVITIES, IS PRODUCED BY HUMAN CD4$^+$ AND CD8$^+$ T CELLS AND CD4$^+$ Th0, Th1 AND Th2 T CELL CLONES., René de Waal Malefyt, John S. Abrams§, Jan E. de Vries and Hans Yssel., Departments of Human Immunology and §Molecular biology, DNAX Research Institute of Molecular and Cellular Biology[1], 901 California Ave, Palo Alto, CA 94304 - 1104

Human Interleukin-13 is a novel cytokine which has been shown to affect human B cell and monocyte functions. In the present study, details of the production of IL-13 by human T cells and T cell clones were determined and compared to those of IL-4. IL-13 was produced by CD4$^+$ and CD8$^+$ T cell subsets isolated from PBMC and CD4$^+$ Th0, Th1 and Th2 clones and CD8$^+$ T cell clones. The production of IL-13 was induced rapidly and was relatively long lasting. IL-13 mRNA was maximally induced 2 hours after activation of the T cell clones and was still detectable at 48 hours, whereas IL-4 mRNA expression was more transient and no longer detectable after 12 hours. These results were confirmed by determining the kinetics of intracellular production of IL-13 protein and its secretion. Moreover, IL-13 could be induced in T cell clones by multiple modes of activation, including activation via a single stimulus like Ca^{++} ionophore A23187, PMA, anti-CD3 mAbs or PHA. Generally, the highest levels of IL-13 were produced following activation of T cells by anti-CD3 mAbs in combination with PMA. Production of IL-13 was inhibited by Cyclosporin A, but not by IL-10. Taken together, these results indicate that IL-13 is expressed in T cell clones more abundantly and independently of IL-4. Interestingly, unlike IL-4, IL-13 did not act as an autocrine T cell growth factor for T cell clones and was not able to induce CD8α on CD4$^+$ T cell clones or CD4$^+$ cord blood T cells, indicating that these cells may not express a functional IL-13R. Its expression pattern and lack of activities on T cells suggest a unique role for IL-13 in human immune responses.

V 365 MOLECULAR MECHANISMS OF IFN-γ GENE REGULATION IN ACTIVATED T CELLS, Don J. Diamond, Danny Z. Pang, and Takuya Tsunoda, Division of Immunology and the Department of Hematology and BMT, City of Hope National Medical Center and the Beckman Research Institute, Duarte, CA 91010

The product of the Interferon γ (IFN-γ) gene is a potent modulator of the immune response. Most T cells when activated in a normal immune response or through autoreactive mechanisms produce IFN-γ as an early response gene product. The goal of this study is to better define the transcriptional component of the activation of the IFN-γ gene. We have shown that agents which transduce signals through the major cell surface receptors of T cells to activate transcription from the IFN-γ promoter have molecular targets within the 284 bp promoter segment. The present studies have been expanded to include investigations of oligonucleotide response elements derived from the nucleotide sequence of the IFN-γ promoter which share sequence similarities with previously identified promoter elements from T cell activation genes. ST (signal transduction) mechanisms which result in T cell activation often involve the protein products of cellular proto-oncogenes. We have investigated whether there are oncogene components of the nuclear complexes which interact with IFN-γ promoter response elements. Comparison of the composition of these complexes from normal, leukemic and autoreactive cells may lead to further insight on how molecular targets of the ST pathways of T cells are disrupted in certain pathogenic diseases. We have also used molecular probes such as the calcineurin (CN) gene product and antibodies against the T cell surface receptors CD3 and CD28 to better understand the molecular differences in the targets of ST resulting from different activation pathways. We have also studied lymphokine (IL-12) mediated transcriptional activation of the IFN-γ promoter, to further understand how different ST pathways induce the synthesis of IFN-γ mRNA. Since IFN-γ enhances transplantation antigen (MHC) expression, it is directly involved in normal and pathogenic immunoactivation that can result in heightened graft rejection or autoimmune disease. Therefore, we have also investigated the mechanism by which immunosuppressive drugs act to inhibit IFN-γ mRNA synthesis. In conclusion, IFN-γ has important antitumor properties, while its dysregulated synthesis may contribute to autoreactivity. Therefore, further studies on the regulation of IFN-γ synthesis may lead to our ability to manipulate its synthesis for therapeutic benefit.

V 366 IL-10, IL-12, AND TNF-α DIFFERENTIALLY INFLUENCE THE PROLIFERATION OF $CD4^+$ OR $CD8^+$ T-CELL CLONES IN THE ABSENCE OF ANTIGEN. J.H.F. Falkenburg, C.A.M. van Bergen, W.M. Smit, W.F.J. Veenhof, M. Rijnbeek and R. Willemze. Lab. of Exp.Hematology, Univ.Med.Center, Leiden, The Netherlands.

Proliferation of T-cells in vitro is stimulated by specific antigens presented in the context of HLA, non-specific mitogens, and various cytokines. The expansion of T-cell clones is generally performed by the addition of a combination of these stimuli. Apart from IL-2, the relevance of many cytokines for the expansion of T-cell clones in vitro is unknown. We investigated the role of IL-10, IL-12 and TNF-α on the proliferation of $CD8^+$ cytotoxic T-cell (CTL) clones, and $CD4^+$ proliferative or CTL clones. The proliferation of these T-cell clones was analyzed one week after the last refeeding in the absence of antigens or mitogens. No proliferation was observed in the absence of IL-2. In the presence of IL-2, IL-10 significantly increased $CD8^+$ CTL proliferation as measured by ^{3}H-Thymidine incorporation on day 2 and 4 of culture by approximately 50%. Only 1 of 5 $CD4^+$ clones was affected. IL-12 induced some proliferation of $CD8^+$ CTL clones in the first 2 days after stimulation, which was followed by a strong inhibitory effect from day 4 to day 7 in the presence of IL-2. Only 2 of 5 $CD4^+$ T-cell clones were late inhibited by IL-12. Thus, IL-12 induced a late inhibitory effect on CTL clone expansion. After treatment with IL-12, the CTL's underwent morphological changes including accumulation of cytoplasmic granulae, suggesting further cytotoxic differentiation. TNF-α inhibited only 2 of 5 $CD4^+$ clones, but strongly inhibited the proliferation of all $CD8^+$ CTL's from day 2 after stimulation. Using PCR, the endogenous cytokine production of the T-cell clones was analyzed. The $CD8^+$ T-cell clones produced no IL-2, IL-10 or IL-12. A valuable amount of TNF-α was produced, but, as measured by Elisa, less than 0.5 ng/ml was released into the medium in a period of 4 days, which was insufficient for effective inhibition. The $CD4^+$ clones only produced IL-2, and low amounts of TNF-α. These results indicate that endogenous production of these cytokines didnot influence the results. In conclusion, antigen independant proliferation of $CD8^+$ T-cell clones can be modulated by the addition of IL-10, IL-12 or TNF-α in the presence of IL-2. These cytokines may be relevant for the clonal selection of proliferative or cytotoxic T-lymphocyte clones.

V 367 IL-2 DEPENDENT PRODUCTION OF ONCOSTATIN-M BY ACTIVATED CD4+ AND CD8+ LYMPHOCYTES. **Lee Ann Feeney and Dewey J. Moody. Applied Immune Sciences, Santa Clara CA 95054.** **Oncostatin M is a product of activated lymphocytes and has been shown to have regulatory as well as growth stimulatory and inhibitory functions. In addition to the inhibition of tumor cell line growth, the growth stimulation of AIDS associated Kaposi's Sarcoma spindle cells has recently been reported . We demonstrate that oncostatin M (OM) is still secreted many days after activation unlike other activation induced cytokines and this secretion is an IL-2 dependent process. Isolated $CD4^+$ and $CD8^+$ lymphocytes were activated with PHA and propagated IL-2. Supernatants were taken 24 hours after cells were put into fresh medium to insure no cytokine accumulation. Both lymphocyte subsets showed sustained production of OM. After 12-16 days of culture, high levels of OM were secreted: $CD4^+$ cells 2.2-3 ng/ml, $CD8^+$ cells from healthy controls 1-1.5 ng/ml and $CD8^+$ cells from HIV seropositive donors 1.5-1.7 ng/ml. A dose dependent reduction in OM was observed when IL-2 levels were decreased over 24 hours. The effect of IL-2 could be blocked by the addition of antibodies to the IL-2 receptor. At 1 ug/ml of anti-IL-2 receptor-a, the OM secreted from cells in the presence of 100 U/ml IL-2 was 14% to 24% of controls. The production of OM from activated $CD4^+$ and $CD8^+$ lymphocytes is dependent on IL-2. There appears to be no differences in the secretory patterns of $CD8^+$ cells from HIV seronegative and seropositive donors.**

V 368 THE EFFECT OF INTERLEUKIN 7 ON FUNCTIONAL AND PHENOTYPIC PROPERTIES OF ANTIGEN-DRIVEN CTL, G. Ferrari., K King, M. Packard, J.A. Bartlett, J. Tartaglia*, J. Toso, and K.J. Weinhold, Departments of Surgery, Medicine and Pathology, Duke University Medical Center, Durham, NC 27710 and *Virogenetics, Inc., Troy, NY 12180

Interleukin 7 (IL 7) is a stromal cell-derived cytokine with multiple biological effects, including the capacity to support maturation of precursor CTL_p into functional effectors. To further explore this activity, a series of studies was initiated to examine the effects of IL 7 in an antigen-driven system of human CTL_p cell activation. CTL_p were obtained from PBMC of fully immunocompetent donors as well as HIV-1 infected patients. CTL_p were stimulated with autologous PBMC acutely infected with either vaccinia/HIV-1 *gag* (vDK1) or vaccinia/HIV-1 env (vPE16) constructs. *In vitro* stimulations were performed in the presence and absence of recombinant human IL 7 (rh-IL 7) and relative CTL reactivity was determined by standard 51-chrominum release assays using autologous EBV-transformed B-cell lines (BLCL) infected with recombinant vaccinia/HIV-1 constructs as targets. Effector cell populations were characterized by extensive flow cytometric analysis. With CTL_p from HIV-1 infected patients, the addition of IL 7 resulted in significant augmentation of CTL reactivities over a broad range of cytokine concentrations (ie, 10U/ml-1000U/ml). However, overall cellular expansion was only evident at the higher (ie, 1000U/ml) levels. In HIV-1 seronegative donor PBMC, IL 7 increased anti-vaccinia CTL activities with no apparent effect on cell proliferation. Maximum cellular yields occurred at 7 days of stimulation while peak CTL activities did not occur until day 10. The IL 7 augmentation of CTL was accompanied by increased expression of CD25 and TcRα,β among the effector cell population. None of the IL 7 effects were observed in the absence of specific antigen activation. Thus, IL 7 may be an important biologic entity to consider as part of future immune-based therapies in which CTL may be an important determinant.

V 369 IL-4 PRODUCTION AND TYROSINE PHOSPHORYLATION OF CD3ζ, Barbara S. Fox*†, Christian R. Engwerda†, and Jerko Barbic†, *ImmuLogic Pharmaceutical Corp., Waltham, MA 02154, and †Division of Rheumatology, University of Maryland School of Medicine, Baltimore, MD 21201

Little is known about signal transduction leading to IL-4 production from helper T cells. We have used a Th0 cell hybridoma, GA15, to define the intracellular signalling pathways associated with IL-4 production. Stimulation of GA15 cells with either anti-CD3 mAb 2C11 or high doses of Ag induced IL-4 mRNA expression and IL-4 secretion. These two stimuli also induced phosphatidyl inositol (PI) hydrolysis; however, the doses of Ag and 2C11 required to induce PI hydrolysis did not correlate with the doses required to induce IL-4 production, arguing that this pathway was not tightly coupled to IL-4 production. The protein tyrosine kinase inhibitor genistein was found to inhibit IL-4 mRNA expression, suggesting that tyrosine phosphorylation was required for IL-4 production. To examine the role of tyrosine phosphorylation, GA15 cells were stimulated with 2C11 or with Ag and APC and CD3-associated proteins were isolated by immunoprecipitation. Western blotting with anti-phosphotyrosine mAbs revealed the presence of a tyrosine phosphorylated protein in the activated GA15 cells. This protein was identified as CD3ζ using 2D gels. To determine whether there was a correlation between CD3ζ phosphorylation and IL-4 production, IL-4 mRNA expression was analyzed from the same cells used for the Western blotting. Interestingly, the same doses of 2C11 and Ag were required to induce both IL-4 production and CD3ζ tyrosine phosphorylation. These data suggest that CD3ζ phosphorylation might either be required for IL-4 production or might be a marker of a required pathway. Experiments are underway to directly test these possibilities.

V 370 Stan-40, A NEW MEMBER OF THE FAS/TNFr SUPERFAMILY EXPRESSED SELECTIVELY ON ACTIVATED, HUMAN CD4+ T CELLS, Wayne Godfrey, David Buck, Marwan Harara and Edgar Engleman, Department of Pathology, Stanford University School of Medicine, Palo Alto, CA 94305.

A monoclonal antibody, designated L106, was prepared by fusion of SP2/0 cells with splenocytes from a mouse repeatedly immunized with human peripheral blood lymphocytes (PBL) that had been activated for 72 hours with PHA. MAb L106 failed to stain resting T cells, B cells, monocytes or NK cells. However, CD4 but not CD8 cells activated with antigens, mitogens or anti-CD3 antibody were brightly stained with this antibody. One-dimensional polyacrylamide gel electrophoresis of L106 immunoprecipitates obtained from activated CD4+ cells revealed a single polypeptide chain of 50 KD. To gain further insight into the structure and function of the antigen recognized by antibody L106, a cDNA library was constructed from PHA activated PBL. The cDNA was ligated into pcDNA-1, a eukaryotic expression vector, and the plasmid library was transfected into COS-7 cells. Transfected cells expressing the L106 antigen were selected by solid phase immunoadsorption, and a plasmid clone was isolated which, upon repeated transfection of COS-7 cells, conferred L106 antigen expression. Sequence analysis of the insert revealed a 1.2 KB cDNA that is homologous (65% identity) to the rat OX-40 antigen. Lesser but still significant homology was observed with TNFr, FAS/APO-1, CD27, CD30, and CD40, all of which (together with OX-40) are members of a recently described superfamily of genes encoding cytokine receptors. These findings indicate that the antigen recognized by Mab L106 is a novel member of this family. Although the function of Stan-40 remains to be determined, its selective expression on CD4+ T cells suggests that it plays a role in the growth, differentiation, or immune functions of this subset.

V 371 A MINORITY POPULATION OF CD4+ T CELLS (MEL-14^{low}) EFFECTS THE DEVELOPMENT OF THE MAJORITY (MEL-14^{high}) INTO IL-4 SECRETING T CELLS, Kenneth J. Gollob and Robert L. Coffman, Department of Immunology, DNAX Research Institute, Palo Alto, CA 94301.

CD4+ T cells can develop into either Th2 or Th1 cells and the developmental pathway taken is influenced by several possible factors, included are T cell receptor signaling, APC type (costimulatory molecules), and initial cytokine encounter. We have been using plate bound anti-CD3 or Vβ to study the development of purified T cells and determine what factors dominantly effect their development into IL-4 secreting cells (IL-4 S.C.) Stimulation of total CD4+ T cells via plate bound anti-CD3 plus IL-2 generates a population of high IL-4 S.C. This contrasts with the development of no detectable IL-4 S.C. in response to anti-Vβ6 or Mls 1^a. It is shown that the responding T cell concentration is critical in determining whether there is development of IL-4 S.C. from whole CD4+ T cells stimulated with anti-CD3 or anti-Vβ6. When the responding cell concentration of anti-Vβ6 stimulated cultures is made equivalent to that of anti-CD3 stimulated cultures, the anti-Vβ stimulus led to the development of IL-4 S.C. Thus, qualitative signaling differences were not the dominant forces in driving the development of IL-4 S.C. Furthermore, the development of IL-4 S.C. from the whole CD4+ T cell population was inhibited by anti-IL-4 mAb in all cases. Thus, some endogenous source of IL-4 was acting to induce the development of IL-4 S.C. When total CD4+ T cells were subdivided into naive and memory/activated populations using the Mel-14 marker, several important differences became apparent. In response to anti-CD3 Mel-14^{high} (naive) T cells did not develop into IL-4 S.C., and had a high proliferative capacity. In contrast, Mel-14^{low} T cells did give rise to IL-4 S.C., but expanded less. To determine if the Mel-14^{low} T cells secreted IL-4 which induced the development of the Mel-14^{high} T cells into IL-4 S.C. we purified Mel-14^{low} cells from C57Bl/6 and Mel-14^{high} from C57Bl/6, Ly5.2 congenic mice. This allowed us to follow each population and to separate them after the primary culture and determine which population of T cells were the precursors of the IL-4 S.C. which develop from the mixed T cells. It was determined that in the mixed population of T cells, the Mel-14^{high} cells develop and account for more than 95% of the IL-4 S.C. in the mixed population. This development required both the presence of Mel-14^{low} cells and IL-4 (determined by adding anti-IL-4 mAb) Thus, a minority population of T cells, Mel-14^{low}, direct the development of the majority population, Mel-14^{high}, into IL-4 S.C.

V 372 VISUALIZATION OF *IN VIVO* CD4 EFFECTOR DEVELOPMENT IN AN ADOPTIVE TRANSFER MODEL, Laura Haynes, Xiao-Hong Zhang, Phyllis J. Linton*, Loretta Mayer* and Susan L. Swain, Department of Biology, University of California San Diego, La Jolla, CA 92093 and *The Scripps Research Institute, Department of Immunology, La Jolla, CA 92037.

While there is an increasing knowledge of the generation of T helper cell effector cells *in vitro*, very little information is available concerning the development of effector T cells after exposure to antigen *in vivo*. Effector cells generated *in vitro* are large blast-like cells which display an activated phenotype (L-selectin$^-$, CD45RB$^+$, CD44^{hi}). They exhibit either Th0, Th1 or Th2 patterns of cytokine secretion depending on the cytokines present during culture.

In order to study *in vivo* CD4 T helper effector cell development, we have developed an adoptive transfer model using naive CD4 T cells from transgenic mice (Tg) with a Vβ3/Vα11 T cell receptor (TCR) that is specific for the 88-104 fragment of Pigeon Cytochrome C (PCC) and IE^k. These antigen-specific CD4 cells are transferred, along with J11d^{lo} B cells, into C3H/HeSnJ-SCID mice. Recipient mice are immunized with soluble PCC on day 0 and day 28 and the phenotype and function of the TCR Tg CD4 T cells are evaluated at various time points. The transferred CD4 T cells undergo a dramatic shift with almost all detectable CD4 cells participating. CD4 effector cells elicited during the primary response (days 4, 7 and 10) express an activated/memory phenotype (L-selectin$^-$, CD45RB$^+$, CD44^{hi}) and a Th0 (IL-2 with a small amount of IL-4 and IFNγ) pattern of cytokine secretion. After the initial response, peaking a 7 days after antigen, the majority of the CD4 population disappears, but the few cells remaining can respond and expand again when antigen when antigen is introduced later. CD4 cells involved in the secondary response (day 35) also come to express an activated/memory phenotype and they secrete a Th1 (IL-2 and IFNγ; no IL-4 or IL-5) pattern of cytokines. These results indicate that this model offers the a way of following the development of effector and memory cells *in vivo* and can be useful for studying the regulation of T effector cell and memory development.

V 373 FUNCTIONAL DIFFERENCES IN CD4 SUBSETS RELATED TO CD45 ISOFORM EXPRESSION IN CATTLE, Chris J. Howard, Gary P. Bembridge, Niall D. MacHugh, Paul Sopp, Bob A. Collins, Keith R. Parsons and Geraldine Taylor, Department of Immunopathology, AFRC Institute for Animal Health, Compton, Nr Newbury, Berkshire RG16 0NN, UK

Several isoforms of the bovine CD45 molecule have been identified with monoclonal antibodies (mAb). On $CD4^+$ T cells a high MW isoform is identified by mAb CC76 (CD45RB?) and a low MW isoform is identified by mAb IL-A116 (CD45RO). Flow cytometric analysis of expression of these isoforms on $CD4^+$ T cells has defined three subsets. The major ones are either $CC76^+$ IL-$A116^-$ or $CC76^-$ IL-$A116^+$. The third subset is $CC76^+$ IL-$A116^+$, ie. co-expresses both isoforms at a high intensity. Cells were sorted from a calf that had been immunised with inactivated respiratory syncytial virus (RSV). In proliferation assays $CD4^+$ IL-$A116^-$ $CC76^+$ cells failed to respond. Both the IL-$A116^+$ $CC76^+$ and IL-$A116^+$ $CC76^-$ subsets within the $CD4^+$ population proliferated in response to RSV antigen indicating two phenotypes of memory $CD4^+$ T cells. Differences in the cytokines produced by subsets were shown by PCR. The different phenotypes had distinct tissue distribution and recirculation patterns. In particular, in the lamina propria from areas of the gut mucosa that was devoid of organised lymphoid structures $CD4^+$ T cells were mostly (95%) of the memory phenotype.

V 374 SELECTIVE TARGETING OF NUCLEAR TRANSCRIPTION FACTORS FOLLOWING ACTIVATION OF T CELLS VIA LY-6, THY-1 OR T CELL RECEPTOR, Vladimir Ivanov, Tony J. Fleming and Thomas R. Malek, Dept. of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101.

Crosslinking of glycosylphosphatidylinositol (GPI)-anchored proteins, such as mouse Ly-6 and Thy-1, leads to IL-2 secretion and T cell activation whereas engagement of Ly-6A/E uniquely inhibits IL-2 production induced via TCR. Little is currently known concerning the molecular basis by which GPI-anchored proteins regulate IL-2 production. In this study, we have examined the ability of an anti-Ly-6A/E mAb to regulate transcription factors controlling IL-2 expression. Stimulation of Ly-6E transfected EL4J cells with anti-CD3ε or anti-Ly6A/E mAbs strongly induced nuclear NF-κB (p65-p50) and AP-1 binding activities and increased NF-AT activity while OCT1, OCT2 and NF-Y binding activities were not significantly changed. CREB and TCF1(α) binding activities were selectively induced by anti-CD3ε but not anti-Ly6A/E mAb suggesting that signaling via TCR and Ly-6 was not identical. Costimulation of these cells with both mAbs produced substantially reduced levels of AP1, NF-AT and especially NF-κB (p65-p50) binding activities whereas CREB and TCF1(α) activities were induced to a level seen after stimulation by anti-CD3ε. In contrast to Ly6A/E, anti-Thy-1 induced only the NF-AT and AP1 binding activities but not NF-κB and did not inhibit anti-CD3ε-induced NF-κB (p65-p50) suggesting that distinct signals are generated via Thy-1 and Ly-6. Using the 2B4.11 T cell hybridoma and a mutated variant revealed a crucial role of ζ chain for Ly6A/E activation of NF-κB. However, the inhibitory effect of anti-Ly-6A/E mAb on anti-CD3ε induction of NF-κB (p65-p50) appeared to be independent from the ζ chain. Taken together, these results strongly support the notion that there is selective targeting of transcription factors following T cell activation via the Ly-6, Thy-1 and CD3ε. The inhibition of anti-CD3ε-induced IL-2 production by anti-Ly6A/E may be a result of competition for a crucial signal transduction element possibly required for activation of NF-κB.

V 375 INTERLEUKIN-4 INDUCES ASSOCIATION OF PROTOONCOGENE PRODUCT OF C-*FES* TO INTERLEUKIN-4 RECEPTOR AND PHOSPHATIDYL-INOSITOL 3-KINASE, [1]Kenji Izuhara, [2]Ricardo A. Feldman, [3]Peter Greer and [1]Nobuyuki Harada. [1]DNAX Research Institute, Palo Alto, CA 94304; [2]Dept. of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201; [3]Dept. of Pathology and Biochemistry, Queen's University, Kingston, Canada, K7L 3N6.

Recently, we have demonstrated that interleukin-4 (IL-4) induces tyrosine phosphorylation of several cellular proteins including IL-4 receptor (IL-4R) itself and association of phosphatidylinositol 3-kinase (PI3-kinase) to the IL-4R. Although PI3-kinase is known to bind a specific amino acid sequence, YXXM, which is tyrosine phosphorylated, the IL-4R does not have this motif in the cytoplasmic domain, suggesting that PI3-kinase may associate with the IL-4R through some other tyrosine phosphorylated protein. Here In this study, we present identification of tyrosine phosphorylated protein with molecular weight of 92 kDa (p92) which associates both PI3-kinase and the IL-4R upon IL-4 stimulation. We have detected p92 in both immunoprecipitates of PI3-kinase and the IL-4R in several different cell types after IL-4 stimulation. Interestingly, p92 was recognized by anti-c-*fes* antibody. Furthermore, c-*fes* associates with the IL-4R in COS7 cells transfected with both IL-4R cDNA and c-*fes* cDNA. These data indicate that IL-4 induces association of c-*fes* with both the IL-4R and PI3-kinase, suggesting that *c-fes* may function as an adapter molecule between the IL-4R and PI3-kinase.

V 376 **Inhibition of Immunopathogenic HTLV-I Specific CD8+ Cytotoxic T Cell Responses in Patients With HTLV-I Associated Neurologic Disease.** Steven Jacobson, Irina Elovaara, Steven Boheme, National Institutes of Health, Building 10, Room 5B-16, Bethesda, MD 20892

High levels of circulating HTLV-I specific, $CD8^+$, HLA class I restricted cytotoxic T cells (CTL) that are known to be restricted to immunodominant regions of the HTLV-I tax protein, have been shown to be present in the peripheral blood and cerebrospinal fluid of patients with HTLV-I associated neurological disease and not in HTLV-I seropositive, asymptomatic individuals. Specific peptides of the HTLV-I tax protein have been defined which are recognized in the context of particular HLA class I alleles. An estimate of the precursor frequency of these HTLV-I tax specific CTL (pCTL) indicated an extraordinarily high frequency of 1 in 75 to 1 in 280. This suggested that HTLV-I specific CTL may contribute to the pathogenesis of HTLV-I associated neurologic disease and may therefore be a good target for immunotherapeutic attempts to eliminate these cells. The addition of the immunodominant peptide specific for these CD8+ CTL to either long term cytotoxic T cell clones or freshly isolated peripheral blood lymphocytes with defined CD8+ cytotoxicity resulted in complete inhibition of peptide-specific cytolysis. Mechanisms for this inhibition are being investigated with the most likely candidate being propriocidal inhibition due to apoptosis. These results suggest that the use of immunodominant peptides may be rational therapy to eliminate these immunopathogenic cells in vivo and potentially ameliorate disease.

V 377 T CELL SUBSET ENGAGEMENT IN ATOPIC DERMATITIS. O. Kilgus, R. Fritsche, L. Braun-Elwert, R. Holzhauser, P. Nemeth, and G. Stingl. Dept. of Dermatology, Univ. of Vienna Med. School, Vienna, Austria

Evidence exists that contact hypersensitivity (CHS) is mediated by TH1 cells. Atopic dermatitis (AD) histologically and clinically resembles CHS and, in some patients, "aeroallergen" patch tests result in eczematous skin lesions resembling AD; on the other hand, AD is also associated with elevated IgE serum levels, a feature suggesting TH2 involvement. The type of T helper cell subsets activated in AD and the signals provided by them in vivo have not yet been unraveled. We therefore investigated whether the cytokine profile in vivo in atopic dermatitis differs in any way from that of non-atopic forms of eczema, e.g. CHS or nummular eczema (NE). RNA extracted from 4 mm punch biopsies of atopic dermatitis (n=7), CHS (5 biopsies from 3 patients), NE (n=1), and normal skin (n=5) was investigated using suitable constructs for competitive quantitative PCR for IL-2, -4, -5, -10, IFN-g, and glycerol-3-phosphate dehydrogenase (G3PDH). In normal skin, we were able to detect low levels of IL-2, -10, and IFN-g, but neither IL-4 nor -5. In AD lesions, the following pattern was observed: IL-4 and/or -5 were positive in most samples (6/7), IL-2 and -10 were upregulated to a variable extent and, remarkably, no significant induction of IFN-g was detected in any of the samples. In contrast, in 4 of the 6 biopsies of non-atopic eczema, marked induction of IFN-g in addition to IL-2, -4, -5, and -10 was observed. These data indicate that AD in vivo has features of a TH2 immune response when compared to CHS or NE and, thus, that an eczematous (type IV) skin reaction can occur on the basis of a TH2 response. However, both in AD and CHS, TH1 and TH2 cytokine expression was not mutually exclusive in vivo; rather, the balance appears to be shifted to either side depending on the individual as well as on the disease.

V 378 PERSISTENT HIGH gagCTLp FREQUENCY AND LOW VIRAL LOAD IN LONG-TERM ASYMPTOMATIC HIV-1 INFECTION. Michèl R. Klein*; C.A. van Baalen, C.A.**, R.J.Bende*, I.P.M. Keet***, J.K.M. Eeftinck Schattenkerk***, and F. Miedema*
*Department of Clinical (Viro) Immunology of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. **Natl. Inst. of Public Health and Env. Protection, Bilthoven. ***Academic Medical Centre, Amsterdam. ****Municipal Health Service, Amsterdam, The Netherlands.

Objective: Long-term survival in HIV infection may be related to an effective host immune response against HIV-1 and/or to viral properties. To investigate the cellular immune response to HIV-1 we analyzed gag-specific CTL activity in long-term asymptomatic HIV-1 infection.

Methods: 15 Longterm asymptomatic individuals with CD4 counts >500 cell/μl after more than 7 years of infection were selected from The Amsterdam Cohort Study on AIDS. CTL activity was measured on ^{51}Cr labelled autologous B-LCL, infected with recombinant vaccinia viruses expressing HIV-1 gag. Both bulk and limiting dilution CTL assays were performed with PBMC after Ag-specific stimulation.

Results: Bulk CTL response after Ag specific stimulation is MHC class I restricted and mediated by CD8+ T-lymphocytes. Longitudinal studies, spanning 7 years, showed high gag CTLp frequencies and a low virus load. CTL response was directed against several epitopes in gag.

Conclusions: Sustained, broad anti-HIV cellular immunity may correlate with maintenance of the asymptomatic state in long-term survival by controlling the viral replication.

V 379 MHC-LIGAND AFFINITY DETERMINES PRODUCTION OF Th1 OR Th2-LIKE LYMPHOKINE SECRETION PATTERN BY THE SAME Th CELL. Vipin Kumar, Vatsala Bhardwaj, Luis Soares, Alessandro Sette* and Eli Sercarz. Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024, *Cytel Corp. San Diego, CA 92121.

Both Th1 and Th2 cells are thought to differentiate from a common pool of precursors as a consequence of activation by the ligand, the MHC/antigen complex. It is not known how the structure of the ligand itself influences the production of lymphokines, for example, IFN-γ (Th1) and IL-4/IL-5 (Th2). A murine CD4$^+$ T cell clone specific for myelin basic protein (MBP) responded to its immunodominant determinant, Ac1-9, by both IL-4 secretion and proliferation. A strong heteroclitic proliferative response to peptide variants of Ac1-9, with hydrophobic amino acid residues at position 4, correlated with their ability to bind strongly to the restricting MHC molecule, I-A^u. We have studied how stimulation of T cells with variants of different MHC-binding affinities resulted in differential lymphokine secretion patterns in vitro. Furthermore, the effect of MHC-binding affinity on the frequency of IL-4/IL-5 or IFN-γ-producing cells was studied in vivo. Our data suggest that MHC-binding affinity of antigenic determinants is crucial for the differential development of cytokine patterns in T cells and therefore represents a potential target for immune manipulation of Th1 or Th2 cells involved in autoimmune as well as allergic responses. Supported by funds from NIH.

V 380 SELECTIVE MODULATION OF CYTOKINE PRODUCTION BY CD4+ T CELL SUBSETS THROUGH CO-STIMULATORY SIGNALS, Riitta Lahesmaa*, Marie-Claude Shanafelt, Carol Soderberg, Andrea Allsup and Gary Peltz, *Dept. of Neurology, Stanford University, Stanford, CA 94305, and Dept. of Leukocyte Biology, Syntex Research, Palo Alto, CA 94304.

The selective activation of CD4+ T cell subsets with distinct profiles of cytokine production has been shown to play an important role in the pathogenesis of human inflammatory or allergic diseases. To determine whether cell adhesion/activation molecules are involved in this process, we have characterized the level of expression of 28 activation or adhesion molecules on the surface of a panel of human CD4+ T cell clones representative of Th1 and Th2 cells. The T cell subsets could not be distinguished by the cell surface markers examined, including isoforms of CD45. To test the possibility that differential activation of costimulatory pathways is another mechanism for selectively modulating cytokine production by CD4+ T cells, the profile of cytokines produced by human CD4+ T cell clones representative of Th1 or Th2 cells, in response to ligation of different costimulatory molecules was measured. Th1-like T cell clones secreted the same profile of cytokines, irrespective of which costimulatory pathway was engaged. However, the profile of cytokines produced by some, but not all, Th2 T cell clones depended upon costimulatory signals activated. The two T cell subsets did not differ in their proliferative response when different costimulatory pathways were activated. These results indicate that the costimulatory pathways activated by antigen presenting cells can selectively influence cytokine production by a CD4+ T cell subset.

V 381 **A THY-1 ASSOCIATED MOLECULE IDENTIFIES A SUBSET OF CD4 CELLS AND IS REQUIRED FOR THY-1 ACTIVATION.** A. Lehuen, L. Beaudoin and R.C. Monteiro
INSERM U25- Hôpital Necker- Paris- France.

A surface protein expressed on T cells has been characterized using natural autoantibodies. This protein has an apparent molecular mass of 100,000 (p100), and appears to be non-covalently associated to two PI-linked molecules, Thy-1 and ThB. The insensitivity to PI-PLC treatment suggests that p100 protein has a transmembrane domain. This protein is expressed by the majority of thymocytes, all peripheral CD8 cells but only ~60% of CD4 cells. In the present study, we show evidence that p100 protein is involved in signal transduction through Thy-1 molecule. Only peripheral CD4 $p100^+$ cells, but not CD4 $p100^-$ cells, proliferate in response to Thy-1 cross-linking by anti-Thy-1 antibodies whereas both populations express high levels of Thy-1 molecules on their surfaces. Control stimulation by anti-CD3 antibodies or concanavalin A induces identical thymidine uptake of both populations of CD4 cells. Similarly as observed for proliferation, only CD4 $p100^+$ but not CD4 $p100^-$cells release cytokines, such as IL-2, IFNγ, and IL-4, after incubation with anti-Thy-1 antibodies. Furthermore, p100 expression is also required for aggregation phenomena observed during Thy-1 mediated activation since CD4 $p100^-$ do not aggregate after Thy-1 cross-linking. However, concanavalin A induces aggregation of both CD4 populations. Finally, immunoprecipitation followed by in vitro kinase assay reveals that anti-Thy-1 antibodies can co-immunoprecipitate several phosphorylated proteins of 90, 60, 56 and 33 kDa, from total CD4 cell lysate but not from CD4 $p100^-$ cells. Altogether, these data stongly suggest that p100 protein provides a link between the PI-anchored molecule, Thy-1, and intracytoplasmic kinase(s).

V 382 ANALYSIS OF ANTIGEN SPECIFIC T CELL RESPONSIVENESS IN TUMOR BEARING MICE. Levitsky, H.I., Staveley-O'Carroll, K.F., Bruce, A.T., and Pardoll, D.M. Johns Hopkins University School of Medicine.

A central question in understanding how tumors evade immunologic rejection is what effect tumor presentation of antigen has on the activation of antigen-specific T cells. Such an analysis has previously been difficult because of the cryptic nature of most tumor antigens, and the inability to identify antigen specific T cells present at a low precursor frequency. We utilized TCR transgenic mice which have 20% of their peripheral T cells specific for influenza HA peptide (aa 111-120) presented on I-E^d. HA was expressed in two H-2d tumor lines after transfection and selection. Renca-HA, an HA transfected renal cell carcinoma model, is MHC class II^-, but can be induced to express class II antigens after exposure to interferon. A20-HA is an HA transfected B cell lymphoma which constitutively expresses high levels of MHC class II antigens. In vitro, naive transgenic T cells proliferate vigorously after stimulation with A20-HA but not A20 wild type, while proliferation is seen in response to Renca-HA only if it has been pretreated with γ-interferon. Remarkably, both Renca-HA and A20-HA grow progressively in transgenic mice after subrenal capsule or i.v. injection respectively. Clonotype$^+$ T cells are not deleted. Histology reveals no significant inflammatory response to the HA bearing tumors relative to non-transfected tumor. Tumor explanted from these mice retain HA expression, and explanted A20-HA cells still stimulate naive transgenic T cells in vitro. Purified T cells from tumor bearing mice are being analyzed for their capacity to respond to HA peptide + APCs in terms of proliferation and pattern of cytokine expression.

V 383 Fc RECEPTORS (FcR) EXPRESSED BY T_H2 CELLS ARE PHYSICALLY ASSOCIATED WITH A TcR-CD3 COMPLEX CONTAINING FcεRI-γ CHAINS. R.P.Lowry, B.Konieczny, T.Takeuchi & P.Selvaraj. Depts. of Medicine and Pathology, Emory University School of Medicine, Atlanta, GA 30322.

Protein tyrosine kinase $p59^{fyn}$ is known to associate with the membrane proximal portion of CD3-ζ and to be essential for PI hydrolysis & Ca^{2+} flux as obtains in Th1 cells upon antigen-receptor occupancy. Conversely, $p59^{fyn}$ is not known to associate with the FcεRI-γ chain which is present in lieu of CD3-ζ in TcR-CD3 of "LGL" and appears to mediate transmembrane signalling (J.Exp.Med. 175:203). Th2 cells flux Ca^{2+} weakly, if at all, following TcR-CD3 crosslinking. We reasoned that FcεRI-γ might also exist in Th2 and mediate transmembrane signalling as a component of CD3. Therefore we examined the structure of CD3 as expressed by Th1 & Th2 cell lines, D1.1 & CDC25, respectively, kindly provided by D.Parker, Worcester MA. Equivalent numbers of metabolically labelled (^{35}S-cysteine/methionine), or unlabelled, Th1 & Th2 cells were solubilized in a digitonin buffer and TcR-CD3 complexes were immunoprecipitated with anti-CD3ε mAb 2C11, electrophoresed in 2D non-reducing/reducing or 1D gradient PAGE gels & submitted to autoradiography or western analysis using anti-ζ,-η or anti-FcεRI-γ antisera provided by Drs. Klausner and Kinet, respectfully. We find that Th1 & Th2 cells both express CD3-ζ,-η heterodimers while only Th2-derived TcR-CD3 immunoprecipitates contain FcεRI-γ homodimers. Additionally, since Th2 cells, and not Th1, are reported to express Fc receptors, a semiquantitative immunoprecipitation assay system was evolved to ascertain whether these FcR are physically associated with the TcR-CD3 complex of Th2. Immune complexes (IC) were prepared by reacting rabbit anti-mouse IgG (MIg) with MIg covalently coupled to Sepharose. Preliminary experiments established that anti-FcγR mAb 2.4G2 precipitable receptor was partially or fully cleared from lysates of a macrophage cell line by one or two exposures to IC, respectively, as determined by western analysis. TcR-CD3 associated FcεRI-γ chains identified in western blots of anti-CD3ε precipitates of Th2 cells were also removed when cell lysates were precleared with Sepharose coupled IC but not by Sepharose alone. These data suggest that the FcεRI-γ chain might represent the primary signal transduction molecule associated with the antigen-receptor of Th2 and that FcR are physically associated with this multichain complex. It is conceivable that CD3-ζ & -η identified in Th2 lysates might be more closely associated with FcR and thus expressed during restricted phases of the cell cycle progression.

V 384 IL-12 AND ANTI IL-4 RESULT IN TH1 PHENOTYPE DEVELOPMENT VIA DIFFERENT MECHANISMS IN A TCR TRANSGENIC MOUSE MODEL: TH1 COMMITTED CELLS REQUIRE COSTIMULATORS FOR MAXIMAL IFNγ PRODUCTION, Steven E. Macatonia[1], Chyi-Song Hsieh[2], Janice A. Culpepper[1], Maria Wysocka[3], Giorgio Trinchieri[3], Kenneth M. Murphy[2] and Anne O'Garra[1]. [1]Department of Immunology, DNAX Research Institute, Palo Alto, CA 94303., [2]Washington University School of Medicine, St Louis, MO 63110, and [3]The Wistar Institute, PhiladelphIa, PA 19104.

Antigen specific CD4+T cells isolated from naive αβ TCR transgenic mice were used to examine mechanisms of Th1 development. T cells stimulated with OVA and either dendritic cells or spleen cells as APC in the presence of IL-12 or anti IL-4 mAbs induced Th1 cells with the capacity to secrete high levels of IFNγ on restimulation with splenic APC. Inductio of Th1 development by these two treatments appeared to operate by separate mechanisms. IL-12 mediated induction of Th1 development required IFNγ to be present during priming. In contrast, anti IL-4 mediated Th1 development was independent of IFNγ. Once Th1 cells were generated, upregulation of IFNγ production required costimulatory signals provided by splenic APC but not from dendritic cells. Th1 cells stimulated by dendritic cells required exogenous IL-12, TNFα and IL-1 to produce levels of IFNγ equivalent to that induced by splenic APC. This requirement for IL-12, TNFα and IL-1 for upregulation of IFNγ secretion from Th1 cells was confirmed by antibody blocking studies. mAb to each of these cytokines inhibited IFNγ secretion following stimulation by splenic APC. These results indicate that mechanisms for induction of Th1 development may occur either by positive signalling mediated through IL-12 which is IFNγ dependant, or via a default pathway by removal of endogenous IL-4. In addition antigen specific stimulation of committed Th1 cells requires additional costimulatory factors such as IL-12, TNF-a and IL-1 to demonstrate optimal IFNγ production. These results illustrate that complex regulatory events control the development of cell mediated immunity.

V 385 DYSFUNCTION OF Th1 CELLS AND OUTGROWTH OF Th2 CELLS AFTER HIV INFECTION,

Linde Meyaard[1], Sigrid A. Otto[1], Ireneus P.M. Keet[2], René A.W. van Lier[1], and Frank Miedema[1].
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Before CD4+ T cells are depleted, in asymptomatic HIV-infected individuals delayed type hypersensitivity reactions are decreased and T cells are non-responsive to activation via the CD3/T-cell receptor complex with low release of Th1 cytokines. In addition, clinically a relative dominance of Th2-mediated responses can be recognized during HIV infection. There is an increased incidence of allergic dermatitis and asthma, and elevation of serum IgE levels together with increased numbers of IgE antibodies against environmental antigens are indicative for allergic disease. We addressed the question whether HIV-infection could be associated with a dysfunction of Th1 and subsequent outgrowth of Th2-like T cells.

We investigated the type of T cells that could be cloned from HIV-infected individuals. T cells randomly cloned from HIV-infected individuals showed a high proportion of IL-4, IL-5 and/or IL-10 producing clones upon activation, whereas from an HIV-negative control, T-cell clones produced only low or not detectable levels of these cytokines. In contrast, T cells cloned from the HIV-negative control produced high levels of IL-2 and IFN-γ, whereas lower IL-2 and/or IFN-γ production was found in clones derived from the HIV-infected individuals. The finding that increased numbers of Th2-like T-cell clones can be isolated after HIV-infection was further substantiated by clones derived in parallel from cryopreserved material from the same individual before infection with HIV and 26 months after seroconversion. Again, higher numbers of clones producing IL-4, IL-5 and IL-10 were found after HIV infection in contrast to the clones derived from the time point before infection.

Thus, upon HIV-infection, clones secreting predominantly Th2-like cytokines are generated. This might be the consequence of HIV infection of macrophages, which occurs during all stages of HIV infection. Infection of antigen presenting cells might lead to impaired signalling to T cells or decreased production of cytokines like IL-12 resulting in dysfunction of Th1 cells and outgrowth of Th2 cells. This might contribute to the attenuation of the immune system and impaired responses to foreign antigens as observed in HIV-infected individuals.

V 386 LIGATION OF CD3 ON POLYCLONALLY ACTIVATED CD8+ CELLS FROM PERIPHERAL BLOOD. Dewey J. Moody, Amy L. Emerson and Lee Ann Feeney, Department of Infectious Diseases, Applied Immune Sciences, Santa Clara CA 95054.

CD8+ cells were isolated from the peripheral blood of HIV seropositive donors using a solid phase capture system. The isolated cells were activated with phytohemagglutinin and propagated with 600 to 1200 IU/ml recombinant human interleukin-2 (rIL-2, Chiron). A dichotomy in responsiveness to CD3 ligation was observed on the cultured CD8 cells. Interferon-gamma secretion was significantly ($p<0.05$) lower with control cells (372±588 pg/ml) than with cells stimulated (24 hr) with anti-CD3 (OKT3, 1927±1058 pg/ml). This effect was not significantly ($p>0.05$) different between CD8+ cells from HIV seropositive (mean, 1460 pg.ml) or seronegative (mean 2161 pg/ml) donors. Ligation of the cultured CD8+ cells, however, also produced negative effects. The proportion of apoptotic CD8+ cells (as detected by flow cytometry) following stimulation with 20 ng/ml of anti-CD3 ranged from 10 to 26% between 24 and 72 hours post-stimulation whereas control cultures ranged from 2-8% positive. Preliminary data indicate that there is a negative correlation ($r=-.7023$) between the dose of rIL-2 and the proportion of cells in S-phase. We hypothesize that the sensitivity to CD3 ligation may be related to the proportion of cultured cells in S-phase. Interleukin-2 dose response experiments with respect to apoptosis induction via CD3 stimulation of propagated CD8+ cells are in progress.

V 387 CYTOKINE EXPRESSION AND CONTROL OF RETROVIRUS-INDUCED IMMUNODEFICIENCY IN THE MOUSE, R. Morawetz, N.Giese, M.Doherty, W.Muller, R.Kuhn, K.Rajewsky, R.L.Coffman and H.C. Morse, Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD 20892, Institute for Genetics, University of Cologne, Cologne, Germany and DNAX Research Institute, Palo Alto, CA 94304

Development of retrovirus-induced immunodeficiency (MAIDS) in disease-sensitive mice (e.g. B6) is associated with a Th2 pattern of cytokine expression while a more Th1-like pattern develops in disease-resistant mice such as strain 129 or B10.D2. To determine if the balance of Th1 andTh2 cytokines is a crucial determinant of resistance or sensitivity to MAIDS, we examined the course of disease in mice in which this balance was disrupted by the administration of anti-cytokine mAb, constitutive expression of a cytokine from a transgene, or by the inability to produce specific cytokines resulting from gene knockouts. We found first, that treatment of B6 mice with anti-IL-4 mAb from the time of infection through 8 weeks or at various intervals beginning 4 weeks post infection had no prominent effect on the development of MAIDS. Furthermore, B10.D2 mice treated with anti-IFN-γ mAb were not rendered susceptible to MAIDS. Second, we observed that B6 mice homozygous for a disrupted IL-4 gene (6 generation backcross) developed disease like normal B6 animals. Finally, we showed that 129 mice bearing an IL-4 transgene were unaltered in their resistance to MAIDS, even though the level of IL-4 expression is sufficient to render them susceptible to Leishmania major. In addition, no signs of MAIDS were observed in IL-4 transgenic mice treated with anti-IFN-γ mAb. However, B6 mice infected with L. major as an inducer of a Th1 cytokine response and treated with anti-IL-4 mAb showed greatly reduced manifestations of MAIDS. These findings suggest that the contributions of cytokines to development of resistance or sensitivity to MAIDS are more comlex than for infections with certain parasites and that MAIDS cannot be explained simply by crossregulatory influences of Th1 and Th2 cytokines.

V 388 MODELING TH1/TH2 CROSS REGULATION

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T helper cells can be subclassified according to the lymphokines they secrete following activation in to two types: Th1 cells which secrete IL-2, IFN-γ and mediate the delayed type hypersensitivity response and Th2 cells which secrete IL-4, IL-5, IL-10 and provide help for specific B cells. Th1 and Th2 cells cross-regulate each other via the action of the lymphokines they secrete. We present the analysis of a mathematical model of the regulation between Th1 and Th2. This model involves Th1, Th2 and the lymphokines IL-2, IL-4, IL-10 and interferon-γ. The different effects of the lymphokines and their couplings have been modeled so that they reproduce *in vitro* results. I.e. they take into account the biological variability between cells, the time delays, the competition (antagonism and synergy) between lymphokines and the role of macrophage in the effect of IL-10. All the components of the Th1 and Th2 system which have been experimentally tested are put together to build a model of the cross regulation between the two kinds of T helper cells. The model has been tested experimentally using a panel of Th1 and Th2 clones. This model is a direct translation in equations of what is assumed or understood about this system.

The model is run on a supercomputer for different immunological conditions. The immediate goal is to compare the predictions of the model with corresponding in vitro situations. The ability of this model to reproduce *in vitro* experiments is important to see whether the Th1/Th2 system dynamics can be reduced to these components or whether it involves other ingredients. A longer term goal is use this model to get insight *in vivo*, in situations where the dynamics of the cross-regulation between Th1 and Th2 seems to play an important role in the regulation of the immune response.

V 389 MECHANISMS OF DIFFERENTIATION OF NAIVE CD4+ T CELLS FROM TCRαβ TRANSGENIC MICE INTO IFN-γ AND IL-4 PRODUCING EFFECTOR CELLS, Victor L. Perez, James A. Lederer, Andrew H. Litchman and Abul K. Abbas, Department of Pathology, Harvard Medical School and Brigham & Women's Hospital, Boston, MA 02115.
The roles of exogenous cytokines, antigen-presenting cells(APCs) and antigen in the differentiation of naive CD4+ T-cells into Th1 and Th2 like subsets has been examined using T-cells from mice expressing a transgenic T-cell receptor (TCR) specific for pigeon cytochrome c + I-E^k. IL-12 and IL-4 are potent inducers of Th1 and Th2 development respectively. Both cytokines act directly on naive T-cells and not on APCs. Quantitative RT-PCR analyses indicate that IL-12 (and probably IL-4) may regulate the transcription of endogenous cytokine genes in response to antigenic stimulation. Experiments are being done to determine if this is due to an effect of the exogenous cytokines on the production of transcription factors specific for IL-2, IFN-γ and IL-4. IL-12 induced Th1-like populations can be converted to IL-4 producers by exposure to exogenous IL-4, but Th2 differentiation is not reversible. Splenic adherent cells (SAC) are more potent inducers of Th1 cells than purified B lymphocytes, because SAC secrete IL-12 and may express costimulators that are required for the production of IL-2 but not IL-4. The concentration of antigen to which naive T-cells are exposed also influences the cytokine phenotypes of the T-cells that develop. We are examining the effect of antigen concentration on the transcription of cytokine genes and on the expansion or inhibition of either subset. Finally, analogs of the cytochrome c (81-104) peptide containing substitutions in TCR contact residues are being used to define the influence of TCR-antigen interactions in the development of Th1 and Th2-like cells.

V 390 **CLONAL POPULATIONS OF CD8+CD28- T CELLS IN NORMAL ELDERLY HUMANS,** David N. Posnett, Rakhi Sinha, Shara Kabak, Carlo Russo. Dep. of Medicine, Cornell University Medical College, and the Immunology Graduate Program, Cornell University Graduate School of Medical Sciences, New York, NY 11021.
To determine whether T cells, like B cells, can become clonally expanded in normal individuals as a function of age, we compared the T cell Vß repertoire of cord blood to that of peripheral blood from normal donors over 65 years of age. T cells from elderly subjects contained expanded subsets (greater than the mean + 3 standard deviations) of TCR Vß populations. These expanded subsets were observed primarily among CD8, but not CD4 cells, represented up to 37.5% of all CD8 cells and are probably present in almost all normal elderly subjects. An expanded Vß5.2/3 CD8 subset and a Vß6.7a CD8 subset from separate donors were analyzed by RT-PCR, cloning and sequencing of the TCR ß chain VDJ junction. They were mono- or oligoclonal while control CD4 subsets were polyclonal. Using 2 color flow cytometry it was possible to identify the expanded oligoclonal subsets as CD8+ CD28- CD11b+ cells. Moreover they lacked a specific proliferative response to the relevant anti-Vß MAb. These cells are similar to human IEL, which are also known to be oligoclonal.

V 391 BIOCHEMICAL AND PHENOTYPIC ANALYSIS OF ICAM -3 INDUCED LYMPHOCYTE ACTIVATION, Mary Poss and Gary Peterman, ICOS Corp., 22021 20th Ave SE, Bothell, WA. 98021
We have examined human T lymphocyte activation in response to monoclonal antibodies specific for CD3 and ICAM-3. For these studies, peripheral lymphocytes were separated into resting and activated subsets by discontinuous gradient centrifugation on Percoll. High buoyant density, resting cells were composed of CD3+ T cells which contained both CD45RA+ and CD45RO+ cells and were negative for CD69, CD25 and CD71. In contrast, medium buoyant density activated cells were composed of CD3+ cells which contained both CD45RA+ and CD45RO+ cells but were variably positive for CD69, CD25 and CD71. Kinetics of response to activation through the T-cell antigen receptor and ICAM-3 was evaluated biochemically and phenotypically for each population. We find that the medium buoyant density fraction appears to be in a primed state and the level of this state of activation was variable among donors whereas the high buoyant density fraction displayed a truely resting phenotype regardless of donor. Cells from the medium buoyant density fraction responded more quickly in protein kinase C and MAP Kinase translocation assays in response to OKT3 than did cells from the high buoyant density fraction. Cells in both fractions were capable of translocating PKC but not MAPK following engagement of ICAM-3 with immobilized antibody for up to 90 min, although this response was seen more consistently with high buoyant density cells. We conclude that resting lymphocytes obtained by Percoll density centrifugation contain both CD45RA+ and CD45RO+ cells may provide a good model for activation mediated by ICAM-3.

V 392 **GENERATION AND FUNCTIONAL ACTIVATION OF ANTIGEN-SPECIFIC T_H1 AND T_H2 CLONES IN THE ABSENCE OF PROTEIN TYROSINE KINASE p59*fyn*** Dapeng Qian, Patrick Fields, David Lancki, Tom Gajewski, and Frank W. Fitch Ben May Institute, University of Chicago, Chicago, IL 60637
The hematopoietic form of protein tyrosine kinase p59*fyn* has been implicated in T-cell signal transduction and activation. In this study, we examined the requirement of Fyn expression for the generation and functional activation of murine T_H1 and T_H2 clones. We derived a panel of antigen-specific CD4+ T-cell clones having the characteristics of T_H1 (producing IL-2 and IFN-γ) and T_H2 (producing IL-4) subsets from *fyn*$^{-/-}$ mutant mice (kindly provided by Drs. P. Stein and P. Soriano) immunized with ovalbumin. These T-cell clones did not express normal Fyn protein as determined by immune-complex kinase reaction using anti-Fyn antibody. Upon antigen stimulation, T cell clones from both normal and *fyn*$^{-/-}$ mice demonstrated comparable levels of functional responses, including lymphokine secretion, proliferation, and antigen-specific cytolysis. Thus, p59*fyn* is not required for differentiation and antigen-induced functional activation of T_H1 and T_H2 subsets. We are currently studying the early signaling events (e.g., tyrosine phosphorylation of several substrates) in these *fyn*$^-$ T-cell clones, and these results will be presented.

V 393 CYTOKINE INVOLVEMENT IN LCM DISEASE OF β_2-MICROGLOBULIN-DEFICIENT MICE. Daniel G. Quinn and Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599.

Intracranial (i.c.) injection of β_2-microglobulin deficient (β2m-) mice with lymphocytic choriomeningitis virus (LCMV) results in a chronic wasting disease, often followed by death. We have previously shown that β2m- mice develop CD4+, MHC class II-restricted CTL following LCMV infection, and that these cells are capable of transferring disease to immunosuppressed, infected animals. We have now investigated the roles of cytokines in LCM disease. β2m- mice were treated with neutralizing antibodies against interferon (IFN)-γ or interleukin (IL)-4 prior to i.c. infection with LCMV. Pretreatment of β2m- mice with anti-IFN-γ prevented weight loss and death following LCMV challenge. In contrast, anti-IL-4 antibody had no apparent effect. This suggests that a T_H1 type T cell response may play a role in LCM disease in β2m- mice. Given the wasting nature of LCM disease in these mice, we measured the production of TNF-α by LCMV-specific, MHC class II-restricted CTL. In response to LCMV, CD4+ T cells from β2m- mice produce TNF-α, and induce bystander lysis of non-infected, TNF-α sensitive target cells. TNF-α, however, is not solely responsible for the cytotoxic effects of these cells, since a neutralizing anti-TNF antibody did not totally inhibit antigen specific lysis. Further, TNF-α resistant target cells are also killed in an antigen-dependent, MHC-restricted manner by CD4+ T cells from LCMV infected β2m- mice. TNF-α production by antigen specific T cells suggest that this cachectic cytokine may mediate weight loss in LCMV infected β2m- mice. Neutralizing antibody experiments *in vivo* will directly address this question.

V 394 **T_H1 DEVELOPMENT OF NAIVE CD4$^+$ T CELLS DEPENDS ON THE COORDINATE ACTION OF IL12 AND IFNγ AND IS INHIBITED BY IL4 AND TGFß1,** Edgar Schmitt, Petra Hoehn, Norbert Palm, Erwin Ruede, and Tieno Germann, Institute for Immunology, 55101 Mainz, Germany.

IL12 was reported to be a macrophage-derived cytokine decisively involved in cellular immune responses, especially by activating CD4$^+$ and CD8$^+$ T cells as well as NK cells. We investigated the influence of IL12 on the development of naive CD4$^+$ T cells activated by immobilized anti-CD3 mAb in the absence of accessory cells. Differentiation of naive CD4$^+$ T cells was monitored by determining the secondary production of IFNγ (T_H1) and IL4 (T_H2).

In combination with a T cell receptor mediated signal IL12 turned out to be a potent inducer of T_H1 development. This effect of IL12 could be abolished by neutralizing anti-murine IFNγ (mIFNγ) mAb but IL12 could not be replaced by exogenous mIFNγ. Further experiments revealed that T_H1 development of naive CD4$^+$ T cells depends on the coordinate action of IL12 and IFNγ. Another cytokine involved in T_H-differentiation is TGFß1. TGFß1 was reported to enhance IFNγ production by CD4$^+$ T cells and to support their differentiation to T_H1 cells. In contrast to these data, we found that TGFß1 strongly inhibited the IL12 induced production of IFNγ by naive CD4$^+$ T cells as well as their development towards T_H1 phenotype. These data were confirmed using neutralizing anti-TGFß1 Ab. IL4, a cytokine known to be essential for T_H2 development of naive CD4$^+$ T cells, completely abolished T_H1 differentiation promoted by IL12. Conversely, IL12 could not prevent IL4 induced T_H2 development even in the presence of relatively low amounts of IL4.

Thus, our data demonstrate that IL12 is a potent differentiation factor for T_H1 cells and that its effect depends on the costimulation by IFNγ. Furthermore, it was found that TGFß1 and IL4 are potent inhibitors of the T_H1 differentiation inducing capacity of IL12.

V 395 EFFECT OF rIL-12 AND CD8$^+$ T CELLS ON THE *IN VITRO* MATURATION OF CD4$^+$ CORD BLOOD T CELLS INTO IL-4 PRODUCING CELLS, Patricia V. Schneider[1], Usha Chitkara[2], Jan E. de Vries[1] and Hans Yssel[1], [1]Human Immunology Department, DNAX Research Institute and [2]Department of Gynecology and Obstetrics, Stanford Medical School, Palo Alto, CA 94304

As has been shown in the mouse, cytokines like IL-4 and IL-12 play an important role in the differentiation of CD4$^+$ T cells into Th2 and Th1 T cell subsets, respectively. In human however, studies on the effects of cytokines on the differentiation of T cells into subsets with different cytokine production profiles have only been carried out in antigen primed T cells and little information is available about the effects on immunologically naive T cells. Therefore, we have investigated the effect of various cytokines on maturation of CD4$^+$, CD45RA$^+$ cord blood (CB) T cells, which produce high levels of IL-2 and low levels of IFN-γ upon primary activation, but no detectable amounts of IL-4 and IL-5 and are thus limited in their capacity to produce Th2 cytokines. Highly purified CD4$^+$ CB T cells were stimulated with anti-CD3 mAb, crosslinked on CD32-expressing L cells that had been cotransfected with B7 and LFA-3, in the presence of recombinant (r)IL-4, rIFN-γ and rIL-12 or combinations thereof. Six days later, the cells were restimulated with anti-CD3 mAb presented by L cell transfectants and the cytokine production was measured by specific ELISA in 48 h culture supernatants. Culturing the cells in the presence of rIL-4 resulted in the production of IL-4 and low levels of IFN-γ upon restimulation. On the other hand, CB T cells cultured in rIL-12 or rIL-4/rIL-12, produced high levels of IFN-γ. Interestingly however, rIL-12 grown cells also produced significant levels of IL-4 upon activation, whereas the addition of rIL-12 to rIL-4-cultured cells had a synergistic enhancing effect on the production of IL-4. The rIL-12-mediated induction of IL-4 production was not enhanced by the addition of a neutralizing anti-IFN-γ mAb, whereas it was only slightly inhibited by the addition of rIFN-γ. These results indicate that IL-12 is not only involved in the development of CD4$^+$ Th1 T cells, but in that of CD4$^+$ Th2-like T cells as well.

Addition of equal amounts of autologous CD8$^+$ T cells to the CD4$^+$ CB T cell cultures was found to inhibit the differentiation into IL-4 producing cells. Results based on experiments using neutralizing anti-IFN-γ mAb indicated that this effect seems to be only in part mediated by IFN-γ. This notion was underscored by the finding that addition of rIL-12 to the culture system, which resulted in the differentiation of IL-4 producing cells, also induced high production levels of IFN-γ. The nature of the inhibitory activity displayed by CD8$^+$ is currently under investigation.

V 396 CYTOKINE AND P24 PROFILES IN GP160, GP120, AND TETANUS SPECIFIC T-LYMPHOCYTE LINES FROM HIV SEROPOSITIVE PATIENTS. K.V. Sitz, Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, MD 20850; S. Ratto^, F. Manca, R.R. Redfield, D.L. Birx, and the MMCARR

We have developed CD-4 + T-lymphocyte lines specific for gp160, gp120, and tetanus toxoid (TT) in HIV seropositive patients enrolled in a double-blinded, placebo-controlled, gp160 vaccine therapy trial. CD4+, antigen-specific, T-lymphocyte lines were developed from the PBMCs of 10 patients, 150 days after beginning either gp160 or placebo injections, by repeated stimulation with autologous APC's pulsed with gp160, gp120, or TT. Supernatants from lines which were specific for antigen after the 3rd stimulation were analysed for the presence of IFN-G, TNF-ß, IL-4 , IL-6, andp24 .

Results: Out of 29 lines from 10 patients:

Observed Frequencies for Antigen, p24

	Not Performed	Undetectable	Positive	Totals
gp160	0	8	1	9
gp120	1	6	3	10
tetanus toxoid	1	6	3	10
Totals	2	20	7	29

Observed Frequencies for TNF-B, p24

	Not Performed	Undetectable	Positive	Totals
Positive	0	6	0	6
Undectectable	2	14	7	23
Totals	2	20	7	29

Observed Frequencies for IL-6, p24

	Not Performed	Undetectable	Positive	Totals
Positive	2	14	6	22
Undetectable	0	6	1	7
Totals	2	20	7	29

Observed Frequencies for IFN-G, p24

	Not Performed	Undetectable	Positive	Totals
Positive	2	14	6	22
Undetectable	0	6	1	7
Totals	2	20	7	29

Conclusions: Although, the numbers are too small for statistically significant comparisons, we are intrigued by the fact that of the supernatants which were positive for TNF-ß, none were simultaneously positive for p24, whereas approximately 45% of the cultures which were positive for either IL-6 or IFN-G were simultaneously positive for p24. Only one supernatant had dectectable IL-4, and it was negative for p24.

V 397 SELECTION OF A PEPTIDE VACCINE FOR LEISHMANIASIS.

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Infection of BALB/c mice with *Leishmania* leads to a fatal progressive disease while C57BL/6 mice undergo spontaneous healing. The immunological basis for this difference is due to the expansion of distinct $CD4^+$ T cell subsets, Th-2 in BALB/c and Th-1 in B6 in response to *Leishmania* antigen. Immunization of the susceptible strain prior to infection can confer either protection or exacerbation of the disease depending on the choice of immunogen and the route of immunization. One of the prominent proteins on the parasite surface, PSP, can confer a good degree of protection when used as an immunogen in BALB/c and CBA mice but peptides derived from this protein can be either innocuous or partially protectice. Using synthetic peptides covering the entire sequence of PSP we show that many T cell determinants exist in the PSP sequence and that several of them are promiscuous and shared almost the identical core amino acid residues in different strains tested. We also show that the choice of peptide within a determinant envelope is essential for the induction of a Th-1 response: there are peptides within the same envelope that induce an undesirable Th-2 component. These findings should be of importance when defining a peptide vaccine for leishmaniasis.

V 398 EFFECT OF PEPTIDE BINDING AFFINITY ON THE DEVELOPMENT OF $CD4^+$ T LYMPHOCYTE EFFECTOR FUNCTION,

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The induction of either humoral or cellular immunity involves the participation of two distinct $CD4^+$ T cell subsets, Th1 and Th2. Th1 cells activate macrophages, mediate delayed-type hypersensitivity reactions, and release the cytokines IFNγ and TNFβ. Th2 cells induce B cell differentiation, are potent mediators of IgE antibody responses, and release IL-4, IL-5. The mechanism controlling generation of Th1 and Th2 CD4 T cells is not fully understood. Previous studies from our laboratory have shown that mice primed with human collagen IV (CIV) developed either humoral (Th2) or cell-mediated (Th1) immune responses depending on the MHC class II genotype. $CD4^+$ T cells primed in $H\text{-}2^b$ were primarily Th2 cells, whereas in $H\text{-}2^s$ they were Th1-like. Identification of an immunogenic peptide of CIV indicated that a 12 amino acid peptide induced the same type of effector response as the protein CIV. Analysis of CIV peptide binding to the two MHC class II molecules indicated that the peptide bound to $I\text{-}A^s$ with higher relative affinity than to $I\text{-}A^b$. Variants of the CIV immunodominant peptide that differed in binding to MHC class II were prepared. The ability of the variant peptides to induce either Th1 or Th2 response was compared. The data indicate that peptides binding to MHC class II with high affinity induce Th1 cells preferentially, whereas peptides binding to MHC class II with low affinity drive Th2 cell differentiation. Thus the peptide:MHC density on the antigen-presenting cell appears to play a role in the generation of Th1 and Th2 cells. Experiments analyzing the priming of CD4 T cells from mice transgenic for the αβ T cell receptor specific for the antigen peptide cytochrome c to peptide variants are currently being performed.

V 399 HUMAN T-CELLS RESPONSIVE TO CNS MYELIN FOCUS ON A MINOR PROTEIN COMPONENT

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Introduction

The activation of human myelin-reactive T cells is widely considered to be of pivotal importance to the development of multiple sclerose (MS). The rational development of antigen-specific intervention in MS requires information on exactly which of the multitude of different myelin proteins trigger autoreactive human T cells. In this study, we aim at identifying such proteins.

Experimental approach

Human peripheral blood T cells from HLA-typed healthy donors as well as from definite MS patients were cultured *in vitro* with delipidated human myelin proteins for two weeks. As antigen source, purified myelin was used from either affected MS brains or control brains. Total CNS myelin proteins from the same sources were also fractionated by reversed-phase HPLC and these HPLC fractions served as test antigen in a standard proliferation assay in which responses of the short-term bulk cultures were assayed.

Results

Proliferative responses of human T-cell cultures were mainly directed against one or a few minor protein components of human myelin. Although the expected large amounts of MBP and PLP were present in both stimulatory and test-preparations (as confirmed by SDS-PAGE and ELISA), both major myelin proteins triggered only marginal proliferative responses. In all HLA backgrounds tested, the strongest proliferative responses were directed at the same protein fraction, levels of which may be different between MS and control brains. Upon reversed-phase HPLC, the highly immunogenic protein fraction did not co-elute with either MBP, PLP, MOG or MAG. We currently aim at the characterization of this protein fraction.

V 400 SINGLE-CELL ANALYSIS OF CYTOKINE PAIRS DURING $CD4^+$ T CELL PHENOTYPE DEVELOPMENT: IMPLICATIONS FOR CYTOKINE GENE REGULATION AND PHENOTYPE HETEROGENEITY.

Casey T. Weaver, Laurel Karr, Guo-qiang Huang, Jimin Li, Kenneth M. Murphy*, and R. Pat Bucy, Departments of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, and Washington University*, St. Louis, MO

Naive $CD4^+$ T cells from αβ-TCR transgenic mice were analyzed for co-expression of cytokine mRNA pairs during phenotype development using a novel double-label *in situ* hybridization technique. The frequency of cytokine-producing T cells in the primary antigenic response was quite limited despite demonstration that all transgenic T cells underwent blast transformation. IL-2 was the most abundant cytokine, though it was expressed by less than 5% of the activated T cells. IFN-γ was significantly less abundant (1.2%), and IL-4, IL-5, and IL-10 were barely, though reproducibly, detected (0.12%, 0.052%, and 0.034 %, respectively). Surprisingly, the frequency of cells that co-expressed two cytokines was quite limited. The frequency of cells that co-expressed IL-2 and IFN-γ in the primary response was <20% of the single-positive IL-2- or IFN-γ-producing cells. Thus, these two "Th1" cytokines show very poor coordinate regulation in individual T cells in a primary antigenic response. On secondary stimulation, the frequency of IL-2/IFN-γ double-positives increased, though it remained a minority of all IL-2 and IFN-γ positive cells. The production of "Th2" cytokines was dramatically increased in the secondary stimulation. IL-4 and IL-10 showed 78- and 250-fold increases, respectively. Coordinate activation of the IL-4 and IL-10 genes in the same cell was the exception rather than the rule, though co-expression was greater than that for IL-2 and IFN-γ. Of all the cytokine pairs examined, IL-4 and IL-5 were the most frequently co-expressed. IL-4 and IFN-γ showed the greatest tendency towards segregation of expression, rarely co-expressed after the primary stimulation.

These data establish that there is tremendous heterogeneity of individual cytokine gene expression during the early antigenic response of $CD4^+$ T cells. Co-expression of any pairs of cytokines, much less "Th1" and "Th2" cytokines, is generally the exception. It appears that the majority of cells that generate a cytokine response, even in a clonal, transgenic population, tend to activate single cytokine genes during a given round of antigenic stimulation, and only a minority of the total cells stimulated generates a cytokine response at all. As the cells undergo additional rounds of activation, there is increased frequency of cytokine-producing cells and evolution of more coordinate expression of different cytokine pairs, but this is in no case absolute. These data suggest that the Th0 phenotype is in fact a *population* phenotype rather than an *individual cell* phenotype, as previously assumed.

V 401 DEVELOPMENT OF CYTOTOXICITY AND PERFORIN GENE EXPRESSION IN CD4+ AND CD8+ CELLS FROM NAIVE MURINE SPLENOCYTES, Noelle S. Williams[1], Mary Beth Graham[2], Thomas J. Braciale[1,3], and Victor H. Engelhard[1], Beirne Carter Center for Immunology Research and the Departments of Microbiology[1], Medicine[2], and Pathology[3], University of Virginia, Charlottesville, VA 22908

While cytotoxic activity is generally considered to reside in the CD8+ population of T lymphocytes and not in the CD4+ population, clear exceptions to this rule exist. CD4+ cells with cytolytic capabilities have been identified, but they are often believed to kill by a mechanism distinct from that used by CD8+ CTL. In the present study, a cytotoxic TH_1 CD4+ clone was shown to express mRNA for perforin, a molecule implicated in the cytotoxic mechanism of CD8+ CTL, while a noncytotoxic TH_2 CD4+ clone was shown to lack the perforin message. This observation demonstrates that CD4+ CTL potentially kill by the same mechanism utilized by CD8+ CTL and poses the question of what regulates the development of cytotoxicity in CD4+ cells. Data from this laboratory have shown that when NK cell depleted murine splenocytes were activated by either allogeneic spleen cells for five days or ConA + PMA for three days, cytotoxicity was restricted to CD8+ cells. In the case of ConA + PMA activated cells, this cytolytic capability could be correlated with the presence of mRNA for perforin. That this message was CD8 specific was shown by immunodepletion of CD8+ cells with anti-CD8 antibodies and complement at the effector stage with the concomitant loss of cytotoxicity and perforin message. However, CD4+ CTL could also be generated in a primary allogeneic culture if they were activated either in the absence of CD8+ cells or with a stimulus that activates only CD4+ cells. This suggests that activated CD8+ cells exert an inhibitory influence on the development of cytotoxicity in the CD4+ population. Experiments are currently in progress to assess the relative role of cell contact versus soluble mediators in this effect using the Costar Transwell™ system and to determine the role of perforin in killing by primary CD4+ CTL.

Superantigens

V 410 STRUCTURAL ROLE OF THE T CELL RECEPTOR ALPHA CHAIN IN SUPERANTIGEN RECOGNITION, Marcia A. Blackman, Daniel Hankley, Phuong Le, David L. Woodland, and Kieran Daly, St Jude Children's Research Hospital, Memphis, TN, 38105. Superantigen specificity of T cells is controlled largely by the Vβ element of the T cell receptor (TCR). However, recent studies have shown that the TCR alpha chain may also contribute to superantigen recognition, but the structural role remains undefined. In addition, whereas superantigen recognition is not classically MHC-restricted, we have previously shown that the specificity of some T cells is influenced by polymorphic differences in the MHC presenting molecule, implicating potential contacts between the TCR and MHC.[1] In an attempt to understand the molecular contribution of the alpha chain to superantigen recognition, we have analyzed the Mls-1 specificity patterns of a panel of T cell hybridomas with limited TCR sequence diversity. The hybridomas expressed TCR with identical beta chains (transgenic Vβ8.1DβJβ2.3Cβ2) and alpha chains containing one of two Vα11 family members, $V\alpha11.1^b$ or $V\alpha11.2^b$, that differ in only 5 amino acids. The Vα11.1 and Vα11.2 hybridomas showed distinct patterns of reactivity to Mls-1: the Vα11.1 hybridomas were independent of MHC effects, whereas the Vα11.2 hybridomas were variably influenced by MHC polymorphism. Sequence analyses are in progress to correlate these differences in fine specificity with Jα and junctional sequences. The data suggest two potential roles for the alpha chain, one mediated by the Vα element and the other by Jα and junctional sequences. The data will be discussed in terms of a proposed 3-dimensional structure of the TCR.[2]

[1]Woodland, D.L. and Blackman, M. A. 1993. Immunol. Today 14:208.
[2]Chothia, C., Boswell, D. R., and Lesk, A. M. 1988. EMBO J. 7:3745.

V 411 AN ACUTELY LETHAL SIMIAN IMMUNODEFICIENCY VIRUS ACTS AS A SUPERANTIGEN, Zheng Wei Chen, Zhong-Chen Kou, David Lee-Parritz, Patricia N. Fultz and Norman L. Letvin, Harvard Medical School, New England Primate Research Center, Southboro, MA 01772 and Department of Microbiology, University of Alabama, Birmingham, AL 35294
Although superantigen-mediated deletion of CD4+ lymphocyte subpopulations has been proposed as a possible mechanism for CD4+ cell loss in AIDS, evidence that an AIDS virus can act as a superantigen has been inconclusive. SIVsmmPBj14, a variant simian immunodeficiency virus isolated from a pig-tailed macaque, stimulates the proliferation of macaque T lymphocytes *in vitro* and in macaques induces an acutely lethal disease characterized, in part, by lymphadenopathy and splenomegaly. To determin *whether* SIVsmmPBj14 acts as a superantigen, in vitro and in vivo studies of T cell receptor (TCR) Vß repertoire were undertaken using a PCR-based quantitative method. While PHA did not cause the perturbation of TCR Vb repertoire, SIVsmmPBj14 stimulated the expansion of T lymphocyte subpopulations expressing the Vß7 and Vß14 gene families. Such Vß7 and Vß14 expansions could be confirmed by multiple RNase protection assay. Furthermore, the expansion of the same lymphocyte subpopulations was also detected in PBL and lymph node cells of virus-infected macaques. These results suggest that SIVsmmPBj14 encodes a superantigen that may contribute to its ability to induce an acutely lethal disease.

V 412 THE STRUCTURE-FUNCTION ANALYSIS OF MOUSE MAMMARY TUMOR VIRUS ENCODED SUPERANTIGEN, Yongwon Choi, Chae-Gyu Park and Gary Winslow*, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Av., New York, NY10021, and Howard Hughes Medical Institute, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

It has recently been shown that open reading frames in the 3' long terminal repeats of mouse mammary tumor viruses encode superantigens. These viral superantigens (vSAGs) stimulate most T cells expressing appropriate Vβs almost regardless of the rest of the variable components of the T cell receptors expressed by those cells. Viral superantigens produce a type II integral membrane protein with a non-essential short cytoplasmic domain and a large glycosylated extracellular COOH-terminal domain, which is predicted to interact with major histocompatibility complex (MHC) class II molecules and the T cell receptor (TCR). The transmembrane region of vSAG also has an internal positively charged lysine residue of unknown significance. A set of chimeric and mutant viral superantigen genes has been used in transfection experiments to show that only the extreme COOH-terminal portion of vSAGs determine their TCR Vβ specificities, and to show that the lysine residue in the transmembrane domain is not essential for the function of vSAG. Site-directed mutagenesis study has also been used to show that proteolytic cleavage of vSAG at amino acid residues 168-171(RKRR) is required for the function of superantigen. Further studies are being carried out to determine how vSAGs interact with MHC class II molecules.

V 413 B CELL SPECIFIC TRANSCRIPTIONAL REGULATION OF MTV SUPERANTIGEN EXPRESSION.

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Mouse Mammary Tumor Virus (MMTV) has been a paradigm for cell type specific expression. It was believed to be only expressed in mammary tissue. Only recently it became clear that infectious MMTV as well as endogenous, inherited Mtv loci are also transcribed in B cells, at a significant level. At least some of these messages seem to get translated into protein since it has been shown that the open reading frame located in the Mtv 3' LTR region encodes for various superantigens that had been functionally defined by mixed lymphocyte reactions. Mtv transcription can be detected at a constant rate throughout B cell ontogeny starting at the pre B cell stage but does not correlate with the presence of any known B cell specific genes or transcription factors. Moreover, Mtv transcription is upregulated during a particularly late stage of B cell activation, when cells start secreting antibody. This phase, as well as upregulation of Mtv transcription can be induced by IL5, IL6 or LPS treatment in the cell line CH12-LBK.

We are currently investigating, which cis acting promoter and enhancer elements of the viral genome are involved in basal as well as IL5, IL6 and LPS induced Mtv transcription and which B cell specific transcription factors are utilized by these viral elements. Preliminary results from stable and transient transfections indicate that a recently discovered second promoter located upstream of the LTR promoter is active in B cells and requires a downstream enhancer as well as a minimal domain of 200bp upstream of its presumed TATA box. This novel promoter is strongly inducible by glucocorticoids.

Our studies of the interaction between MMTV and B cells should also provide new insights into B cell activation.

V 414 ANTAGONISM OF SUPERANTIGEN STIMULATED TH CELLS BY ALTERED PEPTIDE LIGAND,

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T cell activation by peptide: MHC molecules can be antagonized by non-stimulatory analogs of that peptide. Multiple altered peptides, each containing a single substitution for the amino acids identified as T cell contact residues, were used to examine the antagonist response. In addition, we investigated this TCR antagonism using staphylococcal enterotoxin (SE) superantigen to stimulate hemoglobin-specific [Hb(64-76] Th cells because its activation pathway may differ from conventional antigen. Interestingly, superantigen activation of these Th cells was antagonized by hemoglobin peptide analogs even though agonist (superantigen) and antagonist (altered peptide) bind at different sites on the MHC molecule and TCR. The antagonism appeared to be a fundamental block in T cell activation as cytokine production and proliferation were reduced in Th1 or Th2 clones, and cytokine production and apoptosis was inhibited in a T cell hybridoma. When multiple altered peptide ligands were made at a single amino acid position (amino acid 73), peptides with agonist, partial agonist and antagonist characteristics were generated. Interestingly, the peptides that functioned as partial agonists for these Th cells also antagonized T cell responses, and thus were a subset of the peptide antagonists. In summary, our results demonstrate that analogs of immunogenic peptide are potent antagonists for Th cell responses induced by superantigen as well as immunogenic peptide.

V 415 **Mutations in the MHC class II β1 domain affect superantigen induced T cell activation.**

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Superantigens are proteins that activate T cells bearing certain Vβ segments of the TCR. Exogenous superantigens are soluble proteins produced by bacteria such as *Staphylococcus aureus* while endogenous superantigens are encoded by the 3' LTR of mammary tumor proviruses (MTV). Staphylococcal enterotoxin superantigens such as SEA and SEB have been shown to bind to MHC class II molecules and induce strong oligoclonal T cell activation. In addition, I-E molecules bind and present exogenous superantigens better than the I-A isotype. For endogenous MTV encoded superantigens, mice expressing both I-A and I-E delete T cells bearing the appropriate Vβ as part of TCR while mice expressing only I-A show marginal deletion. Amino acid residues 89-92 of the I-A and I-E β-chain differ greatly and we chose to investigate whether mutations in this region would affect superantigen binding and T cell activation. SEB and MTV gene product induced T cell activation was measured with Vβ 8.3 and Vβ 7 bearing T cell clones respectively. It was found that both endogenous and SEB SAg activity was dependant upon the F89 and could only be substituted with Y or M in order to activate Vβ 8.3 (SEB) and Vβ 7 (vSAG M12) T cell hybridomas. SEA on the other hand was less sensitive to substitution in position F89 and pigeon CyC response was not significantly different between the M12.C3 clones mutated in position F89. Mutation of histidine 81 to tyrosine ameliorates SEA activity. This is in accordance with previous findings with the HLA-DR molecule and indicates similar binding requirements for SEA on I-E and HLA-DR. We are currently investigating whether amino acids 82-88 influence SAg interaction with MHC class II and whether a variety of toxins have different amino acid preferences within the 82-88 region.

V 416 MHC CLASS II-POSITIVE CELLS ARE REQUIRED FOR DEVELOPMENT OF MAIDS, N.Giese,T.J.McCarty,C. DeRivera,T.Giese,H.C.Morse, Laboratory of Immunopathology, NIAID and Laboratory of Genetics, NCI, NIH, Bethesda, MD 20892

MAIDS is a murine retrovirus-induced immunodeficiency syndrome with many similarities to AIDS. Progression of this syndrome is strongly dependent on genetic background and in susceptible strains of mice (e.g. C57BL/6) infected with LP-BM5 MuLV, is associated with amplification of an etiologic replication-defective virus (BM5def) and helper MuLV; nonpathogenic replication-competent B-tropic ecotropic (BM5eco), and mink cell focus-inducing (MCF) viruses. Virus spread is accompanied by T-dependent B-cell activation at an early stage, progressive impairment of T- and NK-cell function and development of B- and T-cell lymphomas at later stages of disease. There are two general theories about MAIDS pathogenesis: 1. Neoplastic concept. BM5def is a transforming agent for B cells and the associated immunodeficiency is a paraneoplastic process. 2. Antigenic (or super AG) concept. BM5def genome encodes surface AG itself or activates expression of endogenous SAG's encoded by MMTV's. Thus, APC presentation and T cell recognition of these AG is crucial to MAIDS pathogenesis. Our experiments utilized MHC class-II knockout mice (C2D) that lack cell surface expression of class II molecules and, as a result of failed positive selection in thymus, are depleted of mature $CD4^+$ T cells. The data obtained favor the AG(SAG)-concept, since, despite high expression BM5def (detected by RT-PCR) and BM5eco and MCF viruses (detected by ICT) in lymphoid organs of infected C2D mice, we detected no signs of MAIDS as judged by morphological and histopathologic criteria, FACS analysis, serum IgG levels or a number of functional assays. Reconstitution of C57BL/6 nude or C2D mice with CD4-positive T-cells from C57BL/6 mice resulted in development of MAIDS in nude, but not in C2D mice. Therefore, in the C2D mice, BM5def integrated in the host genome, persisted and amplified in host tissues, but in the absence of MHC class-II molecules was incapable of inducing MAIDS.

V 417 MHC INDEPENDENT STIMULATION OF A T CELL HYBRIDOMA BY STAPHYLOCOCCAL ENTERO-TOXIN B PRESENTED ON A SOLID SUPPORT, Abdel Rahim A. Hamad, Philippa Marrack and John W. Kappler. Howard Hughes Medical Institute, Natl. Jewish Center for Immunology and Respiratory Medicine, Denver CO.

The superantigen Staphylococcal enterotoxin B (SEB) stimulates T cells that bear specific TCR Vß elements. Binding of SEB to MHC class II molecules is a prerequisite for recognition of the complex by TCR irrespective of the allele or isotype of class II. For example T cells can respond to superantigens even when bound to xenogeneic class II molecules. This lack of stringency in the MHC requirement for T cell activation by superantigen suggested to us that plate bound anti-SEB monoclonal antibodies might mimic class II on the surface of antigen presenting cells (APC) and present superantigen to an appropriate T cell hybridoma. To test this hypothesis four antibodies that recognize different epitopes of SEB were generated, characterized and used to present SEB to a T cell hybridoma bearing TCR Vß 8.3. The antibodies include, 2B33, which blocks binding of SEB to MHC class II and a second antibody, B87, blocks the TCR recognition site on SEB. Antibodies were coated onto plates overnight, SEB was then added followed by the T cell hybridoma. Three of the four antibodies which bound SEB led to stimulation of the T cell hybridoma in this assay as measured by IL-2 production. The antibody B87 did not result in T cell stimulation, however, this was not surprising since this antibody prevented T cell responses to SEB even when the superantigen was presented by appropriate APC. Moreover, SEB mutants that have lost their ability to bind to MHC class II molecules can stimulate T cells when presented by anti-SEB monoclonal antibodies. This study shows that plate bound monoclonal antibodies can substitute for MHC class II in presentation of SEB to T cell hybridomas.

V 418 UNDERSTANDING SUPERANTIGEN INDUCED NON-RESPONSIVENESS, M.E. Hamel and A.M. Kruisbeek, Department of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Superantigens (SAs) have in common biological features that distinguish them from conventional antigens: SAs induce vigorous proliferative responses in all T cells expressing selected TCR Vß chains, both *in vivo* and *in vitro*. *In vivo* these responses are followed by partial clonal deletion and induction of nonresponsiveness in mature peripheral T cells.

To study the mechanisms of SEB-induced nonresponsiveness, we developed an *in vitro* model. Purified T cells are cultured for 3 days with Staphylococcal Enterotoxin B (SEB) in the presence of T cell depleted irradiated spleen cells as a source of APCs. Subsequently, the T cells are washed, put to rest in fresh medium for 5 days and restimulated with SEB and APCs. Indeed, nonresponsiveness can be observed 3 days after restimulation, in analogy to previous *in vivo* reports (Rellahan, B.L. *et al.*, *J.Exp.Med.*172:1091 (1990)). The requirements for induction of this nonresponsiveness are addressed by modulation of the primary cultures, through the use of different APCs and selected cytokines or cytokine antagonists. The impetus for these experiments is the strong skewing towards a Th1 cytokine production profile induced by SEB. Indeed, reversal of this skewing during the primary response, through the use of IL4 plus anti-IFNgamma, restores secondary proliferative responses to SEB.

In studying the kinetics of T cells restimulated with SEB, we made an unexpected observation; while the cells displayed a non-responsive phenotype on day 3 after restimulation, a vigorous response was seen on day 1 and, to a lesser extent, on day 2 after restimulation. This is not an *in vitro* artefact as *in vivo* SEB primed T cells proliferate as well on day 1 and 2 after restimulation, though less pronounced. Whether this enhanced proliferation in fact leads to apoptosis (as recently shown in other systems) remains to be examined. Either way, the term anergy clearly requires further qualification, with, for SAs, implying a change in kinetics rather than non-responsiveness in all parameters of T cell activation.

V 419 THE OUTCOME OF Vβ3 T CELL ACTIVATION BY SEB: QUANTITATION OF APOPTOSIS AND THE EFFECT OF ADDED CYTOKINES. Anthony R. Hayward, Mary Cosyns and Yingjian Zhang, Departments of Pediatrics and Immunology and Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Denver CO 80262.

The mean frequency ($\pm$ 1 SD) of $V\beta3^+$ $CD4^+$ T cells in cell cycle following *in vitro* stimulation with staphylococcal enterotoxin B (SEB) increases from 5% $\pm$ 4% on day 1 to 42% $\pm$ 10% on day 4 of culture. This figure is substantially higher than the 1% of $CD4^+$ T cells which is detected in SEB-stimulated limiting dilution culture conditions. To determine whether cell death might contribute to the difference between cells entering cell cycle and the those surviving to expand clonally, we determined the amount of DNA released into the supernatants of SEB-stimulated cell cultures in the presence or absence of added cytokines. SEB stimulated cultures of 10^6 cells contained 5.1 $\pm$ 1 µg/DNA/mL which was significantly greater than the 3.9 $\pm$ 0.6 µg/DNA/mL found in the unstimulated control cultures ($p<0.05$). Addition of 10 u/mL of IL2, IL4 or IL6 increased the fraction of cells entering cycle and reduced the amount of cell death. Our results suggest that the frequency range for $V\beta3^+$ cells entering cell cycle is sufficiently narrow to permit comparisons between different physiologic conditions, as following *in vivo* tolerance induction protocols. As applied to human subjects, statistically significant differences in the frequency of blood Vβ3 cells stimulated by SEB which entered cell cycle were found between severely ill and healthy individuals.

V 420 T CELL RECEPTOR Jβ RESIDUE(S) AFFECT REACTIVITY WITH A SUPERANTIGEN (MAM).
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Superantigens (SAG) are thought to bind to the laterally exposed surfaces of MHC class II and the TCR (CDRIV region), both distant from sites involved with recognition of nominal peptide antigens. MAM can be recognized by T cells using a restricted number of human Vb genes (Vβ17, 8, 5.1, 12.2) as is typical of SAG. However, we have isolated both MAM reactive and MAM non-reactive IL-2 dependent T cell clones expressing exactly the same Vb genes, verified by sequencing the entire Vb region. Reactivity with MAM appears to be strikingly correlated with the absence of the otherwise common Jβ2.1 gene segment. In particular, a residue just upstream of the highly conserved Jβ-encoded PheGly is highly correlated with MAM reactivity: Tyr is associated with MAM reactivity and Phe with non-reactivity. This association holds up with all 4 MAM-reactive Vβ genes listed above and is not dependent on the MHC class II alleles used for MAM presentation since the same results were obtained with 4 MHC disparate EBV-transformed B cell lines used as APC. The Tyr/Phe position which appears to influence MAM reactivity is located at the base of the CDRIII loop. It could indirectly affect the SAG binding site presumably located on CDRIV or it could represent a direct contact residue for either MAM or MHC class II molecules. Thus β-chain V(D)J recombination provides a mechanism to escape from SAG mediated deletion/anergy of an entire Vβ subset.

V 421 STAPHYLOCOCCAL ENTEROTOXINS INHIBIT ANTIGEN-SPECIFIC T CELL ACTIVATION WITHOUT CAUSING ANERGY, David R. Karp, Robert N. Jenkins, and James E Dowd, Simmons Arthritis Research Center, UT Southwestern Med. Ctr., Dallas, TX 75235-8884
As superantigens, the staphylococcal enterotoxins (SE) stimulate polyclonal T cell populations based largely on the expression of responsive T cell receptor Vβ elements. One salient feature of SE action is the specific and high affinity binding to non-polymorphic regions of MHC class II molecules. The effect of this interaction between SE and MHC on T cell activation was studied. CL-1 is an HA307-319 specific, HLA-DR1-restricted human T cell clone. The TCR from CL-1 was cloned from cDNA and sequenced. It expresses Vβ13.1 and Vα 1.3. It responds to SEC2 by secreting IL-2, but does not respond to SEA, SEB, or toxic shock syndrome toxin (TSST). HLA-DR1-expressing B cells were pulsed with antigenic peptide, then cultured with CL-1 in the presence of various concentrations of SE. There was a SE concentration-dependent decrease in the amount of IL-2 released by the T cells. This inhibition of T cell activation was not due to negative signalling via class II on the T cell. CL-1 was non-specifically stimulated by immobilized anti-CD3 antibody, 64.1. When the anti-HLA-DR anitbody, L243 was added, there was a 75% decrease in IL-2 secretion. No similar effect was seen with SE at concentrations that inhibited antigen-specific T cell activation. SE have also been shown to cause T cell anergy that can prevent subsequent antigen-specific T cell activation. CL-1 was cultured in the presence of high concentrations of SE. While culture in SEC2 was able to abolish the subsequent activation of CL-1 by peptide plus APC, no effect was seen with SEA. In addtion, SEC2, but not SEA, caused the expected alteration in CL-1 surface phenotype: decrease in surface CD3 and increase in surface CD25. Finally, SEA was able to competitively inhibit the binding of fluoresceinated avidin to B cells that had been pulsed with biotinylated HA307-319. This suggests that SE and peptide can occupy the same MHC molecule, and implies a topology for SE/MHC interaction. Taken together, these data demonstrate that SE binding to MHC can prevent activation of an SE-non-responsive T cell. This inhibition is probably the result of steric effects and suggests a role for SE or their derviatives as immunomodulatory agents.

V 422 AMINO AND CARBOXYL-TERMINAL DOMAINS OF STAPHYLOCOCCAL ENTEROTOXIN E MEDIATE TCR Vβ SPECIFIC INTERACTIONS, James G. Lamphear, Joseph A. Mollick, Kristin B. Reda, and Robert R. Rich, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030
Staphylococcal enterotoxin A (SEA) and E (SEE) are two closely related bacterial superantigens that bind to major histocompatibility complex (MHC) class II surface antigens, and activate discrete subsets of T lymphocytes bearing certain variable elements of the T cell receptor (TCR) β chain. In order to characterize interactions between bacterial superantigens and TCR, we defined the regions of SEE responsible for mediating Vβ-specific stimulation using a series of chimeric enterotoxins that incorporated specific residues of SEE at analogous positions in SEA. The potential of these chimeras to stimulate purified populations of human T lymphocytes and competitively bind to HLA-DR1 molecules was assessed by flow cytometry. Two regions of SEE located near the amino-terminus (residues 20, 21 and 24) and carboxyl-terminus (residues 200, 206 and 207) were required to restore wild-type stimulatory properties to a chimeric molecule. The amino-terminal residues appear to be important only in the activation of T cells with SEE, while the carboxyl-terminal residues are necessary for both SEA and SEE-mediated activation of T cells. MHC class II binding was not altered by the exchange of either amino or carboxyl-terminal residues, suggesting that the functional domains responsible for TCR specificity and class II binding affinity are distinct. Alignment of the chimeras with SEB places the regions 20-24 and 200-207 on two structurally contiguous loops of SEB thought to partially comprise a TCR binding pocket. Thus the residues of SEE that mediate Vβ-specific interactions reside in domains at either end of the enterotoxin molecule, and may coalesce to form a common TCR binding site with multiple specificities.

V 423 RESPONSIVENESS TO BACTERIAL TOXIN SEB IN THE MINORITY OF Vß6 T CELLS IS ASSOCIATED WITH Vα4 EXPRESSION, Stuart Macphail, Lucas Collazo and Herb Borrero, Department of Surgery, North Shore University Hospital and Cornell University Medical College, Manhasset, NY 11030
Our previous functional analysis of a panel of Mls1^a responsive, Vß6 T hybridoma clones indicated that elements of the T cell receptor (TCR) in addition to Vß determined responsiveness to bacterial superantigen staphylococcal enterotoxin B (SEB) in T cells expressing Vß6, a Vß element not commonly associated with SEB responsiveness. This conclusion was indicated by the finding that while the majority of the T cell hybridomas were not SEB responsive, a minority (approximately 20%) were; the pattern of responsiveness of the Vß6 hybridomas to endogenous superantigen Mls1^a showed that responsiveness to SEB was not due to non-specific effects such as relative levels of TCR or adhesion/accessory molecules. A ß chain cDNA sequencing analysis indicated that ß chain junctional elements did not, autonomously at least, determine SEB responsiveness in the Vß6 T cells; this was confirmed by transfection of ß chain cDNA derived from a responsive T hybridoma into a non-responsive T hybridoma. No transfer of SEB responsiveness was seen in cells transfected with either ß chain from the responsive T hybrid or their own ß chain cDNA despite observation of an increase in TCR expression and Mls1^a responsiveness in both relative to controls transfected with vector alone. An α chain sequencing analysis of the SEB responsive and non-responsive Vß6 T hybridomas indicated (i) that α chain junctional elements did autonomously determine the functional phenotype and (ii) that Vα4 expression was associated with SEB responsivness in Vß6 T cells. A functional analysis of a separate set of T hybridomas expressing Vα4 and various Vß elements provided further evidence for the role of Vα4 in facilitating SEB responsiveness in Vß6 T cells. Transfection experiments will test for the autonomy of Vα in this function.

V 424 FUNCTIONAL CONSEQUENCES OF IN VIVO TCR TARGETTING BY MEANS OF EITHER MONOCLONAL ANTI-Vβ ANTIBODIES OR SUPERANTIGENS. C. Martinez-A., J.A. Gonzalo, A. Garcia, R. Tarazona and G. Kroemer. Centro Nacional de Biotecnologia, CSIC, Universidad Autonoma, Campus de Cantoblanco, 28049 Madrid, Spain.

Two classes of agents that are targeted to certain TCR Vβ gene products, monoclonal antibodies and superantigens, have successfully employed for the experimental prophylasis and therapy of autoimmune diseases. Monoclonal anti-Vβ antibody stimulates T cell proliferation, lymphokine production, anergy and apoptosis in vitro. Superantigens induces a complex response, including the induction of deletion via apoptosis, cytokine release, and anergy. Surprisingly however, in vivo the response elicited by both reagents obey rather different principles. Thus, 1) SEB causes the secretion of a variety of cytokines (interleukins 1,2,3,4,10, GM-CSF, γ-IFN and TNF). In contrast anti-Vβ8 (F23.1)- that efficiently induces these mediators in vitro-fails to do so in vivo; 2) SEB, not F23.1-induced PCD concerns T cells that have passed through the S phase of the cell cycle; 3) deletion of Vβ8$^+$ T cells induced by SEB not by F23.1, can be blocked with RU 486 or retinol, and 4) depletion of Vβ8+ cells by F23.1 not by SEB can be affected by macrophage depletion. These results suggested that the elimination of T cells induced by this means obeys different principles: activation-induced cell death in the case of SEB and passive macrophage mediated elimination in the case of F23.1

V 425 PROTEOLYTIC CLEAVAGE OF VIRAL SUPERANTIGEN INVESTIGATED THROUGH MUTATIONAL ANALYSIS, Christopher W. McMahon, Gary M. Winslow*, and Ann M. Pullen, Department of Immunology, Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, and *Division of Basic Immunology, Howard Hughes Medical Institute, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Endogenous superantigens, encoded by mouse mammary tumor virus (MMTV) proviral integrants, have a profound effect on the shaping of the T-cell repertoire during tolerance induction. Viral superantigen (vSAG) forms a complex with MHC class II products on presenting cells, and is then able to stimulate T-cells bearing a particular Vβ element on their T-cell receptor, in a manner distinct from normal antigen presentation. MTV-encoded viral superantigens are type II membrane glycoproteins, and are detected on the cell surface as a carboxy-terminal fragment- some fraction of which remains non-covalently associated with the membrane-traversing amino-terminal.

To investigate whether this proteolytic cleavage is a requirement for viral superantigen presentation, protease recognition motifs in the vSAG-6 amino acid sequence were disrupted. These mutant constructs were then expressed in a B-cell line and tested for their ability to stimulate Vβ3$^+$ T-cell hybrids *in vitro*. The molecular weight of the vSAG-6 proteolytic fragment predicts cleavage at a tetrabasic protease motif found at amino acid 171. This cleavage motif, recognized by the ubiquitous endoprotease furin, is found at the cleavage site of many viral and endogenous glycoproteins. Surprisingly, mutation of this predicted cleavage site to a sequence that should no longer be recognized by furin or dibasic-specific proteases does not abrogate cleavage of vSAG-6. These mutant molecules are still effectively presented to Vβ3$^+$ T-cell hybrids.

Further mutations of this and other putative cleavage sites are currently underway. Biochemical analyses and the stimulatory activity of these mutants should identify the vSAG-6 cleavage site(s), the substrate specificity of proteases able to mediate this cleavage, and whether any cleavage is necessary for vSAG presentation to T-cells.

V 426 **Early loss of T cell receptor expression upon primary activation of T lymphocytes in vivo.**

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Injection of bacterial superantigens like Staphylococcal enterotoxin B (SEB) in the footpad of mice results in several changes of superantigen reactive Vß8$^+$ T lymphocytes. First, within minutes superantigen reactive T cells of the draining popliteal lymph node loose the expression of L-selectin (MEL-14) on their surface. Second, within 24 hours this subset (normally ~ 28% of all T cells in Balb/c mice) is reduced to around 50% as determined by FACS analysis. Reduction of the Vß8$^+$ T cell subset starts around four hours and is most pronounced 24 hours after injection of SEB. Third, parallel in time increasing amounts of fragmented DNA can be isolated from popliteal lymph nodes indicating that the reduction of Vß8$^+$ T lymphocytes is at least in part mediated by apoptotic mechanisms. Later on surviving Vß8$^+$ T cells expand in vivo as described by others comprising of more than 40% of all T cells between day 2 and day 3 after treatment.

Here, we describe the new finding that a part of the SEB reactive Vß8$^+$ T cell subset downregulate the T cell receptor (TCR) upon primary activation in vivo. This observation is most prominent between 8 and 12 hours after injection of SEB and results in T cells which express CD4 or CD8 molecules at normal densities but 10 to 20-fold reduced expresssion levels of CD3 and the TCR. Later in time cells are generated in the draining popliteal lymph node which are completely negative for CD4, CD8, CD3 or TCR expression. Furthermore, this population can not be stained with mAbs specific for B-cells, natural killer cells, makrophages or granulocytes. If these cells are selected by FACS and cultured for 24 hours in presence of rec. IL-2 reexpression of the TCR, i.e. Vß8, as well as CD3, CD4 and CD8 molecules can be observed. TCR downregulation as well as loss of Vß8$^+$ T cells 24 hours after treatment with SEB is dependent on higher doses of SEB (10 - 1 µg/footpad). With smaller doses like 0.01 µg/footpad we were unable to observe any Vß8$^+$ T cell loss. Instead, this subset expanded to around 37% of all T cells in 24 hours after treatment. We speculate that TCR downregulation leads to the generation of cells which completely lack the T cell phenotype and these cells are prone to apoptotic cell death. This cascade can be interrupted by cultering TCR negative cells in vitro where reexpression of the TCR as well as other surface molecules occurs.

V 427 LACK OF FAS DELAYS ENTEROTOXIN-INDUCED DELETION AND TOLERANCE OF Vβ8 + PERIPHERAL T CELLS IN MRL *lpr/lpr* MICE.

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Mice homologous for the lymphoproliferative mutation *lpr/lpr* accumulate large numbers of peripheral T cells, most of which bear the immature phentoype of αβTCR^{int} CD4- CD8-, while a minority of mature CD4+ or CD8+ cells are also present. The *lpr* mutation a retroviral intron insertion within the *fas* gene, which encodes a cell surface protein (Fas) that induces apoptosis. We examined the possibility that the lack of Fas might alter activation and subsequent deletion of Vβ8 + T cells or tolerance induction by Staphylococcal Enterotoxin B (SEB) in vivo. When compared to normal adult MRL +/+ mice, treatment of MRL *lpr/lpr* mice with a single intraperitoneal dose of SEB leads to delayed deletion of Vβ8 + T cells. Reaction to SEB was apparent in the mature peripheral subpopulations of the MRL *lpr/lpr* mice. Both CD4 + and CD8 + Vβ8 + T cells were activated and expanded proportionally in the first few days after SEB treatment then were deleted. In contrast, the CD4-CD8-Vβ8 + T cells did not expand in this period. When restimulated with SEB in vitro, MRL-*lpr* lymph node cells showed a much greater proliferative response than MRL +/+ cells, indicating impaired tolerance induction. This difference was greater in the first week after SEB treatment in vivo and approached normal level two weeks after treatment. Thus, the lack of Fas impairs superantigen-induced deletion and tolerance of T cells. This may in part explain the progressive accumulation of T cells and autoimmune symptoms in MRL *lpr/lpr* mice.

V 428 EXPRESSION OF A MHC CLASS II TRANSGENE DETERMINES BOTH SUPERANTIGENICITY AND SUSCEPTIBILITY TO MAMMARY TUMOR VIRUS INFECTION.

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Milk-borne mouse mammary tumor virus (MMTV) is a type B retrovirus that induces mammary carcinoma in mice. Infectious MMTV, as well as genomically integrated Mtv proviruses, encode superantigens that, in association with permissive MHC class II molecules, are recognized by T cells expressing an appropriate T cell receptor Vβ product. A series of experiments has been carried out to determine the role of superantigenic stimulation in host susceptibility to MMTV infection. Newborn mice that either did or did not express a transgenic Eα class II product were exposed by foster nursing to milk-borne MMTV, and were then analyzed for MMTV superntigen-induced Vβ-specific clonal deletion of T cells and for MMTV infection. It was found that only mice expressing the Eα transgene undergo Vβ deletion. When MMTV infection was assayed in these mice by ELISA of viral p28 in milk or by PCR analysis of mammary tissue, it was found that susceptibility to infection was similarly dependent upon Eα expression. These results demonstrate that susceptibility to MMTV infection, as well as susperantigen-induced Vβ-specific T-cell deletion, is dependent upon expression of a permissive class II MHC product. This observation is consistent with a permissive role of superantigenic stimulation in MMTV infection and/or viral replication.

V 429 MOLECULAR CHARACTERIZATION AND PHYLOGENETIC DISTRIBUTION OF THE STREPTOCOCCAL SUPERANTIGEN (SSA) GENE FROM *S. PYOGENES*.

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Bacterial superantigens (SAg) are potent immunomodulatory toxins that share the ability to bind MHC class II molecules and interact with specific TCR Vß elements to stimulate T cell proliferation and cytokine release. We recently exploited this immunological specificity to purify a new streptococcal SAg, SSA, from the culture supernatants of a serotype M3 *S. pyogenes* strain causing streptococcal toxic shock syndrome (STSS). The striking N-terminal homology of SSA to staphylococcal enterotoxin superantigens SEB and SEC raised questions regarding the origin and dissemination of the SSA gene. In order to characterize further the relationship of SSA to other bacterial SAg, and to determine the distribution of *ssa* among group A *Streptococcus*, we cloned the SSA gene. *ssa* is a 783 base pair open-reading frame encoding a predicted 260 amino acid protein that is similar in length to several other bacterial SAg. The deduced sequence of SSA is 60.2% identical to SEB, but only 49% identical to streptococcal pyrogenic exotoxin A. Purified recombinant SSA retained the SAg activity attributed to the native molecule and stimulated T cell proliferation in an MHC class II-dependent, Vß-specific manner. Southern blot and PCR analysis of 138 group A streptococcal strains representing 65 M protein serotypes and 15 non-typable strains found *ssa* to be widely disseminated, but restricted to only 10 of the 80 clonal lineages examined. The *ssa*-positive lineages were in several instances phylogenetically diverse and had not shared a recent common ancestor. Analysis of the ET 2-M3 lineage, a clone causing STSS, severe invasive disease, and rheumatic fever, found evidence for temporal variation in *ssa* association. Contemporary ET 2-M3 disease isolates had *ssa*, but two older isolates of this clone recovered in 1910 and 1920 lacked the gene. The clonal and temporal distribution pattern of *ssa* suggest a relatively recent acquisition of this SAg-encoding gene by the ET 2-M3 *S. pyogenes* lineage, perhaps by horizontal transfer and recombination.

V 430 REGULATION OF FUNCTIONAL SUPERANTIGEN EXPRESSION BY MHC CLASS II

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Mouse mammary tumor virus (MMTV) is a murine retrovirus that can be transmitted as an infectious unit or inherited as an endogenous provirus. Increasing evidence suggests that MMTV uses the immune system, particularly B cells, as necessary intermediates in its life cycle. To this end, the virus utilizes existing transcriptional machinery for regulation of gene expression and propagation of the infectious virus. One viral protein has recently been shown to be especially important for viral expansion. Encoded by a unique open reading frame in the 3' LTR, this protein, termed a superantigen (SAg), elicits the stimulation of T cells based primarily on the Vß T cell receptor subunit expressed. Large scale stimulation of T cells results in an increase in both potential host B cells and infectious virus.

To further investigate the role of B cell activation on MMTV gene expression and SAg function, we stimulated B cell lines with a variety of mitogens and cytokines. These experiments demonstrated that levels of MHC class II proteins regulate functional SAg expression. Specifically, stimuli, such as IL-4, which increase surface expression of MHC class II also increase functional SAg. Interestingly, LPS stimulation, which increases MMTV transcript levels in B cells, results in a rapid decrease in SAg function. To characterize the turnover of the SAg-class II complex, we compared the rate of disappearance of SAg stimulation to the levels of surface class II. These experiments showed that the SAg complex turns over much more rapidly than the pool of surface class II. Additionally, we have shown that the rate of disapearance of the SAg complex differs from that of conventional MHC- peptide complexes. Experiments with antigen specific T cell hybridomas have shown that SAg stimulation ceases prior to the loss of peptide based stimulation. Experiments to define the biochemical basis for these observations are ongoing.

(Supported by NIH grant CA 36642)

V 431 STAPHYLOCOCCAL ENTEROTOXIN B INDUCES ANTIGEN-SPECIFIC ACTIVATION, DELETION, AND ANERGY OF THE HUMAN T-CELL REPERTOIRE IN SCID-HU MICE,

Dominique Schols, Deborah Jones and Maria-Grazia Roncarolo, Department of Human Immunology, DNAX Research Institute, 901 California Avenue, Palo Alto, CA, 94304-1104.

SCID-hu mice, transplanted with pieces of human fetal liver and fetal thymus, provide an *in vivo* model for studying intra-thymic development and differentiation of human T cells. We previously demonstrated that *in vivo* administration of staphylococcal enterotoxin B (SEB) in SCID-hu mice causes clonal deletion in both $CD4^+8^-$ and $CD4^-8^+$ single positive (SP) thymocytes bearing the cognate Vβ T-cell receptor. By tri-color FACS analysis, we show here that clonal deletion of SEB-specific Vβs already occurs at the immature $CD4^+8^+$ double positive (DP) stage. In addition, we observed an increase in the percentages of SP and DP thymocytes expressing the activation markers CD25, CD28, CD71 and HLA-DR. *In vivo* treatment with SEB resulted also in an induction of clonal anergy. Thymocytes from SEB-treated SCID-hu mice did not proliferate when restimulated with SEB *in vitro*, but produced significant amounts of IFN-γ, GM-CSF and IL-10. The lack of proliferation corresponded of a failure to secrete IL-2 upon restimulation with SEB *in vitro*. This clonal anergy could be reversed *in vitro* by either IL-2 or anti-CD28 mAb or by B7 transfected P815 cells, resulting in optimal T-cell proliferation and IL-2 production. These data demonstrate that *in vivo* administration of SEB leads to activation, clonal deletion and clonal anergy of SEB-specific thymocytes. The clonal anergy can be reversed *in vitro* by triggering the CD28/CTLA4-BB1/B7 pathway.

V 432 THE ROLE OF SHORT LIVED T CELLS IN ANERGY INDUCTION BY SEB IN RATS, S. Ted Shaikewitz[1], Donald Bellgrau[1], Daniel P. Gold[2], and Karen S. Sellins[1], [1] Barbara Davis Center for Childhood Diabetes, Denver, CO 80262 and [2] San Diego Regional Cancer Center, La Jolla, CA 92121

Superantigens are a class of antigens characterized by their ability to selectively activate T cells bearing particular TCR Vβ elements. Because of this feature, superantigens have profound effects upon the TCR repertoire. Failure to respond appropriately to superantigens could be indicative of defects in thymic selection and peripheral tolerance induction. We therefore assessed the effect of staphylococcal enterotoxins (SEs) on various immune parameters of T cells from Biobreeding diabetes-prone (BB-DP) rats. These rats spontaneously develop an autoimmune illness similar to human type I diabetes mellitus. Although the Vβ families stimulated by various SEs were similar in BB-DP and normal rats, the BB-DP T cells consistently responded less vigorously than did those of normal animals. BB-DP T cells were also more susceptible to anti-CD4 monoclonal antibody blockage of the SE response than that of normal rats. It has previously been shown that *in vivo* administration of staphylococcal enterotoxin B (SEB), an exogenous superantigen, induced deletion and anergy of mouse T cells bearing particular TCR Vβ families. We readily induced *in vitro* non-responsiveness to SEB in normal rat T cells with in vivo injections of SEB; however peripheral T cells from BB-DP rats were more resistant to anergy induction by *in vivo* administration of SEB. About 50% of BB-DP T cells are a short lived population that survives for only a few days to weeks in the periphery. This short lived population can be eliminated from the periphery by thymectomizing the animal. Thymectomy of normal rats reduced their number of T cells by about 20%; whereas BB-DP T cells were reduced by about 50%. T cells from short term thymectomized BB-DP rats remained resistant to anergy induction by in vivo SEB administration. Surprisingly, thymectomy of normal rats resulted in resistance to anergy induction by *in vivo* SEB treatment. We hypothesize that a short lived T cell population in normal rats may be responsible for the induction and/or maintenance of anergy in the long lived T cell population. This regulatory short lived population may be abnormal in the BB-DP rat such that peripheral tolerance is less easily achieved and/or maintained in these animals.

V 433 SUPERANTIGEN PRODUCTION BY THE POSSIBLE AIDS COFACTOR, *MYCOPLASMA FERMENTANS* INCOGNITUS, P. Michael Stuart, Ann M. Pullen and Jerold G. Woodward Department of Ophthalmology, Washington University Eye Center, Box 8096, St. Louis, MO 63110. Howard Hughes Medical Institute and the Department of Immunology, SL-15, University of Washington, Seattle, WA 98195. Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY 40536.

Mycoplasma fermentans incognitus has attracted much attention recently as a possible cofactor in some cases of AIDS disease progression. This organism was initially isolated by Lo et al. from patients with AIDS and later from asymptomatic HIV-seropositive patients and non-HIV-infected individuals how died of a mysterious flu-like illness. In order to better understand how this organism might accelerate HIV disease progression we tested whether antigenic preparations from *Mycoplasma fermentans* incognitus could stimulate murine T cells. We report that a protease-sensitive factor is released from this pathogen that stimulates strong proliferative responses in primary murine T cell cultures. This stimulatory activity, which is primarily found in culture supernatants, is also associated with membrane and cytoplasmic fractions from this organism. Cell depletion and antibody blocking studies have indicated that the primary responding cell is a $CD4^+$ T cell, which requires the presence of MHC class II^+ APCs for responsiveness to *M. fermentans* antigen. However, these APCs need not be MHC identical with the T cells indicating a lack of classical MHC restriction. Evaluation of T cell receptor usage by BALB/c T cells responsive to *M. fermentans* has shown that T cells bearing Vβ 4, 8.1, 8.2, and 10 are significantly enriched after exposure to *M. fermentans* antigenic preparations. These results were confirmed by testing the ability of *M. fermentans* antigenic preparations to activate a panel of T cell hybridomas. Taken together, these data indicate that *M. fermentans* produces antigenic material that has properties consistent with those of T cell superantigens.

V 434 SEPARABLE BINDING SITES FOR BACTERIAL AND ENDOGENOUS RETROVIRAL SUPERANTIGENS ON MHC CLASS II MOLECULES, Jacques Thibodeau, Nathalie, Labrecque, François Denis, Brigitte T. Huber[1] and Rafick-Pierre Sékaly, Laboratoire d'Immunologie, Institut de Recherches Cliniques de Montréal, Montréal, Québec, Canada, H2W 1R7 and [1] Department of Pathology, Tufts University School of Medicine, Boston, MA 02111

Bacterial and retroviral superantigens can interact with MHC class II molecules and stimulate T cells upon binding to the CDR4 portion of the T cell receptor. While these molecules exert similar effects on T cells, they show little homology in their primary structure. Amino acids critical for the binding of bacterial toxins have been identified on class II molecules; little is known however of the molecular interactions between class II and retroviral superantigens. Experiments were carried out to determine if both types of superantigens interact with the same regions of class II MHC molecules. Results from functional assays demonstrate that the addition of saturating amounts of the bacterial superantigens SEA or TSST-1 does not impair the presentation of MTV-7 to Vβ6 bearing T cells. Conversely the presence of mtv-7 does not affect presentation of SEA to T cells. More importantly, MTV-9 and MTV-7 superantigens stimulate very efficiently T cells when presented by mutated class II molecules that have lost the capacity to bind three bacterial superantigens (SEA, SEB, TSST-1). These results clearly demonstrate that bacterial and retroviral superantigens have distinct binding sites on MHC class II molecules.

V 435 RESPONSE OF HUMAN THYMOCYTES TO SUPERANTIGEN Scott C. Todd,* Roberto Baccala,† Joseph A. Hedrick,* Argyrios A. Theofilopoulos,† and Constantine D. Tsoukas* §
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Exposure of human thymocytes to bacterial enterotoxins results in deletion of thymocytes expressing specific TCR V-ß genes. The factors contributing to this deletion may relate to the inherent nature of the T-cell at a given stage of development. Here we demonstrate that phenotypically immature, $CD1^+$, human thymocytes are capable of proliferating in response to SEB in the presence of autologous $CD2^{-/lo}$ thymic APC. The response is triggered by low concentrations of SEB, requires the participation of the TCR and IL-2R molecules, and is sensitive to Cyclosporin A. Thymocytes expressing specific V-ß genes are expanded, including two V-ß genes that have not previously been shown to engage SEB. In view of previous data, our results suggest that the factor(s) determining deletion or expansion of immature T-cells are not inherent to the developmental state of the T-cell.

V 436 MECHANISMS INVOLVED IN THE IN VIVO ELIMINATION OF MATURE T CELLS FOLLOWING EXPOSURE TO Mls ANTIGENS, Sandra M. Torello, and Susan R. Webb, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Previous studies have shown that injection of Mls^a-negative mice with Mls^a- bearing cells leads to a strong proliferative response of Mls^a-reactive $CD4^+$ cells expressing $V\beta6^+$ T cell receptors (TCR). This immune response is followed by the elimination of the majority of $V\beta6^+$ cells. Though some of these cells appear to undergo apoptosis in the spleen, the extent of apoptosis in the lymphoid tissues appears insufficient to account for the large scale disappearance of these responding T cells. To examine whether T cells might first home to peripheral tissues prior to their elimination, we examined the accumulation of $V\beta6^+$ $CD4^+$ cells in cell suspensions prepared from gut tissues. Our results show that substantial numbers of $V\beta6^+$ cells are found in the gut following in vivo immune responses to Mls antigens. The kinetics of the appearance of $V\beta6$ $CD4^+$ cells in the gut correlates with the loss of $V\beta6^+$ cells from the lymphoid tissues. Within two weeks, these cells are eliminated from the gut; we are currently testing whether they are undergoing apoptosis.

Our working model is that T cells responding to Mls antigens in the spleen can be activated to become short-lived cells which home to the gut prior to elimination.

V 437 EPSTEIN-BARR VIRUS INDUCES PROLIFERATION OF HUMAN THYMOCYTES IN A MHC CLASS II - DEPENDENT FASHION, Constantine D. Tsoukas*†, Scott C. Todd*, and John D. Lambris§.
*Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182. †Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, CA. 92307. §Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Addition of Epstein Barr Virus (EBV) to cultures of human thymocytes stimulates proliferation of these cells. The growth response was demonstrated by ^3H-Thymidine incorporation after five days in culture, however, 24 hours of exposure to virus was sufficient to achieve maximum stimulation. The response requires the specific interaction of virus and cells via CD21 as antibodies to CD21 or to the viral coat protein, gp350, inhibited proliferation. Culture of thymocytes with UV inactivated EBV or recombinant gp350 did not induce proliferation. The response was blocked by anti-HLA.DR antibody and by anti-TCR antibody, suggesting that class II presentation is necessary. The capacity of peripheral blood lymphocytes to proliferate in response to EBV was inhibited in the presence of anti-HLA.DR but not anti-TCR antibodies, distinguishing the PBL response from that seen in thymocytes. Proliferation of both PBL and thymocytes was Cyclosporin A sensitive. The thymocyte response requires at least two cell populations, $CD2^+$ and $CD2^{-/lo}$, which appear to act as responder and APC respectively. The responding cells include a population of phenotypically immature, $CD1^+$, thymocytes. In view of the fact that thymocytes are naive to EBV antigens, and the fact that primary responses to conventional antigens are difficult to demonstrate *in vitro*, one possible interpretation is that the proliferation is induced by an EBV-encoded or host-encoded, but EBV-regulated superantigen.

V 438 A COMPARISON OF BACTERIAL SUPERANTIGEN AND T CELL CORECEPTOR INTERACTIONS WITH HLA-DR. Robert G. Ulrich, and Sina Bavari, Department of Immunology and Molecular Biology, USAMRIID, Frederick, MD 21702.

CD4 T-lymphocyte coreceptors bound to MHC class II molecules on antigen-presenting cells constitute the core of the signal transduction complex involved with the immune recognition of peptide antigens. Polyclonal T-cell responses to the bacterial superantigens SEA and TSST1 are also CD4-dependent. Paradoxically, both toxins appear to interact directly with conserved CD4-binding sites on the immunoglobulin-like domain of HLA-DRβ chains. We present evidence supporting the role of the CD4 molecule as an integral part of T-lymphocyte recognition of at least two bacterial superantigens: SEA and TSST1. It is proposed that the superantigen qualities of these toxins may be due, in part, to a molecular mimicry of the CD4 molecule.

V 439 **SUPERANTIGEN MEDIATED PERIPHERAL DELETION OF TCR $V\beta3^+$ $CD4^+$ T CELLS CAN BE BLOCKED IN VIVO BY PRIMING MICE WITH LIPOPOLYSACCHARIDE.** Anthony T. Vella, John Kappler and Philippa Marrack. Howard Hughes Medical Institute, National Jewish Center for Immunology and Respiratory Medicine, Denver CO 80206.

The exogenous superantigen, SEA, mediates peripheral deletion of most mature $CD4^+$ T cells bearing TCR Vβ3 in B10.BR mice within a week of injection. This mimics one of the fates of autoreactive T cells circulating in the periphery of normal individuals. Here we demonstrate that this form of peripheral tolerance can be circumvented in vivo by treating SEA-primed mice with the bacterial endotoxin lipopolysaccharide (LPS). This result is critically dependent upon dose and timing of LPS injection. Additionally, only doses of LPS which activate B cells, as determined by upregulated MHC class II and B7 expression, will rescue $V\beta3^+$ T cells from death. We speculate that the activation state of B cells is intimately involved in both the deletion and rescuing processes. For example, resting B cells express high levels of MHC class II but lack critical cell surface costimulatory molecules. Presentation of SEA by these cells may therefore trigger a deletion program in the partner T cell. In contrast, LPS-primed B cells express the costimulatory molecule B7. These cells can therefore provide a costimulatory signal and thus stimulate their cognate T cell. These results demonstrate that LPS can break tolerance by preventing peripheral deletion and raise the possibility that autoreactive T cells may accumulate in the presence of activated cognate APC.

V 440 KINETIC ANALYSIS OF LYMPHOCYTE ACTIVATION AND OF ANERGY INITIATION BY STAPHYLOCOCCAL ENTEROTOXIN B. Steven M. Vroegop, Roger A. Poorman, and Stephen E. Buxser. Cell Biology and Cancer and Infectious Diseases. The Upjohn Company, Kalamazoo, MI 49001

The initial events of T cell activation by superantigens - such as staphylococcal enterotoxin B (SEB) - involve the binding of SEB to Class II MHC followed by the binding of the T cell antigen receptor through the variable region of the beta chain. The activation is eventually nullified by the elimination or anergization of superantigen stimulated $CD4^+$ T cells. We explored the mechanism of this phenomenon in cultured cells.

We followed for nine days the activation of Balb/c splenocytes at several concentrations of SEB by measuring incorporation of ^{3}H-thymidine. Kinetic analysis of cell division demonstrates that the following simple model is sufficient to account for the data. Activation and suppression are only dependent on the number of cells initially activated by a given concentration of the superantigen, whereas the rates of proliferation of $CD4^+$ and $CD8^+$ T cells, and the ability of $CD8^+$ T cells to kill both themselves and $CD4^+$ T cells are independent of the concentration of SEB. Furthermore, the number of T cells initially activated by exposure to SEB depends in a saturable manner on the concentration of SEB added. The anergic phase following SEB activation may be accounted for by a direct stimulation of cytotoxicity mediated by $CD8^+$ T cells.

V 441 PROCESSING AND CLASS II BINDING OF THE MTV7 SUPERANTIGEN (vSAG7). Gary M. Winslow, Yongwon Choi*, Chae-Gyu Park*, Philippa Marrack and John W. Kappler. Howard Hughes Medical Institute, 1400 Jackson St., Denver, CO 80206; and *Rockefeller University, New York, N.Y. 10021.

We have previously shown that vSAG7 (Mls-1^a) is synthesized in vivo as a 45 kDa precursor protein but the carboxy-terminus is found on the cell surface as an 18.5 kDa protein, possibly a result of proteolytic processing at a tetrabasic amino acid motif ending at amino acid 171 (Winslow et al. Cell 71: 719-730). More recently, using antibodies that recognize both the carboxy- and amino-termini of vSAG7 we have demonstrated that the carboxy- and amino-termini are associated non-covalently, and that the proteolytically processed vSAG binds to class II molecules. Although we detect some binding of the full-length vSAG to class II, proteolytic processing appears to enhance class II binding. Analysis of the carbohydrate structure of vSAG7 reveals that proteolytic cleavage occurs in a golgi or post-golgi compartment. Furthermore, in addition to proteolytic cleavage at amino acid 171 (the putative proximal cleavage site), we have evidence that processing occurs at two additional sites. The sizes of the carboxy-terminal products are consistent with cleavage occuring at basic residues preceding amino acid 192 (the putative distal site) and at amino acid 73 (the putative transmembrane site). Processing at the putative distal site appears to abrogate class II binding. Site directed mutagenesis of the putative proximal site (RKRR to GEEF) eliminates the predicted proteolytic cleavage product and vSAG7 surface expression, suggesting that cleavage at this position is required for vSAG7 function. We are continuing our use of targeted mutagenesis to clarify the locations of and the requirements for proteolytic processing in vSAG7 presentation.

V 442 INFLUENCE OF MHC MOLECULES ON T CELL RECOGNITION OF SUPERANTIGENS. David L. Woodland, Sherri L. Surman, Marcia A. Blackman and Alison M. Deckhut. Department of Immunology, St. Jude Children's Research Hospital, Memphis TN 38105.

Bacterial and viral superantigens are characterized by their ability to activate T cells directly through the Vβ element of the T cell receptor (TcR). Although MHC class II molecules are required, superantigen recognition is not classically MHC restricted in that a single superantigen can be recognized in the context of multiple alleles and isotypes of class II, including xenogenic molecules. However, several recent studies have suggested that polymorphic residues of the class II molecule may influence the recognition of superantigen by some murine T cells. These observations have been interpreted in terms of a direct interaction between the TcR and MHC molecule during superantigen engagement. In order to determine the role of class II polymorphisms in superantigen recognition, we are studying the responses of T cell hybridomas to both bacterial and retroviral superantigens presented in the context of different class II alleles and mutants (Ehrich et al., 1993 JEM 178: 713.). Preliminary data have shown that mutations within the class II molecule affect superantigen recognition by some, but not all, of the relevant T cell hybridomas. These data support the hypothesis that there is a direct interaction between the TcR and MHC molecule during superantigen recognition.

B Cell Development

V 443 ARRESTED B CELL DEVELOPMENT IN TRANSGENIC MICE EXPRESSING A CHIMERIC IgMκ-Iaα-TRANSMEMBRANE MOLECULE AS THEIR ANTIGEN-SPECIFIC RECEPTOR. *Margaret Beckwith, *Cathy O'Connell, †Philip W. Tucker, #Shawn Hill, *Lysa Baginski, #Dan L. Longo, and James J. Kenny, *Program Resources Inc./Dyncorp., & #Biological Response Modifiers Program, NCI-FCRDC, Frederick, MD 21702 and †Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Tx 75235

B cell lines expressing a chimeric phosphocholine-(PC)-specific IgM-(Iaα TM) molecule as their antigen-specific receptor are unable to function in the B cell activation and differentiation events of immediate signal transduction, antigen presentation, or immunoglobulin secretion following binding by anti-id or antigen (J. Exp. Med. 174:1103,1991). Since many stages of early B cell development appear to be regulated via signaling through the μ-membrane receptor complex, it was of interest to see if transgenic mice expressing this "dead" chimeric molecule would develop B cells, and whether these B cells would exhibited allelic exclusion or any other sign of altered B cell development. All the splenic B cells in transgene positive (TG^+) mice express the TG-encoded IgM-Iaα chimeric receptor in association with endogenous IgM. However, B cells expressing only the TG product appear to develop in the bone marrow. This suggests that B cells expressing only the IgM-Iaα receptor can develop in the absence of endogenous IgM but they are not selected into the peripheral lymphoid organs. To test this hypothesis, TG^+ mice were crossed and back-crossed to μ-knock-out (μNT) mice to obtain TG^+ homozygous μNT/μNT mice. These mice, which are unable to produce endogenous IgM, produce the same number of bone marrow B cells as the TG^+ heterozygous μNT/+ littermates; however, these B cells do not exit the bone marrow. These data support the idea that an IgM receptor-mediated signal is required to positively select B cells from the bone marrow into the peripheral lymphoid tissues and that the chimeric IgM-Iaα molecule is unable to transduce the required signal. However, in the TG^+ heterozygous μNT/+ littermates, the expression of the transgene alters the maturation or activation state of the splenic B cells in that fewer B cells develop compared to normal mice and many of these B cells lack CD23 but express sIgD.

V 444 CHARACTERIZATION OF CD40-DEFICIENT MICE.

Debra Cockayne[1], Tony Muchamuel[1], Rich Murray[1], Stacy Fuchino[1], Ralf Kühn[2], Werner Müller[2], Klaus Rajewsky[2], and Maureen Howard[1], DNAX Research Institute, Palo Alto, CA 94304[1] and Institute for Genetics, University of Cologne, W-5000 Cologne 41 Germany[2].

CD40 is a transmembrane receptor molecule that is found on numerous cell types including B cells, interdigitating dendritic cells, follicular dendritic cells, thymic epithelial cells, monocytes and T cells. CD40 has been most widely recognized as a functionally important B cell activation antigen. Signalling through CD40, in the presence of costimulants, is a potent stimulus for inducing B cell proliferation as well as triggering Ig class switching and isotype secretion. The interaction of CD40 on the surface of B cells with its T cell ligand gp39 has been shown to be one of the most important interactions mediating these B cell responses. Recent studies have shown that point mutation defects in the gp39 molecule are responsible for the deficiencies in serum Ig isotypes levels seen in patients with Hyper IgM syndrome. This observation provides strong evidence that the CD40-gp39 interaction is critical, but possibly not sufficient, for efficient T cell-dependent B cell responses. Although very little is known about the function of CD40 on other cell types such as thymic epithelial cells, T cells, monocytes, and early bone marrow subsets (see A. Heath poster, this session), it is possible that CD40 may play previously unrecognized roles in T cell and B cell ontogeny.

In order to extend our understanding of the role of CD40 in vivo, we have generated mice carrying a targeted mutation in the murine CD40 genomic locus. Exons 7 and 8 of the murine CD40 gene were replaced by the neomycin resistance gene, resulting in a homozygous deletion of sequence corresponding to the transmembrane domain of the CD40 molecule. In addition, a stop codon was placed at the splice acceptor site of exon 6, thereby introducing an additional mutation within the extracellular domain of the CD40 molecule. We will present data on our characterization of homozygous CD40-deficient mice.

V 445 CELLULAR GENE ACTIVATION EVENTS IN PRE-B CELLS EXPRESSING THE BCR/ABL TYROSINE KINASE, Lucie Cohen, Roberta Kato and Owen N. Witte, Department of Microbiology and Molecular Genetics, Howard Hughes Medical Institute, University of California at Los Angeles, Los Angeles, CA 90024-1662

The P210 BCR/ABL oncogene is a naturally occuring fusion gene generated by the reciprocal t(9:22) chromosomal translocation, a cytogenetic marker of chronic myelogenous leukemia. To understand the molecular mechanism by which BCR/ABL transforms cells, we chose to analyse the range of cellular genes whose expression is altered by the activated BCR/ABL serine-tyrosine kinase. We have used the technique of *Differential Display of mRNA by PCR* (DD-PCR) (see ref.1) to compare the pattern of gene expression in pre-B lymphoid cell lines, between cells expressing the wild-type P210 BCR/ABL oncoprotein (TX+ cells), and cells expressing a non-transforming mutant P210 BCR/ABL/1294F carrying a mutation at the autophosphorylation site of the protein (tx- cells). From a series of 100 PCR reactions using different sense and antisense primer pairs, 46 partial cDNA sequences observed only in TX+ cells were isolated for further analysis. Five of these sequences were confirmed to be differentially expressed in TX+ cells compared to tx- and normal pre-B cells by Northern blot analysis. The RNA for one particular sequence (DD29C2) undetected in normal cells, was found to be highly expressed in TX+ cells, and in pre-B cells transformed by the v-*abl* oncogene. Comparison of the sequence with sequences in the Genebank showed no significant homology with known sequences in the database, suggesting that the DD29C2 clone may represent a new gene implicated in leukemogenesis.

(Ref. 1: Liang, P. and Pardee, A. Science 257, 967 (1992)).

V 446 CONVERGENCE OF DIFFERENT ACTIVATION PATHWAYS LEADING TO B CELL DIFFERENTIATION, Ronald B. Corley, Troy D. Randall, Joseph W. Brewer, Frances E. Lund, Edwin C. Murphy III, and Randolph Wall. Department of Immunology, Duke University Medical Center, Durham, NC 27710 and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

IL-5, IL-6, and LPS all act as B cell differentiation factors as defined by their ability to stimulate immunoglobulin H and L chain mRNA and induce high rate antibody secretion. We have compared the ability of these differentiation factors to stimulate various genes during B cell differentiation using clonal inducible B cell lymphomas. We find that each stimulation has distinct effects on the gene programs expressed by differentiating B cells. For example, both IL-5 and IL-6 independently stimulate increases in μ_s, kappa, and J chain mRNA. LPS also stimulates μ_s and kappa transcripts, but fails to transcriptionally activate J chain to significant levels. This difference results in definable differences in the type of secreted IgM polymer which predominates following differentiation. There are also significant differences in the activities of the two lymphokines. For example, while IL-6 acts only as a differentiation factor, IL-5 also augments B cell proliferation, indicating that these two lymphokines have different composite activities. Moreover, the differentiation stimulated by IL-5, but not by IL-6, is partially inhibited by IL-4. Inhibition is not at the level of IL-5 receptor expression since IL-4 inhibits differentiation, but not proliferation induced by IL-5. IL-5, but not IL-6, stimulates increases in MMTV proviral gene expression. On the other hand, while neither of these lymphokines activates c-myc expression above constitutive levels, LPS stimulates a pronounced and transient increase in c-myc transcripts. These results demonstrate that while IL-5, IL-6 and LPS act as differentiation factors for B cells, they induce differentiation using at least partially distinct pathways. Since differences in gene expression can be detected within four hours after stimulation, these results imply that early activation events distinguish these modes of stimulation which lead to pronounced differences in gene expression. Nevertheless, each of these different signaling pathways coalesce into the same result, at least with respect to antibody secretion. Experiments to assess the consequences of these differences on the fate of B cells, and to identify genes which distinguish these early differentiative events, are currently underway.

V 447 THE KINETICS OF EXPRESSION OF DNA TRANSFECTED INTO NORMAL HUMAN B LYMPHOCYTES CHANGE RAPIDLY FOLLOWING INFECTION BY EPSTEIN-BARR VIRUS. Toni A. Gahn and Bill Sugden, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Normal human B lymphocytes are considered refractory to expression of DNAs introduced by transfection. By analyzing the expression of a reporter gene transfected into normal human B lymphocytes by electroporation, we have found that the cells are capable of expressing a reporter gene. However, maximum expression was detected only at very early times (6-9 hrs) after transfection and declined rapidly to background levels by 24 hours. These kinetics were in contrast to the kinetics of expression of the same reporter gene in human B cell lines. In cell lines, expression increased steadily reaching maximum levels at approximately 36 hours after transfection and then declined slowly.

To investigate the differences between normal B cells and established B cells that lead to the differing kinetics of expression of transfected genes, we have electroporated cells at various times following infection by EBV. Infection of B cells by EBV causes the cells to proliferate indefinitely in culture, a process referred to as immortalization. The immortalized cells display changes indicative of activation including the secretion of immunoglobulin and a vast increase in CD23 at the cell surface. We have found that a change in kinetics of expression of transfected DNA occurs rapidly (within 3 days) following infection by EBV. We are investigating the factors that influence expression in normal and EBV infected human B cells.

V 448 **THE V-ABL TYROSINE KINASE NEGATIVELY REGULATES NF-κB AND BLOCKS KAPPA GENE TRANSCRIPTION IN PRE B LYMPHOCYTES.**
Sheila J. Gerety[1], Christopher A. Klug[1], Yunn-Yi Chen[2], Nancy R. Rice[3], Naomi Rosenberg[2], and Harinder Singh[1]. [1]Howard Hughes Medical Institute, Univ. of Chicago, Chicago, IL. [2]Tufts University School of Medicine, Boston, MA. [3]NCI - Frederick Cancer Research and Development Center, Frederick, MD.

Transformation of B lineage precursors by the Abelson murine leukemia virus appears to specifically arrest their development. Such cells generally retain unrearranged immunoglobulin kappa alleles that are transcriptionally inactive. We demonstrate that non-transformed pre B cells expanded from the mouse bone marrow efficiently transcribe germ-line kappa alleles. In addition, they contain activated complexes of the transcription factor NF-κB, in contrast with their Abelson-transformed counterparts. Using conditionally transformed pre B cell lines, we show that the viral transforming protein, a tyrosine kinase, blocks germ-line kappa gene transcription and negatively regulates NF-κB activity and the functioning of the kappa intron enhancer. We are exploring the biochemical mechanism by which v-abl inhibits transcription and rearrangement of the kappa locus during pre B cell differentiation.

V 449 CHARACTERIZATION OF A NOVEL REARRANGING MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY IN THE NURSE SHARK, GINGLYMOSTOMA CIRRATUM. Andrew Greenberg and Martin Flajnik, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Fl 33136
The timing in evolution of the emergence of immunoglobulin (Ig) and T cell receptor (TCR) molecules from a common rearranging precursor is unknown. We have isolated a novel Ig superfamily molecule by PCR from the nurse shark which contains one V domain and 5 C1-SET domains. Analysis of several cDNA clones has indicated that 1)The V domain shows great diversity and 2)The C domains are identical in sequence. The V domain differs from canonical V domains found in Ig and TCR molecules in that part of the predicted CDR2 loop is missing, and the region corresponding to CDR3 contains from 1-5 cysteines. Hybridization with a V probe under lowered stringency conditions detect from 2-4 bands; since the diversity of expressed V regions exceeds that of the germline, somatic mutation may also be contributing to the generation of diversity. Data concerning the germline organization of the rearranging gene segments will be presented. We have recently produced a battery of monoclonal antibodies specific for the protein found in nurse shark serum encoded by the cDNA. This protein is dimeric and does not bind to light chains like bona fide Ig, consistent with the deduced amino acid sequence lacking the canonical light chain binding cysteine found in Ig heavy chains. Thus, this new Ig superfamily molecule may represent an intermediate in the evolution of Ig and TCR molecules and may further our understanding on how rearranging receptors have evolved during phylogeny.

V 450 **CD40 AND MURINE B CELL ONTOGENY**
Heath AW, Wu WW, Cyster JG*, Goodnow CC* and Howard M. DNAX Research Institute, Palo Alto, CA and Howard Hughes Medical Institute, Stanford, CA.

While monoclonal antibodies against human CD40 have been available for some time, to date there have been no antibodies available against the murine homologue. We have generated a panel of monoclonal antibodies against murine CD40 (mCD40) by immunising rats with soluble, recombinant mCD40. All antibodies specifically bind recombinant murine CD40 expressed on L cells. At least two functional groups of antibodies have been identified, one group induces B cell proliferation in the absence of co-stimulators, and the other group will not induce proliferation either on its own or with IL4, but will synergise with the agonistic antibody or with anti-IgM to induce proliferation.

These antibodies have been used to determine expression of CD40 in murine bone marrow B cell haematopoesis, with interesting results. CD40 is expressed early in murine B cell ontogeny, on a proportion of $B220^{low}$, IgM^- pro/pre B cells, but is down-regulated on a subset of $B220^{low}$ IgM^+ immature B cells. These $B220^{low}$ IgM^+CD40^- cells differentiate overnight into $CD40^+$ cells, and thus would appear to precede this larger population. Since these immature B cells are thought to be the tolerance susceptible stage, and CD40 is of importance in rescuing cells from apoptosis, we investigated the functional importance of CD40 down-regulation using sorted immature B cells. As expected, both $CD40^+$ and $CD40^-$ subsets of the immature population were induced to programmed cell death by anti-IgM stimulation, and both populations proliferated in response to CD40 stimulation, presumably because of differentiation of the $CD40^-$ cells. However a combination of anti-IgM and CD40 antibody resulted in apoptosis of the $CD40^-$ subset, but proliferation of the CD40 positive subset.

The possible relevance of this down-regulation of CD40 in relation to tolerance induction in response to self antigens will be discussed.

V 451 BIOLOGICAL ROLE OF HUMAN IgM HEXAMERS

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Secreted IgM is classically described as a pentameric molecule assembled from five H_2L_2 monomeric subunits disulfide-bonded together with an additional polypeptide, the J chain. However, studies in the mouse have clearly shown the existence of a second functional polymer form of IgM, an IgM hexamer. Surprisingly, these studies have shown that IgM hexamers never contain J chain and are capable of activating complement up to 20 times more efficiently than IgM pentamers. This increased efficiency of IgM hexamers in activating complement represents a potentially important mechanism by which low affinity IgM antibodies can increase their biological activity. At this time, however, the physiologic functions of IgM hexamers remain unclear. IgM hexamers could be beneficial, contributing to immune responses to antigens where clearance is greatly dependent on complement activation, such as the immune clearance of bacterial pathogens. However, IgM hexamers could be deleterious due to their high efficiency in activating complement, particularly in IgM-mediated diseases. We are particularly interested in the biology of human IgM hexamers. Monoclonal IgM antibodies and IgM autoantibodies obtained from immunodeficient patients and EBV transformed B cells have been screened for the presence of IgM hexamers. A number of these samples contain two polymeric forms of secreted IgM. Analysis of these two polymer types by SDS-PAGE and sucrose density gradients indicates the presence of pentameric and hexameric IgM. The identification of sources for both human IgM pentamers and hexamers has allowed us to begin analysis of these two molecules with respect to J chain content and the ability to bind to the polymeric Ig receptor. Current work is also focused on comparing the ability of both polymer forms to activate complement.

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V 452 CD22 IS AN ACCESSORY SIGNAL TRANSDUCTION MOLECULE THAT REGULATES B CELL ACTIVATION VIA THE ANTIGEN RECEPTOR COMPLEX.

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CD22 is a B cell-restricted transmembrane glycoprotein that has recently been shown to mediate heterotypic aggregation between B cells and other hemopoietic cells. Based on the developmental kinetics of CD22 expression, it is likely that this molecule plays a role in B cell activation. Studies have shown that cross-linking of CD22 decreases the threshold of activation for quiescent B cells. Moreover, expression of CD22 is required for signal transduction events that are initiated by antigen receptor (AgR) cross-linking. We have demonstrated that CD22 physically interacts with the B cell AgR, membrane immunoglobulin (mIg), as well as the protein tyrosine phosphatase (PTP), CD45. Thus, it appears that these receptors interact with one another in the membrane and may exist as a heterotrimeric complex. Additionally, both mIg and CD45 were observed to regulate the tyrosine phosphorylation of CD22 via their associated protein tyrosine kinase and PTP activities, respectively. Cross-linking of either mIg or CD45 resulted in a rapid increase in tyrosine phosphorylation of CD22 (within 30 sec to 1 min). We further demonstrated that tyrosine phosphorylation of CD22 mediates its association with *src* homology 2 (SH2)-containing proteins including phosphatidylinositol 3-kinase. Thus, it appears as though CD22 is part of a multi-receptor complex and that its accessory function in the B cell is regulated by tyrosine phosphorylation. Phosphorylation of CD22 leads to the recruitment of signal transducing molecules containing SH2 domains that presumably associate with CD22 through binding to specific phosphotyrosine motifs. Finally, deletion of CD22 from a B lymphoma cell line using chemical mutagenesis and negative selection was observed to abrogate signal transduction via the AgR, further supporting the conclusion that CD22 functions as an important accessory molecule in the B cell.

V 453 INVOLVEMENT OF THE G-PROTEIN-COUPLED RECEPTOR BLR1 IN MURINE B CELL DIFFERENTIATION

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We have recently described the cloning of a novel lymphocyte-specific G-protein-coupled receptor endowed with the typical hallmark of seven transmembrane-spanning regions, designated BLR1. We report here the isolation of the *blr1* gene, encoding the murine homologue of human *BLR1*. We demonstrate that expression of *blr1* is restricted to certain differentiation stages of lymphoid and neuronal cell lineages, in particular, to mature B cells, and to distinct cell layers of the cerebellum.

Blr1 consists of two exons encoding a protein of 374 amino acid residues which shows an overall homology of 83% to the human receptor. The first extracellular amino-terminal domain is less conserved with an identitiy of about 47%. In addition, comparison of the promoter region of the human and the murine genes revealed that the position of multiple transcription factor binding sites are maintained suggesting a conserved regulation of the *blr1* gene in both species.

Blr1-specific transcripts were observed in secondary lymphatic organs and to a lesser extent in brain of adult mice but not in other tissues. RNA *in situ* hybridization localized *blr1* transcription to primary follicles and to the mantle zone of secondary follicles. SCID mice in which mature B cell development is severely impaired exhibit a strongly reduced level of *blr1*-specific RNA in the spleen. Activation of resting splenocytes of NMRI mice let to down-regulation of *blr1* expression. Interestingly, the *blr1* gene is also expressed during late embryogenesis in fetal liver and brain. In view of the remarkable expression pattern in the B cell lineage we suggest that murine BLR1 may represent a cytokine/ neuropeptide receptor exerting regulatory functions on recirculating mature B lymphocytes.

V 454 A NOVEL PRE-B CELL GROWTH SUPPORTING GP I-ANCHORED MOLECULE IN RHEUMATOID ARTHRITIS BONE MARROW,

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Stromal cells are essential for B cell growth and development in the bone marrow (BM). However, molecular mechanisms of the interaction between B cells and stromal cells remains to be clarified. We have previously suggested that the BM as well as joints are affected in rheumatoid arthiritis (RA), because the pre-B cell growth supporting ability of BM stromal cells was observably augmented in RA. Unknown surface molecules on BM stromal cells were involved in the stromal cell-dependent growth of preB cells. To further elucidate the molecular nature of the stromal cell abnormality, we established two monoclonal antibodies (RF3 and SG2) against RA BM stromal cells and molecularly cloned a novel human BM stromal cell surface molecule, BST-1, recognized by the mAbs. The predicted amino acid structure of BST-1 suggested that it is anchored to the cell membrane by a GPI-linkage. BST-1 gene was located to Chr 14q32.3. Surface and mRNA expression of BST-1 was enhanced in RA BM stromal cell lines as compared to those derived from healthy donors, indicating the presence of an abnormality in the BM of RA. Compared with the parent BALB3T3 cells, human BST-1 expressing transfectants showed twofold enhanced ability to support the stromal cell-dependent growth of a murine pre-B cell line, DW34. Furthermore, soluble form of BST-1 could suppress the stromal cell-dependent growth of DW34. These results suggest that a novel stromal cell GPI-anchored molecule, BST-1, is involved in pre-B cell growth and that its enhanced expression in the BM may be in part responsible for polyclonal B cell abnormalities in RA.

V 455 VIRUS NEUTRALIZING ANTIBODIES WITHOUT SOMATIC POINT MUTATION, Ulrich Kalinke, Etienne Bucher, Annette Oxenius, Stephan Geley[$], Reinhard Kofler[$], Andre Traunecker[+], Rolf M. Zinkernagel and Hans Hengartner, Institute of Experimental Immunology, University of Zürich, Schmelzbergstr. 12, CH-8091 Zürich, Switzerland, [+]Basel Institute for Immunology, Grenzacherstr. 487, CH-4005 Basel, Switzerland, [$]Department of Molecular Biology, Institute for General and Experimental Pathology, University of Innsbruck Medical School, Fritz-Pregel-Strasse 3, A-6020 Innsbruck, Austria.

Mice infected with vesicular stomatitis virus (VSV) mount a protective T_h-dependent IgG response. As early as day 6 or 12 after infection hybridomas can be isolated secreting neutralizing IgG antibodies with high affinity for the unique antigenic site on the glycoprotein of VSV. Nothern dot blot analysis of RNA extracts of the hybridomas revealed a polyclonal IgG response with a prevalent usage of VH elements belonging to the J558, Q52 and 7183 VH families. Sequence analysis of VH and VL genes of a series of hybridomas defined by characteristic VH, JH and JK hybridization patterns showed that these clones used unique VH elements and that only few or no point mutations were found in the VH and VL segments. The point mutations did not add significantly to affinities since in such a group affinities differed not more than a factor of 2-3 and were comparably high. The V region genes of a particular hybridoma bearing 5 point mutations in the VH and no point mutation in the VL were linked and ligated to a C-kappa domain to be expressed as a monovalent single chain antibody (FVCK). This FVCK antibody showed a binding affinity comparable to the native antibody implying that a single binding site of the bivalent native antibody is responsible for the affinity of the antibody. Presently we are generating FVCK antibodies in germline configuration by site directed mutagenesis to evaluate the influence of single amino acid positions on the antibody affinity.

V 456 EXPRESSION OF IMMUNOGLOBULIN μ HEAVY CHAIN WITH V_H81X REGIONS IN PRE-B CELLS

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Studies of V_H gene usage during B cell differentiation demonstrated that a member of the immunoglobulin V_H 7183 family, V_H81X, is the predominantly used V_H region in pre-B cells, but it is very rarely expressed in mature B cells. Recently, it has been shown that immunoglobulin μ-chains, the heavy (H) chains of IgM, in pre-B cells must associate with two proteins, (λ5 and VpreB), and that the formation of this complex is required for the development of pre-B cells into mature B cells. Based upon these findings, we contend that pre-B cells that express μ-chains with V_H81X regions are not activated to become mature B cells, because these μ-chains do not associate with λ5 and VpreB. I will test this model using a subclone of the pre-B cell line 18-81. This subclone (F) expresses μ-chains with the V_H81X region only in the cytoplasm but not on the cell surface. We also isolated a surface μ-chain positive variant of F, FM10. We hypothesize that FM10 has acquired a mutation that allows surface expression of μ in this cell line. Genetic and biochemical analysis of the surface μ-negative clone, F, and the surface μ-positive variant, FM10, will be discussed.

V 457 THE RELATIONSHIP BETWEEN LEVELS OF SERUM AND MEMBRANE BOUND IgD IN NORMAL INDIVIDUALS. Gillian A. Kingsbury, Fauzia Rana and Richard P. Junghans. Biotherapeutics Development Laboratory, New England Deaconess Hospital, Harvard Medical School, Boston MA 02215.

Membrane IgD is co-expressed with IgM on the surface of mature B cells. Antigenic stimulation of surface IgD+ cells does not lead to increased IgD expression and antibody-secreting plasma cells no longer express surface IgD. The serum concentration of secreted IgD has been shown to vary over 100 fold between individuals, ranging from <2 to 250 ug/ml. It is known that IgD catabolism does not vary between individuals with low and high serum levels (Rogentine *et al* J. Clin. Invest. 45. 1966), therefore differences must lie in the rates of secretion of IgD. Two forms of IgD mRNA, encoding the secreted and membrane bound immunoglobulin, can be found during all stages of B cell differentiation.

Serum IgD concentrations were quantified in 15 normal donors using a radial diffusion assay. PBMCs were purified using ficoll gradients and the percentage of $CD19^+$ and surface IgD^+ B cells assayed by cytofluorometry. The fluorescence intensity of anti-IgD stained cells was used as a measure of relative levels of expression of surface IgD. The lack of correlation between serum IgD and number of IgD^+ B cells was confirmed (Fraser and Schur, Clin. Immunol. Immunopath. 19. 1981). In addition no correlation was found between serum IgD and the relative level of surface IgD expression. Current studies are examining the relative steady state levels of the two forms of delta mRNA in B cells from individuals with high and low serum IgD levels to elucidate the molecular basis for differences in IgD secretion.

V 458 MHC CLASS II EXPRESSION DISTINGUISHES FETAL AND ADULT-TYPE B CELL DEVELOPMENTAL PATHWAYS, Kong-Peng Lam and Alan M. Stall, Department of Microbiology, College of Physicians & Surgeons, Columbia University, New York, NY 10032

All mature B cells co-express MHC class II molecules, I-A and I-E, which are restriction elements required for antigen presentation to $CD4^+$ T-cells. However, the expression of class II during the early stages of B cell development has been unclear. Some studies indicated that B cells acquire class II and surface (s)IgM concurrently during development, while other reports suggested that IgM^+, Ia^- cells are present in young mice and in long term bone marrow cultures. We demonstrate that there is a fundamental difference in the expression of class II molecules during B cell development in the fetal liver and adult bone marrow. Pre-B and immature IgM^+ B cells generated in the fetal liver initially lack all surface class II expression. In contrast, in the adult bone marrow, I-A is expressed from the pre-B cell stage while I-E is first expressed on mature B cells. These results demonstrate that there are at least 2 distinct B cell developmental pathways in ontogeny and that the cell surface expression of I-A and I-E are not co-ordinately regulated. Interestingly, fetal-type B cell development can be found to occur in various organs up to 4-6 weeks of age. This has implications for the generation of $CD5^+$ B cells in the early neonatal environment. The differences in class II expression on B cells generated at different times in ontogeny could potentially play a role in determining the immune repertoire and thus the funtions of B cell subsets and lineages.

V 459 STRUCTURE OF THE HUMAN B-CELL ANTIGEN RECEPTOR COMPLEX ON NORMAL AND MALIGNANT B CELLS. A.C. Lankester, G.M.W. van Schijndel, C.J.M. van Noesel and R.A.W. van Lier. Dept. of Clin. Viro-Immunolgy, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, The Netherlands.

On human B cells the antigen receptor complex (BCR) is composed of the membrane immunoglobuline molecule (mIg) and the Igα/Igβ heterodimer. We have previously shown that mIgM, mIgD and mIgG are associated with similar heterodimers. Recently it has become evident that the isotype related differences between the heterodimers are posttranslationally defined. In order to further investigate the architecture of the BCR, we biochemically analyzed the intermolecular interactions in mIgM, mIgD and mIgG complexes. Following disruption of the disulfide mediated intermolecular interactions, Igα was found no longer associated with Igβ nor the mIgH chain. In contrast, the association between Igβ and the mIgH chain was largely unaffected. These findings indicate that Igβ primarily facilitates the interaction between the heterodimer and the mIgH chain. Igα may function in stabilizing this interaction and/or mediate the association with other membrane molecules.

Several B-cell specific transmembrane molecules (eg. CD19 and CD22) have been described to be associated with the BCR. CD5 is an accesory molecule expressed on T cells, a subpopulation of normal B cells and characteristically on B-CLL cells. On T cells CD5 has been shown to be physically and functionally coupled to the TCR/CD3 complex. We found that CD5 is similarly associated with the BCR complex on normal and malignant B cells, and serves as substrate for BCR induced tyrosine kinase activity. These findings suggest that T and B cells use a similar mechanism to associate with and activate accesory molecules. Moreover, $CD5^+$ B cells have an exclusive potential to modulate antigen receptor mediated signals.

V 460 BLIMP-1, A NOVEL ZINC-FINGER CONTAINING PROTEIN INVOLVED IN THE DIFFERENTIATION OF MATURE B CELLS, David H. Mack, C. Alex Turner, and Mark M. Davis, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305

Subtractive cloning techniques have been used to isolate cDNAs corresponding to transcripts which are induced during B cell maturation. Blimp-1 (B lymphocyte induced maturation protein) transcripts are rapidly induced during activation of B cells to secretion and are present exclusively in late B and plasma cell lines and predominantly in tissues which contain plasma cells. Sequence analysis of full length Blimp-1 cDNAs predicts a putative 856 amino acid open reading frame which contains five tandemly arranged C_2H_2 Krüppel-type zinc finger motifs and proline rich and acidic regions similar to those observed in the activation domains of known transcription factors. Antisera raised against the predicted protein recognizes a ~100 kD protein which is specifically localized to the nucleus. Stable or transient transfection of Blimp-1 into mature B cell lines leads to the expression of multiple phenotypic changes associated with B cell differentiation including increased accumulation of J chain message, upregulation of the plasma cell marker Syndecan-1, and the onset of immunoglobulin secretion. Thus Blimp-1 appears to be a pleiotropic regulatory factor involved in terminal differentiation of B cells.

V 461 **Regulation and Function of bcl-2 During B Cell Development.**

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Homeostasis in the central and peripheral B cell pools is controlled by a continous production of B cell precursors in the bone marrow, as well as by the rate of survival and death of B cells. The high rate of cell death during B cell development is due, in part, to a selection process by which B cells that fail to display surface IgM or those that express antigen receptors for self are clonally eliminated by apoptosis. The intracellular signals that control the survival of developing B cells are largely unknown. A clear candidate for such a role is the bcl-2 proto-oncogene, whose product promotes cell survival by inhibiting apoptotic cell death. The physiological role of the endogenous Bcl-2 protein is unclear because its expression in developing B cells and its ability to modulate apoptosis are undetermined. We have studied in detail the expression of Bcl-2 during B cell development by three-color flow cytometric analysis. Our results shown a striking developmental regulation of the Bcl-2 protein in B lymphocytes. Bcl-2 is highly expressed in pro-B and mature B cells but downregulated at the pre-B and immature B cell stages of development. Importantly, expression of Bcl-2 perfectly correlates with susceptibility to apoptosis mediated by dexamethasone in vivo. Targeting of Bcl-2 to pre-B cells and immature B cells rescued the cells from dexamethasone-induced apoptosis. In order to assess Bcl-2 in the process of clonal deletion of self-reactive immature B cell precursors, neonatal C57BL/6 mice were injected intraperitoneally with $F(ab')_2$ goat anti-mouse IgM polyclonal antibody which mimmics the delection process. Two days following injection, there was a selective elimination of IgM^+IgD^- immature B cells expressing low levels of Bcl-2. In contrast, Bcl-2^{high} IgM^+IgD^- immature B cells and IgM^+IgD^+ mature B cells largely survived anti-IgM treatment. Importantly, overexpression of a bcl-2 transgene in Bcl-2^{dull} IgM^+IgD^- immature B cells failed to prevent their anti-IgM-induced cell death . These results strongly suggest that downregulation of Bcl-2 at the pre-B and immature B cells stages facilitates appropriate selection of developing B cells following physiological cell death signals. Yet, clonal deletion at the immature B cell stage but not glucocorticoid-induced apoptosis is independent of Bcl-2. After or during clonal selection, Bcl-2 is upregulated in mature B cells and maintains peripheral B cell survival.

V 462 A DEVELOPMENTALLY REGULATED, LYMPHOCYTE-SPECIFIC FACTOR BINDING THE IGK 3' ENHANCER, Kerstin B. Meyer and John Ireland, The Wellcome/CRC Institute of Developmental Biology and Cancer, and the Department of Pathology, Cambridge University, Tennis Court Road, Cambridge CB2 1QR, UK

Developing B lymphocytes pass through a number of well characterised developmental stages which can be defined on the basis of the rearrangement state and expression of the immunoglobulin (Ig) genes. The Igκ light chain locus is activated at the transition from the pre-B to the B cell stage. Regulated rearrangement and expression of the Igκ gene requires multiple transcriptional control regions: the V-gene promoters, the κ intron enhancer and the κ3' enhancer. In pre-B cells activity of both enhancers is inducible by the mitogen LPS. While NF-κB is responsible for the induction of the intron enhancer, the 3' enhancer is controlled by a region that acts to repress transcriptional activity. We have now identified a novel nuclear factor which binds to a site within this repressor region. The factor, which has tentatively been named KSF (kappa silencing factor), is lymphoid specific. It cannot be detected in cell types such as fibroblast, osteosarcoma, monocytes or myeloid cells. In lymphoid cells on the other hand, KSF exists in multiple forms. Slowly migrating complexes can be detected in pro-B cells, T cells and weakly in B cells, while a complex with high electrophoretic mobility is seen in pre-B cells and T cells. Importantly the high mobility form is down-regulated upon LPS stimulation of pre-B cells, with 50% of the protein having disappeared after approx. 12 hrs. The tissue-distribution as well as the developmental regulation of KSF, especially of the high mobility from, is thus compatible with KSF performing a function as a transcriptional repressor. It is interesting to note that this complex is seen in those cell types that express the recombinase activating genes RAG1 and 2 (i.e. pre-B cells and cells of the T lineage) but nevertheless do not rearrange the κ light chain gene, raising the possibility that KSF regulates the rearrangement process. Functional studies and further biochemical characterisation of KSF are currently under way.

V 463 GENERATION OF MEMORY B CELLS SPECIFIC FOR CYTOCHROME *c* EARLY IN THE IMMUNE RESPONSE, Jeanne M. Minnerath, Carolyn Mueller, and Ronald Jemmerson, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455

Previously, we showed that resting, mature memory B cells specific for cytochrome *c* (cyt) can be tolerized in splenic fragment cultures using polymerized cyt in the absence of T cell help. It has been technically difficult to test whether newly arising memory B cells may be even more susceptible to tolerance induction than resting memory cells. For example, the very low frequency of cyt-specific precursor cells has prevented our ability to observe memory B cells early in the response (prior to day 10). By modifying the immunization protocol, i.e. first priming mice with OVA, followed 3-4 weeks later with an injection of cyt-OVA, it is now possible to observe cyt-specific memory B cells in splenic fragment cultures as early as 3 days after injection with cyt-OVA, if not even earlier. Since primary cyt-specific B cells are not readily observed and re-exposure to cyt *in vitro* is required for Ab production, the cyt-specific B cells activated in the cultures are memory cells. The memory B cells responding in the cultures 3 days after cyt-OVA challenge secrete primary mAb as indicated by: 1) the high frequency of the IgM isotype among these mAb (78%), 2) the low affinities of the mAb compared to mAb obtained at later times in the response, and 3) similar affinities of the mAb to mAb from hybridomas, prepared 3 days after *in vivo* challenge with cyt-OVA, whose V_H and V_L genes maintain the germline sequence. The ability to observe memory B cells early in the response without the contamination of primary cells may allow this system to be used to address a number of questions regarding the development and maturation of memory B lymphocytes, in addition to the relative tolerance susceptibility of these cells at different stages in the immune response.

V 464 CHARACTERIZATION OF B220$^-$ B CELL PRECURSORS WITH THE MONOCLONAL ANTIBODY AB8, Chantal Moratz, Kathryn Reese, and John F. Kearney Department of Microbiology and Division of Developmental and Clinical Immunology, University of Alabama at Birmingham, Birmingham, Al 35294

In mouse and man, haematopoietic stem cells can generate differentiated progeny of all haematopoietic lineages as well as having the potential for self renewal. As stem cells begin to differentiate to lineage committed progeny, a combination of characteristic cell surface markers identify stages of this developmental process along the various lineages. The earliest committed B cell progenitors so far defined are those that express the cell surface molecule CD43, have nuclear expression of Tdt, and express B220. We have isolated a monoclonal antibody, AB8, which reacts with a subpopulation of mouse bone marrow and other tissues. These cells coexpress Fall 3, Pgp-1, transferrin receptor as well as high levels of M169 (HSA). Of the AB8$^+$ only a subset of the population coexpress the CD43 antigen and a very low or undetectable levels of B220, IgM, Gr-1, or CD3. Preliminary analysis of early neonatal tissues, spleen, liver, and peritoneal cavity, suggests that the percent of AB8$^+$ cells detected by immunofluorescence analysis is initially high and decreases with advance of age. AB8$^+$ cells sorted from the bone marrow did not give rise to granulocyte macrophage colonies, erythroid burst colonies or pluripotent colonies in Dexter type in vitro cultures. Also AB8$^+$ bone marrow cells transplanted into irradiated scid mice led to the appearance of donor IgM in the serum followed by donor B cells in the scid host peripheral lymphoid organs. The preliminary data suggests that contained within the AB8$^+$ population is a more immature lymphoid precursor cell than has been previously described.
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V 465 THE MURINE IMMUNOGLOBULIN HEAVY CHAIN 3'α ENHANCER IS A TARGET SITE WITH REPRESSOR FUNCTION FOR THE B CELL LINEAGE-SPECIFIC TRANSCRIPTION FACTOR BSAP (NF-HB, Sα-BP), Markus F. Neurath, Warren Strober and Yoshio Wakatsuki, Mucosal Immunity Section, National Institute of Health, Bethesda, MD 20895

Using electrophoretic mobility shift assays (EMSAs) we have identified several target sites for nuclear proteins in the murine immunoglobulin 3'α enhancer. Two of these sites, denoted oligo H and oligo K, were shown by several criteria, including cell distribution and stimulation experiments, EMSA cross-competition studies and proteolytic clipping bandshift assays, to bind to the same protein, identical to the transcription factor BSAP (NF-HB, Sα-BP). As shown by quantitative EMSA, one of these binding motifs (oligo H) bound BSAP with higher affinity (k_f= 1.8·10^5) and formed a stable complex with BSAP in vitro, whereas the second binding site (oligo K) showed a weaker affinity for BSAP (k_f= 7.3·10^4). Genomic footprinting with recombinant BSAP and a labelled 3'α enhancer fragment identified two protected areas between nucleotides 664-680 (TGTTGAGCCACCCAT CC) and 771-786 (CATGGACCCCCAGTCC), respectively. To assess the possible functional role of these BSAP binding sites in the 3'α enhancer, we transiently transfected a construct containing a 314bp-3'α enhancer fragment upstream of a luciferase reporter gene in MOPC-315 cells, a plasmocytoma line lacking BSAP. In these cells, co-transfection with a vector expressing recombinant BSAP led to significant reduction in the activity of the 3'α enhancer fragment. Conversely, in the mature B lymphoma cell line CH12.LX, a cell line which expresses BSAP and has a less active 3'α enhancer, selective BSAP-downregulation by an anti-sense phosphorothioate-substituted oligonucleotide overlapping the translation start site of murine BSAP was sufficient to considerably upregulate 3'α enhancer activity as were mutations of both binding sites that prevented binding of BSAP to the 3'α enhancer. Conclusions: Our findings thus suggest that the natural loss of BSAP expression in terminally differentiated plasma cells contributes to the activation of the murine immunoglobulin 3'α enhancer. Furthermore, the lack of BSAP expression in plasma cells might mediate high-rate immunoglobulin production by those cells.

V 466 LYMPHOPOIESIS IN MICE LACKING THE HEAT STABLE ANTIGEN (mCD24), Peter J. Nielsen, Roland H. Wenger, Manfred Kopf, Lars Nitschke, Marinus C. Lamers and Georges Koehler, Max Planck Institute for Immunobiology Freiburg, Germany

In the mouse, the Heat Stable Antigen (HSA) is a heavily glycosylated, GPI-anchored surface protein expressed on several immature cell types including lymphocytes. For B-cells, HSA expression begins very early in pro-B cells, reaches a maximum at the pre-B stage in the bone marrow, is reduced in peripheral resting B-cells, and is absent on memory and plasma cells. For T-cells, HSA expression begins very soon after entry of the T cell precursor into the thymus and reaches a maximum around the CD4/CD8 double positive stage. Subsequently, HSA is reduced and peripheral T-cells are negative. Published data suggest that HSA is involved in cell adhesion and can deliver a costimulatory signal during the activation of peripheral T-cells by antigen presenting cells.

In order to learn more about the function of HSA, we have disrupted the HSA gene by homologous recombination. Mice homozygous for this mutation survive and are fertile. They possess peripheral B and T-cells in normal numbers suggesting that HSA is not absolutely required for lymphopoiesis. Thymocyte subpopulations also appear normal. Changes were seen however, in the bone marrow. There was a 2-3 fold reduction in B-lymphocyte precursors, particularly in pre-B cells of the B220$^+$,BP1^{hi} stage. Results will be presented from experiments which examine the functionality of lymphocytes in the HSA deficient mouse.

V 467 A REPRODUCIBLE CULTURE SYSTEM FOR SELECTIVE GROWTH OF HUMAN B CELL PROGENITORS: GENE EXPRESSION AND RETROVIRAL GENE TRANSFER, David J. Rawlings, Shirley Quan, and Owen N. Witte, Department of Microbiology and Molecular Genetics, Howard Hughes Medical Institute, University of California at Los Angeles, Los Angeles, CA 90024-1662

B cell ontogeny is characterized by an orderly expression of regulatory genes, surface markers, and response to activating signals. Studies of murine pre-B cell development were accelerated by the establishment of long term bone marrow culture systems. More recently, distinct gene expression patterns and growth requirements of B lineage restricted progenitors (pro-B cells) have been evaluated using a culture system to selectively grow this cell population. While there has been some success in maintaining purified human B cell populations on stromal support, study of human B lymphopoiesis has been limited by the lack of equivalent reproducible culture models. As a model for preclinical gene therapy for X-linked agammaglobulinemia (XLA) we have developed a culture system to study transfer of the B cell tyrosine kinase, Btk, into early human B cell progenitors. $CD34^+$ enriched mononuclear cells from human cord blood were seeded onto the murine stromal line, S17, under conditions favoring B lymphopoiesis to establish a biphasic culture system. Cultures were characterized by an early transient growth of myeloid cells followed, at 3-4 weeks, by the outgrowth of a population enriched for lineage restricted B cell progenitors. A 50-100 fold increase in cell numbers consisting predominantly of populations expressing early B-lineage markers including CD10, CD19, CD38 and CD45, but lacking CD20 and surface IgM was observed and could be maintained for greater than 8 weeks in culture. Gene expression patterns, growth factor responses, and Btk retroviral gene targeting of these cells will be described.

V 468 XLA RESULTING FROM MUTATIONS OUTSIDE THE KINASE DOMAIN OF BTK: IMPLICATIONS FOR B CELL SIGNALLING, Douglas C. Saffran, David J. Rawlings, Qili Zhu, Ming Zhang, Shi-Han Chen, Hans D. Ochs*, Owen N. Witte, Department of Microbiology and Molecular Genetics, Howard Hughes Medical Institute, University of California at Los Angeles, Los Angeles, CA 90024-1662; *Department of Pediatrics, University of Washington, Seattle, WA 98195

X-linked agammaglobulinemia (XLA) is an inherited immunodeficiency disease characterized by a severe deficit of circulating B cells and profound hypogammaglobulinemia. The disease results from mutations of the cytoplasmic tyrosine kinase gene Btk (Bruton's tyrosine kinase) and is selective for the B cell lineage. Reported mutations in the Btk gene either knock out catalytic activity or result in deficient expression of Btk. We have used a strategy to search for new mutations in Btk which occur naturally in XLA and may be informative for Btk function. Btk expression and kinase activity is analyzed in EBV transformed B cell lines derived from patients with either phenotypically typical or atypical XLA. Atypical XLA is defined by less dramatic reduction in circulating B cells and serum immunoglobulin levels. This phenotype is likely to be associated with more subtle mutations in the Btk gene. In patient B cell lines which express functional Btk, the entire gene is sequenced using an RT-PCR technique with overlapping primer sets spanning the Btk gene. Using this strategy we identified several mutations in Btk outside of the catalytic domain. The range of mutations and their functional implications will be discussed.

V 469 EFFECTS OF THE IMMUNOSUPPRESSANT RAPAMYCIN ON B-LYMPHOPOIESIS, Walter Schuler, Meike Lorenz & Valerie Quesniaux, Preclinical Research, Sandoz Pharma Ltd, CH-4002 Basle, Switzerland

Rapamycin (RPM) is an immunosuppressant which exerts its effect by blocking the interleukin(IL)-2-driven proliferation of T cells. However, it has been shown that the anti-proliferative effect of RPM is not restricted to T cells/IL-2: RPM inhibits the proliferative response of cells of various lineages to a variety of growth factors. RPM is thus considered a general inhibitor of growth factor-driven cell proliferation. Among the interleukins, IL-7 plays an essential role in B-lymphopoiesis in the bone marrow. We could show that RPM also inhibits the IL-7-driven *in-vitro* proliferation of a stroma cell-dependent pre-B cell line. Therefore, we expected an adverse effect on B-lymphopoiesis after *in-vivo* administration of RPM. Treatment of C57Bl/6 mice for 9 or 27 consecutive days with an immunosuppressive dose of 50mg RPM per kg per day reduced the number of B220-positive/IgM-negative (pre-B) cells in the bone marrow to about 50% of the control; the frequency of mature B cells in spleen remained unaffected. In contrast to the effect on steady-state B-lymphopoiesis, a 9-day treatment with 50mg RPM/kg/day completely suppressed development of pre-B cells in regenerating bone marrow following treatment with 5-fluorouracil (5-FU). Likewise, RPM inhibited the *in-vitro* development of B220-positive cells in long term bone marrow cell cultures. Considering that it takes about 8 - 9 days for a committed B-lymphoid cell to develop into a mature B cell (which then leaves the bone marrow), the effect of RPM on steady-state B-lymphopoiesis is surprisingly small. In conclusion, RPM has a different effect on B-lymphopoiesis in a steady-state situation compared to a situation, as after 5-FU treatment, where development starts with the recruitment of normally quiescent stem cells. Either RPM is not a general inhibitor of growth factor-driven proliferation, or growth factor requirements are different in steady-state lymphopoiesis.

V 470 GENETIC ANALYSIS OF OCT-2 AND PU.1 FUNCTION DURING B-CELL DEVELOPMENT

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B-cell development is characterized by an ordered progression of DNA rearrangements that result in the sequential expression of the immunoglobulin (Ig) genes. We are studying two transcription factors, Oct-2 and PU.1, implicated in expression of B-cell specific genes. Oct-2 is a B-cell specific POU-domain transcription factor that binds to an octamer DNA element in Ig promoters. Although Oct-2 was implicated as a developmental regulator of Ig gene transcription in B-lineage cells, gene targeting in mice as well as in the B-cell line WEHI 231, indicate that it is not required for transcription of Ig genes [1,2]. Instead, Oct-2 appears to be required for terminal differentiation of B-cells. Oct-2 function in lymphoid maturation will be determined by *Rag* $2^{-/-}$ blastocyst complementation[3] with *Oct-2* null embryonic stem (ES) cells. The chimeric nature of these animals should overcome the lethal phenotype of *Oct-2* null mice, thus allowing the determination of the role of Oct-2 in B-cell development. We have been able to successfully generate *Oct-2* null ES cells by deleting the exons which encode the POU and Homeo box domains of the protein. The *Oct-2* null cells have been used to generate chimeric animals with *Rag-2* null blastocysts. Our analysis will be presented. PU.1 function is to be examined both at the level of blastocyst complementation and germ line transmission to produce a *PU.1* null animal. Multiple ES cell clones which have one allele of *PU.1* disrupted by the *neo* gene have been generated. Male chimeras have been produced from four independent clones. These males are currently being bred for germline transmission which will allow us to investigate the function of PU.1 in the development of the organism. *PU.1* null ES cells are being generated by the targeting of the second wild-type allele with a *Hyg* based vector. *Hyg* and *neo* resistant clones are currently being screened for disruption of the second allele. *PU.1* null ES cells will be used in the *Rag* $2^{-/-}$ blastocyst complementation assay to determine the role of PU.1 in B-cells.

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2. Feldhaus, A.L. et al. EMBO J., 12,2763 (1993)
3. Chen, J. et al. PNAS, 90,4528 (1993)

V 471 REGULATORY REGIONS 3' OF THE Eμ ENHANCER DIFFERENTIALLY AFFECT EXPRESSION OF ANTIBODY HEAVY CHAIN TRANSGENES, Dagmar Sigurdardottir, Jeongwon Sohn, Jamie Kass and Erik Selsing, Immunology Program, Sackler School of Graduate Biomedical Sciences and Department of Pathology, Tufts University School of Medicine, Boston, MA 02148

We have compared the expression patterns of three immunoglobulin heavy chain transgenes. The three constructs differ only by deletion of J-C intron sequences located downstream of the Eμ enhancer region. When transfected into a myeloma cell line, all three constructs are expressed at comparable levels. However, transgenic mice carrying the three constructs show dramatic differences in transgene expression. Our results indicate that, within the J-C intron, at least two regions, other than the Eμ enhancer, influence transgene expression. Reg1, located directly downstream of the Eμ enhancer, is important for upregulation of transgene expression after LPS activation of splenocytes. Reg2, located within or downstream of the Sμ switch region, is required for the high levels of expression normally observed in splenocytes of heavy chain transgenic mice. Our analyses demonstrate that the Eμ enhancer region is not sufficient to provide normal expression of an immunoglobulin heavy chain transgene.

V 472 FUNCTIONAL RECONSTITUTION OF LYMPHOID DEVELOPMENT IN RAG-1 NULL MICE BY THE INTRODUCTION OF ANTIGEN RECEPTORS.

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The RAG-1 and RAG-2 V(D)J recombination activating genes were identified by virtue of their ability to activate recombination of an artificial substrate in fibroblast lines. In order to understand the role of RAG-1 *in vivo*, we have generated mice that are homozygous for a null allele of the RAG-1 gene. Mutant mice are immunodeficient since lymphoid development is arrested at an early stage precisely prior to the recombination of the TcR and Ig loci. Thus, RAG-1 appears to have an indispensable role in V(D)J reconbination.To further elucidate the potential role of RAG-1 at later stages of lymphoid development and address the question of whether functional transgenic antigen receptors can reconstitute the RAG-1 null phenotype, as well as study the regulatory role of antigen receptors at different stages of the lymphoid differentiation, we established RAG-1 deficient mouse lines transgenic for either: the membrane form of a μ heavy chain protein, or the same μHC and an additional κ light chain, an antibody that recognizes an H-2^k MHC class I peptide, or finally a TcR specific for an influenza peptide, restricted by class I. Analysis of the above mice revealed that: the mere expression of a functional rearranged antigen receptor is able to allow complete reconstitutiton of the RAG-1 null phenotype. Expression of the membrane form of the μ chain enables transition from the $B220^{dull}/CD43^+$ to the $B220^{dull}/CD43^-$ small pre-B cell stage, arresting prior to the rearrangement of the κ loci. RAG-1 deficient mice expressing the anti-H-2^k antibody develop fully mature B-cells in the periphery and are able to eliminate the self-reactive B-cells when present in the deleting haplotype (H-2^k or H-2^b). Similarly, the TcR transgene alows complete reconstitution of the T-cell lineage and the generation of fully mature $CD8^+$ cells with cytolytic activity. In these $TcR^+/RAG\text{-}1^{-/-}$ mice T-cell development is skewed towards the $CD8^+$ phenotype, however, a small percentage of $CD4^{high}/CD8^{low}$ thymocytes could be detected suggesting that a "stochastic" rather than "instructive" mode operates during positive selection in these mice.

V 473 CREATION OF PLASMIDS FOR ASSAYING SWITCH RECOMBINATION, Janet Stavnezer, Department of Molecular Genetics and Microbiology, Univ. of Massachusetts Medical School, Worcester MA 01655

When mature B lymphocytes, which express IgM and IgD on their surface, are activated by antigen and receive accessory signals, they switch to express downstream heavy chain constant region (C_H) genes while maintaining the same expressed variable region domain. Antibody class switching is effected by a DNA recombination event called switch recombination, which occurs within or near 2 to 10 kb switch (S) regions containing tandemly repeated sequences located 5' of each C_H gene, except Cδ. The means by which the DNA is cut, aligned and rejoined is unknown, as are the roles of several nuclear proteins that have been shown to bind to S regions. Switch recombination appears to be effected by an error-prone DNA synthesis event since the sequences surrounding the junctions have nucleotide substitutions, deletions and insertions.

In order to examine the DNA sequences, cell type specificity and the genes and proteins involved in switch recombination, we are developing plasmids that can undergo switch recombination. Results from an extensive series of experiments using transiently transfected plasmids indicate that they do not undergo switch recombination in a cell type specific manner nor do the sites of recombination appear to correlate with sites used in vivo, unless the use of other sites are selected against. Therefore, we have constructed plasmids that integrate, and which undergo switch recombination using the tandem repeat sequences at a high frequency and which appear to have cell type specificity. Plasmids with fragments containing transcription activator elements, i.e. the μ intron enhancer and Ig V_H promoter and/or the germline Cα promoter recombine more frequently than those that do not. These plasmids recombine more frequently in a B cell line that undergoes switching (I.29μ) than in the J558L myeloma line, that does not undergo switching of its endogenous genes.

V 474 DEFECTS OF CD40-MEDIATED SIGNALLING IN B-CELLS FROM PATIENTS WITH HYPER IGM SYNDROME, Toshio Tanaka, Osamu Saiki, Yoshinori Katada, Hiroshi Ochi, Masakazu Aitani, Shoji Hashimoto, Akio Tawa, Hitoshi Kikutani, Masaki Suemura, and Tadamitsu Kishimoto, Department III and Mol. Cell. Institute of Osaka University, Suita 556, Japan

Immunoglobulin (Ig) class-switching, through which biological function of Ig is altered, is believed to be exerted by deletional recombinational event between unique repeatable sequences located at 5' portion of constant exon of IgH gene (Switching region :S). In vitro studies using anti-CD40 antibody or CD40 ligand and demonstration of deletions or mutations of CD40 ligand in patients with X-linked hyper IgM syndrome, in whom serum level of IgA, IgG or IgE is extremely diminished, have indicated that signals through CD40 expressed on B cells might induce a machinery of switching recombination (S-S recombination). Since a clinical heterogenity exists in the hyper IgM patients, we asked whether or not there might be an intrinsic defect in B cells from patients with hyper IgM. In one acquired patient, anti-CD40 induced little B cell proliferation and the stimulation of peripheral blood mononuclear cells (PBMC) with anti-CD40 in the presence of IL-4 or IL-10 failed to stimulate IgA, IgG or IgE synthesis. In two patients with an aquired form and suprisingly a X-linked hyper IgM syndrome, the incubation of PBMC with anti-CD40 in the presence of IL-10 did not cause an induction of IgG and IgA whereas IgM production was found. Moreover, little IgE synthesis was induced in response to anti-CD40 plus IL-4 by the patients' PBMC. Reverse Transcription-PCR showed that the expression of germline transcript of Cε gene by the latter patient's PBMC in response to anti-CD40 plus IL-4 was found but the expression of its productive transcript was not. It was found that defect of Sμ-Sε recombination was a cause of failure of IgE secretion by Digestion Circularization PCR. The addition of IL-10 could restore its IgE synthesis by IL-4 plus anti-CD40-stimulated PBMC from the latter two patients but not from the former patient. These results indicate that defects of CD40-mediated signalling may exist in some patients with hyper IgM-syndrome.

V 475 POSITIVE SELECTION OF V_H12-EXPRESSING B CELLS RESULTS IN A HIGHLY RESTRICTED V_HCDR3 REPERTOIRE IN THE ADULT PERIPHERY AND OCCURS AT THE PRE-B CELL STAGE, Jian Ye, Suzanne McCray, and Stephen Clarke. Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

B cells specific for phosphatidyl choline (PtC) comprise approximately 5 to 10% of the B-1 cell repertoire in unmanipulated B10.H-2^aH-4^b mice. These B cells express either V_H11 and $V\kappa9$ or V_H12 and $V\kappa4$, and cells of both types are represented about equally in the peritoneum. These cells are extraordinarily restricted in V_HCDR3, indicating that they are present in mice in such large numbers because of antigen driven clonal selection. Selection of V_H12-expressing B cells is evident at birth. In the neonatal liver two-thirds of the productive rearrangements encode V_HCDR3s that resemble those of PtC-specific B cells of the adult. This restriction is not evident in the day 18 fetal liver. We propose that this restriction is the result of positive selection; V_H12-expressing B cells with V_HCDR3 of a particular length and sequence receive a positive selection signal and continue differentiation, whereas V_H12-expressing B cells with different V_HCDR3 sequences do not receive a positive selection signal and consequently die. We have now examined V_H12-CDR3 diversity in the adult bone marrow, spleen, and peritoneum and find that regardless of the J_H gene segment rearranged, productive V_H12 rearrangements have V_HCDR3 sequences indistinguishable from PtC-specific B cells. Since splenic B cells are predominantly B-2, the restriction imposed by positive selection of V_H12-expressing B cells affects both B-1 and B-2 repertoires. Sorted IgM^- cells from adult bone marrow show the same restriction as unsorted bone marrow suggesting that selection has occurred at the pre-B cell stage when the H chain is complexed with surrogate L chain on the cell surface. Therefore, this selection appears to be based on the structure of the H chain and suggests that there is not a common ligand responsible for pre-B cell positive selection.

Alloreactivity

V 476 AGE RELATED DIFFERENCES IN THE CAPACITY TO DEVELOP CELL MEDIATED IMMUNE RESPONSES IN C57BL/6J MICE. G. Alfaro[1], E. Verástegui[2], G. Nava[1] and V. Díaz[3]. 1: Instituto de Investigaciones Biomédicas, UNAM, 2: Instituto Nacional de Cancerología, and 3: Instituto Nacional de la Nutrición. México 04510, D.F., México.

It is now well established that a decline in the capacity to develop normal humoral and cellular immune responses is associated with old age. Cytotoxic T lymphocytes (CTL) and natural killer cells (NK) appear to be more severely affected than B cells. It is possible that these defects may be partially responsible for the increment in autoimmune diseases and the development of malignant tumors in aged populations.

This work demonstrates that young (8-10 weeks) and healthy aged (70-87 weeks) females of the C57BL/6J (H-2^b) inbred strain of mice differ in their capacity to develop an effective and protective immune response against the H-2-deficient cell variant LR.4, a derivative of the T cell lymphoma L5178Y (H-2^d). Young mice grew and rejected the tumor in 99% when the tumor was transplanted into the peritoneal cavity; such animals remained resistant to subsequent challegnes 100 days after the tumor had been at a faster rate and invariably killed the mice. Furthermore, at the cellular level it was observed that immune responses mediated by CTLs were less efficient in aged C57BL/6J that those detected in young animals. NL and LAK activities were similar in both groups of mice; however, old females expressed higher levels of the IL-2 receptor. The role of sex hormones in the regulation of the immune response was investigated.

V 477 P91A TRANSGENIC MICE: MODEL FOR MINOR HISTOCOMPATIBILITY RESPONSES, SKIN GRAFT REJECTION AND PERIPHERAL TOLERANCE, A. Antoniou, A. Mellor*, H. Yeoman*, D. Scott, P. Chandler and J. Dyson, CRC, Transplantation Biology, Watford Road, Harrow, Middlesex, U.K. *NIMR, The Ridgeway, Mill Hill, U.K.

Evidence indicates that minor H responses require at least two epitopes, one that is MHC class I restricted and recognised by CD8+ cytotoxic T lymphocytes and one that is MHC class II restricted and recognised by CD4+ helper T lymphocytes which provide the necessary 'help' for cytotoxic T cell maturation.

To establish the nature and requirements for minor H antigen responses, we are using the tumour minus mutated gene P91A. The P91A protein has a single amino acid substitution in comparison with the product of the endogenous, non-mutated gene. This leads to a unique aggretope that is L^d restricted and recognised by CD8+ cytotoxic T cells. A P91A transgenic DBA/2 mouse line has been generated. To assess whether a P91A disparity is sufficient to elicit a minor H response, skin grafting experiments have been performed using DBA/2 mice as recipients. These experiments have shown that a P91A disparity does not lead to skin graft rejection even after immunisation. The introduction of the male specific antigen H-Y (for which DBA/2 is a non-responder with respect to skin graft rejection and the generation of CTLs), in association with P91A leads to skin graft rejection. This suggests that a helper effect has been introduced by the recognition of the H-Y antigen.

To investigate the mechanism of skin graft rejection and immunisation to the P91A and H-Y antigens, recipients were immunised with H-Y+P91A+ cells and grafted with two donor skin grafts, one P91A+ and the other H-Y+. An analogous experiment was performed by immunising recipients with cell populations expressing these antigens alone and grafting with H-Y+P91A+ donor skin grafts. Rejection was observed with mice that had been immunised with H-Y+ P91A+ cells and grafted with H-Y+P91A+ donor grafts. This suggests that immunisation and skin graft rejection require the expression of both the P91A and H-Y antigens on the same antigen presenting cell.

The above observations were utilised to test the possibility of inducing peripheral tolerance to P91A. This was performed by immunising with P91A alone without H-Y i.e. a 'helper' second signal, followed by a second immunisation with P91A+H-Y+ cells and subsequent skin grafting with P91A+H-Y+ donor grafts. Initial experiments indicate that tolerance can be induced by initially immunising with P91A+ alone. This result seems to support one of the predictions of the '2 signal hypothesis'.

V 478 ALLOREACTIVE T CELL RECOGNITION OF MHC-PEPTIDE COMPLEXES. Ned S. Braunstein, Dominique A. Weber, Nancy K. Terrell, and Yuan Zhang. Department of Medicine, College of P&S, Columbia University, New York, NY 10032

Although alloreactivity has been extensively studied, the fine molecular specificity of alloreactive T cells for MHC alone or for MHC-peptide complexes remains controversial. To better characterize the role of peptide in alloreactivity and to identify peptides important in these responses, we developed a model system using the antigen processing-defective mutant cell line (.174 x CEM)T2. Mouse class II MHC (I-A) genes were transfected into T2, its phenotypically normal counterpart, T1, and mouse L cells. Immunoprecipitation and SDS-PAGE demonstrated that, as expected, T2 transfectants expressed only SDS-unstable I-A; the other cells expressed both stable and unstable I-A conformers. Limiting dilution analyses of alloantigen-triggered, IL-2 producing, $CD4^+$ T cell activation showed that the response to cells expressing only SDS-unstable I-A was markedly lower than the response to cells with SDS-stable I-A. Priming with normal allogeneic spleen cells increased the frequencies of T cells responding to stable but not unstable class II MHC. Priming to T2 transfectants, in contrast, was either ineffective or induced a small increase in the number of anti-T2-I-A alloreactive precursors. Although T2 transfectants constitutively express only SDS-unstable I-A, the addition of peptide Eα 56-72 to T2-I-A^b transfectants generated SDS-stable I-A-peptide complexes on the cell surface as determined by immunoprecipitation with Y-Ae, an antibody specific for this MHC-peptide complex. Peptide-loaded T2-I-A^b cells were no more effective than untreated cells in stimulating primary allo responses. However, after priming with spleen cells that constitutively express this I-A^b-Eα peptide complex, limiting dilution analysis demonstrated an increase in the frequency of T cells that respond to Eα 56-72-loaded T2-I-A^b but no change in the frequencies of T cells that respond to untreated T2-I-A^b or to the same cells loaded with other peptides. These studies not only demonstrate that the SDS-stable, peptide-containing conformer of class II MHC is the relevant ligand for alloreactive T cells but importantly indicate the presence of $CD4^+$ alloreactive T cells specific for at least one such known, common I-A-peptide complex. Peptide-loaded T2-I-A^b cells are being used to clone alloreactive T cells with unique MHC-peptide specificities.

V 479 HOST-GRAFT INTERACTION IN DISCORDANT CARDIAC XENOTRANSPLANTATION: INHIBITION OF THE INDUCED ANTIBODY RESPONSE IN TLI TREATED RECIPIENTS, RJ Brewer, MS Roslin, MJ Del Rio, I Alexandropoulos, M Sadeghian, JN Cunningham & AJ Norin, Departments of Anatomy & Cell Biology, Surgery, Medicine and the Transplant Immunology and Immunogenetics Laboratory, SUNY Health Science Center at Brooklyn & Maimonides Medical Center, Brooklyn, NY 11203

A major obstacle to xenotransplantation (XT) is the induction of high levels of antibody directed against the donor species (XAb) that are not suppressed by standard therapies used in allotransplantation. We have previously demonstrated that total lymphoid irradiation (TLI) prevents the generation of XAb in concordant monkey to baboon cardiac XT. We now report studies on the effect of TLI in a discordant swine to baboon model. Recipients received 800 rads of TLI administered over four weeks prior to XT. Preformed antiswine XAb was adsorbed by perfusion of pig spleen or liver with recipient whole blood or plasma followed by heterotopic cervical cardiac XT. XAb levels were determined by complement-dependent cytotoxicity or flow cytometry. Preformed XAb titers in naive baboons were variable, with both IgG and IgM ranging from 1:64 to >1:1000. TLI had no effect on the level of preformed XAb. Adsorption lowered but did not eliminate these XAb so the induced response is assessed against this background. Rejection was defined as cessation of graft function. Mean graft survival was 7.5 days (n=6). Animals which underwent specific adsorption on the day of XT maintained XAb levels within one dilution of the initial titer (eg. 1:256-1:512) until graft rejection; XAb levels then rose to much higher levels after rejection. However, animals which underwent specific adsorption on the day prior to XT demonstrated a significant rise in XAb titer by the day of rejection (eg. 1:256→1:2048). Additional experiments suggest that donor-species organ perfusion releases a substantial amount of porcine xenoantigen into the recipient thus inducing a humoral immune response. A TLI-treated baboon which underwent adsorption but did not receive a xenograft had an IgG XAb titer of 1:32000 on day 17 after the procedure. TLI clearly had an inhibitory effect on XAb formation in the presence of a functioning xenograft. In contrast, exposure to xenoantigen without XT induced very high levels of XAb after TLI treatment. Antigenic exposure even 24 hours prior to XT apparently negates the inhibitory effect of TLI thus suggesting an interaction between the TLI-altered immune response and the vascularized graft.

V 480 INDUCTION OF ALLOGRAFT TOLERANCE BY AN HLA CLASS I DERIVED PEPTIDE, Carol Clayberger, Steve Nisco, Grant Hoyt, Patrick Vriens, Bruce Reitz, and Alan M. Krensky, Departments of Cardiothoracic Surgery and Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

T cell recognition of foreign major histocompatibility complex (MHC) molecules initiates a cascade of events resulting in allograft rejection. Cytotoxic T lymphocytes (CTL) damage the graft by targeting nonself MHC class I molecules. We and others have previously shown that small synthetic peptides corresponding to regions of certain MHC class I molecules can inhibit the CTL response against MHC class I alloantigens in vitro. Here we report that rat heart allografts survived indefinitely when transplanted into recipients treated with a synthetic peptide corresponding to residues 75-84 of the human HLA-B7 molecule (B7.75-84) in combination with a subtherapeutic dose of cyclosporine A (CsA). Furthermore, this treatment induced long-term donor specific tolerance, indicating that such peptides have potential as therapeutics for human organ transplantation.

V 481 THE IMMUNOSUPPRESSIVE ROLE OF NITRIC OXIDE DURING GRAFT VERSUS HOST DISEASE, Rosemary A. Hoffman, Jan M. Langrehr,* David A. White, LuAnn M. Berry, Richard L. Simmons, Susan A. McCarthy; Department of Surgery, University of Pittsburgh, PA 15261 and *Department of Surgery, Free University of Berlin, Germany D-13353

Cytokine-induced nitric oxide (NO) production during lymphoproliferative assays results in inhibition of lymphocyte proliferation. Addition of an inhibitor of NO synthesis, N^G-monomethyl-L-arginine (NMA) results in promotion of lymphocyte proliferation coincident with inhibition of NO synthesis. To determine if the global immunosuppression of immune responses occurring during GvHD was due to NO, a parent to F1 (C57BL6 → C57BL/6 x DBA2/J F1) model was utilized. A transient increase in serum NO_2^-+NO_3^- levels was observed on days 11-12 post GvHD induction. NO synthesis by GvHD splenocytes in response to the mitogens ConA and LPS was greatly enhanced compared to control F1 mice. In addition, peritoneal macrophages from animals with GvHD become primed for NO synthesis in response to LPS. Administration of a 1% solution of the NO synthesis inhibitor, aminoguanidine (AG), in the drinking water of mice with GvHD resulted in statistically significant inhibition of the elevation of serum NO_2^-+NO_3^- levels noted in GvHD animals that did not receive the inhibitor. Splenocytes obtained from GvHD animals that received AG demonstrated enhanced spontaneous proliferation as well as an enhanced response to ConA compared to splenocytes from GvHD animals that did not receive AG. Phenotyping of the splenocyte population for H2 antigen expression revealed that by week 3 post-GvHD induction, animals treated with AG contained 70% host cells and 30% donor cells. In contrast, in GvHD animals that did not receive AG, 50-80% of the splenocytes typed as donor cells. These results demonstrate that administration of an NO synthesis inhibitor during GvHD does not exacerbate the disease process by promoting donor anti-host responses. Rather, inhibition of NO synthesis during GvHD seems to promote hematopoiesis and improve immune function of the host.

V 482 DONOR-SPECIFIC CYTOTOXIC T LYMPHOCYTES INFILTRATING THE ALLOGRAFTED HUMAN HEART EXPRESS A LIMITED NUMBER OF T-CELL RECEPTOR Vß GENE FAMILIES, Hu H.Z., de Jonge N., Gmelig-Meyling F., van Reijsen F., Tilanus M., Schuurman H.J., de Weger R.A., Department of Pathology, University Hospital, 3508 GA Utrecht, The Netherlands

We analyzed the early entering T cells in biopsies from 4 allografted human hearts by specificity, function and phenotype. Biopsies were taken once in a week or two weeks in the first 3 months after transplantation. T cells were propagated in culture medium containing IL-2 and IL-4 (bulk cultures). The donor specific cytotoxicity of the T cells correlated with rejection reactions. T cells from two bulk cultures were cloned. About 70% of the T-cell clones were cytotoxic, all of which were $CD8^+$ and donor-specific. Two to three bulk T cell cultures from each patient and all donor specific CTL clones were analyzed for the T cell receptor Vß gene family expression at mRNA level. In all bulk cultures a biased usage of TCR Vß gene families was observed, whereas the Vß expression in the blood from the same patients showed a normal distribution. The cloned T cells expressed a limited number of TCR Vß families. Sequence analysis of these CTL clones disclosed that some clones used the same Vß, Dß, Jß, Cß gene segments and V-D-J junction region, indicating that they originated from the same precursor. Using a specific primer complementary to the V-D-J junction region of a CTL clone in combination with a corresponding Vß primer, T-cells bearing the same ß chain were found in bulk cultures from other biopsies of the patient, and also in blood before but not after transplantation. Our data show that donor-specific CTLs infiltrating allografted human heart use restricted TCR Vß gene families. Precursors of these CTLs circulate in blood and after the transplantation they home to the graft without recirculation.

* supported by Dutch Heart Foundation

V 483 RAPID CELLULAR TISSUE REJECTION IN A MARINE SPONGE: FOUNDATIONS OF IMMUNE RECOGNITION, Tom Humphreys, University of Hawaii, Honolulu HI 96822

Histocompatibility was examined by apposing branches of the red beard sponge. Most (114/116) individuals are incompatible, with rejection first evident at about two hours when a yellow line contrasting with the bright orange tissue becomes visible along the boundary of contact. The line represents a pus-like accumulation of "gray cells", a wandering, multi-granular cell type of previously unknown function. Within 3 days an encapsulation-like reaction leads to the formation of a collagenous barrier between the incompatible tissues. Cytotoxic processes are not evident and the response occurs upon both allogeneic and xenogeneic contact. The rapid accumulation of gray cells suggests cellular recognition and activation reactions inducing the release of cytokine(s). Only small numbers of cells are required since individual-specific aggregates, reconstituted from about 5×10^5 randomly mixed, dissociated cells including a few hundred gray cells, also carry out immediate rejection evidenced by gray cell accumulation. The intimate involvement of the gray cells, one of the 12 or so cell types of the sponge, in the rejection process suggests that they may be sponge immunocytes. Rapid cellular reactions which can determine individual specificity without prior sensitization raises fundamental questions concerning the conceptual basis of invertebrate immune recognition. Burnet [NATURE (1971) 196, 230] suggested that invertebrate immune specificity was based on the ability of immunocytes to recognize self as self and reject all foreign cells because they do not display the self marker. How do immunocytes learn to specifically recognize only the histocompatibility marker allotypes the individual has inherited from the many polymorphic allotypes it might have inherited. The problem is formally similar to self-restriction in T-cell biology and I suggest that positive selection may have evolved for self recognition in invertebrates and only later have been co-opted by T cells.

V 484 T CELL RECEPTOR AND T CELL ACTIVATION PROFILES IN GVHD IN MAN.

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We studied the T cell repertoire reconstitution and T cell activation after BMT, and focused our attention on patients developing GvHD.
Peripheral blood mononuclear cells (PBMC) were obtained from the HLA matched sibling donors and the thalassaemic patients before BMT, and at day 30, 60 and 180 after BMT. This study was performed by four colour immunofluorescence analysis, using a panel of 24 monoclonal antibodies, whose specificities were directed at the TCR and activation markers.
Following treatment 3 patients had marked preferential expansion of a single Vβ in the CD8+ subpopulation (Vβ2 in one donor, and Vβ8 in the other two). In 2 of these patients this expansion was related to the development of an acute or chronic severe Graft versus Host Disease (GvHD). In these two patients we also observed a sizable and significant increase of CD3+ CD4- CD8- αβ+ T cells after BMT.
After BMT nearly all the T cells, both CD4+ and CD8+, express the CD45RO isoform, other activation markers, such as CD25, CD54, CD69 and HLA-DR, presented a less consistent and homogenous profile of expression. However the most consistent increases were observed in the patients developing GvHD. These overexpressed markers may become a suitable target to control GvHD.

V 485 GENETIC ANALYSIS OF THE CHROMOSOMAL REGION ENCODING EPITOPES RECOGNISED BY H-Y SPECIFIC T CELLS, Diane Scott, Ingrid Ehrmann, Alexander Agulnik*, Michael Mitchell†, Elizabeth Simpson, Beth M. Simpson° and Colin Bishop†, Transplantation Biology, CRC, Harrow, U.K., *University of Tennessee, Memphis, †INSERM U242, Marseille, France and °Jackson Lab, Maine.

In the mouse, genes involved in the expression of the male-specific minor H antigen, H-Y (*Hya*), spermatogenesis (*Spy*), primary sex determination (*Sry*), zinc finger containing genes (*Zfy*-1, *Zfy*-2) and a Y-linked ubiquitin activating homologue (*Ube*ly-1), have been mapped to the short arm of the Y chromosome. In particular, *Hya* and *Spy*, *Zfy*-2 and *Ube*ly-1 have been sub-localized to a deletion interval defined by Sxr^b. H-Y stimulates T cell responses and several class I and class II restricted T cell clones have been raised against different H-Y epitopes. Deletion mapping and chromosomal inactivation data show that these H-Y epitopes can be expressed independently. Here, we have transfected several overlapping cosmid clones spanning a small region of the Sxr^b deletion into antigen presenting cells with the appropriate MHC restriction element. We have examined the transfectants for expression of different H-Y epitopes and have identified cosmids that appear to specifically encode one or more H-Y epitopes. Further results on the identification of specific sequences that encode H-Y epitopes will be presented.

V 486 IMMUNODOMINANCE IN THE T-CELL RECOGNITION OF MINOR HISTOCOMPATIBILITY ANTIGENS. A STUDY ON NATURALLY ELUTED PEPTIDES. Wolpert E , Franksson L and Kärre K. Microbiology and tumorbiology center, Karolinska Institute, Stockholm, Sweden.

Minor histocompatibility antigens cause rejection of grafts transplanted between MHC-matched individuals. In this study we try to define minor histocompatibility antigens as peptides bound to MHC Class I molecules. Splenocytes from Balb.B mice were lysed, immunoaffinity purified for MHC Class I K^b and D^b and eluted under acid conditions. The material was fractionated with a hydrophobic gradient on a reversed phase HPLC. The different fractions were incubated on the peptide loading deficient cell line RMA-S and tested against T-cells from C57BL mice (B6) primed with either Balb.B or different B6-mice congenic for single minor antigens from Balb.B. T-cells primed with Balb.B spleen cells (i.e. all minor antigens) consistently recognized 3 fractions: nr 15 restricted to D^b, and nr 25 and 28 restricted to K^b. Those fractions seem to contain peptides that constitute dominating epitopes in the B6 anti Balb.B reaction.

B6 T-cells primed with spleen cells from the mouse B6.C-H-8c, congenic for a single minor H antigen from Balb.B, did not recognize any of the above mentioned fractions, but a new fraction in the Balb.B eluate, nr 20. This fraction was not recognized by B6 T-cells primed with Balb.B, although Balb.B cells apparently contain this antigen. This fraction seem to contain a peptide representing a dominated/ cryptic minor histocompatibilty antigen, which fails to elicit an immune response when other stronger antigens are present, but that is immunogenic when present alone.

This system may thus prove interesting to study the molecular mechanisms for immunodominance , and also to further characterize the proteins that constitute minor histocompatibility antigens.

Tolerance During Lymphocyte Development

V 487 CALCIUM-MEDIATED CELL DEATH SIGNALING IN IMMATURE THYMOCYTES, Sofija Andjelic, Nada Jain and Janko Nikolic-Zugic, Immunology Program, Sloan-Kettering Institute, New York, NY 10021.

Programmed cell death (apoptosis) eliminates a large number of cells during thymocyte development. These cells are either potentially autoreactive and are therefore clonally eliminated or die of neglect due to a lack of positive selection. During clonal elimination, TcR-generated, Ca^{++}-mediated signals lead to the activation of the Ca^{++}-dependent endogenous endonuclease and subsequent oligonucleosomal DNA fragmentation. The signaling events mediating this process are poorly understood. We investigated the reactivity of early precursor-containing thymocytes to Ca^{++}-induced signals, and discovered a breakpoint in their sensitivity to calcium-mediated cell death (CMCD). $CD25^+$ $CD8^-4^-TcR^-$ (triple-negative, TN) thymocytes stimulated with a calcium ionophore maintain their viability and precursor activity. By contrast, their immediate progeny, $CD25^-CD8^{lo}$ $4^{lo}TcR^{lo}$ (triple-low, TL) cells react to calcium elevation by abrogation of precursor activity and apoptotic cell death. This developmental difference is specific for CMCD, since both $CD25^+$TN and $CD25^-$TL cells are susceptible to steroid-induced apoptosis. The presence of bcl-2 mRNA correlates directly to the resistance of CMCD - $CD25^+$TN cells express it and $CD25^-$TL cells do not. These experiments show that thymocytes become sensitive to Ca^{++}-induced apoptosis as soon as they begin to express molecules that mediate antigen-recognition, and suggest that a concomitant downregulation of bcl-2 may mediate this phenomenon. Further investigation of the CMCD revealed the presence of two putative signaling pathways in distinct thymocyte subsets. These pathways are differentially sensitive to cyclosporin A. At present, we are studying the mechanism of the CsA-dependent pathway in the $bcl\text{-}2^+$ thymocyte subsets.

V 488 APOPTOSIS AND BCL-2 EXPRESSION IN POST-THYMIC T CELLS, H. Elizabeth Broome, Eileen F. Bessent, and Catherine M. Dargan, Department of Pathology University of California, San Diego 92093

Constitutive bcl-2 expression in T lymphocytes decreases their susceptibility to apoptosis while the absence of bcl-2 expression increases their susceptibility to apoptosis (1, 2, 3). Therefore, we decided to further investigate the regulation of bcl-2 expression in post-thymic T cells and to investigate how that expression affects their susceptibility to apoptosis. Murine splenic T cells have relatively high levels of bcl-2 compared to thymocytes, and about 50% remain viable in culture for over 4 days without activation. However, over the 4 days, there is a gradual decline in cell numbers by apoptosis without any change in the overall bcl-2 levels as determined by immunoblotting. Concanavalin A (Con A) activated splenic T cells undergo IL-2 dependent proliferation. Con A alone without IL-2 results in all the cells undergoing apoptosis within 2 days. There is a direct correlation between the dose of IL-2 supplied to Con A activated T cells and the level of bcl-2. With high levels of IL-2 (100 units/ml) for 5 days, the bcl-2 reaches a maximum of 3 fold above pre-activation levels. However, when the IL-2 is withdrawn from these cells, there is rapid death by apoptosis resulting in about 80% cell death by 24 hours. Again, the overall level of bcl-2 remains unchanged. Therefore, the overall level of bcl-2 in post-thymic T cells does not correlate with their susceptibility to apoptosis as measured by their rate of apoptosis with time in culture. Immunostaining and flow cytometry shows that there is a heterogeneous expression of bcl-2 at 2-4 days after Con A plus IL-2 activation. By 5 days after activation with Con A plus high IL-2, there is a very homogeneous population of cells with high bcl-2 expression. The presence of the low bcl-2 expressing cells within the first 2-4 days after activation correlates with the presence of an apoptotic population. Therefore, within the first few days of Con A activation, there are cells with low levels of bcl-2 that undergo apoptosis spontaneously in culture even in the presence of IL-2. At all times after activation, the cells with high or low levels of bcl-2 remain susceptible to apoptosis after IL-2 withdrawal.

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V 489 SELF TOLERANCE ALTERS BUT DOES NOT ELIMINATE THE T CELL RESPONSE TO A VIRAL ANTIGEN THAT IS A CLOSE HOMOLOG OF SELF. Douglas Cerasoli, Simon Carding@, Fei F. Shih, Barbara Knowles* & Andrew J. Caton, The Wistar Institute, Philadelphia, PA 19104, @ Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104, *Present Address: The Jackson Laboratory, Bar Harbor, ME 04609

We have analyzed the T cell response to a viral antigen that differs from a self peptide by a single conservative amino acid interchange. Transgenic mice that express the influenza virus A/PR/8/34 hemagglutinin (PR8 HA) as a neo-self antigen in the thymus (HA Tg mice) were immunized or infected with a mutant influenza virus (K113) that contains an Arg to Lys interchange in the PR8 Th determinant site 1. The majority of the T cell hybridomas isolated from BALB/c mice in response to K113 react equally well with the native site 1 determinant, and many use a characteristic Vα/Vβ gene segment combination. In contrast, most of the hybridomas isolated from HA Tg mice in response to K113 show undetectable reactivity with the neo-self site 1, and the characteristic Vα/Vβ gene segment combination is not used. Indeed, many highly diverse and distinct TCRs were used by the K113-specific hybridomas from HA Tg mice. Therefore, both the specificity and the genetics of the response to K113 in the HA Tg mice were altered by tolerance to the neo-self site 1 determinant. Interestingly, a few of the K113-specific hybridomas from HA Tg mice can clearly react with the neo-self site 1, in one case requiring only a 20-fold higher dose of the self antigen than K113 to achieve half-maximal IL-3 secretion. However, these hybridomas generate nearly undetectable levels of IL-2 in response to the neo-self site 1, even though they produce IL-2 in response to K113. These T cells appear to have evaded negative selection because they received only a partial activation signal in response to self.

V 490 DELETION OF T CELLS IN THE THYMUS IS DEPENDENT ON THE LEVEL OF MOUSE MAMMARY TUMOR VIRUS (MMTV) SUPERANTIGEN EXPRESSION AND THE PRESENCE OF *env* GENE PRODUCT. A.Chervonsky *, T.Golovkina, S.R.Ross and C.Janeway, Jr.* *Yale University School of Medicine and Department of Biochemestry, University of Illinois at Chicago

Expression of different portions of C3H MMTV genome as transgenes leads to different consequences. Open reading frame (*orf*) expressed alone causes deletion of V_β 14 T cells. This deletion is age dependent and accumulation of Tcr ^{high} double positive cells can be detected in the adult animals. These cells also show elevated levels of CD69 expression, suggesting that they are undergoing activation prior to deletion. The density of ligand appears to be important for the level of deletion, since animals homozygous for *orf* delete larger population of V_β14 T cells than the heterozygous ones. Combination of *orf* and *env* genes makes deletion more profound and independent of age or gene copy number. The role of *env* product is not clear yet, but the findings here are in concordance with the previous observation on the necessity of *env* for T cell activation by superantigen *in vitro*.

V 491 Identification of genes induced during clonal deletion of immature T cells in the thymus . Jang-Won Choi, and Yongwon Choi, Howard Hughes Medical Instate, The Rockefeller University, 1230 York Av., New York, NY10021.

During T cell development, immature T cells are clonally deleted in the thymus upon interaction with self antigen/major histocompatibility complexes. Molecular mechanisms involved in deletion process of immature T cells is not clear except that cells are going through apoptosis. To understand how T cell receptor engagement in immature T cells induce apoptosis and clonal deletion, we have isolated several cDNA clones of which expression is differentially controlled during this process. By differential display PCR, we have isolated 4 genes which are specifically induced upon TCR engagement of immature thymocytes. Two of these cDNA clones are already identified genes whose expression is controlled by either serum factors or UV. We have also identified 2 genes which are specifically down-regulated during this period. The role of these genes during T cell development is currently being investigated.

V 492 ABERRANT TRANSCRIPTIONAL REGULATION OF THE IL2 GENE MAINTAINS THE ANERGIC STATE OF MRL-*LPR* CD4-8- T LYMPHOCYTES. James L. Clements and Ralph C. Budd, Department of Medicine, University of Vermont College of Medicine, Burlington, VT 05405

The anergic state of freshly isolated MRL-*lpr* (*lpr*) peripheral CD4-8- T cells is manifest primarily as an inability to produce IL2 in response to antigenic or mitogenic stimulation. This stands in contrast to intact receptor-mediated early signalling events, such as IP_3 generation and a normal calcium flux. We therefore sought to analyze the activation state of the IL2 promoter in freshly isolated as well as stimulated *lpr* CD4-8- T cells. The IL2 promoter contains several conserved regions which mediate transcriptional regulation, including AP-1, NFIL (Oct), and NF-κB sites. The NFAT and CD28RE sites appear unique to the IL2 gene and play a pivotal role in activation of the IL2 gene under conditions of physiological stimulation. The NFIL, NF-κB, and CD28RE sites appear to be regulated normally in *lpr* CD4-8- T cells upon stimulation in the presence of mitogens and anti-CD28 mAb, as assessed by gel-mobility shift assays. However, AP-1 expression is constitutively elevated in these cells, and cannot be induced to higher levels upon stimulation. Furthermore, nuclear extracts from freshly isolated as well as stimulated *lpr* CD4-8- T cells display a novel NFAT-site specific binding activity, which correlates with their anergic state. This binding activity is not detectable in extracts from normal mouse T lymphocytes. Culturing *lpr* CD4-8- T cells in the presence of mitogens, anti-CD28 mAb, and an exogenous source of IL2 induces cell cycling and results in acquisition of functional capacity in terms of ability to proliferate and produce IL2 upon repeat stimulation. Analysis of nuclear extracts from cultured (i.e., functional) *lpr* CD4-8- T cells reveals normal regulation of the IL2 promoter at the NFAT site. The abnormal expression of AP-1 and NFAT may therefore be secondary to the *fas* mutation in these mice, but appear to play a primary role in maintaining the inert state of *lpr* CD4-8- T cells.

V 493 ARRESTED DEVELOPMENT OF IMMATURE SELF-REACTIVE B CELLS ON ENCOUNTER WITH ANTIGEN IS REVERSED BY REMOVAL OF ANTIGEN BUT NOT BY T CELL HELP. Jason G. Cyster, Suzanne B. Hartley, and Christopher C. Goodnow. Howard Hughes Medical Institute, Beckman Center B163, Stanford University Medical Center, Stanford, CA 94305.

Deletion of self-reactive B cells following encounter with membrane antigen is preceded by an arrest in development at an immature stage (Hartley et al.,1993; Cell 72,325). The existence of short-lived autoreactive B cells in the bone marrow raises questions regarding their ability to receive T cell help and break tolerance, a significant possibility given the ease with which SLE type syndromes can be induced in experimental graft versus host disease, and is also relevant to the issue of receptor editing. These questions have been examined here using immunoglobulin (Ig) transgenic mice producing B cells specific for Hen Egg Lysozyme (Hel) and transgenic mice expressing a membrane bound form of Hel (mHel). In Ig x mHel double transgenic mice all Ig expressing B cells are arrested at a short lived immature stage in the bone marrow. Following transfer of arrested immature B cells to recipients lacking mHel the cells differentiate at a rate similar to bone marrow B cells that were not arrested, indicating that encounter with antigen does not induce an irreversible or long lasting change in the B cells. However, in the presence of mHel the arrest in development is not overcome by placing the cells in an environment with large numbers of activated T cells. Cyclosporin A and FK506, while supporting partial differentiation, are also unable to fully overcome the anti-differentiation signal transmitted by the antigen receptor. Factors which may contribute to the low T cell responsiveness include a lack of CTLA4 ligand and other accessory molecule upregulation following antigen receptor crosslinking and little or no CD40 expression at this stage of differentiation. The relevance of these findings to systemic autoimmunity and self-reactive B cell receptor editing will be discussed.

V 494 THE PERIPHERAL DELETION OF $V\beta5^+CD8^+$ T CELLS IN MTV-6^+9^+I-E^- MICE IS ACCOMPANIED BY THE APPEARANCE OF A POPULATION OF FUNCTIONALLY COMPROMISED $CD8^{low}TCR^{low}$ CELLS, Stacey Dillon, Gail Turk and Pamela Fink, Department of Immunology, University of Washington, Seattle, WA 98195

$V\beta5^+$ T cells are deleted intrathymically in I-E^+Mtv-9^+ mice, and are more highly represented in $CD8^+$ relative to $CD4^+$ peripheral T cells. Our previous studies have shown that $CD4^+V\beta5^+$ T cells are slowly depleted in the lymphoid periphery of Mtv-6^-9^+I-E^- (B6) nontransgenic and $V\beta5$ transgenic mice. Prior to depletion, these $CD4^+$ cells are activated and then rendered anergic to signals through their TCR. We have recently found that in Mtv-6^+9^+ I-E^- (B6 x BXD15)F_1 mice, both $CD4^+V\beta5^+$ and $CD8^+V\beta5^+$ cells are subject to peripheral deletion, such that $V\beta5$ transgenic mice bred onto this genetic background are severely depleted of both $CD4^+$ and $CD8^+$ T cells by 5-6 months of age. Mtv-$6^+9^+V\beta5$ transgenic mice possess a striking population of $CD8^{low}V\beta5^{low}$ peripheral T cells. These $CD8^{low}$ cells can be detected in the peripheral blood and spleen, but not in lymph nodes from the same animals. These cells also appear in the population of $CD8^+V\beta5^+$ cells in nontransgenic F_1 mice, indicating that their occurrence is not transgene-dependent. Furthermore, $CD8^{low}$ cells are not present in the thymus, and are found in thymectomized F_1 mice, suggesting that the signal leading to the downregulation of CD8 is probably delivered in the lymphoid periphery. The $CD8^{low}V_{\beta}5^{low}$ cells express uniformly high levels of the memory cell marker CD44, high levels of VLA-4, and low levels of L-selectin (MEL-14), indicating their prior activation. However, the $CD8^{low}$ population is not enriched for blasts, as these cells are smaller than $CD8^{high}$ cells from the same animals. We have assessed the functional capabilities of $CD8^{low}$ cells by plating FACS-sorted $CD8^{low}$ and $CD8^{high}$ cells from F_1 PBL or spleen in limiting dilution to determine CTL precursor frequency. These analyses have revealed that, compared to the $CD8^{high}$ compartment, there is a severe reduction in the number of alloreactive CTL precursors present in the $CD8^{low}$ population. However, $CD8^{low}$ splenocytes freshly isolated from the F_1 mice do not exhibit the internucleosomal DNA fragmentation that is indicative of apoptosis. Thus, we have identified what seems to be an intermediate population of T cells targeted for peripheral deletion which, although functionally compromised, have not yet undergone programmed cell death.

V 495 DIFFERENT BEHAVIOUR OF Vß8-EXPRESSING CD4+8- and CD4-8+ THYMOCYTES FOLLOWING SEB INJECTION IN ADULT MICE, Gino Doria, Andrea Fattorossi* and Selene Baschieri, Laboratory of Immunology, ENEA C.R.E. Casaccia, and *D.A.S.R.S., Pratica di Mare, Rome, Italy

Adult (C57BL/10 x DBA/2)F1 male mice were injected i.p. with 10 or 100 µg Staphylococcal Enterotoxin B (SEB) and, at different time intervals, their thymocytes were analyzed for the Vß8+ and Vß10+ TCR distributions in the four CD4/CD8 cell subsets by three colour flow cytometry. SEB induces significant modifications only in single positive (CD4+8- and CD4-8+) Vß8+ thymocytes. At day 2 after SEB injection, both the percentage and absolute number of CD4-8+Vß8+ cells are increased while those of CD4+8-Vß8+ cells are reduced, as compared to PBS-injected controls. At day 3, also the CD4-8+Vß8+ cells are reduced under control values. Results from overnight cultures of thymocytes from SEB-injected mice at day 2 suggest that reduction in the percent of CD4+8-Vß8+ cells may be due to intrathymic death whereas the decrease in the percent of CD4-8+Vß8+ cells may be due to migration to the periphery. Thymocytes from SEB-injected mice were also evaluated, at different time intervals, for their ability to proliferate in vitro upon stimulation by SEB or SEA. The mitotic responses appear to be correlated with the percentages of CD4+8- and CD4-8+ cells, expressing either Vß8 or Vß10, in culture. In conclusion, the present results demonstrate, at variance with what has previously been found for peripheral lymphocytes, a different behaviour of CD4+8-Vß8+ and CD4-8+Vß8+ thymocytes in response to SEB injection.

V 496 EFFICIENCY OF THYMIC DELETION ON PEPTIDE VERSUS V-SAG LIGANDS, P. Julian Dyson, Andrew. Mellor*, James I. Elliott and Elizabeth Simpson, Transplantation Biology, CRC, Watford Road, Harrow, HA1 3UJ, UK and * National Institute for Medical Research, Mill Hill, The Ridgeway, London, NW7 1AA, UK

Thymic deletion of endogenous superantigen responsive thymocytes is rarely a complete process, the reactive subsets (defined by TCR beta chain expression) being diminished in relative percentage of the peripheral population rather than totally eliminated. The remaining cells may express TCRs which have only low affinity for the particular vSAG and hence may simply not be subject to thymic deletion or their presence may reflect an inefficiency in the process of superantigen dependent deletion. In order to distinguish between these alternatives a TCR transgenic was produced utilising the TCR from a T cell clone derived from the non-deleted $V\beta11$ T cell pool present in the vSAG-8^+,-9^+ CBA mouse strain. The $CD8^+$ T cell clone C6 expresses $V\beta11$ and $V\alpha8$ and recognises H-Y in the context of K^k. Male CBA mice transgenic for the C6 TCR α and β chain genes exhibit very small thymi which lack the CD4, CD8 single and double positive $V\beta11$ positive populations. Female CBA mice showed thymic deletion of the single positive populations. In the periphery, however, substantial numbers of $V\beta11$ positive cells are found in females. *Ex vivo*, these cells are initially unresponsive to H-Y, but on *in vitro* culture they respond well to H-Y. These results suggest the C6 T cell receptor does have reactivity to vSAG -8,-9 but apparent deletion of the single positive thymic subsets does not prevent export to the periphery. Extrapolation to deletion of $V\beta$ subsets by endogenous superantigens suggests the residual peripheral pools do have reactivity but a stochastic aspect of thymic deletion operates which makes the process inefficient.

V 497 CTL from β_2m Deficient Mice Lack Tolerance for Self H-2^b Expressed at Normal Ligand Density. Rickard Glas, Claes Öhlén, Petter Höglund and Klas Kärre. Laboratory of Tumorbiology, Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, S-171 77 Sweden.

By in vivo priming and in vitro restimulation with allogeneic cells it could be shown that CD8$^+$ TCR $\alpha\beta^+$ CTL could be generated from β_2m deficient (H-2^b) mice. In contrast to CTL from the β_2m^+ littermates they were found to lack tolerance for self H-2^{b}expressed in β_2m^+ cells. This additional specificity of the β_2m deficient CTL was distributed also at the single cell level, since the two specificities competed for eachother in cold target competition experiments. The results could not be attributed to specific crossreactivity between H-2^b and allogeneic H-2 since 1) It never observed using β_2m^+ CTL, 2) Killing of H-2^b expressing cells was performed by β_2m deficient CTL generated against several different stimulators. It could be shown by modulating the H-2^b expression on RMA-S cells that different ligand densities of self H-2^b could modulate sensitivity to β_2m deficient CTL. Furthermore, β_2m deficient CTL specific could also be generated in primary MLC, i.e. without in vivo priming, but only when stimulated with H-2 syngeneic, H-2^b expressing cells. We believe that these results show a general bias for self H-2^b, among the CTL in the β_2m deficient mice. This is consistent with model that low levels of free heavy chains in the β_2m deficient mice mediate T cell selection, and that the "selection window" is shifted to fit very low densities of MHC class I, which would result in a T cell repertoire that is reactive against H-2^b when expressed at normal density. The results suggest a role for MHC class I ligand density in T cell selection.

V 498 CENSORING OF SELF-REACTIVE B CELLS WITH A RANGE OF RECEPTOR AFFINITIES IN TRANSGENIC MICE EXPRESSING HEAVY CHAINS FOR A LYSOZYME-SPECIFIC ANTIBODY, Suzanne B Hartley and Christopher C Goodnow, Department of Microbiology and Immunology, and Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

Transgenic mice carrying a rearranged immunoglobulin (Ig) gene encoding the heavy (H) chain of a lysozyme-specific antibody were used to examine the effect of antigen binding affinity on elimination of self-reactive B cells. In H chain transgenic mice, B cells expressed surface IgM and IgD composed of lysozyme-specific H chains and many different possible light (L) chains from endogenous L chain genes. Immunofluorescent staining and flow cytometry revealed a distinct subset comprising approximately one percent of spleen B cells that bound lysozyme with a high affinity (relative K_d ~ 10^{-9} M) comparable to the original lysozyme-specific antibody. Additional subsets accounting for a total of 5-6 % of spleen B cells bound lysozyme more weakly, with the weakest exhibiting a relative K_d of approximately 10^{-5} M. When the various B cell subpopulations were allowed to develop in animals expressing lysozyme as a membrane-bound antigen on autologous cells, both low and high affinity lysozyme-binding B cells were undetectable in peripheral lymphoid organs. These findings demonstrate the efficacy with which low affinity self-reactive B cells can be eliminated in vivo. consistent with the notion that this cellular mechanism accounts for the absence of natural IgM antibodies against self antigens on the surface of blood cells. The data also illustrate the potential use of Ig transgenic mice for analyzing and selecting novel receptor-ligand interactions.

V 499 THYMOCYTE DELETION: IS ONE SIGNAL SUFFICIENT ? Patrice Hugo, Gary Winslow, John Kappler and Philippa Marrack. National Jewish Center for Immunology and Respiratory Medicine, Denver, CO. 80206.

Fixed antigen presenting cells (APC) or immobilized MHC molecules have been shown to activate T cell hybrids, but not T cell clones. This has been interpreted as an indication that the delivery of one signal was sufficient to activate T cell hybrids, while an additional signal(s) was required for the activation of T cell clones (Schwartz 1990). Fixed thymic epithelial cells were tested for their capacity to present superantigens and induce the deletion of CD4$^+$CD8$^+$ thymocytes bearing the appropriate TcR Vβ elements. It was found that these fixed APCs strongly deleted CD4$^+$CD8$^+$ thymocytes from TcRV$_\alpha$11.1$_\beta$3 transgenic mice in the presence of Staphylococcal enterotoxin A (SEA). This observation suggests that no active role from the APC was needed to mediate SAG-induced thymocyte deletion. The capacity of purified MHC class II molecules immobilized on a solid surface to present SEA and induce the deletion of thymocytes from TcRV$_\alpha$11.1$_\beta$3 transgenic mice support this notion. These data indicate that one signal might be sufficient to trigger the deletion of thymocytes with SAG.

V 500 BCL-2 PREVENTS THE CLONAL DELETION OF PHOSPHOCHOLINE-SPECIFIC B CELLS IN $\mu\kappa$ M167 TRANSGENIC XID MICE, James J. Kenny*, Randy T. Fischer#, John C. Reed+, and Dan L. Longo#, *Program Resources Inc./Dyncorp., & #Biological Response Modifiers Program, NCI-FCRDC, Frederick, MD 21702 and +La Jolla Cancer Research Foundation, La Jolla, CA. 92037

We have previously shown (J. Immunol.146:2568,1991) that the combined expression of the M167 $\mu\kappa$ anti-phosphocholine (PC) transgenes (TG) with the x-linked immunodeficiency gene, *xid*, results in an almost total failure to develop B cells in the peripheral lymphoid organs of such mice. Since TNP-specific splenic B cells develop normally in Sp-6 $\mu\kappa$ *xid* transgenic mice and PC-specific B cells develop normally in the bone marrow of M167 $\mu\kappa$ TG$^+$ *xid* mice, the lack of peripheral PC-specific B cells appears to be due to an Ig-receptor-mediated clonal deletion of these B cells. M167 $\mu\kappa$ TG$^+$ female mice homozygous for the *xid* gene were crossed to BCL-2 transgenic mice to determine whether or not over-expression of BCL-2 would prevent the apparent clonal deletion of PC-specific B cells in the BCL-2$^+$:V$_H$1$^+$ double transgenic F1 male progeny. The number of splenic B cells increased > 20 fold in V$_H$1$^+$ *xid* mice in the presence of BCL-2 going from < 2 x 10^6 B cells in the BCL-2$^-$:V$_H$1$^+$ males to ~ 50 x 10^6 in the BCL-2$^+$:V$_H$1$^+$ male mice. As previously shown by other laboratories, the over-expression of BCL-2 caused a 3 to 4 fold increase in splenic B cells in both phenotypically normal female and *xid* male mice lacking the M167 $\mu\kappa$ transgenes. Over-expression of BCL-2 had no effect on the bone marrow pre-B cell pool in any of the F1 progeny; however, the mature, recycling, CD23$^+$:δ^+ B cells increased 4 to 10 fold in the bone marrow of both BCL-2$^+$:V$_H$1$^+$ and BCL-2$^+$:V$_H$1$^-$ mice. Studies are in progress to determine whether or not BCL-2$^+$:V$_H$1$^+$ *xid* mice can respond to immunization with *Streptococcus pneumoniae*, a PC-containing thymus independent antigen.

V 501 ANALYSIS OF IMMUNOLOGICAL SELF-TOLERANCE IN THE B CELL COMPARTMENT. Julie Lang, University of Colorado Health Sciences Center, Department of Immunology, Denver, CO 80220 and David Nemazee, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Because autoantibodies can cause and exacerbate disease, we have been analyzing the extent of self-tolerance in the B-cell compartment. Autospecific B lymphocytes are subject to negative regulation through the complementary, and, perhaps, partially overlapping mechanisms of deletion and functional inactivation. We have observed deletion of autoreactive B cells specific for class I antigens in mice transgenic for genes encoding the IgM and IgD forms of the heavy and light chains of an anti-K^k antibody, 3-83. In the absence of self antigens to which the 3-83 antibody reacts, the B-cells in these mice are virtually monoclonal, with >95% bearing the 3-83 specificity. By contrast, in the presence of H-$2K^k$ the autoreactive clone is completely eliminated from the peripheral lymphoid tissues. To analyze the sensitivity of this self-tolerance mechanism to antigen-antibody affinity, we have screened the affinity of the 3-83 antibody for different allelic forms of H-2K and found the following hierarchy of binding: K^k (Ka ~10^8)>>K^b>K^{bm8}>K^{bm3}. We then introduced the various allelic forms of K to our trangenic mice by breeding and analyzed the extent of B-cell deletion. Remarkably, in all cases the autoreactive B-cells were apparently completely deleted in all the mice tested. We conclude that in normal individuals self-tolerance in the B-cell compartment is remarkably sensitive, at least for cell surface autoantigens.

V 502 REGULATION OF ANTI-DNA B CELLS IN KAPPA KNOCK-OUT MICE TRANSGENIC FOR AN IMMUNOGLOBULIN HEAVY CHAIN. Nina Luning Prak and Martin Weigert. Department of Molecular Biology. Princeton University. Princeton, N.J. 08544. Autoreactive B cells can escape deletion by editing their surface receptors. This phenomenon of receptor editing has been observed recently in mice transgenic for anti-MHC class I antibodies (Tiegs, S. et. al.) as well as in animals transgenic for the anti-DNA antibody heavy chain 3H9 (Gay, D. et. al., Radic, M. (b) et. al.). The 3H9 heavy chain binds DNA when associated with a variety of different light chains including Vλ1 (Radic, M. (a) et. al.) and Vλ2 (Erikson, J.). However, animals which are transgenic for the 3H9 heavy chain can still make non-self reactive B cells by pairing this heavy chain transgene with certain endogenous light chains (Erikson, J. et. al. and Radic, M. (b) et. al.). We interpret this to mean that light chains which contribute to DNA binding are replaced by light chains that, with 3H9, do not bind DNA.

To assess receptor editing as a mechanism of tolerance in the κ locus, we are characterizing B cells from 3H9 transgenic animals which are hemizygous for a κ locus deletion generated by gene targeting (Chen, J. et. al.). These κ hemizygotes (3H9/kdel/wt) are useful for studying κ editing because clones with multiple κ rearrangements must have undergone successive κ rearrangement, as opposed to a non-productive rearrangement on the other κ allele. A prediction of κ editing is that the frequency of Jκ1 usage in 3H9kdel/wt hybridomas will be reduced because secondary κ rearrangement requires the removal of Jκ1 by deletional or inversional recombination to downstream Jκ segments. We in fact observe a reduced frequency of Jκ1 rearrangements, consistent with receptor editing by sequential κ locus rearrangements (or with selection acting upon particular Vκ-Jκ combinations).

To assess receptor editing in the λ locus, we are studying 3H9 heavy chain transgenic mice which are homozygous for the κ locus deletion (3H9/kdel/kdel). Because Vλ1 and Vλ2 combined with the 3H9 heavy chain give rise to anti-DNA antibodies, a 3H9/kdel/kdel animal is left with a very limited system in which to maintain self tolerance. The frequencies of Vλ1 and Vλ2 rearrangement among splenic B cell hybridomas in 3H9/kdel/kdel are reduced by 75% and the frequency of clones with rearrangements to Vλx is increased 5 to10-fold compared to non-transgenic kdel/kdel animals. As successive rearrangements to Vλx do not inactivate Vλ1 or Vλ2, the consequence of light chain editing in 3H9/kdel/kdel is failed allelic exclusion at λ. Therefore we are assessing the functional status of λ rearrangements in 3H9/kdel/kdel clones which harbor multiple λ rearrangements.

V 503 THYMOCYTE SELECTION, INACTIVATION AND ELIMINATION; REQUIREMENTS FOR SELF MHC EXPRESSION. Andrew L. Mellor, Peter Tomlinson, Anne-Marit Sponaas, Jane Antoniou, Ruth Hartley, Anne-Marie Schmitt-Verhulst[1], Nathalie Auphan[1], Bernard Malissen[1] & Dennis Loh[2]. Molecular Immunology, Natl. Inst. for Med. Res., The Ridgeway, Mill Hill, London NW7, UK; [1]Centre d'Immunologie de Marseille-Luminy, 13288, Marseille, France and [2] Howard Hughes Med. Inst., Washington Univ. , St. Louis, USA.
Promoters from α-lactalbumin (a milk protein gene, KAL mice), β-globin (Kβ mice) and CD2 (CD2K^b mice) genes were used to drive expression of the H-$2K^b$ MHC class I gene in transgenic mice. By mating these mice to T cell receptor (TCR) transgenic mice we have studied the effect of limited expression of self H-$2K^b$ molecules on selection processes in the thymus. Three different TCR transgenic mice have been used to date; two carry TCR genes from H-$2K^b$ specific, CD8 dependent or CD8 independent CTL clones and another carries TCR genes which confer H-$2L^d$ specificity but which are subject to H-$2K^b$ dependent positive selection in the thymus. Our studies indicate that tolerance can be induced in the thymus even when self MHC class I expression on thymic cells is very low (KAL mice) and that deletion of self reactive (TCR^+) thymocytes occurs late in thymocyte development and is not totally effective when few thymic cells express self MHC (KAL and Kβ mice). In contrast, thymocytes from CD2K^b are eliminated at an early developmental stage prior to differentiation into $CD4^+CD8^+$ thymocytes when thymocytes are the only cells expressing self MHC class I molecules in the thymus, although this does not prevent the accumulation of large numbers of functionally unresponsive $TCR^+CD4^-CD8^-$ T cells in the peripheral lymph nodes of such mice (CD2K^b mice). We have also shown that positive selection fails to take place in KAL and CD2K^b mice despite the fact that thymocytes express relatively high levels of H-$2K^b$ in CD2K^b mice. This suggests that thymocytes are unable to affect thymocyte selection. However, positive selection does take place in Kβ mice. We are investigating which cell types from these mice are responsible for positive selection.

V 504 ***Differential expression of Mtv loci in MHC class II^+ thymic dendritic cells.*** Nel C. Moore, Graham Anderson, John J. T. Owen and Eric J. Jenkinson, Department of Anatomy, University of Birmingham, Birmingham, B15 2TT, U.K.
Despite the important role endogenous superantigens play in shaping the T cell repertoire, little is known concerning the expression of the different Mtv loci in the cells of the thymic microenvironment involved in selection. To monitor the tissue specific expression of the different Mtv loci and their effects on development of the TCRVβ repertoire in Balb/c mice we have used highly purified preparations of thymic stromal cells and dendritic cells (DC), in combination with reaggregate and normal organ cultures and PCR analysis using primers able to distinghuish *Mtv-6* (255 bp) from *Mtv-8* and *-9* (280 bp). *Mtv-6* and *Mtv-8/9* mRNA's are expressed in thymus lobe organ cultures from Balb/c mice throughout a 12 day culture period. However, when expression in individual MHC class II^+ components of the thymus was examined, all Mtv's were found to be absent from epithelial cells. In contrast, a striking pattern of differential expression was observed in thymic DC which were devoid of *Mtv-8/9* but expressed *Mtv-6* mRNA. This pattern of expresion correlated well with further studies which showed that Vβ3 cells, normally deleted by *Mtv-6,* were effectively deleted in organ cultures when DC were present but not in reaggregate cultures where the only MHC class II^+ cells were purified epithelial cells. On the other hand, $V\beta11^+$ cells were not effectively deleted in organ cultures, perphaps refecting the absence of *Mtv-8/9* expression in DC. Our study suggest the influence Mtv's have on shaping the T-cell repertoire not only depend upon availability within the particular strain but also on their tissue specific pattern of expression in relation to MHC class II which is necessary for the presentation of these superantigens.

V 505 VARIABLE DELETION OF SELF REACTIVE THYMOCYTES REFLECTS A DIFFERENCE IN VIRAL SUPERANTIGEN mRNA EXPRESSION LEVELS,
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When developing thymocytes bearing particular Vβ elements within their T cell receptor interact with endogenous viral superantigens (vSAGs) in the thymus, they undergo negative selection. Thus, mice carrying endogenous vSAGs are characterized by the absence of these reactive Vβ bearing T cells in their periphery. In order to study the factors that affect the process of negative selection, we have used two strains of mice in which the mouse mammary tumor virus (Mtv)-1 or -6 genes encoding vSAG-1 or -6 are expressed singly on a B10.BR background. While the amino acid sequences of vSAG-1 and -6 have been shown to be identical, the reactive Vβ3+ thymocytes are deleted to different extents. This reflects the peripheral phenotype: B10.BR-Mtv-1 mice have approximately 1% of their total peripheral T cells bearing the Vβ3 element, while B10.BR-Mtv-6 mice have virtually none (J.Exp.Med. (1992) 175:41). Our studies show that the developmental stage at which Vβ3+ thymocytes are deleted from B10.BR-Mtv-6 mice is earlier than that of B10.BR-Mtv-1 mice. In addition, the two strains have different time courses of deletion.

Using RNase protection assays, we find that a distinct hierarchy of vSAG mRNA expression exists. For example, adult B10.BR-Mtv-6 animals have a higher level of vSAG expression in thymus compared to B10.BR-Mtv-1 mice. We therefore conclude that, in this system, a higher level of vSAG mRNA expression correlates with a more complete deletion of reactive thymocytes. Quantitative PCR will be used to delineate the cell types involved in tolerance induction to these endogenous vSAGs.

V 506 EFFECT OF CsA ON *IN VIVO* CLONAL DELETION BY A SOLUBLE ANTIGEN. Stephan Oehen, Frank Eidelman and Stephen Hedrick, University of California, San Diego, 9500 Gilman Dr., Dept. of Biology, La Jolla, CA 92093-0063

It has been suggested that the immunosupressant cyclosporin A (CsA) interferes with T cell ontogeny as evidenced by the appearance of graft versus host (GVHD) disease and organ-specific autoimmunity after cessation of CsA treatment. Previous studies have shown that thymocytes from mice treated with CsA for a short time period were made resistant to the induction of apoptosis by anti-CD3. In some model systems clonal deletion of T cells bearing Vß elements reactive to endogenous superantigens escaped clonal deletion after CsA treatment, however, other reports investigating different Vß elements were unable to confirm these findings. Therefore, it is still unclear as to how CsA interferes with negative selection. Using T cell receptor (TCR) transgenic mice specific for pigeon cytochrome *c* (PCC), we find that short term treatment with CsA did not prevent clonal deletion in the adult by administration of soluble PCC or SEA superantigen. In contrast, long term treatment with CsA deleted 80% of the CD4+CD8+ (DP) thymocytes. The remaining DP could not be deleted by soluble PCC. Surprisingly, these remaining cells that escaped clonal deletion had downregulated their transgenic TCR. Other studies have shown that TCR downmodulation may represent a reversible process. Thus, self-reactive T cells can escape clonal deletion in CsA-treated mice by TCR downmodulation and may give rise to a pool of potentially self-reactive T cells by reexpression of their TCR.

V 507 **MEDULLARY THYMIC EPITHELIAL CELLS INDUCE TOLERANCE TO SELF-PEPTIDES PRESENTED BY THE MHC CLASS I AND CLASS II MOLECULES.**
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INSERM U 267. Lab. de Génétique des Mammifères, I.P. INSERM U 345.

Negative selection of the T cell repertoire in the thymus reflects contact of immature thymocytes with bone marrow (BM) derived cells. Whether thymic epithelial cells (TEC) contribute to tolerance is controversial. BM chimeras and TG mice for MHC molecules exclusively present in the thymic epithelium accepted tolerogen expressing skin graft but mounted in vitro tolerogen specific CTL and proliferative responses in the presence of IL_2. These in vitro responses might be directed against peptides derived from proteins present in the peripheral APC but not in the TEC. The best way to define the tolerogenic capacity of TEC would be to study whether TG mice expressing a foreign protein in the thymic epithelium can mount a proliferative and a CTL response against this protein. That is why we have examined the state of tolerance toward β-galactosidase (β-gal) peptides complexed with MHC class I and class II molecules in a H-2^b mouse that expressed β-gal in the nucleus of medullary TEC, cutaneous epithelial cells and Purkinje cells. Our results have shown that TG β-gal mice did not develop a β-gal specific CTL response even of the presence of exogenous IL_2; in contrast, they developped a weak β-gal specific proliferative response when high doses of β-gal were used for in vitro stimulation; this partial reactivity was completely restored in the presence of exogenous IL_2. The tolerance to β-gal was due to the β-gal^+ medullary TEC; TG β-gal thymus grafted nude mice failed to mount an anti-β-gal CTL response while they mounted a weak anti-β-gal proliferative response. These results clearly show that TEC induce a complete tolerance toward the β-gal peptides presented by MHC class I molecules and a partial tolerance toward β-gal peptides presented by MHC class II molecules. This partial tolerance could be explained by the hypothesis that processing of a nuclear protein, such as the β-gal, by the endogenous pathway give rise to a number of MHC class II/ peptides that is sufficient to tolerize the high affinity but not the low affinity β-gal specific T helper cells. We are actually studying this hypothesis in our laboratory.

V 508 DIFFERENT LEVELS OF TOLERANCE TO MAJOR T CELL DETERMINANTS FROM THE SAME SELF ANTIGEN, Fei F. Shih, Douglas Cerasoli, Barbara Knowles*, and Andrew J. Caton, Wistar Institute, Philadelphia, PA 19104, *Present address: The Jackson Laboratory, Bar Harbor, ME 04609

We are interested in defining how self tolerance is established among developing thymocytes that recognize different determinants generated from the same self antigen. To address this issue, we have generated transgenic mice that express the influenza virus A/PR/8/34 hemagglutinin (PR8 HA) as a defined self antigen under control of the SV40 early region promoter/enhancer (HA Tg mice). These mice express the HA in the thymus (and also the spleen and kidney). Previous studies have identified eight CD4+ T cell determinants in the response of BALB/c mice to the PR8 HA. Of these, the majority of HA-specific T cells from BALB/c mice are directed to two determinants, termed Site 1 (IE^d-restricted, residues 110-119) and Site 2 (IA^d-restricted, residues 126-138). In order to evaluate how these determinants are recognized as self antigens, normal BALB/c and HA Tg mice were infected with PR8 virus and immune lymph node cells were expanded *in vitro* in response to GST fusion proteins containing the Site 1 and Site 2 determinants. T cell hybridomas were generated and analyzed for their specificity for Site 1 or Site 2. Site 1- and Site 2-specific hybridomas were isolated from normal BALB/c mice (5.5±1% and 20±5% of total hybridomas, respectively). In the HA Tg mice, Site 2-specific hybridomas were readily isolated, although at a lower frequency than in normal BALB/c mice (1.2±0.4% of total hybridomas). By contrast, no Site 1-specific hybridomas have been isolated from HA Tg mice. These findings suggest that the HA Tg mice may differ in their level of tolerance to these major T cell determinants, such that T cells that are directed to Site 2 but not Site 1 can be induced by infection with PR8 virus. We are currently evaluating factors that could account for differences in how these two determinants are recognized as self antigens.

V 509 INDUCTION OF THYMIC AND PERIPHERAL T CELL TOLERANCE BY A COGNATE PEPTIDE IN CLASS I RESTRICTED T CELL RECEPTOR TRANSGENIC MICE

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There is evidence that peripheral antigens can travel to the thymus and can be presented by Class II molecules inducing *in vivo* apoptosis of immature thymocytes bearing a class II restricted TCR specific for this antigen. However, it is not yet clear what the effect is of peripheral antigens that are presented by Class I and recognized by Class I restricted T lymphocytes.

We have used a TCR transgenic mouse whose T cells recognize an antigenic peptide of SV40 large T antigen in the context of Class I molecule K^K to study *in vivo* the effect of peripheral antigens in the induction of T cell tolerance.

A 9mer peptide of the SV40 large T antigen protein (Aa 560-568), an 18mer antigenic peptide (Aa 559-576), the native SV40 large T ag, and fusion protein containing the SV40 antigenic epitope or PBS were administered intraperitoneally daily for up to 6 days. Thymocytes and peripheral lymphocytes were harvested at day 4 and 7 and total cell number was evaluated and Facscan analysis was performed after staining with CD4, CD8, Vβ8 and Pgp-1 specific antibodies. We have found that only the 9mer induces a reduction of the total number of thymocytes (up to 90% of thymocytes) with a clear depletion of Vβ8+/CD8+ thymocytes at days 4 and 7. The percentage of Vβ8+CD8+ reactive T cells present in PBS treated control mice is 40-60% of the total and this is reduced to <5-20% in 9mer treated mice. This reduction preferentially affects these cells at the immature CD4+CD8+ stage. Furthermore, the 9mer also apparently induces a reduction of Vβ8+CD8+ reactive mature T cells in the periphery which starts at day 3 and is more evident 6 days after administration of the cognate peptide. Pgp-1 staining shows that in the 9mer treated mice, peripheral CD8+Vβ8+ T cells have encountered the antigen by day 3 (they become Pgp-1 high) and they are much decreased by day 7. The molecular mechanisms involved both in thymic and peripheral depletion as well as the kinetics of the phenomena described are currently being investigated.

V 510 SELF ANTIGEN PRESENTATION FOR INDUCTION OF TOLERANCE OR AUTOIMMUNITY IN MHC CLASS II T CELL RECEPTOR TRANSGENIC MICE.

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We have generated transgenic mice expressing an I-E^K restricted T cell receptor specific for the serum protein C5 . Mice expressing this T cell receptor on a C5 deficient background show positive selection in the thymus, skewing to CD4+ single positive cells with virtually no CD8+single positive cells and high functional activity. Peripheral T cells from these mice produce IL-2 and γIFN upon culture with APC and as little as 10ng of intact C5 protein. Crossing these mice with a C5 expressing congenic mouse strain results in negative selection of the TcR. Interestingly, thymi of these mice are not drastically reduced in cellularity and the CD4+CD8+ compartment is virtually unaffected. This is consistent with a delayed presentation for negative selection due to the failure of thymic macrophages and epithelial cells to present C5.Antigen presentation requirements for negative selection of circulating self protein which requires internalization and processing before it is presented with class II is currently under investigation. However, negative selection by deletion is not complete in these mice. Mature T cells in the periphery make substantial amount of IL-2 and γIFN upon stimulation in vitro. Experiments to test their functional behaviour in vivo are in progress.

V 511 LACK OF T CELL TOLERANCE TO HOST ANTIGENS IN RAT -> SCID MOUSE CHIMERAS, Charles D. Surh, and Jonathan Sprent, Dept. of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Provided that adult C.B-17 SCID mice are first lightly irradiated, transplantation of rat fetal liver (FL) cells leads to generation of rat -> mouse chimeras with full differentiation of rat T and B cells. Unlike chimeras prepared with allogeneic FL cells, rat FL -> mouse chimeras develop severe lethal chronic graft-vs-host disease (GVHD). The rat T cells generated in these rat -> mouse chimeras are incompletely tolerant to host mouse antigens as determined by various parameters including MLR and CTL assays in vitro, adoptive transfer of T cells to secondary SCID hosts and the lack of Vβ deletion to endogenous host Mtv antigens. Interestingly, the severity of GVHD and the degree self-reactivity of the rat T cells appear to be donor rat strain dependent. Thus, chimeras generated with Lewis FL cells are more severely affected than chimeras established with Fisher 344 or Wistar Furth FL cells. The inability of newly-formed rat T cells in rat -> mouse chimeras to become fully tolerant to host mouse antigens appears to be due to depletion of host APC by irradiation. Thus, rat -> mouse chimeras generated by transplanting rat fetal liver cells into unirradiated neonatal SCID mice fail to develop GVHD, and the rat T cells display self tolerance. Since allogeneic mouse -> SCID chimeras display strong self tolerance, presumably through recognition of host antigens on thymic epithelial cells (TEC), the implication is that mouse TEC are tolerogenic only for mouse and not for rat T cells. This may reflect that the accessory molecules on TEC required for tolerance induction are poorly recognized by the complementary accessory molecules on rat T cells. The identity of the accessory molecules concerned is under investigation.

Recognition by Natural Killer Cells

V 520 CD16 AND CD45 STIMULATE IFN-γ PRODUCTION IN IL-2-ACTIVATED NATURAL KILLER (NK) CELLS.

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Little is known about the nature of molecules that regulate cytotoxic activity or cytokine production in IL-2 activated NK cells. We here report that, in addition to CD16, cross-linking of CD45 can stimulate IFN-γ production in IL-2 activated NK cells. We first tested the ability of a panel of antibodies to directly stimulate or co-stimulate NK cells. Only anti-CD16 and anti-CD45 mAbs could directly stimulate IFN-γ production. Northern blot analysis revealed that these antibodies increased the levels of mRNA for both IFN-γ and TNF-α. Phenotypic analysis revealed that fresh peripheral NK cells express the CD45RA, but IL-2 activated NK cells express the CD45RO. Antibodies directed at CD45RO could also stimulate IFN-γ production, but anti-CD45RA antibodies did not. The ability of CD45 to directly stimulated cytokine production in NK cells was unexpected since cross-linking of CD45 has *never been reported* to directly stimulate T cells.

We next performed experiments to determine whether engagement of CD16-Fc receptor was necessary for the stimulatory activity of CD45. First, we used CD45 antibodies of the IgG1 isotype to stimulate NK cells, since CD16 reportedly does not bind IgG1. Second, the combination of CD16 and CD45 mAbs were not more stimulatory, and in some experiments were less stimulatory, than individual antibodies alone. Third, $F(ab)_2$ of CD45 could stimulate IFN-γ production (although not as efficiently as intact CD45). Finally, cross-linking of CD45 induced tyrosine phosphorylation of endogenous substrates that were qualitatively non-identical to CD16 cross-linking. The kinetics tyrosine phosphorylation induced by CD16 and CD45 mAbs were also different; CD16 induced maximum phosphorylation at 5 mins whereas CD45 induced maximum phosphorylation at 15 mins. Collectively, the data suggests that the stimulatory activity of CD45 is independent of CD16. These observations lead us to speculate that CD45 (or CD45RO) on IL-2 activated NK cells associates either directly or indirectly with tyrosine kinases; the cross-linking CD45 induces the activation of tyrosine kinases which then initiates a signaling cascade that results in transcription of IFN-γ and TNF-α. Studies are on-going to define molecules involved in this CD45-triggered signaling.

V 521 GENERAL ROLE OF HLA CLASS I MOLECULES IN THE PROTECTION OF TARGET CELLS FROM LYSIS BY NK CELLS. EVIDENCE THAT THE TRIMERIC FORM OF CLASS I MOLECULES IS REQUIRED FOR THE PROTECTIVE EFFECT.

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Some HLA-C alleles have been shown to exert a specific protective effect preventing target cells from lysis by groups of NK clones displaying a defined specificity. In this study we analyzed whether class I mediated protection is a more general phenomenon involving all NK cells. First, we utilized two anti-class I mAbs (6A4 of IgG1 isotype and A6-136 of IgM isotype), which had been shown to induce lysis of protected target cells by "group 1" and "group 2" NK clones. Addition of 6A4 $F(ab')_2$ or A6-136 mAbs resulted in lysis of all protected target cells by all NK clones analyzed. Target cells were represented by a panel of HLA homozygous B-EBV cell lines while NK clones were representative of clones displaying different GL183/EB6 surface phenotypes and/or different ability to lyse allogeneic cells. Unselected NK clones derived from 7 different individuals were tested against autologous target cells represented by PHA-induced blasts or B-EBV trasformed cell lines. In both instances, addition of a mixture of 6A4 $F(ab')_2$ and A6-136 mAbs resulted in lysis of autologous target cells, thus suggesting that class I molecules prevent lysis of normal cells by self NK cells. We further investigated whether the class I-mediated protection requires the trimeric form of class I molecules (composed of α chain, β_2 microglobulin and peptide) or rather the free α chain. Acidic treatment of the C1R ($Cw4^+$) target cells or 81.22 ($Cw3^+$, $Cw4^+$) at pH2.2 resulted in loss of reactivity with 6A4, A6-136 and W6-32 mAb (known to react with the assembled form of class I molecules) and in the de novo reactivity with L31 mAb (specific for the HLA-C free chain). While the untreated $Cw4^+$ C1R cells were resistant to lysis by the Cw4-specific group 1 NK clones, the pH2.2-treated cells became highly susceptible to lysis by the same clones. These data indicate that, at least for the NK clones analyzed, the protection of target cells requires class I molecules in the form of a trimeric complex.

V 522 NK-CELL MEDIATED LYSIS REQUIRES MORE THAN MHC-I DEFICIENCY, I. Correa, L. Corral, W. Wu, J. Dorfman and D. Raulet. Department of Molecular and Cell Biology. U.C. Berkeley. Berkeley, CA-94720.

A variety of evidence indicate that MHC class-I molecules play a role in regulating natural killer cell activity. The finding that NK cells lyse syngeneic nontransformed lymphoblast target cells from class-I deficient mice indicated that recognition by NK cells does not require the presentation of foreign or tumor antigens and it might be thought that class-I deficiency by a cell is a sufficient condition for it to be lysed by NK cells. Here, we describe experiments to address whether class I deficiency in general, or the failure to express the specific inhibitory MHC alleles in the case of $Ly49^+$ NK cells, is a sufficient condition to render lymphoblast target cells or tumor cells sensitive to NK cell-mediated lysis. Our results suggest that other specific properties of the target cell are necessary to activate NK-mediated lysis. But all types of NK-mediated lysis, of lymphoblasts, of tumor cells and of almost any target by ADCC, can be inhibited by appropriate class I gene expression in the target cell. These results suggest a model in which lysis by NK cells must be triggered by any one of several distinct target cell ligands, but that all of these signals can be overruled by class I-mediated inhibition.

V 523 "MISSING SELF" RECOGNITION OF TUMOR CELLS BY NK CELLS FROM β_2-MICROGLOBULIN DEFICIENT MICE, Petter Höglund, Lars Franksson and Klas Kärre, Microbiology and Tumor Biology Center (MTC), Laboratory of Tumor Biology, Karolinska Institutet, Stockholm, Sweden.

Mice deficient in β_2m gene expression (β_2m -/-) have greatly reduced levels of MHC class I molecules and a compromised NK cell repertoire. In contrast to normal (i.e. β_2m +/- or β_2m +/+) mice, β_2m -/- mice are unable reject β_2m -/- and allogeneic bone marrow grafts. NK cells from β_2m deficient mice are also unable to kill β_2m -/- Con A blasts in vitro, although the latter are killed by NK cells from normal mice. This suggested that β_2m -/- NK cells are regulated to maintain nonresponsiveness toward their own self (or rather "missing self") phenotype. We show here that NK cells from β_2m -/- mice can distinguish between class I expressing and deficient cells in two lymphoma models: the TAP-2 mutant RMA-S (sensitive) and its control RMA (resistant), as well as the β_2m deficient $C4.4\text{-}25^-$ mutant (sensitive) and its β_2m expressing control EL-4 (resistant). One explanation to account for the sensitivity of β_2m deficient $C4.4\text{-}25^-$ cells versus the resistance of β_2m -/- Con A blasts is that the blasts but not the tumor cells express readily detectable levels of free MHC class I heavy chains at the cell surface. We propose that NK cells developing in β_2m deficient mice are calibrated to recognize such low levels of free class I heavy chains as a self-associated turn off signal. The reason that β_2m -/- tumor cells but not the β_2m -/- Con A blasts are killed would thus be because the tumor cells fail to express such free heavy chains at adequate levels.

V 524 ANTITHROMBOEMBOLIC GLUCOSAMINOGLYCANES DRAMATICALLY DEPRESS NATURAL KILLER CELL ACTIVITY *IN VITRO*.

Sabine Johann, Christine Zoller, Günther Blümel and Sylvia Haas, Institut für Experimentelle Chirurgie der TUM, München, FRG

Glucosaminoglycanes (GAGs) are used as protective drugs in humans against pre- and postoperative thromboembolic processes. Despite its common clinical use little is known about the influence of GAGs on human immune competent cells. The present study shows alteration of natural killer cell (NK) activity *in vitro* by four different GAGs: two naturally derived GAGs (unfractionated heparin, low molecular weight heparin), half synthetic GAG (Pentosan-Polysulphate, PPS) and one synthetic GAG.

Peripheral blood mononuclear cells (PBMC) of healthy donors were separated from whole blood and were cocultured for 4 hrs with K562 human erythroleukemic cells at target:effector ratios ranging from 1:6.25 to 1:25. GAGs were added at clinically relevant concentrations and natural killer cell activity was analysed.

PPS led to a significant decrease of NK-activity at all cell ratios and at all concentration tested. NK-activity was reduced to 72%, to 30% or to 10% of control at concentrations of 0.01, 0.1, and 1 mg/ml, respectively. Unfractionated heparin depressed NK-activity at concentrations used clinically for acute thromboembolic therapy whereas low molecular weight heparin and synthetic GAG reduced NK-activity at concentrations only reached in extra corporal circulations. Our results show that GAGs depress NK-activity *in vitro* at concentrations clinically used for thromboembolic prophylaxis and therapy.

Further studies must be done to investigate whether GAGs bind directly to NK cells or induce suppression via cytokine release. However, it seems that GAGs have profound influence on immune competent cells as further studies at our laboratory demonstrate that these substances also dramatically increase the oxidative burst in polymorphonuclear leukocytes.

V 525 GENERATION OF LAK CELLS IN RODENTS IS NITRIC OXIDE DEPENDENT,

Antonio Juretic, Giulio C. Spagnoli, Konrade von Bremen, Heidi Hörig, Luis Filgueira, Urs Lüscher, Reto Babst, Felix Harder and Michael Heberer, Departments of Surgery and Research, University of Basel, Basel, Switzerland.

The role of nitric oxide (NO) in the generation of lymphokine-activated killer (LAK) cells was investigated. We here report that L-arginine analog N^G-monomethyl-L-arginine (NMMA), a specific inhibitor of nitric oxide synthase (NOS), prevents LAK cell generation from cultured rat splenic cells. Accumulated NO endproduct nitrite (NO_2^-), as measured in the supernatants of rat splenic cells, correlated well with the generation of LAK cells. In contrast cell proliferation induced by rIL-2 or by Con A was not affected by NMMA. Similarly, phenotypic expression of CD25 in rIL-2 stimulated cultures was unaffected. LAK cell generation could no longer be blocked if NMMA was added to the rat cell cultures 24 hours after rIL-2 stimulation. To further confirm the role of NO in LAK cell generation, rat splenic cells were cultured in medium without L-arginine. Under such conditions rIL-2 could not induce LAK cell generation. Hemoglobin, which is a scavenger of NO, also inhibited LAK cell generation. Finally, addition of sodium nitroprusside (SNP) which releases NO in cultures was able to overcome blocking effects of NMMA. To attempt the identification of NO producing cells, lysosomotropic agent, L-leucine methyl ester (LME), was used. Generation of LAK cell activity was virtually abolished in cell cultures treated with LME. Addition of SNP to cultures however sufficed to restore LAK cell generation. These results suggest that LAK cell precursors depend on a exogenous NO supply from other cell types, in order to display their full cytotoxic potential. Similar results were also obtained by using mouse splenocytes as responder cells. In contrast NMMA did not affect generation of LAK cells from human peripheral blood or spleen mononuclear cells.

V 526 NATURAL KILLER CELLS MAY RECOGNIZE HLA-BOUND PEPTIDE AND HLA SURFACE RESIDUES.

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Natural killer (NK) lymphocytes do not rearrange or express T cell receptor or immunoglobulin genes and represent 5-15% of peripheral blood mononuclear cells (PBMC). NK cells kill allogeneic cells, tumor cells and some virally infected cells without prior immunization. NK-mediated killing is often inhibited by target cell HLA class I molecules. It has been proposed, that mutations in the HLA class I peptide binding groove can reverse HLA protection from NK-mediated killing. We introduced single point mutations in and around the HLA-B7 peptide binding groove. HLA-B7 variants are expressed in HLA class I negative 721.221 lymphoblasts at similar levels. These cells are used as targets for bulk PBMC NK cells, NK lines and NK clones, generated from 7 healthy human donors. Bulk PBMC show distinct patterns of killing which are largely independent of the donor HLA type. Seven of eleven peptide binding groove mutations allowed significantly increased NK mediated killing, as compared to the unmutated HLA-B7. Similiarly, four mutations at residues oriented towards the T cell receptor also allowed signigficantly increased NK mediated killing. These data suggest that peptide bound to HLA class I as well as surface residues are important for NK recognition. The recognition of the HLA class I by NK cells may be similar to Tcell recognition with regard to the importance of peptide binding groove and surface HLA class I residues. These patterns of recognition are not entirely reproduced by NK lines and clones from the same donors. Our data suggest that although the specificty of NK cells may be broad, clonal distribution of specificities may be selected for in vitro and may depend on the mode of stimulation. Additionally, certain specificities may predominate in bulk PBMC.

V 527 *AUGMENTATION OF NATURAL KILLER ACTIVITY FOLLOWING IN VITRO MYCOPLASMA PNEUMONIAE INFECTION,*

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NK activity of human peripheral blood mononuclear cells (MNC) was significantly increased within 18 hr of contact with clinical isolates of *M. pneumoniae*. As few as one colony forming unit (CFU) of *M. pneumoniae* per MNC caused a significant augmentation of NK activity. An initial rise in NK activity was observed after 5-6 hours of contact with the mycoplasmas, and optimal increased NK activity was obtained within 24 hours. Immunofluorescent analysis revealed that, after a minimum of four hours of incubation with MNC, *M. pneumoniae* colonies specifically bound to a subset of cells that were morphologically large and granular. The majority of these MNC were $CD14^+$. HLA-DR was also expressed on a significant portion of the mycoplasma-bound large, granular MNC. In contrast, very few *M. pneumoniae* organisms bound to any small, resting MNC. Mycoplasma-cleared supernates from *M. pneumoniae*-infected MNC cultures induced increased NK activity in cultures of fresh MNC to a level equivalent to that observed after physical contact of MNC with organisms. *M. pneumoniae* did not increase the NK activity of erythrocyte-rosette $(ER)^+$ cells (> 90% $CD3^+$ cells), but did induce increased NK activity in the ER^- cell population. Supernates from mycoplasma-infected, or non-infected, ER^+ cells induced only slight increases in basal NK activity of fresh MNC cultures. In contrast, supernate from mycoplasma-infected ER^- cells induced significantly higher NK activity of freshly isolated MNC than did supernate from non-infected ER^- cells. Supernates from mycoplasma-infected cultures of non-adherent MNC and adherent MNC both induced increased NK activity equally. Additionally, *M. pneumoniae*-treated cultures contained higher levels of TNF-α than did untreated cultures, as assessed by bioassay. However, recombinant human TNF-α did not effect a strong NK response in total human MNC cultures. In conclusion, *M. pneumoniae* binds to large, granular MNC and induces those cells to release a soluble factor(s) (i.e. cytokines). This factor then either acts directly on NK cells, or induces the production of other activating factors that result in increased NK activity.

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V 528 E1A ONCOGENE EFFECT ON EXPRESSION OF MHC CLASS-I AND OTHER PLASMA MEMBRANE PROTEINS: SUSCEPTIBILITY TO NK CELL CYTOTOXICITY. Q Liu, M.O Mondragon, S Tao, B Das, JL Cook and AJ Norin, Departments of Anatomy & Cell Biology and Medicine, SUNY Health Science Center, Brooklyn, NY 11203 and National Jewish Hospital Ctr. for Immunol. and Resp. Med. Denver, CO 80206

E1A oncogene transfection of NK resistant tumor cells can induce NK susceptibility (PNAS 88:6491, 1991). The observation of increasd NK susceptibility was reproduced in a human fibrosarcoma cell line HT 1080 (H4) and its E1A transfectant, p2AHT2a (P2) (Frisch et al, Oncogene 5:75, 1990). P2 could be lysed by naive human NK cells (28% cytotoxicity, E:T ratio 100:1) whereas parent H4 cells were resistant. In contrast both E1A and non- E1A transfected cells were highly susceptible to IL-2 activated killer cells (LAK), 77-85% cytotoxicity E:T ratio 100:1. To examine possible reasons for E1A induced NK-susceptibility cell surface molecules of both H4 and P2 were investigated. Flow cytometry analysis of MHC class I expression was determined using an antibody (W6/32) that reacts with a monomorphic determinant on HLA-A,B,C molecules. P2 expressed nearly twice as many class I molecules on their surface as H4 cells indicating increased NK susceptibility is not associated with decreased MHC class 1 expression in this system. This finding supports the suggestion of Litwin et al (J.Exp. Med. 178: 1321, 1993) that NK cells may possess cytotoxic mechanisms that are both MHC-dependent and MHC-independent. Additional studies were performed to compare cell surface molecules which bind to an NK cell line (NK 3.3). Surface biotinylated plasma membrane proteins of H4 and P2 were isolated, solubilized and reacted with NK 3.3. The coated cells were washed extensively to remove non-specifically bound proteins. SDS-PAGE and Western-blot analysis demonstrated an identical pattern of approximately 20 proteins that bound to NK 3.3. H4 proteins of about 80 KD and 100 KD bound to a greater extent on NK 3.3 as compared to proteins from P2. E1A-induced alterations in expression of these proteins may enhance NK susceptibility. Alternatively these differences may be inconsequential and increased NK susceptibility may result from intracellular changes due to the E1A oncogene.

V 529 PROTECTIVE ROLE OF MHC CLASS I ALLELES ON TARGET CELL RECOGNITION BY NK CLONES, Mauro S. Malnati, and Eric O. Long, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852.

The presence of class I MHC molecules at the surface of target cells has often been correlated with protection from lysis by NK effectors cells. We have studied the protective role of class I molecules by analyzing the recognition of several MHC class I transfectants (HLA-A2, A3, Aw68, B7 and B27) of the partially class I-negative cell line Hmy-C1R by a panel of 40 NK clones derived from 2 different individuals. Different specificities of lysis by NK clones were observed, in that some clones were not blocked by any class I allele expressed by the transfected cells, some clones were specifically blocked by a given class I allele, and other clones were blocked by several class I alleles. These results clearly demonstrate a clonal heterogeneity in the specificity of NK clones with regard to recognition of individual class I alleles. The potential role of peptides bound to class I molecules in the protection from NK lysis has been analyzed with synthetic peptides known to bind to particular class I alleles, and with target cells transfected with mutated class I molecules.

V 530 A NOVEL RECOGNITION STRUCTURE ON NK SUSCEPTIBLE TUMOR CELL LINES. M.O. Mondragon, B. Das, S. Tao, A.J. Norin, Departments of Medicine, Surgery and Anatomy & Cell Biology , SUNY Health Science Center of Brooklyn NY 11203

Despite progress in the identification of molecules involved in the interaction of cytolytic lymphocytes and tumor cells an NK specific recognition structure has not been established. To study target structures in NK mediated cytolysis, we examined membrane proteins from an NK susceptible cell line (K562) that bind to T lymphocytes and NK cells utilizing a direct adsorption method. Membrane proteins of surface biotinylated tumor cells were adsorbed to freshly isolated lymphocytes, solubilized and identified on western blots by streptavidin alkaline phosphatase reaction. Immunomagnetic bead fractionated CD 3^-16^+ cells bound a 38.5KD K562 protein while $CD5^+16^-$ did not adsorb this protein. Purified p38.5 membrane protein retained selective binding property to CD 3^-16^+ and exhibited concentration dependent inhibition of cytolysis of K562 cells. Further, purified p38.5 conjugated to sepharose 4B preferentially bound a 72 KD membrane protein from $CD3^-16^+$ cells (but not from $CD3^+16^-$cells). To determine the expression of p38.5 on various tumor cell lines we developed an affinity purified antibody obtained by repeated immunization of rabbits with wet gel slices of protein from preparative SDS PAGE. Anti-p38.5 reacted with the surface of NK susceptible cell lines K562, Molt-4, and Jurkat as compared to NK resistant cell lines Raji and A549 by flow cytometry and western blot analysis. These data suggest that p38.5 from the K562 plasma membrane preferentially binds to NK cell enriched preparations in contrast to T cell enriched lymphocyte fractions and is selectively expressed on the plasma membranes of NK susceptible tumors compared to NK resistant tumors. These properties are consistent with those expected for a NK cell specific tumor recognition structure, and the involvement of a unique pair of receptor-ligand proteins in NK cell mediated cytotoxicity.

V 531 REGULATION OF THE PUTATIVE MHC RECEPTOR LY-49 ON NATURAL KILLER CELLS OF H-2D^d EXPRESSING MICE, Mats Y. Olsson, Charles L. Sentman, Klas Kärre, Laboratory for Tumor Biology, Microbiology and Tumor Biology Center, Karolinska Institute, 171 77 Stockholm, Sweden

It has been proposed that natural killer (NK) cells receive inhibitory signals that prevent their lysis of normal cells after recognition of self-MHC class I molecules. The Ly-49 antigen is expressed on a subset of NK cells in mice. The lytic function of Ly-49+ NK cells from C57Bl/6 (H-2^b) mice has been shown to be inhibited by the expression of H-2 D^d on tumor cells. It has also been suggested that the Ly-49+ NK subset is deleted or not allowed to develop in mice expressing H-2 D^d. We have investigated the expression of Ly-49 on NK cells from inbred and transgenic mice which express H-2 D^d. Our data indicate the percentage of NK cells which express Ly-49 in these mice is similar to that of mice which do not express H-2 D^d, however, the NK cells express 30 to 50% less Ly-49 than C57Bl/6 mice. This reduced expression may be functionally important for adequate performance of Ly-49+ cells in an environment expressing H-2 D^d.

V 532 MICE DEFICIENT FOR THE 55-kDa RECEPTOR FOR TUMOR NECROSIS FACTOR ARE RESISTANT TO ENDOTOXIC SHOCK YET SUCCUMB TO *Listeria monocytogenes* INFECTION, Klaus Pfeffer, Toshifumi Matsuyama, Thomas M. Kündig, Andrew Wakeham, Kenji Kishihara, Arda Shahinian, Katja Wiegmann[&], Pamela S. Ohashi, Martin Krönke[&], and Tak W. Mak, the Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Princess Margaret Hospital, the Amgen Instiute, 500 Sherbourne Street, Toronto, Canada M4X 1K9, [&] and the Institute for Medical Microbiology and Hygiene, Technical University of Munich, Trogerstr. 4a, 8000 Munich 80, Federal Republic of Germany.

The multiple biological activities of tumor necrosis factor (TNF) are mediated by two distinct cell surface receptors of 55 kDa (TNFRp55) and 75 kDa (TNFRp75) apparent molecular mass. We have generated by gene targeting a mouse strain deficient for TNFRp55. Cells from TNFRp55-/- mutant mice lack expression of TNFRp55 but display normal numbers of high-affinity TNFRp75 molecules. Thymocyte development and lymphocyte populations are inconspicuous and clonal deletion of potentially self-reactive T cells is not impaired. However, TNF signaling is largely abolished as judged by the failure of TNF to induce the nuclear transcription factor κB (NF-κB) in T lymphocytes from TNFRp55-deficient mice. The loss of TNFRp55 function renders mice resistant to lethal dosages of either lipopolysaccharides (LPS) or Staphylococcus aureus enterotoxin B (SEB). In contrast, TNFRp55-deficient mice are severely impaired to clear *Listeria monocytogenes* and readily succumb to infection. Thus the 55-kDa TNF receptor plays a decisive role in host defense against micro-organisms and their pathogenic factors.

V 533 DIFFERENTIAL EFFECTS OF H-2 CLASS I TRANSGENES ON MURINE NK CELL REACTIVITY. Charles L. Sentman, Urban Lendahl*, and Klas Kärre. The Microbiology and Tumor Biology Center and the Center for Cell and Molecular Biology*, Karolinska Institutet, 171 77 Stockholm, SWEDEN.

The expression of an H-2D^d transgene in C57BL/6 mice (D8 mice) has been shown to alter the NK cell recognition of D8 bone marrow cells (BMC). To investigate the ability of other H-2D alleles to alter NK cell recognition of BMC, we have examined the effects of an H-2DP transgene in C57BL/6 (H-2^b) mice (B6DP mice) and an H-2D^b transgene in B10.D2 (H-2^d) mice (KL/B10D2 mice). Although the nontransgenic littermate was strongly resisted by NK cells from both B6D2F1 and D8 strains of mice, the H-2DP transgene provided complete protection from rejection in B6D2F1 mice and partial protection in D8 mice. The presence of H-2DP also resulted in strong resistance to the transgenic BMC by C57BL/6 mice. In contrast, the H-2D^b transgene did not alter the BMC rejectability or NK cell reactivity of B10.D2 mice. The KL/B10D2 mice were unable to mediate resistance to BMC from DBA/2 mice, and their BMC were no more resistant to rejection by B6D2F1, CB6F1, C57BL/6, or D8 mice than BMC from nontransgenic littermates. These data indicate that an H-2DP transgene can protect C57BL/6 BMC in a similar manner as an H-2D^d transgene and directly demonstrate the degeneracy of H-2D class I products to alter NK cell recognition. These data indicate that it is not sufficient for BMC merely to express all "self" H-2D alleles to provide protection from NK mediated BMC rejection, but they suggest that it is the expression of H-2 associated motifs or "NK-topes", which are influenced by H-2 class I expression, that determines resistance or susceptiblity of BMC to NK mediated killing.

V 534 DIFFERENTIAL REQUIREMENT OF PROTEIN KINASE C AND PROTEIN TYROSINE KINASE IN THE ACTIVATION OF IL-2-INDUCED AND αCD3-INDUCED ACTIVATED KILLER CELLS, Chou-Chik Ting, Myrthel E. Hargrove, and Jie Wang. National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

This study has examined the requirement of protein kinase C (PKC) and protein tyrosine kinase (PTK) in the generation of IL-2-induced LAK cells and αCD3-induced CD3-AK cells. For the generation of proliferative response, PTK but not PKC is required for both the LAK cells and CD3-AK cells. This is shown by the inhibition by PTK inhibitors herbimycin A or genistein of the proliferation of both LAK and CD3-AK cells, and PKC inhibitor calphostin had little effect on their proliferation. PTK inhibitors are found to inhibit the αCD3-induced IL-2 production and thus addition of exogenous IL-2 restores the CD3-AK proliferative response. For the generation of cytotoxic response, the results are quite different. Herbimycin A and genistein inhibit the generation of cytolytic LAK cells but calphostin had no effect on the LAK cytolytic activity. In contrast, PKC inhibitors staurosporine or calphostin abrogates the generation of cytolytic CD3-AK cells but herbimycin A or genistein have no effect on their cytolytic activity when exogenous IL-2 is supplemented. These findings indicate that PTK but not PKC is essential for the generation of cytolytic LAK, and the opposite is true for CD3-AK cells. The inhibitory effect of staurosporine or calphostin on the generation of CD3-AK cells is reversed by IL-4, indicating that IL-4 mediates an PKC independent pathway for CD3-AK generation. In tyrosine phosphorylation study, pretreatment of activated killer cells with genistein causes reduced phosphorlyation of 100-kDa protein which is enhanced by IL-2, and reduced phosphorylation of 40-kDa protein which is enhanced by αCD3 stimulation. Our results show that PTK is essential for the initial activation of both LAK and CD3-AK cells to induce proliferation. However, in the later stage of differentiaiton into killer cells, PTK is required for LAK cells but PKC is required for CD3-AK cells.

Biochemical Events in Lymphocyte Activation

V 535 INTERACTION OF HUMAN p56^{lck} SH2 DOMAIN WITH THE ZAP-70 TYROSINE KINASE, Oreste Acuto, Pascale Duplay, Margot Thome and Frédérique Hervé. Institut Pasteur, Laboratory of Molecular Immunology, Department of Immunology, 25 rue du Dr Roux, 75015 PARIS, France.

There is increasing evidence that the protein tyrosine kinase p56^{lck} plays a key role in signal transduction during T cell activation. p56^{lck} contains an SH_2 domain found in a number of proteins involved in intracellular signaling including the src family of tyrosine kinases. This domain mediates protein-protein interaction by specifically recognizing phosphorylated tyrosines in a particular sequence context on target proteins.

To evaluate further the function of p56^{lck} and, in particular, to identify its potential substrates, we used a recombinant p56^{lck} SH2 domain to analyze the SH2-binding proteins from lysates of Jurkat cells before and after activation. In non-activated Jurkat, we detected two tyrosine phosphorylated proteins which bound specifically to p56^{lck} SH2. Upon activation for 1-2 minutes with anti-CD3 mAbs, seven additional tyrosine phosphorylated SH2-binding proteins were found. We identified the ζ-associated tyrosine kinase ZAP-70 as one of the proteins capable of specifically binding to the p56^{lck} SH2 domain after CD3 stimulation. The significance of this interaction was further investigated *in vivo*. In agreement with the *in vitro* findings, p56^{lck} could be coprecipitated with the ζ/ZAP-70 complex and conversely, ZAP-70 was detected in p56^{lck} immunoprecipitates in activated Jurkat cells.

In addition to ZAP-70, another tyrosine phosphorylated protein of 74 kDa was found *in vivo* to co-precipitate with p56^{lck} following Jurkat activation. Studies are in progress to identify this p56^{lck} -associated protein.

V 536 EFFECT OF A DOMINANT-NEGATIVE *ras* TRANSGENE ON MAP KINASE ACTIVATION IN MOUSE THYMOCYTES.

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Stimulation of T cells through the T cell receptor induces rapid activation of p21*ras*, which is essential for lymphocyte activation. We have used transgenic mice expressing a dominant-negative *ras* (*ras*N17) under the control of the *lck* proximal promoter to examine the downstream biochemical events regulated by *ras* during T cell activation. Our results show that *ras*N17 completely abrogates MAPK and p90S6K activation induced with anti-CD3 mAbs. In contrast, MAPK activation induced with phorbol esters was not affected, either in SP or in total thymocytes, despite an over twenty fold overexpression of *ras*N17. Since phorbol esters are also able to activate *ras* in a PKC dependent way, we tested the effects of PKC inhibitors (staurosporine) on phorbol ester-induced MAPK activation. Surprisingly staurosporine was also unable to block phorbol ester induced MAPK activation at concentrations able to inhibit other PKC-mediated events. Therefore phorbol ester mediated activation of MAPK in thymocytes seems to be independent of PKC activation and not inhibitable by a dominant negative *ras*, indicating that there exists an alternative mechanism whereby phorbol esters can stimulate the MAP kinase cascade.

V 537 THE CD45 PHOSPHATASE REGULATES KINASE-MEDIATED TYROSINE PHOSPHORYLATION OF T-CELL ANTIGEN RECEPTOR COMPONENTS, Denis R. Alexander and Mark Biffen, T-cell Laboratory, Department of Immunology, AFRC Babraham Institute, Cambridge, CB2 4AT, U.K.

The T-cell antigen receptor complex (TCR) couples to intracellular signalling pathways as a result of increased phosphorylation in Tyr residues found in the Antigen Receptor Activation Motifs present in the TCR ζ, ε, δ and γ chains. The role of the CD45 phosphotyrosine phosphatase in regulating this process was investigated using a novel CD3+veCD4+veCD8+ve T-leukaemia cell-line (CB1) established from a patient with Acute Lymphoblastic Leukaemia. The primary transformed cells of this patient were found to comprise CD45-ve and CD45+ve cell populations and permanent cell-lines were established which maintained these two phenotypes. The CD45-ve and CD45+ve sub-clones were shown to be clonal in origin by analysing TCR-β chain rearrangements. A novel technique was established for measuring the activities of the specific pools of tyrosine kinase associated with CD4/CD8 or TCR molecules located at the plasma membrane. Using this technique it was shown that the CD4-p56-lck and TCR-associated tyrosine kinase activities were markedly increased in the CD45+ve cells compared to the CD45-ve cells. Analysis of the components phosphorylated in TCR immunoprecipitates from CD45+ve cells following *in vitro* kinase analysis revealed that the major substrates were the TCR ζ, ε and γ chains, which were phosphorylated in Tyr residues. No phosphorylation of these components was detected in TCR immunoprecipitates from CD45-ve cells. These results indicate that CD45 plays a critical role in regulating the Tyr phosphorylation of the TCR chains that couple the receptor to intracellular events.

Supported by the Leukaemia Research Fund.

V 538 ALTERED T CELL SIGNAL TRANSDUCTION IN RHEUMATOID ARTHRITIS. M.E.Allen, S.P.Young, R.H.Michell & P.A.Bacon. Dept of Rheumatology and Clinical Centre for Research in Immunology and Signalling, University of Birmingham, Birmingham. B15 2TT. U.K.

Synovial and peripheral blood (PB) T lymphocytes from rheumatoid arthritis (RA) patients show functional abnormalities which include reduced *in vitro* responses to recall antigens or mitogens, and depressed helper and suppressor function. These responses can be normalised by intense stimulation with a combination of phorbol ester and Ca^{2+} ionophore. This suggests an underlying signalling defect. Our initial spectroflurometric studies indicated a 5-fold diminution in the rate of calcium influx in PB mononuclear cells which prompted further investigation.

We compared intracellular calcium $[Ca^{2+}]_i$ changes in T cells, purified by negative selection from patients and healthy controls. Alterations in $[Ca^{2+}]_i$ in response to PHA-P and to cross-linked anti-CD3 were monitored by flow cytometry of indo-1 loaded cells. Rheumatoid T cells showed smaller $[Ca^{2+}]_i$ responses to both stimuli ($p < 0.005$). Since fluorescence changes induced by Ca^{2+} ionophore were similar in RA and control cells, this difference was not due to differential indo-1 loading.

All cell populations showed intercellular variation in the kinetics of the $[Ca^{2+}]_i$ response. The responsive rheumatoid cells responded less well than those in the normal cell samples, but the RA population may also have included a larger proportion of non-responding cells. $[Ca^{2+}]_i$ responses to anti-CD3 correlated with disease activity, as assessed by concurrent C-reactive protein levels. T cell proliferation to PHA-P or anti-CD3 was also impaired, with the lowest $[Ca^{2+}]_i$ responses often accompanying low proliferation. The RA T cells showed normal CD2 and CD3 expression, but an elevated proportion of DR +ve (i.e. pre-activated) cells ($p < 0.05$). The observed defect in signal transduction may contribute to the functional deficiencies described in RA.

V 539 CLASS I MOLECULES ARE IMPLICATED IN CO-SIGNAL TRANSMISSION DURING T CELL ACTIVATION, Nathalie Amirayan and Patrick Machy, Centre d'Immunologie de Marseille-Luminy, Case 906, 13288 Marseille CEDEX 9 FRANCE.

Mouse MHC class I-specific mAbs recognizing the $\alpha1/\alpha2$, but not those directed against the $\alpha3$ domain of the molecule, inhibited RNA, protein and DNA synthesis in response to stimulation via the TCR/CD3 complex. Similar inhibition was seen with LFA-1-specific mAbs under the same stimulation conditions. The effect of class I- and LFA-1-specific mAbs reflected a decrease both of IL-2 and IFN γ synthesis, and of expression of low affinity α chain IL-2 receptor. IL-2, α chain IL-2 receptor, IFN γ, c-*fos*, c-*jun*, and c-*myc* mRNAs were not transcribed. However, although bulk phosphorylation was inhibited, early tyrosine phosphorylation as well as calcium ion influx were normally induced. Protein phosphatase inhibitors did not reverse this inhibition, ruling out an enhanced activation of these enzymes for the inhibition observed. Cell surface expression of the early protein kinase C activation marker CD69 was also inhibited. Phorbol esters that directly activate protein kinase C prevented inhibition. Thus, like LFA-1 molecules, class I molecules are implicated in signal transduction involved at an early stage in T cell activation and contribute to the regulation of protein kinase C activity. Inhibition induced by class I- and LFA-1-specific mAbs was dependent on the stimulus that activated T cells: inhibition was observed when activation was induced by CD3-specific mAb in soluble but not in immobilized form, in primary but not secondary MLR or by mAbs directed against some epitopes of human CD2 (transgenic mice) or Thy-1 molecules. This suggested the existence of at least two activation pathways, one dependent on and another independent of a co-stimulatory signal implicating class I molecules. In agreement with this, class I-negative T cells from β_2 microglobulin knock-out mice were able to be stimulated, although to a lesser extent than class I-positive T cells. This stimulation was inhibited by LFA-1- but not class I-specific mAbs. Thus, in the same way that the LFA-1 molecule binds to ICAM-1 and -2 ligands, we propose that in normal situation, class I molecules could bind to a ligand other than CD8 since stimulation of both CD4+ and CD8+ T cells was inhibited and because class I $\alpha3$ domain-specific mAbs did not inhibit activation. Experiments in progress are designed to establish whether or not class I molecules would be part of either the TCR/CD3 or the CD28/B7-BB1 activation modules or of an independent one.

V 540 RECONSTITUTION OF TCR RESPONSIVENESS IN p59*fyn*^{null} THYMOCYTES. Mark W. Appleby., Hai U. Wang., Katherine A. Forbush and Roger M. Perlmutter. Department of Immunology and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195.

Engagement of the T cell receptor for antigen initiates a number of cellular responses including mitogenesis, the production of lymphokines and the execution of cytolytic function. The mechanisms whereby these events are mediated are enigmatic. However the observation that they are preceded by the phosphorylation of a number of cellular proteins upon tyrosine has implicated tyrosine kinases in these processes. One member of the *src* family of tyrosine kinases in particular, p59^{fyn}, appears crucial for satisfactory signaling from the TCR. We have previously described the generation of transgenic animals overexpressing p59^{fyn} and of *fyn*^{null} mice, produced by gene targeting. These animal models have permitted a partial dissection of TCR signaling processes. The overexpression of p59^{fyn} under the control of the *lck* proximal promoter results in the production of thymocytes that are acutely sensitive to stimulation through the TCR. In comparison, thymocytes from mice which lack p59^{fyn} as a consequence of gene targeting are refractile to TCR stimulation. The availability of embryos derived from *fyn*^{null} mice has permitted examination of the functions of the discrete domains within p59^{fyn}. Using a direct reconstitution approach, these studies demonstrate that features of both the kinase catalytic domain and of other structures are required to confer upon p59^{fyn} its unique function in thymocyte signaling.

V 541 Thy-1, CD26, CD45, p56^{lck}, AND p59^{fyn} FORM MULTIMERIC COMPLEXES IN THE MEMBRANE OF A T LYMPHOMA CELL.

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Thy-1 and CD26 surface glycoproteins are both capable of delivering proliferative signals to T cells upon cross-linking with appropriate antibodies. The tyrosine phosphatase CD45 is a key regulator of T cell proliferation as it controls the activity of *src* family kinases p56^{lck} and p59^{fyn}. Associations of Thy-1 with CD45, and of CD26 with CD45 have been reported after chemical cross-linking of intact cells and it is likely that such associations constitute part of the structural basis for the T-cell stimulating capacity of Thy-1 and CD26 accessory molecules.

We have developed an extraction protocol to isolate multimolecular complexes of membrane proteins by centrifugation through a sucrose gradient containing TX-100 and define the nature of molecules associated with immunoprecipitated surface glycoproteins.

Thy-1, CD26 and CD45 are specifically cross-linked at the cell-surface and form multimolecular complexes with a 78 kDa protein that is recognized by an anti-BiP (Grp78) antibody. The 78 kDa protein is labelled with ^{125}I as well as biotin esters and forms oligomers in the plasma membrane.

In vitro kinase assays on anti-Thy-1 and anti-CD45 immunoprecipitated molecules show the presence of kinase activity due to p56^{lck} and p59^{fyn}. The two *src* family kinases are selectively associated with Thy-1 and CD45 contained in multimolecular complexes.

The multimolecular structure we describe could represent a transducing unit that incorporates surface receptors and regulators of tyrosine phosphorylation that are stabilized through interactions with an oligomeric scaffold of membrane proteins.

V 542 RECONSTITUTION OF THE FUNCTIONAL IL-2 RECEPTOR COMPLEX: INVOLVEMENT OF THE IL-2 RECEPTOR γ CHAIN IN SIGNAL TRANSDUCTION

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Interleukin 2 (IL-2) receptor γ chain is indispensable for the formation of the receptor complexes with high- and intermediate-affinities to IL-2. The present study demonstrated that the $\alpha\beta\gamma$ heterotrimer complexes of IL-2 receptor reconstituted on human T cell line have the ability to transduce IL-2-mediated signals for activation of tyrosine kinase, for induction of c-myc, c-fos and c-jun expression and for ^{3}H-thymidine uptake. A mutant of the γ chain which has deletion of carboxy terminal 68 amino acid residues showed loss of such signal transducing ability of the IL-2 receptor complexes. Another mutant carrying carboxy terminal 30 amino acids deletion retained the ability to activate a tyrosine kinase, to induce c-myc expression and to promote ^{3}H-thymidine uptake, but lost the ability to induce c-fos and c-jun expression. Moreover, the mutant with src homology 2 (SH2) subdomain deletion fails to induce such signal transduction. On the other hand, a mutant constructed by substitution of the SH2 like subdomains for the equivalent SH2 subdomains of p56^{lck} showed the uptake of ^{3}H-thymidine in an IL-2 dose dependent manner. These results suggest that at least two distinct signals, one for c-myc induction, which parallels tyrosine kinase activation, and the other for c-fos and c-jun induction can be transduced from the IL-2 receptor complexes; in particular the SH2 subdomains should have a pivotal role for signal transduction from IL-2 receptor complex.

V 543 UTILIZATION OF PMA AS AN INTRACELLULAR ACTIVATION PROBE FOR PRIMED HUMAN T LYMPHOCYTES. Bruce Babbitt, Joseph Goodwin, Jenny Zhang and Barry Caplan. Cellcor, Inc., Newton, MA 02159.

An in vitro assay that measures the activation level of ex-vivo activated (EVA) T cells currently being used in the adoptive immunotherapy of metastatic renal cell carcinoma has been developed. This assay is based on the ability of activated, but not resting, T cells to proliferate and produce cytokines in response to the protein kinase C activator phorbol myristate acetate (PMA). Although phorbol esters have previously been used with mitogens or calcium ionophores to costimulate resting T lymphocytes, we have instead utilized PMA by itself as an "intracellular activation probe" to quantitate the functional activation level of previously activated or "primed" cells. EVA cells, generated by stimulating human PBMC with OKT3 and autologous cytokines, had greatly enhanced capacity to proliferate and produce cytokines in response to further in vitro stimulation with 1 ng/ml PMA. Intrapatient, but not interpatient, there was a linear correlation between the magnitude of the PMA response and the degree of activation as measured by the percentage of cells that expressed CD25. The EVA cells proliferating in response to PMA were predominantly polyclonally activated T lymphocytes including both $CD4^+$ and $CD8^+$ T cell subsets as well as both $CD45RO^+$ "memory" and $CD45RA^+$ "naive" T cells. The enhanced responsiveness of EVA cells to PMA was inhibited by culturing the cells in the presence of staurosporin, a protein kinase C inhibitor. PMA responsiveness was maintained in cells that had been frozen and stored for greater than 6 months in liquid nitrogen suggesting that distinct and stable biochemical changes had occurred in the cells as a result of their prior activation. These results suggest that EVA cells may possess lowered activation thresholds in vivo as a result of their effective priming in vitro and that the degree of activation can be quantitated by responsiveness to PMA.

V 544 Chimeric TCRs with Altered Signalling Properties. B. Thomas Bäckström, B. Jaureguiberry, V. Appel, Georg Tiefenthaler, and Ed Palmer. The Basel Institute for Immunology, CH-4005 Basel, Switzerland.

The TCR is constituted of either α/β or γ/δ disulphide-linked heterodimers which are non-covalently associated with the γ, δ and ε subunits of the CD3 complex and the unrelated ζ chain. These TCR-associated polypeptides play an important role in both TCR cell-surface expression and transmembrane signalling after binding of the TCR with its ligand.

In order to generate TCRs with altered signalling properties, we constructed chimeric TCR α chains by replacing the transmembrane and cytoplasmic domains of Cα with homologous sequences from the TCR δ chain. Similar chimeras were made for the TCR β chain using donor sequences from the Cγ. The chimeric TCR chains were introduced into a TCR-negative T cell hybridoma and analysed for responsiveness to allo-antigen (I-A^{bm12}), superantigen (SEB), and to anti-TCR mAbs. A single combination of chimeric TCR chains produced a TCR which responded normally to anti-TCR antibodies but was markedly deficient in its response to allo and superantigens. The response of this chimeric TCR to superantigen could be restored by increasing the amount of SEB presented by >1000 fold. Thus, we have generated a mutant TCR with altered recognition or signal transduction properties.

V 545 INVESTIGATION OF THE MECHANISM OF ANALOG PEPTIDE ANTAGONISM OF T CELL PROLIFERATION IN A CLONE SPECIFIC FOR AN Eα PEPTIDE PRESENTED BY I-A^b. Avlin Barlow and Charles Janeway, Jr., Section of Immunobiology, Yale University, New Haven, CT 06510

A phenomenon has been described in which analog peptides, created by amino acid substitutions in antigenic peptides, antagonize the response of a T cell clone to its antigenic peptide. This antigen specific antagonism is believed to be mediated through interaction with the T cell receptor rather than competition for binding to the MHC molecule. Evavold et al. (Science,1991,252:1308) showed that analog peptides inhibit proliferation, but not cytokine production suggesting inadequate signalling through the T cell receptor. De Magistris et.al. (Cell, 1992, 68:625) found that analog peptides inhibit proliferation, IL-2 production, and early T cell activation events suggesting a lack of signalling through the T cell receptor.

We have found two analog peptides that inhibit the proliferation of an Eα peptide specific T cell clone. In preliminary experiments, coincubation with anti-CD28 monoclonal antibody (mAb), previously shown to induce costimulation, prevents this inhibition of proliferation. Furthermore, the agonist peptide, at high concentrations, inhibits proliferation of the T cell clone. Again, anti-CD28 mAb prevented inhibition of proliferation normally induced by high concentrations of the antigenic peptide. One possible explanation is that high doses of peptide stimulate killing of the antigen presenting cells before they can provide adequate costimulation needed to induce proliferation of the T cells. Anti-CD28 mAb may provide this costimulation, thus restoring the proliferative response. We are also investigating two possible mechanisms of the inhibition induced by the analog peptides. The analog peptides may 1) stimulate T cell killing of the antigen presenting cells and/or 2) aberrantly signal through the T cell receptor.

V 546 ANALYSIS OF THE MURINE T CELL SPECIFIC TYROSINE KINASE, TSK, Leslie J. Berg, Stephanie D. Heyeck, Christine Gurniak and Stephen Bunnell, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Using a PCR approach based on degenerate oligonucleotides to conserved regions of all tyrosine kinases, we have cloned novel tyrosine kinases from neonatal mouse thymoyctes. One of these clones, TSK (T cell-specific kinase) is T cell specific, and is expressed throughout T cell ontogeny starting as early as fetal day 14 thymocytes. This gene is also expressed at 10-20-fold higher levels in thymocytes than in resting peripheral T cells. TSK is a member of a new family of tyrosine kinases which includes the B cell kinase, BTK, found to be the gene deficient in the immune deficiency disesases X-linked agammaglobulinaemia (XLA) in humans and *xid* in mice. In XLA patients completely lacking BTK activity, B cell development is arrested at the pre-B stage in the bone marrow. Interestingly, TSK and BTK are about 60% identical in amino acid sequence, and in particular, share very strong homology in the amino terminal pleckstrin homology region found to be the site of the *xid* mutation. Together with the expression data, the homology to BTK strongly suggests a role for TSK in T cell development. To test this, we are currently generating a knockout of the TSK gene in mice. In addition, together with Nancy Jenkins at NCI/Frederick and David Housman at MIT, we have mapped the mouse TSK gene to chromosome 11, closely linked to a cluster of lymphokine genes, and the human gene to chromosome 5q.

V 547 CELL CYCLE-SPECIFIC INDUCTION OF Cdk2 EXPRESSION IN B LYMPHOCYTES STIMULATED THROUGH THE ANTIGEN RECEPTOR, Thomas C. Chiles, Department of Biology, Boston College, Chestnut Hill, MA 02167

Cross-linking of the B cell antigen receptor complex (BCR) by mitogenic amounts of anti-IgM leads to cell cycle progression and mitosis. In order to understand the molecular mechanisms underlying BCR-dependent cell cycle entry, the role of specific cell cycle regulatory proteins were investigated. The level of cyclin-dependent kinase 2, Cdk2, expression was found to be restricted to specific cell cycle phases in primary murine B cells insomuch that G_0 or G_1 phase B cells, or cells arrested at or near the G_1/S boundary contained no detectable Cdk2 or associated histone H1 kinase activity. In contrast, B cell entry and progression through S phase was accompanied by the *de novo* synthesis of Cdk2. Cyclin A was found to associate in a cell cycle-dependent manner with Cdk2 and resulted in the catalytic activation of Cdk2-specific histone H1 kinase activity during S phase. These results suggest that, in addition to cyclin-dependent regulation of Cdk2, an additional level of regulation exists in primary B cells that tightly couples the availability of Cdk2 to S phase. Further, the identification of active Cdk2 suggests that the BCR may stimulate S phase progression by assembling active Cdk2/cyclin A complexes. Present studies are directed at ascertaining whether Cdk2 functions to stimulate entry into- and/or maintain S phase progression.

V 548 TYROSINE KINASE REGULATES CA^{2+} CURRENT ACTIVATION IN JURKAT T CELLS. S. Clare Chung, and Phyllis Gardner. Stanford University, Stanford, CA 94305.

During T cell activation, an increase in $[Ca^{2+}]i$ is achieved by the release of intracellular Ca^{2+} stores and by activation of Ca^{2+} channels in the plasma membrane. Ca^{2+} channels appear to open as a result of emptying of Ca^{2+} stores, but the biochemical mechanism is unknown. To investigate the role of tyrosine kinase(s) in Ca^{2+} current activation, we performed Indo-1 fluorescence and whole-cell patch clamp studies on Jurkat cells transfected with human muscarinic receptor1(HM1). Cells were treated with one of three agents, anti-CD3 antibody (UCHT-1), carbachol (CCh), or thapsigargin (TG) (a Ca^{2+} ATPase inhibitor). Each agent depletes intracellular stores and activates Ca^{2+} current (McDonald et al, J. Biol.Chem. 268:3889,1993, Zweifach and Lewis, Proc. Natl. Acad. Sci. 90:6295, 1993). After stimulation of cells with UCHT-1, TG, or CCh, we observed by means of Indo-1 fluorescence a biphasic Ca^{2+} response, with an initial peak representing stores release followed by a sustained plateau representing Ca^{2+} influx. Indo-1 loaded Jurkat T cells pretreated with genestein (25μM) had no influx or sustained plateau. The whole-cell patch clamp recordings also showed that pretreatment with genestein abrogated Ca^{2+} current activation by UCHT-1, TG, or CCh. Furthermore, Ca^{2+} current previously activated by UCHT-1, TG, or CCh was inhibited by Lavendustin A (20μM), a structurally dissimilar tyrosine kinase inhibitor over a ten minute period (CCh: 92% ± 5.7, mean ± S.D., n=6; TG: 60% ± 6.8, n=8; UCHT-1: 95% ± 3.0, n=7). By contrast, Lavendustin B (20μM), an inactive structural analog of Lavendustin A, did not inhibit previously activated Ca^{2+} current. Redox reagents including diamide (thiol-oxidizing reagent) and N-ethylmaleimide (sulfhydryl reagent), as well as the tyrosine phosphatase inhibitor orthovanadate, increase tyrosine kinase phosphorylation in lymphocytes. We found that these reagents activated Ca^{2+} current in whole-cell patch clamp recordings. Thus, tyrosine kinase(s) may play a role in Ca^{2+} current activation.

V 549 IMMUNOGLOBULIN SIGNAL TRANSDUCTION GUIDES B CELL-T CELL INTERACTIONS AND IS BLOCKED IN TOLERANT SELF-REACTIVE B CELLS

Michael P. Cooke*, Andrew W. Heath!, Kevan M. Shokat*, Yongjun Zeng*, Maureen Howard! and Christopher C. Goodnow*
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The specificity of antibody responses depends on focusing helper T lymphocyte signals to suitable B lymphocytes capable of binding foreign antigens, and away from nonspecific or self-reactive B cells. To investigate the molecular mechanisms which prevent the activation of self-reactive B lymphocytes, the activation requirements of B cells specific for the antigen hen egg lysozyme (HEL) obtained from immunoglobulin (Ig) transgenic mice were compared with those of functionally tolerant B cells isolated from Ig transgenic mice which also express soluble HEL. Nontolerant Ig-transgenic B cells required both allogeneic helper T cells and binding of soluble HEL for efficient activation and antibody production. By contrast, tolerant self-reactive B cells responded poorly to the same combination of allogeneic T cells and soluble HEL. Tolerant B cells responded normally to stimulation with IL-4 and anti-CD40 antibodies in vitro, suggesting that they retained the capacity to respond to mediators of T cell help. However, the tolerant B cells exhibited a proximal block in the sIg signaling pathway which prevented activation of receptor-associated tyrosine kinases in response to the binding of soluble HEL. The functional significance of this sIg signaling defect was confirmed by using a more potent membrane-bound form of HEL capable of triggering sIg signaling in tolerant B cells, which markedly restored their ability to collaborate with allogeneic helper T cells and produce antibody. These findings indicate that antigen-specific B cells require two signals for mounting a T cell dependent antibody response and identify regulation of sIg signaling as a mechanism for controlling self-reactive B cells.

V 550 RAPID ACTIVATION OF $p72^{syk}$ IN T LYMPHOCYTES

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Activation of resting T lymphocytes by ligands to the T cell antigen receptor/CD3 complex is initiated by rapid tyrosine phosphorylation of cellular proteins. Protein tyrosine kinases (PTKs) of the *src* family are known to be important, but the mechanism of their recruitment and their interactions with PTKs of other families are incompletely understood. We have found that a member of another family of PTKs, the $p72^{syk}$ kinase, is constitutively bound to the TCR/CD3 complex and becomes tyrosine phosphorylated and activated within 1 min after TCR/CD3 stimulation. This activation did not depend on the presence of $p56^{lck}$ in T cells and in transfected COS cells $p72^{syk}$ was active and tyrosine phosphorylated in the absence of co-transfected *src*-family PTKs. In both cases, however, the phosphorylation of cellular substrates was augmented by *src*-family PTKs. We propose that $p72^{syk}$ may act as an immediate receptor-activated kinase up-stream of the related $p70^{zap}$ PTK and the *src*-family PTKs $p56^{lck}$ and $p59^{fyn}$ in T cells, and that these *src*-family PTKs act as signal amplifiers. This notion is supported by our findings that $p56^{lck}$ binds, via its SH2 domain, to phosphorylated $p72^{syk}$ and that $p72^{syk}$ phosphorylated $p56^{lck}$ at Tyr-192 in the SH2 domain and activated $p56^{lck}$.

V 551 T CELL $p56^{lck}$ AND $p59^{fyn}$ ARE DOWNREGULATED IN GRAFT-VERSUS-HOST REACTION-INDUCED ANERGY, Julie Desbarats*, André Veillette§, Marielle Fournel§, and Wayne S. Lapp*, Department of Physiology* and the McGill Cancer Center§, McGill University, Montreal, QC. Canada H3G 1Y6.

The graft-versus-host reaction (GVHR) results in profound, long-lasting immunosuppression characterized by T cell unresponsiveness to antigenic and mitogenic stimuli. We have investigated the roles of the protein tyrosine kinases (PTKs) $p56^{lck}$ and $p59^{fyn}$ in GVHR-induced T cell anergy. GVHR was induced by the transfer of parental lymphoid cells into F1 hybrid recipient mice. The recipient mice were examined in the early acute and late chronic phases of the disease to determine spleen and lymph node T cell function, phenotype, and expression of detergent soluble PTKs involved in T cell activation. Early in the reaction, mild increases in splenic $p56^{lck}$ and $p59^{fyn}$ were observed, perhaps as a consequence of the massive anti-host lymphoproliferation; however, the levels of both PTKs detected by immunoblotting declined dramatically as the reaction progressed and a profound immunosuppression developed. In long-term chronic GVHR $p56^{lck}$ and $p59^{fyn}$ were barely detectable in the anergic splenic T cells. In contrast, no changes were observed in $p56^{lck}$ and $p59^{fyn}$ expression in mature thymocytes, suggesting that the peripheral PTK downregulation was a post-thymic event. No decreases were seen in cell surface levels of CD3/TCR, CD4 and CD8 concurrently with the decrease in $p56^{lck}$ and $p59^{fyn}$ in the spleens and lymph nodes of GVH-reactive mice. The downregulation in membrane-associated PTKs normally required to amplify T cell activation signals may account for the defective function of the T cells in the periphery of GVH-reactive mice.

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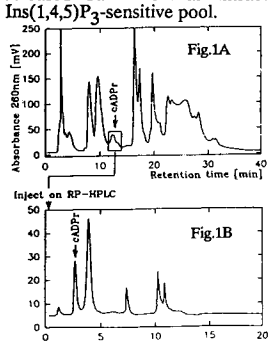
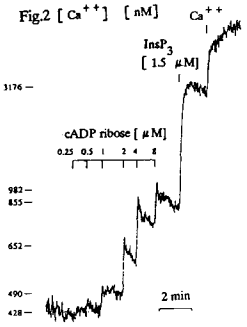
V 552 CYCLIC ADP-RIBOSE: PRESENCE AND CA^{++}-RELEASE ACTIVITY IN JURKAT T-LYMPHOCYTES,

Frank Emmrich, Cristina P. da Silva*, Edith Roth and Andreas H. Guse, Max-Planck-Society, Schwabachanlage 10, D-91054 Erlangen, Germany; *Center for Cell Biology and Dept. of Biochemistry, University of Coimbra, Portugal

Cyclic ADP-ribose (cADPr) is formed by a specific cyclase from NAD^+ and has been shown to release Ca^{++} from sea urchin egg microsomes, probably by interaction with the ryanodine receptor. We were able to detect cADPr in neutralized perchloric acid extracts from human T-lymphocytes (Jurkat cell line) by a newly developed 2-step HPLC procedure. In the first step SAX-HPLC employing a TFA gradient was used (Fig.1A). Material co-eluting with standard cADPr was collected and further chromatographed on reverse-phase/ion-pair HPLC (Fig.1B). A single peak co-eluting with standard cADPr in this system was collected and analyzed for functional identitity in Ca^{++}-release experiments. Standard cADPr or material purified as described above induced a dose-dependent Ca^{++}-release (Fig.2) in permeabilized Jurkat T cells in the presence of Fura2/free acid. Therefore, cADPr appears to be a novel, potent intracellular messenger involved in the regulation of the free cytosolic Ca^{++}-concentration in human T-lymphocytes since cADPr (i) was present in these cells and (ii) released Ca^{++} from an intracellular store different from the $Ins(1,4,5)P_3$-sensitive pool.





V 553 CHARACTERIZATION OF THE LYMPHOCYTE-SPECIFIC G-PROTEIN-COUPLED RECEPTOR BLR1 BY FLOW CYTOMETRY AND HETEROLOGOUS HIGH LEVEL EXPRESSION

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The Burkitt's lymphoma receptor 1 (BLR1) originally identified in Burkitt's lymphoma cell lines by subtractive hybridization has all structural features common to the superfamily of G-protein-coupled receptors, including seven hydrophobic putative membrane spanning domains. It is the first member of this receptor family expressed in lymphocytes showing a differentiation-specific expression pattern; BLR1 is expressed in mature B cells but not in pre B-cells or plasma cells.

To further characterize structure and function of the receptor protein BLR1 was epitope-tagged and expressed in human embryonic kidney 293 cells. Flow cytometry was used to determine the efficiency of transient transfections and to establish 293 cell clones showing stable high level expression of BLR1. Analysis of permeabilized versus non-permeabilized 293 cells transfected either with an amino-terminally or a carboxyl-terminally tagged receptor demonstrated that BLR1 is an integral plasma membrane protein, topologically orientated therein as predicted for other members of this class of seven pass membrane receptors.

By the use of stably BLR1 expressing cell lines we have generated a monoclonal antibody against BLR1 which recognizes a protein of about 60 kDa on transfected 293 cells. FACS analysis of leukocytes revealed that BLR1 is expressed on recirculating mature B cells and additionally on a certain subpopulation of T cells.

BLR1 shows significant homologies to receptors with peptide ligands including those for chemokines (IL-8; MIP-1α) and neuropeptides (Angiotensin II). The heterologous overexpression of BLR1 in 293 cells and its easily assayable expression by the use of mAbs provides a suitable system to investigate the binding affinities of potential peptide ligands to BLR1 and offers an efficient screening tool for the identification of unknown ligands and receptor-associated proteins.

V 554 HERPESVIRAL TRANSFORMED HUMAN T LYMPHOCYTES EXPRESS THE VIRAL ONCOGENE AND A VIRAL lck ASSOCIATED PROTEIN, BOTH REGULATED LIKE T CELL ACTIVATION GENES, Helmut Fickenscher°, Brigitte Biesinger°, Barbara Bröker§*, Alexander Tsygankov+, Frank Emmrich§, Joseph Bolen+, and Bernhard Fleckenstein°, °Institut für Klinische und Molekulare Virologie, Loschgestr. 7, D-91054 Erlangen, Germany; §Max-Planck-Gesellschaft, Klinische Arbeitsgruppen für Immunologie, Schwabachanlage 10, D-91054 Erlangen, Germany; *present address: Bernhard-Nocht-Institut für Tropenmedizin, D-20359 Hamburg, Germany; +Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton NJ 08543.

Herpesvirus saimiri, a T cell tumor virus of New World monkeys, transforms human T lymphocytes to permanent IL-2 dependent growth, without further need for antigen or mitogen. The immortalized cells do not shed virus particles and resemble activated, mature human T cells in many respects. For example, the transformed cells retain patterns of early signal transduction and the antigen specificities of parental clones. Whereas many viral genes remain silent in the growth transformed human T cells, a 1.7 kb transcript from the left terminus of the episomally persisting genome is transcribed predominantly. This viral transcript encodes the oncoprotein STP-C and a 40 kD protein that associates with the tyrosine protein kinase lck. Upon unspecific stimulation by the phorbolester TPA, the transcript is quickly and heavily induced. As the induction is not suppressed, but even increased by cycloheximide, this type of regulation resembles that of early lymphocyte activation genes. STP-C protein levels reflect the transcriptional induction. stp-C promoter regulatory sequences show a T cell specific activity of reporter genes in transient experiments. The regulated expression of the viral genes might contribute to the T cell tropism of Herpesvirus saimiri transformation and lead to the activated phenotype of the immortalized human T cells.

V 555 **INTERLEUKIN-2 (IL-2) SIGNAL TRANSDUCTION IN THE ABSENCE OF PROTEIN TYROSINE KINASE $p59^{fyn}$.** **Patrick E. Fields, Dapeng Qian, David W. Lancki, Thomas Gajewski, and Frank W. Fitch. Ben May Institute, University of Chicago, Chicago, Il. 60637.** **Upon interaction of the IL-2 receptor with its ligand, there is rapid phosphorylation of several protein substrates on tyrosine residues. Although the identity of the responsible kinase(s) is currently unknown, protein tyrosine kinase $p59^{fyn}$ has been implicated in signal transduction through the IL-2 receptor in lymphoid cells. In this study we examine IL-2 signal transduction in a panel of T cell clones lacking normal expression of $p59^{fyn}$. These OVA-specific clones that we derived from fyn $^{-/-}$ mutant mice (kindly provided by Drs. P. Stein and P. Soriano) were shown to proliferate and produce normal levels of lymphokine in response to antigen (Qian, et al., these proceedings). Furthermore, we found that these cells proliferated in response to IL-2 comparably to the wild type clones, suggesting that Fyn is not required for normal functional responsiveness to IL-2 in T cells. When IL-2-induced tyrosine phosphorylation patterns were assessed, we found no significant differences from wild type clones. Similarly, IL-2-induced tyrosine phosphorylation of Shc and IL-2 receptor β were found to be no different from wild type. Thus, $p59^{fyn}$ is not required for phosphorylation of these substrates in response to IL-2 stimulation. Tyrosine phosphorylation of other substrates is currently being studied and the results will be presented.**

V 556 T CELL RECEPTOR-ζ CHAIN ASSOCIATES WITH THE CYTOSKELETON UPON ACTIVATION OF DEVELOPMENTALLY MATURE T LYMPHOCYTES. Terri Helman Finkel and Moshe M. Rozdzial, National Jewish Center for Immunology and Respiratory Medicine, Dept. of Pediatrics, 1400 Jackson St., Denver, CO 80206.

The events that follow T cell receptor (TCR) engagement of antigen and couple the transmission of the activation signal from the cell surface, leading to cell proliferation and lymphokine secretion, remain unknown. The cytoskeleton, a complex network of various filamentous systems, may, by virtue of acting as a physical linker between the plasma membrane and the nucleus, be the underlying substrate which connects the early and late events of cellular activation. Indeed, our preliminary evidence shows that as a consequence of TCR ligation, developmentally mature T cells translocate TCR-ζ chain to the detergent insoluble cell fraction, the cell fraction containing the cytoskeleton. This association appears to be specific, as other components of the TCR, such as the αβTCR chains, are not involved. In immature murine T cells, which are unable to respond to TCR stimulation by dividing and producing lymphokines, the translocation of TCR-ζ with the cytoskeleton is abrogated, suggesting that the cytoskeletal interaction is developmentally regulated and correlated with the ability of T cells to respond to activation. Because tyrosine phosphorylated TCR-ζ is a predominant form of the ζ chain associated with the cytoskeleton, phosphorylation of TCR-ζ by a specific tyrosine kinase may be the regulatory mechanism by which TCR-ζ becomes cytoskeleton-associated after cell activation. Using cell lines with engineered TCR-ζ chains, we have attempted to define the molecular site involved in the cytoskeletal interaction. Preliminary evidence suggests that at least one of the ARH-1 signaling motifs is necessary for this cytoskeletal interaction. Standard methods of immunocytochemistry and biochemistry are currently being used to identify the cytoskeletal protein(s) that mediates TCR-ζ translocation. Biochemical reconstitution of this TCR-ζ-cytoskeleton interaction, in vitro, will then be performed to dissect the processes involved in TCR-ζ-cytoskeleton interaction and consequently the activation cascade leading to cellular differentiation.

V 557 DUAL ROLE OF THE TYROSINE ACTIVATION MOTIF OF THE Ig-α PROTEIN DURING SIGNAL TRANSDUCTION VIA THE B CELL ANTIGEN RECEPTOR,
Heinrich Flaswinkel and Michael Reth, Max-Planck-Institut für Immunbiologie Stübeweg 51, 79108 Freiburg, Germany

The B cell antigen receptor (BCR) is a multimeric protein complex consisting of the ligand binding immunoglobulin molecule and the Ig-α/β heterodimer that mediates intracellular signalling by coupling the receptor to protein tyrosine kinases (PTK). Transfection of the Ig-α deficient myeloma cell line J558Lμm with expression vectors coding for mutated Ig-α allowed us to test the function of the tyrosines in the cytoplasmic region of Ig-α in the context of the BCR. Furthermore we expressed Ig-α mutations as chimeric CD8/Ig-α molecules on K46 B lymphoma cells and tested their signalling capacity in terms of PTK activation and release of calcium. We show here that the conserved tyrosine residues in the cytoplasmic portion of Ig-α have a dual role. Firstly they are required for efficient activation of PTKs during signal induction and secondly one of them is subject to phosphorylation by activated src-related PTKs. We present data suggesting that Ig-α and Ig-β interact with each other and that Ig-β can restore activity of mutated Ig-α. Phosphorylation on tyrosine in the cytoplasmic portion of Ig-α may be a mechanism to couple the BCR to SH2 domain-carrying molecules.

V 558 ADMINISTRATION OF BIOLOGICAL RESPONSE MODIFIERS (BRM) INDUCES TRANSIENT T-CELL SIGNAL TRANSDUCTION MOLECULE ALTERATIONS IN NORMAL MICE, José L. Franco[1], Robert H. Wiltrout[1], Nobuaki Momozaki[2], John J. O'Shea[1], Dan L. Longo[3], and Kristin L. Komschlies[4], [1]Laboratory of Experimental Immunology and [2]Biological Response Modifiers Program, [4]Biological Carcinogenesis and Development Program, PRI/DynCorp; NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201; and [3]Oncotherapeutics, Cranbury, New Jersey 08512

Previously, we showed that the BRM flavone 8-acetic acid (FAA) plus rhIL-2 cures 80% of mice bearing the murine renal cell cancer Renca via a T-cell dependent mechanism. The T cells from these Renca-bearing mice exhibit a loss of the zeta chain (ζ) of the T cell receptor (TCR) and $p56^{lck}$. These proteins return to normal levels of expression after successful treatment with FAA and rhIL-2. Our current findings show that normal mice given FAA plus rhIL-2 also have significant reductions in ζ and $p56^{lck}$ in their T cells, similar to those generated in Renca-bearing mice. However, unlike Renca-bearing mice, the T-cell alterations in FAA plus rhIL-2 treated mice are only transient and return to normal levels by 2 weeks. Moreover, if FAA alone is given to normal mice, ζ levels are reduced, absent and re-expressed by 8, 24 and 72 hours, respectively. Based on Northern blot analysis, these changes are not regulated at the RNA level. Thus, induction of these alterations is not unique to tumor presence, but are also induced in normal mice by BRM and may represent a mechanism by which T-cell responses are regulated.

V 559 SPECIFICITY OF TYROSINE ACTIVATION MOTIF RESIDUES FOR p59^{fyn} BINDING. Lisa K. Timson Gauen*, Yuexin Zhu*, Francios Letourneur, Richard D. Klausner, and Andrey S. Shaw*, *Department of Pathology, Washington University, St. Louis, MO 63110.

We previously demonstrated that p59^{fyn} associates with chimeric proteins which contain the cytoplasmic domain of several T cell receptor proteins, each of which contains at least one copy of a signalling motif known as the tyrosine activation motif (TAM). Here we demonstrate that p59^{fyn} specifically requires the TAM sequences of the CD3 ε cytoplasmic domain for association. To determine if ε TAM residues that are critical for T cell signalling are critical for p59^{fyn} binding, we generated 21 chimeric ε mutants whose cytoplasmic portions were previously tested for the ability to signal in T cells. All mutants which were capable of signalling in T cells retained the ability to bind p59^{fyn}. Surprisingly, only mutants containing a substitution of the second tyrosine of the TAM lost the ability to bind p59^{fyn}. These results suggest that p59^{fyn} may specifically recognize a structural feature of the TAM. This hypothesis will be tested by additional mutagenesis of TAM sequences and by analysis of these mutants for the ability to bind p59^{fyn}.

V 560 ALTERATIONS IN NFκB/REL FAMILY PROTEINS IN SPLENIC T CELLS FROM TUMOR-BEARING MICE AND REVERSAL FOLLOWING THERAPY, Paritosh Ghosh*, Antonio Sica*, Howard A. Young*, Jianping Ye*, Jose L. Franco*, Robert H. Wiltrout*, Dan L. Longo*, Nancy R. Rice†, and Kristin L. Komschlies‡, *Biological Response Modifiers Program; †Applied BioScience Laboratories, Inc.; and ‡BCDP, PRI/DynCorp, NCI-FCRDC, Frederick, MD 21702. Impaired immune system function has been documented in cancer patients and in tumor-bearing mice. One of the reasons for this immune impairment may be due to the suppressed T-cell function. It has recently been demonstrated that T cells from tumor-bearing animals may have abnormalities in their signal transduction molecules. Since a critical outcome of signal transduction is activation of specific genes under the influence of particular transcription factors, we examined the expression of NFκB/Rel family proteins in murine tumor models, as these transcription factors play a crucial role in transcription of genes involved in immune and inflammatory responses. When we examined the splenic T cells from mice bearing Renca, a renal carcinoma, T cells had undetectable levels of nuclear c-Rel, NFκB p65, and NFκB p50; however, two shorter forms of p50 (p48 and p46), truncated at the N-terminus, were present exclusively in the nucleus. These T cells have altered DNA-protein interactions, demonstrated by gel shift analysis with a κB site. When Renca-bearing mice are treated with flavone-8-acetic acid plus rhIL-2, 80% of mice experience long-term, disease-free survival. In successfully treated mice, the T cells expressed normal levels of all NFκB/Rel family members. These results suggest that alterations in transcription factors may accompany changes in signal transduction molecules in T cells from tumor-bearing animals; however, the abnormalities are reversed with successful treatment.

V 561 **BIOCHEMICAL CHARACTERIZATION OF A NOVEL CYTOKINE IL-15 AND ITS RECEPTOR.** Judith G. Giri, Minoo Ahdieh, Kenneth H. Grabstein, June Eisenman, Kurt Shanebeck, Charles Rauch, Satoru Kumaki, Linda S. Park, David Cosman and Dirk M. Anderson. Immunex Research and Development Co., 51 University St. Seattle WA 98101.

IL-2, one of the best characterized growth factors, has a central role in regulation of antigen induced T cell proliferation. We have identified a novel cytokine with similar T cell stimulatory activity to IL-2, which we have designated IL-15. The 14-15 kDa glycoprotein was purified from cultures of a simian kidney epithelial line, cloned based on protein sequence and expressed in yeast. The purified recombinant protein was radiolabeled and retained activity in a CTLL assay. Specific binding of ^{125}-I IL-15 to receptors on a variety of T cell lines and clones as well as some non-lymphoid cells such as fibroblasts and endothelial cells was detected. On human PHA activated PBT for example, between 100-500 high affinity binding sites (Ka of 1.2-3.5 x10^{10} M) were estimated by Scatchard analysis. Although IL-15 shares some biological activities and receptor distribution with IL-2, we found that some cells which have functional IL-2 receptors, neither bind nor respond to IL-15. There is no significant sequence homology between IL-2 and IL-15, but IL-15 appears to belong structurally, like IL-2 and IL-4, to the four helix bundle family of cytokines.

V 562 INDUCTION OF THE IgH 3' ENHANCER BY THE SURFACE IMMUNOGLOBULIN RECEPTOR INVOLVES RECRUITMENT OF THE TRANSACTIVATING COMPLEX NFAB. Patrick A. Grant and Sven Pettersson. Center For Biotechnology, Karolinska Institute, 141 57 Huddinge, Sweden.

The immunoglobulin heavy chain (IgH) 3′ enhancer has been demonstrated to be a control element tightly regulated in its temporal and spatial activity during B-lymphocyte differentiation. Analysis of transgenic mice bearing a β-globin reporter gene driven by the IgH 3' enhancer has revealed that the enhancer is tissue specific in its activity, being active in splenic and peritoneal cells and that the enhancer is active only in large, activated splenic cells and not in resting B-cells. The mechanism by which the enhancer is activated has not been discerned. We found that it is possible to activate the IgH 3' enhancers expression in a transformed B-cell line using the mitogens ionomycin and phorbal ester (TPA) or via crosslinking the immunoglobulin surface receptor. We have identified a lymphoid specific DNA binding complex, called NFAB (Nuclear Factor of Activated B-cells), which can be activated using such stimulation and which is sufficient to confer activated transcription on a reporter gene construct. The components of the NFAB complex and its relative importance in the IgH 3' enhancers activation will be presented.

V 563 THE EXTRACELLULAR DOMAIN OF CD4 REGULATES THE INITIATION OF T CELL ACTIVATION IN A CIS MANNER , Sophie Gratton, Lori Haughn, Michael Julius and Rafick-Pierre Sékaly, Clinical Research Institute of Montreal, Montréal, Québec, Canada, H2W 1R7.

We have previously shown that CD4 can sequester lck away from the TcR and that the CD4-lck association can down-regulate anti-TcR induced proliferation if it is not in close contact with the TcR. To further characterize the role of the domains of CD4 in the regulation of T cell activation, we have designed a chimeric molecule which consists of the extracellular domain of the epidermal growth factor receptor (EGFR) and the transmembrane and cytoplasmic domains of CD4. This chimera was transfected into the CD4 negative murine T cell clone 2.10. Immunoprecipitation experiments have demonstrated that this chimera associates with lck as efficiently as wild type CD4. Moreover, binding of EGF induces an increase in lck autophosphorylation activity. Surprisingly, although the chimera associates with lck, it was not able to downregulate anti-TcR stimulation. The chimera thus lacks a regulatory function that the extracellular domain of CD4 possesses and that is independent of its association with lck. This suggests that both the extracellular domain of CD4 and the CD4-lck association are required in order to get downregulation of TcR-induced stimulation. Complementation experiments using CD4 mutants are being performed to test that hypothesis.

V 564 VOLTAGE GATED AND NON-VOLTAGE GATED CALCIUM CHANNELS IN JURKAT T LYMPHOCYTE ACTIVATION, Lloyd S. Gray and John J. Densmore, Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908

Increased intracellular Ca^{2+} is a critical component of the activation response in T lymphocytes. Although the influx of extracellular Ca^{2+} is responsible for a large part of this increase, the mechanism of Ca^{2+} entry is unknown. Using the perforated patch variant of the patch clamp technique, we have examined some of the properties of two Ca^{2+} currents in Jurkat T lymphocytes, one voltage gated (VG) and the other non-voltage gated (NVG). Both currents are inhibited by 1mM $NiCl_2$. The two Ca^{2+} currents displayed different sensitivities to $MnCl_2$, however, with the NVG current completely blocked by 30μM Mn^{2+} and the VG current only moderately inhibited by 1mM Mn^{2+}. Because the receptor-linked Ca^{2+} entry pathway in T cells is permeable to Mn^{2+}, these results suggest that the NVG current is not primarily responsible for Ca^{2+} influx during T cell activation and support a role for the voltage gated Ca^{2+} current in T cells.

V 565 METABOLITES OF VITAMIN A AS ESSENTIAL COFACTORS FOR ACTIVATION AND GROWTH OF LYMPHOCYTES, Ulrich Hämmerling, Felix Grün and Jochen Buck, Department of Immunology, Memorial Sloan-Kettering Cancer Center and Department of Pharmacology, Cornell University Medical College, New York, NY 10021

Lymphocytes, like other hemopoietic cells, require vitamin A (retinol) as an essential growth factor. When omitted from medium, resting cells cannot be activated, nor can cycling cells continue to proliferate. To determine the intracellular mediators of this growth-promoting effect, we have analyzed the retinol metabolites of lymphocytes. Two of these are of interest: 14-hydroxy-retro-retinol (14HRR) and anhydroretinol (AR). Both belong to the class of retro retinoids that for the first time has been linked to biological function. Despite structural similarities, their effects on lymphocyte physiology are strikingly different. While 14HRR can support activation and growth of lymphocytes and therefore can be regarded as an agonist, AR strongly suppresses lymphocyte activation. The pharmacological relationships (i.e., reversible inhibition) can best be explained by assuming competition for binding to a common receptor site, possibly a nuclear receptor. In summary, retinol can be regarded as a prohormone, that is converted in lymphocytes to an agonistically acting mediator, 14HRR, as well as to an antagonist, AR. This pair of mediators is involved in the regulation, yin-yang fashion, of lymphocyte proliferation.

References:
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V 566 STIMULATION OF THE B CELL RECEPTOR INDUCES TYROSINE PHOSPHORYLATION OF A 75 kDa PROTEIN WHICH ASSOCIATES WITH THE INTERLEUKIN 4 RECEPTOR, [1]Nobuyuki Harada, [1]Leopoldo Santos-Argumedo, [2]Warren J. Leonard and [1]Kenji Izuhara, [1]DNAX Research Institute, Palo Alto, CA 94304; [2]Section on Pulmonary and Molecular Immunology, National Heart, Lung, and Blood Institute, National Institute of Health, Bethesda, MD 20892

Interleukin 4 (IL-4) was originally found to be important for optimal stimulation of B cells with anti-B cell receptor (BCR) antibodies. IL-4 is also known to rescue some B cell lymphoma cell lines from anti-BCR antibody-induced apoptosis. These data suggest that IL-4 receptor (IL-4R) and BCR signaling pathways may crosstalk. However, no biochemical studies have addressed this issue. In this study, we have now analyzed tyrosine phosphorylated proteins which associate with IL-4R upon BCR stimulation using LPS-treated murine splenic B cells, human EBV-transformed B cells, WEHI-231 cells and Daudi cells. In each of these cells, we observed that BCR stimulation induces tyrosine phosphorylation of a 75 kDa protein which can be immunoprecipitated with an anti-IL-4R antibody. We are now characterizing this protein. This finding may provide clues into the relationship between BCR and IL-4R signaling pathways.

V 567 INCREASED INTRACELLULAR Ca^{2+} INDUCES Ca^{2+} INFLUX VIA A CALMODULIN-DEPENDENT PATHWAY IN JURKAT CELLS, Doris M. Haverstick and Lloyd S. Gray, Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908

One current hypothesis for the initiation of Ca^{2+} entry into non-electrically excitable cells proposes that Ca^{2+} entry is linked to the state of filling of intracellular Ca^{2+} stores. In the human T lymphocyte cell line Jurkat, stimulation of the antigen receptor leads to release of Ca^{2+} from internal stores and influx of extracellular Ca^{2+}. Similarly, treatment of Jurkat cells with the tumor promoter thapsigargin induced release of Ca^{2+} from internal stores and also resulted in influx of extracellular Ca^{2+}. Initiation of Ca^{2+} entry by thapsigargin was blocked by chelation of Ca^{2+} released from the internal storage pool. The Ca^{2+} entry pathway could also be initiated by an increase in the intracellular concentration of Ca^{2+} following photolysis of the Ca^{2+}-cage, nitr-5. Thus, three separate treatments that caused an increase in the intracellular concentration of Ca^{2+} initiated Ca^{2+} influx in Jurkat cells. In all cases, Ca^{2+}-initiated Ca^{2+} influx was blocked by treatment with any of three phenothiazines or W-7 suggesting that it is mediated by calmodulin. These data suggest that release of Ca^{2+} from internal stores is not linked capacitatively to Ca^{2+} entry, but that initiation is linked instead by Ca^{2+} itself, perhaps via calmodulin.

V 568 THE CYTOPLASMIC TAIL OF THE T CELL RECEPTOR ζ CHAIN IS DISPENSABLE FOR ANTIGEN-MEDIATED T CELL ACTIVATION, Mirjam Hermans and Bernard Malissen, Centre d'Immunologie INSERM/CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France

The T cell antigen receptor consists of an antigen binding αβ heterodimer and a group of invariant polypeptides denoted CD3-γ, CD3-δ, CD3-ε and CD3–ζ. Whether antigen responsiveness is dependent on the expression of functional CD3-ζ subunit remains controversial. For instance, transfection of a ζ/η negative variant of the 2B4.11 T cell hybridoma with mutated ζ cDNA that encoded a ζ protein truncated at residue 108, restored the surface expression of TcR complexes with, however, impaired antigen responsiveness (Science 1990, 249: 174). In marked contrast BW5147 transfectants that expressed TCR devoid of functional ζ subunits were still able to trigger the production of interleukin-2 in response to antigen (Cell 1992, 68: 83).

To assess if the above discrepancies may have resulted from the use of different recipient T cells, we transfected a ζ/η-deficient variant of 2B4.11 (MA5.8) with the very same truncated ζ cDNA *we previously* used in BW5147. Consistent with our initial observations in BW5147, the cytoplasmic tail of the ζ polypeptide was found dispensable for antigenic responsiveness. However, a difference between the two recipient T cells was detected when cells were challenged via the Thy-1 and Ly-6 molecules. Once expressed in MA5.8, but not in BW5147, TCR complexes devoid of functional ζ subunits were able to sustain activation initiated via Thy-1 and Ly-6 molecules.

V 569 HIGH LEVEL EXPRESSION AND PURIFICATION OF THE CYTOPLASMIC REGION OF HUMAN CD45 FROM YEAST, Terry Higgins‡[1], Anne Pacitti[1], Mark Evans[3], Ian Trowbridge[3], and Panayiotis Stevis[2], Department of Immunopharm.[1], Department of Mol. Biol. and Protein Chem.[2], Sterling Winthrop Pharm. Res. Division, Collegeville, PA, 19426-0900 and Department of Cancer Biology[3], The Salk Institute, San Diego, CA 92186-5800

The importance of CD45 for both T-cell and B-cell receptor signaling has been well documented and it has now been demonstrated that the cytoplasmic portion of CD45 alone is sufficient for intracellular signaling. For these studies, the cytoplasmic region of CD45 corresponding to residues 584-1281 was inserted downstream of the alcohol dehydrogenase promoter and transfected into a haploid strain of yeast. Expression of recombinant CD45 in yeast reached as high as 5% of the soluble protein. Following removal of cellular debris by centrifugation and ammonium sulfate precipitation, enzymatically active material with a purity of ≥ 98% was obtained with a yield approaching 50% using four chromatography steps. The final product gave a Km of 5.5 mM and a Vmax of 87.5 U/mg with p-nitrophenylphosphate and a Km and Vmax of 0.167 mM and 185 U/mg, respectively, with a phosphotyrosine peptide. The native enzyme purified from Jurkat cells showed comparable Kms with both substrates but displayed substanually lower Vmax values for both substrates possibly representing a loss of regulation by the recombinant enzyme. The large quantities of cytoplasmic CD45 produced by the yeast expression system will facilitate detailed characterization of the enzymatic and signal transduction properties of CD45 and has already provided sufficient material for crystallography trials.

V 570 ANALYSIS OF COMPLETE AND PARTIAL GENETIC DEFICIENCIES OF CD45 IN TRANSGENIC MICE, N. Holmes, K. Byth, J. May, S. Howlett and A.J.H. Smith#, Division of Immunology, Dept of Pathology, Cambridge University, Cambridge CB2 1QP,U.K. and #MRC Laboratory of Molecular Biology, Cambridge, U.K.

CD45 is a transmembrane protein tyrosine phosphatase which is expressed on most haemopoietic cells including precursor stem cells. A variety of protein isoforms, resulting from RNA splicing of exons 4,5 and 6, are expressed in a cell-type and developmentally regulated fashion. Studies on long-term cell lines have indicated that CD45 is required for antigen-receptor signal transduction in T and B lymphocytes. Furthermore the derivation of transgenic mice homozygous for a partial deletion encompassing exon 6 of the CD45 gene was recently reported (Kishihara et al. Cell 74, 143). These mice expressed greatly reduced but detectable levels of CD45 as well as lacking any exon 6-inclusive forms. The development of T cells but not B cells was inhibited and B cell signalling was impaired.

We have also addressed the question of the role of CD45 in haemopoietic cell development and lymphocyte signalling *in vivo* using gene targeting. We have derived ES cell clones from the R1 line (generously provided by Dr A. Nagy) which carry 2 distinct mutations. Firstly stop codons have been introduced into exon 9 which should result in the complete ablation of the functional CD45 protein. Correctly targeted clones heterozygous for this mutation have been used to generate germline chimaeras. Thus far we have confirmed that the heterozygous phenotype is normal in respect of development and results in a reduction in the level of CD45 cell surface expression. Breeding experiments are in progress in order to produce homozygous deficient animals and the consequences of complete ablation of CD45 expression for the development of B and T cells will be determined. Our second mutation produces premature termination of isoforms including exon 4 (expressed in the B lineage and a subset of mature CD8 T cells). 2 lines of chimaeric mice have been generated but so far no germline transmission obtained. We are currently making chimaeras with ES cells homozygous for this mutation which will confirm the effect of our mutation and demonstrate whether exon 4+ forms have any unique role in lymphoid development

V 571 THE SEQUENTIAL INTERACTIONS OF T CELL ANTIGEN RECEPTOR WITH TWO DISTINCT CYTOPLASMIC TYROSINE KINASES

Makio Iwashima*, Bryan A. Irving*, Andrew C. Chan@ and Arthur Weiss*#@, Howard Hughes Medical Institute*, Departments of Microbiology and Immunology# and of Medicine@, University of California, San Francisco, San Francisco, CA 94143

The T cell antigen receptor (TCR) initiates signal transduction by interacting with cytoplasmic protein tyrosine kinases (PTKs) through a 17 residue sequence motif (termed the antigen recognition activation motif; ARAM) that is contained in the TCR ζ and CD3 chains. TCR stimulation induces the tyrosine phosphorylation of a number of cellular substrates including the ARAMs.

Three PTKs have been implicated in TCR signal transduction: lck, fyn and ZAP-70. lck is not generally found directly associated with the TCR, but interacts with the co-receptors CD4 and CD8 which colocalize with the TCR during antigen recognition. Studies of an lck-deficient T cell line or a T cell clone have demonstrated that expression of lck is essential for TCR signal transduction including tyrosine phosphoprotein induction. Another src family member, fyn, has been shown to associate, albeit at low stoichiometry, with the TCR ζ chain. Studies of fyn-deficient mice have revealed a TCR signal transduction defect in the most mature thymocyte subsets, although the signal transduction function of TCRs in mature peripheral T cells was not as strikingly affected. We recently identified a cytoplasmic PTK, ZAP-70, which upon TCR stimulation, rapidly associates with the ζ and CD3 chains. ZAP-70 has greatest structural homology with the syk PTK. Recent studies of chimeric receptors in which ZAP-70, syk, lck or fyn were linked to transmembrane receptors suggest that the ZAP-70 or syk kinases can regulate the function of downstream signal transduction molecules.

The precise mechanisms by which the TCR interacts with the distinct families of cytoplasmic PTKs and the interactions between these cytoplasmic PTKs are not well understood. Here, we demonstrate that the kinase activity of lck is required for phosphorylation of two conserved tyrosine residues within an ARAM. This phosphorylation leads to the recruitment and phosphorylation of a second cytoplasmic PTK, ZAP-70, via both of its SH2 domains. These results suggest that initiation of signaling by the TCR is mediated by a sequential interaction between two PTKs through ARAM phosphorylation.

V 572 T- AND B-LYMPHOCYTE ANTIGEN RECEPTOR ACTIVATION OF A NOVEL FAK ISOFORM, Steven B. Kanner, Alejandro Aruffo, Gena S. Whitney and Po-Ying Chan, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Stimulation of T- and B-lymphocytes through their respective antigen receptors results in the activation of protein tyrosine kinases and the phosphorylation of cellular substrates on tyrosine residues. In this report, we describe a novel tyrosine kinase substrate fakB, a homologue of the focal adhesion kinase pp125^{FAK}. Rapid tyrosine phosphorylation of fakB was observed in both T- and B-cells after antigen receptor cross-linking with antibody, whereas pp125^{FAK} was not affected by these stimuli. Costimulatory activation of the T-cell receptor complex (TCR/CD3) with the accessory receptors CD2 and CD4 induced synergistic tyrosine phosphorylation of fakB in normal T-cells. Furthermore, TCR/CD3 engagement with antibody induced the stable association of fakB with ZAP-70, the TCR/CD3 zeta-chain associated tyrosine kinase involved in antigen receptor-induced lymphocyte signaling. Both inducible and pre-formed complexes of fakB/ZAP-70 were observed in T-cells associated with the TCR/CD3 zeta-chain. Taken together, these results indicate that fakB is a new type of focal adhesion kinase-related protein that is differentially regulated from that of pp125^{FAK}, and may play a role in T- and B-lymphocyte signaling through the antigen receptors.

V 573 ACTIVATION OF DISTINCT RECEPTOR-ASSOCIATED TYROSINE KINASES BY INTERLEUKIN-2 AND PROLACTIN IN T LYMPHOCYTES: ROLE OF JAK FAMILY KINASES. Robert A. Kirken, Hallgeir Rui, William L. Farrar. Cytokine Molecular Mechanisms Section, LMI, BRMP, National Cancer Institute, FCRDC, Frederick, Maryland 21702, USA.

Interleukin-2 (IL-2) and prolactin (PRL) receptors belong to the hematopoietin receptor superfamily. Cytoplasmic tyrosine kinases of the JAK family mediate effects of several of these receptors, and we have recently shown that JAK2 constitutes the 120-130 kDa PRL receptor-associated tyrosine kinase. In contrast, a 116 kDa candidate, IL-2 receptor-associated tyrosine kinase (Kirken et al 1993, JBC 268, 22765) with characteristics similar to JAK2, was not recognized by antibodies to any of the known JAK family tyrosine kinases, including JAK2, JAK1 or TYK2. The PRL receptor-associated JAK2 and the IL-2 receptor-associated p116 elicited similar rapid and transient tyrosine phosphorylation kinetics upon ligand binding, reaching peak values within 5 min. In vitro IL-2 receptor-complex tyrosine kinase assays demonstrated that p116 was the main receptor-associated protein to undergo tyrosine phosphorylation, analogous to the autophosphorylation observed with PRL receptor-associated JAK2. Furthermore, phosphorylated p116 and JAK2 migrated with similar charge in two-dimensional NEPHGE/SDS polyacrylamide gel electrophoresis. Highly corresponding one-dimensional phosphopeptide maps of the two proteins following V8 protease digestion furter substantiated the notion that p116 and JAK2 are related proteins. Based on these results, we propose that the IL-2 receptor-associated p116 may represent a novel JAK family kinase utilized by IL-2 receptors and possibly other members of the hematopoietin receptor family.

V 574 STRUCTURE AND FUNCTION OF THE HETEROTRIMER SUBUNIT COMPLEX OF IL-2 RECEPTOR

Motonari Kondo[1)], Toshikazu Takeshita[1)], Naoto Ishii[1)], Masataka Nakamura[1)], Sumiko Watanabe[2)], Ken-ichi Arai[2)] and Kazuo Sugamura[1)]

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IL-2 regulates growth and differentiation of various types of cells in the immune system via its interaction with IL-2 receptor (IL-2R). IL-2Rs are classified into three isoformes differing in IL-2 binding affinity; high-, intermediate- and low-affinity receptors, which are composed of the αβγ heterotrimer complex, the βγ heterodimer complex and the α chain alone, respectively. The high- and intermediate-affinity receptors harbor the function of the intracellular signal transduction, indicating that the β chain and the γ chain which was cloned by us in 1992 are indispensable for the signal transduction but not the α chain. The reconstitution studies of IL-2Rs with the α, β and γ chain genes demonstrated that each subunit has potential for altering the affinity of the receptor, and the cytoplasmic domains of the β and γ chains participate in signal transduction in terms of cell growth, activation of a tyrosine kinase and enhancement of c-myc, c-fos and c-jun transcription. The region containing the SH2 homologous sequence of the γ chain should have a critical function for signal transduction.

Common subunits are known to be shared among receptors; a β chain is shared in receptors for IL-3, IL-5 and GM-CSF, and gp130 functions as a common subunit of receptors for IL-6, OSM, CNTF and LIF. We have demonstrated that a monoclonal antibody specific for the IL-2Rγ chain completely inhibited cell growth not only induced by IL-2 but also induced by IL-4 and IL-7, suggesting that the γ chain is possibly shared among IL-2R, IL-4R and IL-7R.

V 575 **THE CLONING AND CHARACTERIZATION OF THE MOUSE T-CELL RECEPTOR CD3 ZETA-ASSOCIATED PROTEIN (ZAP-m),** **Grace Ku and Bernard Malissen, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, 13288 Marseille Cedex 9, France.**

The T-cell antigen receptor (TCR) complex is an oligomeric structure composed of the α and β chains, which recognize peptide-antigen bound to a major-histocompatibility complex molecule, noncovalently associated with four other polypeptides CD3-γ,-δ,-ε and -ζ, which are required for efficient surface expression. Several groups, including ours, have shown that the CD3-ε or -ζ proteins directly couple antigen-recognition by TCR to intracellular signal transduction events via their respective intracytoplasmic protein domains. Recent work has shown that a 70kd protein-tyrosine kinase (PTK) forms a tight association with CD3-ζ or -ε following cellular activation via the TCR (reviewed in Weiss 1993 Cell, 73:209-212). This ζ-associated PTK (ZAP-70) is composed of two N-terminal Src homology-2 domains and a C-terminal tyrosine kinase domain, is expressed exclusively in T cells and NK cells and may be implicated in TCR function. (Chan et al., 1992 Cell, 71:649-662). In order to characterize the genetic counterpart of ZAP-70 in mice (ZAP-m), we have cloned the genomic and cDNAs which encode this PTK, determined its chromosmal location and examined its expression in different T-cell subpopulations. In parallel, to study the function of ZAP-m in vivo we are inactivating the corresponding gene by homologous recombination in embryonic stem cells.

V 576 TYROSINE KINASE LYN AND SYK REGULATE THE B CELL RECEPTOR-COUPLED CALCIUM MOBILIZATION THROUGH THE DISTINCT PATHWAY

Tomohiro Kurosaki,[1] Minoru Takata,[1] Hisataka Sabe,[2] Akiko Hata,[2] Tetsuya Inazu,[3] Yoshimi Homma,[4] Toshihide Nukada,[5] and Hirohei Yamamura,[3]

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Stimulation of B lymphocytes through their antigen receptor (BCR) results in rapid increases in tyrosine phosphorylation on a number of proteins, and induces both an increase of phosphatidylinositol (PtdIns) and mobilization of cytoplasmic free calcium. The BCR associates with two classes of tyrosine kinase: Src-family kinase (Lyn, Fyn, Blk or Lck) and Syk kinase. To dissect the functional roles of these two types of kinase in the BCR signaling, *lyn*-negative and *syk*-negative B cell lines were established from chicken B cell line DT40 by homologous recombination. Syk-deficient B cells abolished the tyrosine phosphorylation of phospholipase C (PLC)-γ2, resulting in no inositol 1,4,5-trisphosphate (IP_3) generation, as well as calcium mobilization upon receptor stimulation. Cross-linking of BCR on Lyn-deficient cells evoked a profoundly delayed and slow Ca^{2+} mobilization, despite the normal kinetics of IP_3 turnover. This abnormal Ca^{2+} mobilization was restored by the transfection of *lyn*, *lck*, and *fyn* but not by *src* cDNA. These results demonstrate that Syk mediates IP_3 generation, whereas Lyn regulates Ca^{2+} mobilization through a process independent of IP_3 generation.

V 577 **A SELECTIVE SIGNALING DEFECT IN THE FUNCTIONAL ACTIVATION OF ANTIGEN-SPECIFIC T LYMPHOCYTE CLONES IN THE ABSENCE OF PROTEIN TYROSINE KINASE $p59^{fyn}$.** David W. Lancki, Dapeng Qian, Patrick Fields, Thomas Gajewski, and Frank W. Fitch. Ben May Institute, University of Chicago, Chicago IL. 60637.

The GPI-linked cell surface molecule Thy-1 has been implicated in the differentiation and functional activation of T cells and can function as an accessory molecule in the activation of some T cell clones. The mechanisms by which these signaling events are coupled to functional responses are incompletely understood. In this study, we examine the requirement for protein tyrosine kinase $p59^{fyn}$ expression in signaling through Thy-1 using a panel of antigen-specific T cell clones that we derived from *fyn*$^{-/-}$ mutant mice (kindly provided by Drs. P. Stein and P. Soriano). These clones do not express normal Fyn protein as determined by immune-complex kinase reaction using anti-Fyn antibody. Stimulation through the TCR for antigen, either by APC bearing antigen or by anti-CD3ε monoclonal antibody (mAb), resulted in comparable levels of proliferation, lymphokine production, and cytolysis by clones from both normal and *fyn*$^{-/-}$ mice (Qian et al., these proceedings). In contrast, stimulation through Thy-1, using soluble (or cross-linked) anti-Thy-1 mAbs, was deficient in these Fyn$^-$ clones as measured by these responses. Thy-1 expression on the Fyn$^-$ clones, as determined by flow cytometry, was comparable to that observed on normal clones. Thus, $p59^{fyn}$ appears to be selectively required for the activation of these T cell clones through Thy-1. We are currently investigating the early signaling events in these Fyn$^-$ T cell clones.

V 578 Assocation of human Syk with the B cell antigen receptor and its potential function in sIgM signaling. C. -L. Law*, S. P. Sidorenko*, K. A. Chandran*, K. E. Draves*, A. C. Chan†, A. Weiss†Δ, T. W. LeBien¥, and E. A. Clark*. Dept. Microbiol.*, Uni. Washington, Seattle, WA 98195, Dept. Med.† and HHMIΔ, Uni. California, San Francisco, CA 94143, Dept. Lab. Med. Path¥. Uni. Minnesota, MN 55455.

B cell antigen receptors (BCR) are multi-component complexes consisting of the surface immunoglobulin (sIg) and accessory molecules with associating protein tyrosine kinases (PTKs). A spleen tyrosine kinase, Syk, in porcine B cells and a 72 kDa PTK, PTK72, in murine B cells associate with the BCR. We have isolated a full length cDNA encoding the human homologue of Syk. This cDNA predicted a polypeptide consisting of two N-terminal SH2 domains and a C-terminal tyrosine kinase domain. Syk is highly conserved between human and swine and is homologous to the T cell-associated PTK ZAP-70. Both Syk mRNA and protein were detected in cells derived from multiple hematopoietic lineages. Within the B cell compartment, Syk was expressed from early B lineage cells to plasma cells, suggesting that Syk may participate in signaling cascades other than that of the BCR. Tyrosine phosphorylation of Syk associating with the BCR complex in human was rapidly augmented subsequent to sIgM cross-linking. Immunoprecipitation experiments revealed that in human B cells Syk can associate with the Src-family kinase Lyn and additional phosphoproteins of 76 and 120 kDa in size. GST fusion proteins consisting of the SH2 domains of human Syk were generated to examine the interaction of Syk with the BCR. We detected phospho-tyrosine-dependent binding to components of the BCR with a fusion protein with both SH2 domains, but not fusion proteins with only one SH2 domain. We have also identified EBV-transformed B lymphoblastoid cell lines that could not generate any increase in intracellular free Ca2+ upon sIgM cross-linking. The potential role of Syk in this BCR signaling defect will be discussed.

V 579 CD45 AND CD4 REGULATION OF CA++ FLUX IN INDIVIDUAL T CELLS FOLLOWING STIMULATION THROUGH THE T CELL RECEPTOR. D. Leitenberg, T. Novak, D. Farber, B.R. Smith, and K. Bottomly. Departments of Laboratory Medicine and Immunobiology, Yale University School of Medicine, New Haven CT 06510.

An early consequence of T cell activation is an increase in intracellular calcium concentration. This is usually studied using a population of cells and the changes observed represent an average of that population. Recent advances in video laser microscopic techniques enable the examination of individual cells over time following stimulation. Using a wide variety of cell types it has been shown that cells do not simply increase intracellular calcium but often have characteristic oscillations in calcium concentration which can vary in amplitude and frequency. These observations suggest the hypothesis that different patterns of calcium flux may be associated with different late events in cell activation, such as proliferation or apoptosis, or may correlate with a specific pattern of lymphokine secretion. Experiments were done using normal mouse CD4+ T cells (including CD45RBhi and CD45RBlo subsets), mouse Th1 and Th2 clones, as well as various mutant BW5147 cell lines which have been transfected with different individual isoforms of CD45, or a GPI-linked form of CD45 which lacks the cytoplasmic tyrosine phosphatase domains. Following cross-linking of CD3 (using soluble anti-CD3 followed by a second antibody crosslinker, or using plate bound anti-CD3) normal mouse T cells, Th-clones, and CD45-transfected BW cells flux calcium in a similar manner. However, BW cells which do not express CD45, or express the GPI-linked form of CD45 do not increase intracellular Ca following CD3 cross-linking. These cells do, however, secrete IL-2. When normal CD4+ T cells are separated into CD45RBhi and CD45RBlo cells the frequency of responding cells is higher in the CD45RBhi subset although the pattern of Ca++ flux is not significantly different. When anti-CD4 and anti-CD3 are simultaneously added, followed by secondary antibody to crosslink CD4 and CD3 together the calcium flux in individual T cells is quite prolonged with an extended plateau phase as compared to cross-linking with anti-CD3 alone which generally consists of a single calcium spike of relatively short duration. This occurs in both CD45RBhi and RBlo subsets. Further work is being done using Th clones and T cell receptor transgenic mice and antigen presenting cells to simultaneously examine cell-cell contact and early activation events using antigen-pulsed antigen presenting cells and allogeneic stimulator cells.

V 580 CARE-LASS (CALCEIN RELEASE ASSAY), AN IMPROVED FLUORESCENCE-BASED TEST SYSTEM TO MEASURE CYTOTOXIC T LYMPHOCYTE ACTIVITY, Rudolf Lichtenfels, Hildegard Schulz, Anne B. Vogt and Roland Martin, Department of Neurology, Medical School, University of Tübingen, Hoppe-Seyler Str. 3, 72076 Tübingen, F.R.G. and Research Center for Medical and Basic Sciences, University of Tübingen, Ob dem Himmelreich 7, 72074 Tübingen, F.R.G.

Although numerous efforts have been made to develop alternative methods, the standard ^{51}Chromium (^{51}Cr) release assay is still most commonly used to measure cell-mediated cytotoxicity, despite obvious disadvantages such as handling of radioisotopes and extended processing time for counting large numbers of samples. Here we describe the CARE-LASS system, that is closely related to the conventional ^{51}Cr release assay but takes advantage of a fluorescent target cell label. CARE-LASS is a highly sensitive, fast, simple and safe fluorometric micro assay. Using automated fluorescence scanners the processing time is reduced to one second per sample.

We tested the CARE-LASS system using various target cell lines, different effector to target (E:T) ratios and by titration of antigen. The CARE-LASS system provides a reliable and sensitive method to measure cell-mediated cytotoxicity.

V 581 REGULATION OF THE IMMUNOGLOBULIN γ1 GERM LINE PROMOTER, Mats Lundgren, Janet Stavnezer and Eva Severinson, Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden and Department of Molecular Genetics and Microbiology, University of Massachusetts, Massachusetts.

Immunoglobulin (Ig) heavy chain switching is mediated by a DNA rearrangement that replaces the expressed constant region gene with a downstream constant gene. There is a positive correlation between transcription of the germ line (GL) constant gene and switching to the corresponding gene. We have studied the effect of activation of the GL γ1 promoter in an electrophoretic mobility shift assay (EMSA). Induction of a B cell line, L10A6.2, with phorbol ester plus IL-4, changes the pattern of binding of C/EBP family proteins to the promoter.

To define the role of proliferation on GL γ1 transcription, the effect of DNA synthesis inhibitors was tested. Incubation of lipopolysaccharide (LPS) plus IL-4 activated B cells with hydroxyurea reduced the amount of GL γ1 RNA. Activated B cells were separated according to cell cycle phase by elutriation. In these experiments GL γ1 RNA was much reduced in cells in G1 phase, as compared to S phase. We have also tested wether this S phase restriction is mediated by induction of factors binding to to the GL γ1 promoter.

V 582 IDENTIFICATION, PURIFICATION AND MOLECULAR CLONING OF BLK, LYN AND FYN(T) SH2 BINDING PROTEINS FROM B LYMPHOCYTES, Sami N. Malek and Stephen Desiderio, Department of Molecular Biology and Genetics and Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Blk, Lyn and Fyn(T) are tyrosine kinases of the Src family that have been implicated in signalling through the B cell antigen receptor (sIg). Using an in vitro assay for phosphoprotein binding to GST-SH2 fusion proteins, we found that the SH2 domains of Blk, Lyn and Fyn(T), when tested under conditions that favor high-affinity interactions, preferentially bind distinct sets of phosphoproteins from B cells. A subset of the proteins recovered from a Blk SH2 affinity matrix are substrates for sIg-activated tyrosine kinase(s). [Malek, S. N. and Desiderio, S. (1993) J. Biol. Chem. 268:22557-22565] By SH2 affinity chromatography, we have obtained sufficient amounts of selected Blk SH2 binding proteins for isolation of peptides and determination of amino acid sequence. We have isolated molecular clones for two of these phosphoproteins, pp90 and pp76. (1) An oligonucleotide based on peptide sequence from pp90, which preferentially binds the Blk SH2 domain, was used to isolate a partial cDNA from spleen, which then served as a probe to clone full-length pp90. As the larger cDNA encodes several other peptides obtained from pp90, it appears to represent the pp90 transcript. The predicted sequence of 535 amino acid residues is very acidic (pI= 4.4) and has no known homologues. (2) Degenerate, synthetic oligonucleotides, based on peptide sequences from pp76, were used to amplify a partial cDNA. This probe was used to isolate a cDNA clone containing an open reading frame of 749 codons; by sequence, this clone appears to encode a serine/threonine kinase. The in vitro translation product of this cDNA clone migrates anomalously slowly on SDS-PAGE gels. Antibodies against this putative kinase detect a major species with an apparent size of 130 kDa and a minor species with an apparent size of 76 kDa in B cell lysates and among proteins eluted from the Blk SH2 affinity matrix, suggesting that the kinase interacts, directly or indirectly, with the Blk SH2 domain.

V 583 INVESTIGATION OF THE BIOLOGICAL EFFECTS AND BIOCHEMICAL EVENTS FOLLOWING LIGATION OF AN IG-ASSOCIATED SIGNALLING MOLECULE ON MURINE B CELLS, Stuart Marshall-Clarke and Lynn Tasker, Department of Human Anatomy and Cell Biology, University of Liverpool, Liverpool, U.K.

Monoclonal antibody LR-2 defines a cell-surface signaling molecule expressed on murine B cells. We have previously shown that the LR-2 molecule associates with sIg on the B cell surface and that its ligation with mAb inhibits the growth of cells of the WEHI-231 but not the CH31 or CH33 lymphoma lines. The proliferation of normal B cells driven by LPS or anti-Ig + IL-4 is also inhibited in the presence of mAb. Resting B cells are LR-2 -ve, but expression of the molecule is induced following activation with LPS. Levels of LR-2 expression have been investigated under various conditions of stimulation and found not to correlate with changes in sIg or class II expression.

Here we report on the biochemical characterisation of the LR-2 molecule and on the nature of the molecules with which it associates in the B cell membrane. The effects of LR-2 ligation on B cell differentiation have been analysed and our findings suggest that inhibitory signals are delivered to virgin but not memory B cells. Our studies also indicate that the signals transduced after LR-2 ligation differ from those which follow the cross-linking of sIg. The implications of these findings for the regulation of B cell proliferation and the induction of tolerance will be discussed.

V 584 STRUCTURE AND EXPRESSION OF THE NOVEL *csk*-LIKE TISSUE SPECIFIC TYROSINE KINASE, *lsk*, Daniel W. McVicar, Tiziana Musso, Brajesh K. Lal, Andrew Lloyd, Masura Kawamura, Yi-Qing Chen, Xiaoying Zhang, J. Erin Staples, John R. Ortaldo and John J. O'Shea, Laboratory of Experimental Immunology, BRMP and BCDP, PRI/DynCorp, NCI-FCRDC, Frederick, MD 21702-1201.

The critical role of both receptor and *src*-family non-receptor tyrosine kinases in cellular signal transduction and cell growth regulation is now well documented. Regulation of the activity of *src* kinases is thought to occur, in part, through the phosphorylation of highly conserved carboxyl-terminal tyrosine residues. Although the *src*-family includes several molecules with tissue or cell type restricted expression, the only kinase implicated in the regulatory phosphorylation of these enzymes is $p50^{CSK}$. Here we report the structure and expression of the first leukocyte specific $p50^{CSK}$-related kinase, *lsk*. Similar to $p50^{CSK}$, the deduced protein sequence of *lsk* includes a single tyrosine kinase catalytic domain, SH2 and SH3 domains, a short amino terminus, and no autophosphorylation or carboxyl-terminal tyrosine residues (*src* Y416 and Y527, respectively). In addition, neither *lsk* or $p50^{CSK}$ contain the amino-terminal myristylation site characteristic of the *src*-family kinases. Unlike *csk*, which is ubiquitously expressed, *lsk* mRNA is expressed constitutively only in brain and natural killer cells. Significant *lsk* expression can be induced in peripheral T cells and monocytes with mitogens and cytokines, respectively. Polyclonal antisera reactive with the predicted C-terminus of *lsk* specifically recognizes a 55 kDa polypeptide in immunoblots of NK but not T-cell lysates. In agreement with the mRNA expression pattern, $p55^{lsk}$ is induced upon activation. The discovery of a putative kinase such as $p55^{lsk}$ raises the possibility of tissue specific *src*-regulatory pathways.

V 585 THE ACTIVATION SIGNAL THROUGH T CELL RECEPTOR INDUCES GROWTH ARREST BY REDUCING G1 CYCLINS, Shoichiro Miyatake, H. Nakano, S. Y. Park, T. Yamazaki and T. Saito, Division of Molecular Genetics, Center for Biomedical Science, School of Medicine, Chiba University, Chiba, Japan

During the development of thymocytes, certain signals through T cell receptor (TCR) induce apoptotic cell death to eliminate autoreactive clones. Activation of T cell hybridoma also induces cell death. Growth arrest at G1 phase of the cell cycle precedes the cell death. Activation of mature T cells through TCR also induces growth arrest and subsequent cell death if these cells have been preactivated. In order to study cell cycle regulation by the signal through TCR and the link between the growth arrest and the induction of cell death, the regulation of G1 cyclins, cdk2 and cdc2 genes upon activation of T cells were studied. The expression of cyclin D3, E and cdk2 reduced immediately after stimulation while cyclin D2 increased. The expression of cdc2 reduced gradually. The induction of growth arrest at G1 phase and subsequent cell death were observed with cloned T cell lines upon the activation through TCR when these cells were proliferating in the presence of IL2. The down-regulation of cyclin D3, E and cdk2 and up-regulation of cyclin D2 were also detected. In an attempt to study the role of down-regulated genes to the cell cycle, transfectants of T cell hybridomas expressing high levels of cyclinD3, E or cdk2 were established. Overexpression of cyclinD3 or E made cells less sensitive to the signal that induces growth arrest while the overexpression of cdk2 had no effect, indicating that cyclinD3 and E play a crucial role for the growth arrest induced by the activation of T cells.

V 586 INDUCTION OF COMPETENCE STIMULATES EXPRESSION OF CDK4 IN T CELLS, BUT EXPRESSION OF CDK2 REQUIRES AN IL-2-DEPENDENT SIGNAL, Jaime F. Modiano, Joanne Domenico, Joseph J. Lucas, and Erwin W. Gelfand, Department of Pediatrics, National Jewish Center For Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

Recently, several new members of the cyclin-dependent kinase (cdk) family of proteins have been identified and postulated to be important for cell cycle progression. Our goals are to define the events that control growth and proliferation of *normal* T cells, particularly those signals that render these cells competent and those that control progression or passage through the Restriction (R) point. Competent T cells are those that exit G_0, but stop their passage through the cell cycle at a defined point before entry into S phase (i.e., before the R point). These cells have been termed competent due to the fact that the addition of a second signal such as interleukin-2 (IL-2) will allow the cells to progress into S phase and undergo cell division. Our data show that expression of cdk4, but not of cdk2, was induced in competent T cells independent of an IL-2-dependent signal. A further increase in cdk4 mRNA expression was seen upon stimulation of competent T cells by IL-2. IL-2 also induced the expression of cdk2 mRNA in these cells. Thus cdk4 may be necessary, but not sufficient for progression through G_1. Furthermore, the absence of cdk2 expression may be responsible, at least in part, for the growth arrest seen in competent T cells. An alternative possibility is that cdk2 expression is blocked in these cells due to the growth-arrest occurring prior to the transcriptional activation of the cdk2 gene.

V 587 IL-6-INDUCED HOMODIMERIZATION OF gp130 AND ASSOCIATED ACTIVATION OF A TYROSINE KINASE.

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The biological functions of interleukin-6 (IL-6) are mediated through a signal-transducing component of the IL-6 receptor, gp130, which is associated with the ligand-occupied IL-6 receptor (IL-6R) protein. Binding of IL-6 to IL-6R induced disulfide-linked homodimerization of gp130. Tyrosine kinase activity was associated with dimerized but not monomeric gp130 protein. Substitution of serine for proline residues 856 and 858 in the cytoplasmic motif abolished tyrosine kinase activation and cellular responses but not homodimerization of gp130. The IL-6-induced gp130 homodimer appears to be similar in function to the heterodimer formed between the leukemia inhibitory factor (LIF) receptor (LIFR) and gp130 in response to the LIF or cliary neurotrophic factor (CNTF). Thus, a general first step in IL-6-related cytokine signaling may be the dimerization of signal-transducing molecules and activation of associated tyrosine kinases.

V 588 CD45 IS A SUBSTRATE FOR THE p50^{csk} KINASE.

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Src-family protein tyrosine kinases (PTKs) play an essential role in antigen-receptor initiated lymphocyte activation. Their activity is largely regulated by a negative regulatory tyrosine which is a substrate for the activating action of the CD45 phosphotyrosine phosphatase (PTPase) or, conversely, the suppressing action of the cytosolic p50^{csk} PTK. We have found that CD45 is phosphorylated by p50^{csk} *in vitro* on two tyrosine residues, one of them identified as Tyr-1193. This residue was not phosphorylated by the T cell PTKs, p56^{lck} or p59^{fyn}. Tyr-1193 was phosphorylated in intact T cells, and phosphorylation increased upon treatment with PTPase inhibitors, indicating that this tyrosine is a target for a constitutively active PTK. Co-transfection of CD45 and p50^{csk} into COS cells also lead to tyrosine phosphorylation of CD45 *in vivo*. Tyrosine-phosphorylated CD45 bound p56^{lck} through the SH2 domain of the kinase and p50^{csk}-mediated phosphorylation of CD45 caused a several-fold increase in its PTPase activity. We have also found that p50^{csk} is activated in Jurkat T cells within 1 min after stimulation of the cells with anti-CD3 mAbs. In parallel with this activation, p50^{csk} formed a stable complex with one major 72 kDa tyrosine phosphorylated protein and minor polypeptides at 90 and 110 kDa. The isolated SH2 domain of p50^{csk} specifically bound the 72 kDa protein in lysates from activated, but not resting, T or B cells. By several criteria, the associated proteins were not tyrosine kinases themselves and they did not react with antibodies to a panel of known proteins. Our results suggest that p50^{csk} is activated and engaged via its SH2 domain during T cell activation and may also have CD45 as a physiological target.

V 589 Ca^{2+} DEPENDENCE OF GENE EXPRESSION IN SINGLE T LYMPHOCYTES, Paul A. Negulescu, Nilabh Shastri* and Michael D. Cahalan, Department of Physiology and Biophysics, University of California, Irvine, CA 92717,* Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

In T lymphocytes, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ rises within seconds of T-cell antigen receptor (TCR) stimulation and initiates the synthesis and secretion of interleukin-2 (IL-2), a cytokine essential for T cell proliferation and the immune response. Using video-imaging techniques, we tracked $[Ca^{2+}]_i$ signals in individual T cells and measured subsequent expression of a ß-galactosidase reporter gene (*lacZ*) controlled by the NFAT element of the IL-2 enhancer. TCR activation by immobilized αCD3 caused $[Ca^{2+}]_i$ spikes which were positively correlated with gene expression but varied widely between individual cells and were therefore difficult to relate quantitatively to *lacZ* expression. The $[Ca^{2+}]_i$ dependence of NFAT-regulated gene expression was determined by elevating $[Ca^{2+}]_i$ with either thapsigargin or ionomycin and then "clamping" $[Ca^{2+}]_i$ to various, stable levels by altering either $[Ca^{2+}]_i$ or $[K^+]_o$. Using the Ca^{2+} -clamp we elicited either $[Ca^{2+}]_i$ oscillations or sustained $[Ca^{2+}]_i$ increases. Raising $[Ca^{2+}]_i$ from resting levels of 70 nM to between 200 nM and 1.6 µM increased the fraction of cells expressing *lacZ* with a K_d of ≈1 µM, indicating a wide $[Ca^{2+}]_i$ threshold for activation between different cells. Activation of protein kinase C enhanced the $[Ca^{2+}]_i$ sensitivity of gene expression (K_d = 210 nM), whereas stimulation of protein kinase A inhibited $[Ca^{2+}]_i$ dependent gene expression, indicating the convergence of at least three signalling pathways on the regulation of the NFAT element of the IL-2 promoter. The experiments described here provide the first single-cell measurements linking a second messenger to gene expression in individual cells.

V 590 ACTIVATION OF CaM KINASE VIA T CELL RECEPTOR SIGNALLING AND IL-2-SPECIFIC TRANSCRIPTIONAL BLOCK. Paul Nghiem, Phyllis Gardner and Howard Schulman, Departments of Pharmacology and Medicine, Stanford Medical School, Stanford, CA 94305.

T cell receptor-mediated Ca^{2+} influx, depending on the presence of costimulation, can lead either to activation via calcineurin or to a prolonged state of interleukin-2 (IL-2) non-responsiveness via unknown signalling mechanisms. Ca^{2+}-mediated negative regulation of IL-2 transcription can be induced in Jurkat T cells by pretreatment with a Ca^{2+} ionophore causing a significant and specific block of inducible IL-2 transcription. Calcineurin does not appear to mediate this block as transfection of constitutive calcineurin in the absence of costimulation potentiates rather than diminishes later IL-2 activation.

Multifunctional Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase) is a mediator of calcium signals in diverse signalling pathways. Because of its potential involvement in T lymphocyte signal transduction, we have cloned and characterized an isoform of CaM kinase from human T cells (Nghiem, et al JBC 268:5471). Lymphocyte CaM kinase displayed two phases of autophosphorylation characteristic of CaM kinases, including the phase which converts it to a partially Ca^{2+}-independent species.

By following conversion of CaM kinase to a calcium-independent enzyme we found that T cell receptor signalling potently activates endogenous multifunctional CaM kinase. Transfection of a partially constitutive mutant of the T lymphocyte CaM kinase blocks 90% of PMA + ionomycin-inducible IL-2 reporter gene activity. Presence of the constitutive CaM kinase did not however affect basal transcription from a β-actin promoter or PMA-stimulated c-*fos* transcription. Moreover, in the same cells in which IL-2 is blocked and two endogenous promoters are unaffected, an RSV promoter is activated 300% by constitutive CaM kinase. Analysis of the critical transcriptional regulatory sites in the IL-2 enhancer revealed that CaM kinase exerts effects at several sites, partially blocking stimulated transcriptional activity from multimers of NFAT (69% block), AP-1 (37%), and NFIL-2A (53%) DNA elements linked to reporter genes. Constitutive CaM kinase also attenuates IL-2 activation via constitutive calcineurin plus PMA. These findings suggest that these two Ca^{2+}/calmodulin responsive enzymes, CaM kinase and calcineurin, could mediate the divergent effects of Ca^{2+} signals in T lymphocyte regulation.

V 591 REGULATION OF ANTIGEN-SPECIFIC T CELL PROLIFERATION BY MICROTUBULE FUNCTION AND T CELL SURFACE-EXPRESSED MHC CLASS II, Quoc V. Nguyen, Rebecca L. King. Department of Pediatrics, SUNY Health Science Center, Syracuse, NY 13210.

We have characterized the regulation of antigen presentation by microtubule function and by T helper surface-expressed MHC class II molecules in a tetanus toxoid (TT)-specific, class II-restricted antigen presentation system. We have used nocodazole (ND), and taxol (TX), to treat either antigen presenting cells (APC) or T clones, and pre-incubation of T cells with whole or Fab of 9.3F10, an anti-class II monoclonal antibody (mAb), before T cells proliferation assay. When APC's were treated with microtubule disrupters during TT pulse, T clones' proliferation was markedly reduced compared to parallel untreated experiments. Both drugs also decreased T clones' proliferation when only T cells were pre-treated. The inhibitory effect of ND was partially reversible while TX's action was irreversible. When T helper cells were preincubated with 9.3F10 mAb (both for 1 hr on ice, or by overnight incubation at 37°C then washed) before incubation with TT-pulsed irradiated APC, there was a reduction of T clones' proliferation. Similar decrease in T cell proliferation was obtained with in papain-digested incubation Fab of 9.3F10. Since MHC class II processing and transport were independent of microtubule function (at least on B cells), we are testing the hypothesis that the disruption of microtubule function and ligand binding to surface-expressed MHC class II on T cells both inhibit transduction of secondary messenger signals crucial for T cell activation and proliferation.

V 592 PROTEIN TYROSINE PHOSPHORYLATION IN ANERGIC HUMAN T-CELLS, Jeffrey P.Novack, G.T. Nepom, and William Kwok, Virginia Mason Research Center, 1000 Seneca St., Seattle, WA 98101

Protein tyrosine phosphorylation has been demonstrated to be essential for signal transduction by the T-cell receptor. Anergic T-cells are unable to signal through the T-cell receptor after an initial anergizing event. Signalling through the T-cell receptor without a co-stimulatory signal is thought to induce anergy. Anergic cells undergo a number of activation events, but fail to produce IL-2 or proliferate when re-challenged with APC and antigen. Protein tyrosine phosphorylation in anergic mouse cells (in vivo and in vitro)has been shown to be impaired(Bhandoola, A., J. Immun. 151:2355 and Cho,E.A., J. Immun. 151:20 1993). We confirm this observation in human T-cell clones, PHA blasts and Jurkat cells anergized with anti-CD3 antibody or ionomycin. Also, we compare the effects on tyrosine phosphorylation of anergy achieved by stimulating only through the T-cell receptor with stimulation of both the T-cell receptor and Class II. Further, we find a tyrosine phosphorylated protein that is present only in anergized cells and not in control cells. We are attempting to identify this protein in order to determine it's possible role in T-cell anergy.

V 593 DISTINCT EFFECTS ON T CELL ACTIVATION INDUCED BY OVEREXPRESSION OF p56^{lck} AND p59^{fyn} IN A T CELL HYBRIDOMA. Michael W. Olszowy, Kenneth M. Murphy, Suzanne J. Szabo, and Andrey S. Shaw, Department of Pathology, Washington University, St. Louis, MO 63110.

To examine the role of src-family kinases in T cell activation, we overexpressed p56^{lck}, p59^{fyn} and p60^{src} in a murine, ovalbumin-specific, CD4^{+}, class II -restricted T cell hybridoma. Clones overexpressing wild-type and constitutively active forms of p59^{fyn} exhibited increased peptide-dependent interleukin-2 (IL-2) release. Control cells or cells expressing p60^{src} were unaffected. Clones overexpressing wild-type and constitutively active forms p56^{lck} released IL-2 in a peptide-independent major histocompatability (MHC)-restricted fashion. Moreover, clones expressing a p56^{lck} mutant which is incapable of associating with CD4 expressed a similar phenotype. The p56^{lck} overexpressing clones were also distinct biochemically in that they could be stimulated to release IL-2 through activation of protein kinase C alone. These data extend the overall evidence supporting a positive role for p59^{fyn} in T cell activation and demonstrate that p59^{fyn} and p56^{lck} have distinct roles in T cell activation. In addition, these studies demonstrate a CD4-independent mechanism of p56^{lck} signalling and link p56^{lck} to a Ca^{++}-dependent pathway of signal transduction.

V 594 THE LATENT MEMBRANE PROTEIN 1 OF EBV INDUCES FULL ACTIVATION, DNA SYNTHESIS AND BCL-2 IN HUMAN B-CELLS

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The Epstein-Barr Virus (EBV) immortalizes human primary B-cells *in vitro*. The Latent Membrane Protein 1 (LMP1) is one of 10 EBV genes expressed in immortalized B cells. LMP1 is made of six transmembrane domains, a cytoplasmic 25aa amino-terminal tail and a 200aa carboxyl-terminal cytoplasmic tail. LMP1 forms discrete patches (0.2-1μm diameter) on the plasma membrane and associates with the cytoskeleton and thus makes a bridge between the plasma membrane and the cytoskeleton. We have transfected the LMP1 gene, together with a truncated rat CD2 gene as co-transfection sortable marker, in human primary B-cells and made the following observations (within 48 hours of transfection): 1) Transient LMP1 gene expression results in normal LMP1 protein distribution, i.e. formation of discrete patches on the plasma membrane; 2) LMP1 induces dramatic cell size increase, and upregulation of the surface activation markers ICAM-1, LFA-1, CD21, CD23 and CD71 (the transferrin receptor; a marker of readiness for S-phase); 3) LMP1 transient expression induces transient DNA synthesis (peak at around 48 hours post-transfection); 4) The induction of CD71 by LMP1 is inhibited by co-transfection of the Rb gene (showing that LMP1 does not bypass this G1/S transition check-point); 5) LMP1 induces bcl-2 expression. We conclude that LMP1 expression is sufficient to reconstitute the essential early events of B-cell immortalization by EBV. Elucidating the precise biochemical activity of the LMP1 protein, which shares no sequence homology with any known protein, should reveal key aspects of B-cell growth regulation.

V 595 IDENTIFICATION OF PKC DEPENDENT AND ATP DEPENDENT CYTOLYTIC PATHWAYS IN THE YT CYTOTOXIC TUMOR CELL LINE. Joanne Pocsidio, Ballabh Das and Allen J. Norin. Departments of Medicine, Surgery, and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

Evidence for three mechanisms of lymphocyte mediated destruction of tumor cells have been demonstrated involving; DNA breakdown, granule exocytosis and osmotic shock. The latter process undoubtedly requires energy to transport extracellular H+ across the plasma membrane. It is possible that the β subunit of H+ transporting ATPase (found on the surface of tumor cells) may provide this source of energy by hydrolysis of extracellular ATP. Previous studies suggested the utilization of alternate cytolytic pathways by a human lymphoid tumor cell line (YT). YT destruction of the EBV transformed tumor line (Raji) was inhibited by low doses of antibody directed against the ATP binding region of β-H+ ATPase (anti-β). Cytolytic activity of YT was, in fact, elevated by high concentrations of anti-β though similar concentrations of this antibody blocked LAK and NK cytotoxicity by 75% to 100%, respectively. Since β H+ ATPase is expressed on YT as well as the target cells, high antibody concentrations may serve to crosslink these cells resulting in β-H+ATPase independent destruction. To examine this hypothesis we studied cytotoxic killing of PMA treated YT cells in the presence of high concentrations of anti-β. Pretreatment of YT cells with PMA exhausts PKC activity required for granule exocytosis (JI 143:2120,1989). We reasoned, therefore, that under this latter condition, elevated cytolytic activity would not be observed with high doses of anti-β if crosslinking of effector-target cells utilized the granule exocytosis pathway. PMA treatment alone inhibited YT cytotoxicity by 50%. Anti-β treatment of YT increased cytotoxicity by 50%. Cytolytic activity of PMA treated YT cells was not enhanced by incubation with high concentrations of anti-β (ie. stimulation of cytotoxicity by anti-β is prevented by preincubation with PMA). We conclude that anti-β augmented killing of YT is due to a PKC dependent cytolytic pathway (eg. granule exocytosis). It is not clear, whether the PKC dependent pathway and the ATP dependent pathway function simultaneously.

V 596 CLONING, MODELING AND COMPARATIVE ANALYSIS OF THE PROTEIN TYROSINE KINASE *SYK*, Ellen Puré, Phoebe Stewart, Julie Famiglietti, Roger Burnett and Mark S. Forman, The Wistar Institute, Philadelphia, PA 19104

Several non-receptor protein tyrosine kinases (PTK) are implicated in the BCR signaling pathway by virtue of their association with mIg and induction in response to receptor crosslinking. These include *lyn*, *fyn*, *blk* and *lck*, members of the src family of PTK as well as *syk*, a member of a novel family of PTK. In addition, in an *in vitro* phosphotransferase assay, *syk* is one of the predominant tyrosine phosphorylated substrates and activities induced by cross-linking mIg. Anti-Ig and IL-4 act synergistically to induce this response.

We have isolated a 2607 bp full length cDNA clone of human *syk*. This clone has an open reading frame of 1890 bp encoding a 630 amino acid polypeptide with a calculated molecular weight of 71,632 daltons. The deduced amino acid sequence contains two src homology region 2 (SH2) domains as well as a conserved PTK domain. The amino acid sequence has 93.5% identity with the previously characterized porcine *syk*. In the kinase and SH2 domains, *syk* has sequence identity of 62% and 55% respectively with ZAP-70. This sequence identity is generally around 30-40% for other PTK. Based on the known structures of the SH2 domains of *src* and *abl*, as well as the modeling program "profile", we generated model structures of the N and C terminal SH2 domains of *syk*. For each SH2 domain, there is a predicted well conserved phospho-tyrosine binding pocket and a less well conserved hydrophobic specificity pocket. However, the surface of the regions bridging these two pockets is significantly different from each other and from *src* and *abl* suggesting different substrate specificities.

Using these predicted structures as well as predicted regulatory regions in the kinase domain, we targeted mutations that are likely to effect functions specific to *syk*. In conjunction with domain specific antibodies, these transfectants are being used to elucidate the role of *syk* in BCR-mediated signaling.

V 597 **THE QUINOLONE CP-115,953 STIMULATES CYTOKINE TRANSCRIPTION,** K. Riesbeck and A. Forsgren, Department of Medical Microbiology, Lund University, Malmö General Hospital, S-214 01 Malmö, Sweden

The cytotoxic quinolone CP-115,953 specifically excerts its inhibitory effect upon eukaryotic topoisomerase II. CP-115,953 stimulates DNA cleavage mediated by topoisomerase II with a potency approximately 600 times greater than ciprofloxacin, a quinolone antibacterial agent that currently is in clinical use. Since ciprofloxacin has been reported to strongly enhance interleukin-2 (IL-2) production, we considered it important to study the effect of CP-115,953 (6.25 and 25 μM) on IL-2 and interferon-γ (IFN-γ) mRNA and protein expression in mitogen stimulated human peripheral blood lymphocytes. Furthermore, IL-2 gene transcription was investigated by transfection of the human T-cell lymphoma cell line Jurkat with CAT-reporter gene constructs. CP-115,953 enhanced IL-2 mRNA levels up to 8-fold and IFN-γ mRNA concentrations up to 6.5-fold. In contrast, ciprofloxacin strongly induced mRNAs for IL-2 (20-fold) and IFN-γ (7.8-fold). However, CP-115,953 showed a more prolonged kinetics of IFN-γ mRNA when compared to ciprofloxacin. IL-2 transcription (IL-2-CAT-expression) was increased by both CP-115,953 (1.8-fold) and ciprofloxacin (4.5-fold). Ciprofloxacin was a greater inducer of IL-2 production, but performed an equally low stimulatory action as CP-115,953 on IFN-γ synthesis. mRNA levels for the oncogenes c-jun and c-fos were unchanged in the presence of CP-115,953 suggesting a topoisomerase II inhibition and not an activation pathway resembling the DNA damaging response reported in the presence of cytostatic drugs or x-ray irradiation. The promising stimulative effect of T cell cytokines in combination with a cell inhibiting activity by CP-115,953 warrants further investigations.

V 598 HETEROGENEITY OF Mg^{2+} MOBILIZATION RESPONSE IN PERIPHERAL BLOOD MONONUCLEAR CELLS, Ger T. Rijkers, Marleen M. Voorhorst-Ogink, Nic Henriquez and Ben J.M. Zegers.Dept of Immunology, University Hospital for Children and Youth "Het Wilhelmina Kinderziekenhuis" Nieuwegracht 137, 3512 LK Utrecht, The Netherlands.

We previously have shown that activation of human lymphocytes with the Ca^{2+} ionophore ionomycin induces an increase $[Mg^{2+}]_i$. In order to obtain more insight into the relevance of a Mg^{2+} mobilization response in the process of lymphocyte activation, we have studied the dose dependency (0.05-10 μM ionomycin) of this response in subsets of MNC. To that end, human peripheral blood MNC were loaded with the Mg^{2+} indicator mag-indo-1, cell surface stained with phycoerythrin or FITC conjugated CD4, CD8, CD14, CD16, or CD20 antibodies and analyzed by flow cytometry. A significant Mg^{2+} response is observed with 0.25-0.5 μM ionomycin in $CD20^+$ B cells; $CD4^+$ or $CD8^+$ T cells require 1-2.5 μM, $CD16^+$ NK cells 2.5-5 μM ionomycin. CD14 "bright" monocytes show increased $[Mg^{2+}]_i$ at relative high ionomycin concentrations (5 μM), CD14 "dull" monocytes already respond at 0.25 μM. At intermediate ionomycin concentrations (e.g. 2.5 μM in the case of $CD4^+$ T cells), a proportion of cells does show a vigourous Mg^{2+} mobilization response, while $[Mg^{2+}]_i$ in the remainder of the cells remains at resting levels. We will study the impact of this differential Mg^{2+} response for ultimate cell activation by cell sorting on basis of $[Mg^{2+}]_i$, followed by functional analysis.

V 599 DISSECTION OF CA^{2+}-MOBILIZATION AND IL-2 PRODUCTION UPON TCR-TRIGGERING IN A MOUSE T-CELL HYBRIDOMA

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We have previously described the isolation of a collagen type II (CII) specific human autoreactive T-cell clone. To establish a stable read-out system for investigating the structural requirements for CII-recognition, we have transfected chimeric TCR α- and β-chains, consisting of human variable regions and mouse constant parts, into a mouse T-cell hybridoma devoid of endogenous TCR-chains but expressing transfected human CD4.

This cell line can be stimulated for IL-2 production by co-crosslinking of anti-TCR with anti-CD4 antibodies as well as by the specific antigen and antigen derived-peptides. Co-crosslinking but not crosslinking of either anti-TCR or anti-CD4 alone resulted in tyrosin phosphorylation of several intracellular proteins.

Surprisingly, activation of the transfectant with TCR-ligands does not lead to an increase of cytosolic Ca^{2+}-concentration. It was interesting to note, that the transfected T-cell hybridoma is not undergoing apoptosis after TCR/CD4-triggering. In conclusion, early activation steps may exist, that are able to circumvent the IP_3/Ca^{2+}-pathway in TCR-mediated stimulation of IL-2 transcription.

V 600 ASSOCIATION OF FCγRII OF B-CELLS WITH PROTEIN TYROSINE KINASE FYN AND SERINE/THREONINE KINASE (PKC α), Gabriella Sarmay, Israel Pecht and Janos Gergely, Lab. of Immunoregulation, Vienna Int. Res. Coop. Center at SFI, Vienna, Austria; Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel; and Dept. of Immunol. of L. Eotvos Univ. God, Hungary.

We have reported that FcγRII are one of those proteins that undergo serine phosphorylation shortly after B-cell activation. Upon affinity isolation of FcγRII, several molecular entities are co-isolated from the lysates of BL41 Burkitt lymphoma line and tonsil B-cells which undergo "in vitro" phosphorylation in the immune complex-associated kinase assay. The majority of this phosphorylation occurred on ser/thr residues. Furthermore, several of the proteins co-isolated with FcγRII from the lysates of BL41 cells activated by sIgM cross-linking undergo phosphorylation on tyr residues in the intact cells. One of these, corresponding to the 59 kDa co-precipitated component, is identified as the protein tyrosine kinase (PTK) fyn, further two molecules were found to co-migrate with ras GAP and PLC γ, respectively. Clustering the surface IgM molecules on BL41 cells enhanced the in vitro phosphorylation of all molecules co-precipitated with FcγRII as well as that of the exogenously added PTK substrate, enolase. Kinase renaturation assays suggest that at least two major renaturable protein kinases associate with FcγRII. While the 59 kDa component co-migrate to the PTK fyn, the 85 kDa one corresponds to the protein kinase C α. Activation of PKC induces an immediate desensitization of FcγRII, possibly as a consequence of its phosphorylation on ser residue.

V 601A ROLE FOR *c-MYC* IN APOPTOSIS OF NORMAL B LYMPHOCYTES: EFFECT OF ANTISENSE OLIGOS, David W. Scott, Marinus Lamers, Georges Köhler and Rita Carsetti. Max Planck Institute for Immunobiology, Freiburg, Germany and Division of Immunology, Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642 USA.

The induction of deletional tolerance presumably results from the crosslinking of IgM receptors on specific B cells by antigen. Carsetti *et al.* recently reported that Sp6 transgenic mice expressing an IgM receptor specific for TNP showed apoptosis in a subset of splenic B cells after exposure to TNP-dextran *in vivo* or *in vitro* (*Eur. J. Immunol.* **23:** 168, 1993). In addition, previous work by one of us (DS) indicated that anti-μ crosslinking of IgM receptors on a subset of murine B-cell lymphomas led to growth arrest at G1:S border and subsequent apoptosis. Recent data indicate that growth arrest and apoptosis in these lymphomas can be prevented by treatment with antisense oligonucleotides against *c-myc*. This approach was based on the fact that anti-μ causes levels of *c-myc* message and protein to transiently rise and then disappear; antisense oligos interfere with growth arrest and apoptosis by preventing the loss of *c-myc* message (G. Fischer, *et al. J. Exp. Med.*, in press, 1994). In the current studies, we tested the effect of antisense for *c-myc* on the induction of apoptosis in transgenic and normal B cells. Interestingly, high concentrations of polyclonal anti-μ were able to induce apoptosis within 24-32 hours in both adult normal and transgenic B cells *in vitro*. Antisense for *c-myc* prevented both spontaneous and anti-μ-induced apoptosis. The implications of this result on the ability of anti-μ to signal for B-cell deletion in the presence or absence of co-stimulation will be discussed. (Supported by NIH AI29691 and The Max-Planck Gesellschaft).

V 602 TYROSINE KINASES AND GTP-BINDING PROTEINS: EVIDENCE FOR THEIR COOPERATIVITY IN T CELL ACTIVATION, Jack Stanners* and Constantine D. Tsoukas*†, *Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA. 92182. †Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, CA. 92307

Stimulation of T cells via the TCR/CD3 complex results in the activation of phospholipase C and the subsequent hydrolysis of phosphotidy inositols yielding IP_3 and DAG. These second messengers, in turn, are responsible for the mobilization and activation of intracellular calcium stores and PKC respectively.

Of the PLC isoforms expressed in T cells, only γ is known to be activated via tyrosine phosphorylation, an early event following TCR engagement, while the β isoforms are activated by association with the α subunit of the heterotrimeric class of GTP-binding proteins comprising the G_q family.

Perturbation of purified plasma membranes from the Jurkat T cell line with anti-TCR monoclonal antibodies, results in enhanced binding of ^{32}P-GTP to several membrane associated proteins. The apparent molecular weights of the proteins that bind GTP, the specificity of nucleotide binding and the ability of an antibody directed against the common GTP-binding motif to block enhanced binding, suggest that the responding proteins of approximately 50, 40 and 30 kDa represent α subunits of the heterotrimeric class of G proteins. An additional protein displaying enhanced GTP binding upon anti-TCR/CD3 triggering appears to be the ζ chain of the CD3 complex.

Pretreatment of membranes with the protein tyrosine kinase inhibitor genistein or incubation in the presence of a non - cleavable analog of ATP, significantly reduces or abolishes the enhanced ^{32}P-GTP binding observed upon antibody stimulation.

Taken together, these data suggest that G proteins are induced to exchange bound GDP for GTP as a result of engagement of the TCR/CD3 complex and that this exchange is regulated by tyrosine kinase activity. This represents a novel mechanism by which tyrosine kinases and G proteins may operate in concert to effectively initiate a T cell response.

V 603 DIFFERENT MECHANISMS OF EARLY GENE EXPRESSION IN T LYMPHOCYTES

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Activation of T lymphocytes is a complex process requiring recognition of antigen by the T cell antigen receptor as well as concomitant signals from antigen presenting cells. These events lead to the activation of second messenger pathways such as tyrosine kinases and protein kinase C and to an increase in intracellular Ca^{2+}. These signals finally initiate a sequencial activation of a group of genes that in turn give rise to proliferation and immunologic function. Detail mechanisms of these events, however, are still unclear. In order to investigate the regulation of early phase gene expression of T lymphocytes, we have constructed luciferase reporter plasmids for IL-2 gene and c-fos gene expression, respectively, and established each stable transformant of Jurkat cells. Reporting luciferase activity after stimulation, we have compared their sensitivity against several inhibitors ; Staurosporine (kinase inhibitor), K252a (kinase inhibitor), UCN-01 (PKC selective inhibitor), Calphostin C (PKC specific inhibitor), Herbimycin (tyrosine kinase inhibitor), Okadaic acid (phosphatase(PP1, PP2A) inhibitor) and Cyclosporin A. Kinase inhibitors inhibited both IL-2 and c-fos gene expression. On the other hand, Cyclosporin A inhibited IL-2 gene expression but not c-fos gene expression. Interestingly, Okadaic acid activated c-fos gene expression at concentration of 100 nM despite showing inhibitory activity against IL-2 gene expression at the same dose. These results indicate that the regulation of IL-2 gene expression is quite different from that of c-fos gene expression.

V 604 TYROSINE PROTEIN KINASE MEDIATES ANTIGEN RECEPTOR-INDUCED JUN-B EXPRESSION IN MURINE B CELLS, Debra A. Tanguay[1], Brian J. McManus[1], Thomas L. Rothstein[2], and Thomas C. Chiles[1], [1]Department of Biology, Boston College, Chestnut Hill, MA 02167 and the [2]Department of Medicine and Microbiology, Boston University Medical Center, Boston, MA 02118

B cell antigen receptor (BCR) engagement by anti-IgM induces the transient expression of the immediate-early gene, *jun-B*. Studies herein examine the expression of the protein encode by *jun-B*. Two polypeptide forms of Jun-B corresponding to 39 and 43 kDa were rapidly induced and remained elevated for at least 10 h after BCR cross-linking; however, they differ with respect to the kinetics of expression. The increase in Jun-B expression was attributed to *de novo* protein synthesis within the first hour of anti-IgM stimulation and reached maximal levels by 3 h. Studies designed to ascertain the coupling of Jun-B to known signals generated via BCR, indicate that down regulation of PKC reduces subsequent BCR-induced Jun-B expression. Inactivation of BCR-associated protein tyrosine kinases by pre-treatment with either herbimycin A or tyrphostin inhibits subsequent BCR-mediated Jun-B expression. Immunoreactive Jun-B was detected in native c-Fos protein complexes and in BCR-induced nuclear proteins eluted from the *cis*-acting AP-1 sequence. These results suggest that Jun-B may function in the BCR signal transduction cascade by coupling second messenger molecules to nuclear gene expression. We are currently developing antisense methodologies to test this hypothesis.

V 605 DEVELOPMENT OF NOVEL AND SPECIFIC PHARMACOLOGIC INHIBITORS OF INTERLUKIN 2 SIGNAL TRANSDUCTION William Tino, Stuart Bursten, Mary Valentine, Reitha Weeks, James Bianco, Jack Singer, and Glenn Rice Cell Therapeutics, Inc., Seattle, WA 98119.

Recent reports suggest that the breakdown of inositol phosphate glycan (gly-PI) and the induction of the lipid second messenger molecules myristylated diaglycerol (mDAG) and possibly myristylated phosphatidic acid (mPA) are involved in the signal transduction events mediated by IL2. We show here that CT2576, representative of a novel class of synthetic small molecule compounds, inhibits the IL2 induced breakdown of gly-PI and the subsequent generation of mDAG and mPA in an IL2 dependent cytotoxic T-cell clone, CT6. CT2576 inhibits IL2 induced proliferation in CT6 cells as well as the proliferation of anti-CD3-activated thymocytes or anti-CD3-activated thymocytes or splenic T-cells in response to IL2 (IC-50's 200-850nM). Flow cytometric analysis reveals that CT-2576 does not inhibit CD3-mediated upregulation of the p55 IL2 receptor (CD25). In ConA-activated thymocytes CT2576 has no effect on IL2 release, but markedly inhibits release of interferon-γ and IL-4. CT2576 can induce antigen-specific T-cell anergy in a primary mixed tumor-lymphocyte culture using responding C57BL/6 spenocytes (H-2b) and 2PK3 B-cell tumor cells (H2d). Following a five day co-culture with CT2576 and antigen, splenocytes were unable to respond to a rechallenge with the primary antigen but could respond normally to anti-CD3 stimulation. Preliminary data using lysates from IL2 stimulated CT6 cells shows that CT2576 has no effect on the IL2-induced MAP kinase activity, nor does it inhibit IL-2 induced c-myc expression. CT2576 also showed little effect on p70S6 kinase activity. These data suggest that the mechanism of immunosuppression of CT2576 is distinct from that of rapamycin as well as the calcineurin inhibitors cyclosporin A and FK506. CT2576 may have significant utility in unraveling the mechanisms of IL2 signaling, and more importantly, may be representative of a new class of immunosuppressive compounds.

V 606 DETERMINATION OF THE OPTIMAL Ca^{2+} REQUIREMENTS FOR T CELL PROLIFERATION, Alain Trautmann, Emmanuel Donnadieu and *Georges Bismuth, Laboratoire de Neurobiologie, E.N.S., 46, rue d'Ulm, 75005 Paris, and *Laboratoire d'Immunologie Cellulaire et Tissulaire, Groupe Hospitalier Pitié-Salpêtrière, 75013 Paris, France.

Using a human T cell clone (P28D) and various stimuli, we have measured the magnitude of the augmentation of intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$ (measured in single cells with fura-2 on an imaging system) which was optimal for eliciting T cell proliferation. All stimulations were done in the presence of 10 nM PMA. The relation between T cell proliferation and the increase in $[Ca^{2+}]_i$ following stimulation with ionomycin was bell-shaped, maximum proliferation occuring for $[Ca^{2+}]_i$ increases of 400-900 nM. The $[Ca^{2+}]_i$ oscillations elicited by an anti-CD3 antibody (UCHT1, 1/1000) precisely fall in the same range. However, because of the asynchrony between the different cells, the average $[Ca^{2+}]_i$ response induced by UCHT1 (1/1000) was always smaller than 400 nM. In addition, very low UCHT1 concentrations (1/50.000) giving rise to sparse $[Ca^{2+}]_i$ transients of small amplitude in single cells, and to an almost undetectable $[Ca^{2+}]_i$ response in a cell population, still efficiently induce T cell proliferation. The minimum $[Ca^{2+}]_i$ response required for T cell proliferation following ionomycin stimulation could be much higher than following stimulation with UCHT1 because one type of $[Ca^{2+}]_i$ response is oscillating (after UCHT1) whereas the other is not (after ionomycin). Various experiments (namely, using low thapsigargin concentrations to elicit $[Ca^{2+}]_i$ oscillations) show that this hypothesis is incorrect. The alternative explanation is that, in combination with other metabolic pathways activated by UCHT1, Ca^{2+} requirements become very low. Their nature remains to be determined. A puzzling observation is that with very low concentrations of UCHT1 (1/50.000), tyrosine phosphorylations are almost undetectable.

V 607 SPECIFIC ASSOCIATION OF THE 116-kDa AND THE 82-kDa PROTEINS WITH THE TYROSINE PROTEIN KINASE P60*FYN* IN T CELLS, Alexander Y. Tsygankov, Carl Spana, R. Bruce Rowley, Robert C. Penhallow, Anne L. Burkhardt and Joseph B. Bolen. Signal Transduction Laboratory, BMS PRI, Princeton, NJ 08543-4000

The tyrosine protein kinase $p60^{fyn}$ is involved in signaling through the T-cell antigen receptor (TcR). However, the interactions of $p60^{fyn}$ with other proteins participating in TcR-signaling remain to be understood. We previously demonstrated that $p60^{fyn}$ became enzymatically activated following TcR-triggering and that this activation was accompanied by tyrosine phosphorylation of two proteins coprecipitating with $p60^{fyn}$, p82 and p116. Now we demonstrate that this association is mediated by the *src*-homology domains 2 and 3 of $p60^{fyn}$. It appears that the 82-kDa protein binds SH2 as well as SH3, whereas binding of p116 is strictly SH2-specific. Neither $p56^{lck}$ nor $p62^{c\text{-}yes}$ were able to coprecipitate these proteins in significant quantities from cell lysates. However *in vitro*, following kinase assay in the $p60^{fyn}$ immune complexes, p82 and p116 could be reprecipitated by GST-fusion proteins containing sequences of $p60^{fyn}$ or $p62^{c\text{-}yes}$, but not that of $p56^{lck}$. The SH2 domains of all three T-cell *src*-related kinases bound p116 and p82, and the SH3 domains bound p82, thus demonstrating a low selectivity of binding. It has been shown that phosphorylation of the $p60^{fyn}$-associated proteins occurs in various kinds of T cells and several other hematopoietic cell lines. The p116 protein has been shown to become tyrosine phosphorylated *in vivo* following ligation of the TcR. Stable association of p116 and p82 with $p60^{fyn}$ as well as the wide distribution of 115-120-kDa $p60^{fyn}$-associated proteins in hematopoietic cells may suggest that p116 and p82 play a role as physiological substrates and/or regulators of $p60^{fyn}$.

V 608 SIGNAL TRANSDUCTION THROUGH MHC CLASS I AND CLASS II MOLECULES LEADS TO POSITIVE AND NEGATIVE REGULATION OF HOMOTYPIC ADHESION IN HUMAN LYMPHOCYTES, Norbert Wagner*, Pablo Engel*, Miguel Vega• and Thomas F. Tedder*, *Division of Tumor Immunology, Dana-Farber Cancer Institute, and the Departments of Medicine and Pathology, Harvard Medical School, Boston, MA 02115-6084 and the •Servicio de Immunología, Hospital de la Princesa, Universidad de Autónoma de Madrid, 28006 Madrid.

Engagement of multiple lymphocyte cell-surface molecules, including MHC class I and class II antigens, can activate an array of intracellular signal transduction pathways that upregulate the activity of cell-surface LFA-1/ICAM-1 and other unidentified adhesion receptors resulting in homotypic cell-cell aggregation. In this study, engagement of MHC class I and class II molecules with specific mAb was shown to also generate signals that could result in inhibition of lymphocyte homotypic adhesion. Two mAb reactive with class II Ag, HAB-2 and HAB-3, and one mAb reactive with class I Ag, HAB-4, were developed that inhibited homotypic adhesion of B and T cell lines induced through class II, CD19, CD20, CD39, CD40, and Leu-13 as well as through phorbol esters. In addition, the spontaneous adhesion exhibited by some cell lines was also abrogated by binding of these mAb. The HAB-2 and HAB-4 mAb were inhibitory at concentrations as low as 0.1 μg/ml. Crossblocking experiments demonstrated that anti-class I mAb that either induce, block or have no effect on adhesion bind to distinct epitopes. Similar experiments with the anti-class II mAb revealed recognition of epitopes that are either distinct or overlap in part. MAb binding to mutant class I molecules lacking most of the cytoplasmic domain transfected into class I negative B cell lines were able to generate the appropriate signals that either induced or inhibited homotypic adhesion. In contrast, mAb binding to a chimeric molecule in which the transmembrane and cytoplasmic domains of class I were exchanged with those of the HB15 molecule did not induce or inhibit homotypic adhesion. Thus, engagement of distinct epitopes on class I or class II molecules generated different signals which either upregulated or inhibited homotypic adhesion. The signal transduction through class I molecules required the transmembrane domain, but not the cytoplasmic domain. In addition to demonstrating complexity in the signal transduction pathways activated, these results suggest that MHC class I and class II molecules as signalling molecules may be involved in the regulation of adhesive events during lymphocyte activation.

V 609 DIFFERENT SH2 DOMAINS BIND OVERLAPPING SETS OF TYROSINE PHOSPHORYLATED PROTEINS FROM ACTIVATED B AND T CELL LINES.

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The antigen receptors on B and T cells transmit their activation signals to the cell interior by associating with and activation of specific non-receptor tyrosine kinases. Most of these intra-cellular kinases contain at least one of the non-catalytical src homology region 2 (SH2) domains. By binding tyrosine phosphorylated proteins SH2 domains connect different cytoplasmic components of the signal transduction cascade. To test the binding specificity of SH2 domains, Glutathione S-transferase (GST) fusion proteins were produced encompassing SH2 domains of src-related tyrosine kinases, of the ZAP-70 tyrosine kinase and of the GTPase-activating protein GAP. The GST-fusions were used in a Western Blot assay to probe anti-phosphotyrosine precipitates from non-stimulated and stimulated B and T cell lines. From each cell type only subsets of antigen receptor-regulated phosphoproteins were detected. In this assay the phosphoproteins are presented to the SH2 domains in a denatured form. To test for direct protein-protein interaction the GST-fusions were used for affinity purification. Most of the GST-fusions precipitated a set of stimulation-dependent phosphoproteins similar to that obtained with anti-phosphotyrosine antibodies. The GST-fusion containing the C- and N-terminal SH2 domains of ZAP-70 bound only components of the T cell receptor. Structurally, the results show that the three-dimensional conformation of an SH2 ligand contributes to the binding specificity of SH2 domains. Functionally, the restricted binding capacity of the SH2 domains from ZAP-70 suggest a model of how the ZAP-70 kinase is recruited specifically to the activated antigen receptor.

V 610 THE ROLE OF INTERLEUKIN-2 RECEPTOR BETA AND GAMMA CHAIN IN INTERLEUKIN-2 DEPENDENT SIGNAL TRANSDUCTION, Peter Williamson, Isabel Merida and Glen Gaulton. Department of Pathology and Laboratory Medicine, University of Pennsylvania. Philadelphia PA 19104.

Growth and differentiation signals induced by interleukin-2 (IL-2) are mediated through the heterotrimeric IL-2 receptor (IL-2R) complex. Both the β- and γ-chain subunits are essential for formation of intermediate and high affinity receptors, and are required for signaling. IL-2Rβ has a 286 amino acid cytoplasmic domain in which two regions, the S-region and A-region, have been implicated in signal transduction. IL-2Rγ has an 86 amino acid cytoplasmic domain that contains an SH2-like domain that has also been implicated in signal transduction. Mutations which affect the function of IL-2Rγ are responsible for X-linked SCID. To examine the molecular interactions involved in IL-2 dependent signaling we have employed two cellular systems. In the first we are studying the role of subregions of IL-2Rβ using a series of IL-2Rβ chain mutants transfected into the pro-B cell line Baf-B03. Using these mutants we have identified an IL-2Rβ functional domain that is responsible for activation of tyrosine kinase and PI-3 kinase activities. The results also suggest that src-kinase family members interact with IL-2Rβ between amino acid residues 323 and 350. Despite activation of these kinases the cells were not able to proliferate in response to IL-2. To study the role of IL-2Rγ, we have employed a series of cell lines derived from patients with X-linked SCID. We have characterized signaling defects in these cells, and shown that there are several proteins absent from the IL-2R signaling complex following IL-2 treatment. Studies are in progress to reconstitute IL-2 dependent signaling in these cells by gene transfer, and to identify the molecules that are associated with the renewed presence of IL-2Rγ.

V 611 IDENTIFICATION OF ETS ASSOCIATED PROTEINS,

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The v-ets oncogene was originally identified as a component of the 5'-gag-myb^{E}-ets-3' fusion protein from the avian leukosis virus E 26 (ALV-E 26). C-ets-1 the cellular counterpart of v-ets is a member of a family of genes including ets-2, erg, elk-1, elk-2, fli-1, elf-1, PU-1, SAP, and GABPα. These genes have in common a highly conserved DNA binding domain, the ets box, which binds purine rich DNA sequences. Recently a group of these Ets transcription factors have been implicated in the developmental regulation of T cell specific genes. High levels of Ets-1, Fli-1, GABPα, and Elf-1 are expressed selectively in T cells. Moreover functional Ets binding sites have been identified in the regulatory regions of many lymphoid specific genes including the T cell receptor α and T cell receptor β enhancers, and the promoters of IL-2, Lck and the IL-2 receptor β chain. Given that the binding sites recognised by the Ets proteins are short and may be recognised by more than one Ets protein, discrimination between binding sites probably occurs at the level of specific protein-protein interactions. We have used a modified yeast two hybrid system to identify and study cofactors interacting with the members of the Ets family expressed in T cells.

V 612 CALCINEURIN DEFICIENT T CELLS ARE DEFECTIVE IN T CELL RECEPTOR/CD3 SIGNALING AND REMAIN SENSITIVE TO IMMUNOSUPPRESSIVE DRUGS CsA AND FK506, Wei Zhang, J.G. Seidman, Frederick W. Alt, and Jianzhu Chen, Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115

We have tested the hypothesis that calcineurin (*CN*), a calcium and calmodulin dependent serine/threonine protein phosphatase, plays a role in T cell activation and in mediating the immunosuppressive effects of CsA and FK506. We generated $CN^{-/-}$ T cells by injection of $CN^{-/-}$ ES cells into *RAG-2*-deficient blastocysts. In the *RAG-2*$^{-/-}$ chimeras, $CN^{-/-}$ ES cells gave rise to phenotypically normal and mature T cells as assayed by surface expression of T cell receptor (TCR), CD3, CD4, CD8, and Thy-1. Both wild type and $CN^{-/-}$ T cells proliferated in response to PMA plus ionomycin and ConA. However, the $CN^{-/-}$ T cells showed a dramatically reduced proliferation in response to anti-CD3 stimulation. Both $CN^{+/+}$ and $CN^{-/-}$ T cells produced IL-2 and IL-4 and increased IL-2 receptor expression in response to all three stimuli. The defect of CD3 driven proliferation in $CN^{-/-}$ T cells could not be fully reconstituted with PMA, ionomycin, or IL-2. These findings show that distinct signals are required for T cell proliferation and lymphokine production, and that calcineurin is required for TCR/CD3 mediated T cell proliferation, but not for IL-2, IL-2 receptor, or IL-4 expression. The data also suggest that PMA plus ionomycin and ConA can stimulate T cell proliferation via alternative signaling pathways that are *CN* independent. Furthermore, when compared with normal T cells, $CN^{-/-}$ T cells remained sensitive to CsA and FK506 with respect to proliferation and lymphokine production. Whether calcineurin itself is a physiological target for these drugs remained uncertain. We conclude that there are other physiological targets for CsA and FK506 in T cells.

Cell Interactions and Memory

V 613 MICRONUTRIENT NUTRITION INFLUENCES DELAYED HYPERSENSITIVITY RESPONSES TO RECALL ANTIGENS

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The activation of lymphocytes by antigens is heavily dependent on nutritional status. Failure to react to delayed hypersensitivity skin testing (DHST) with recall antigens is common in older people and has been associated with an increased incidence of infectious diseases and with greater mortality from all causes. There is evidence that compromised micronutrient nutrition may be one factor that results in depressed cellular immune functions in older people. A placebo-controlled, double-blind trial of the effects of daily micronutrient supplements on blood micronutrient concentrations and DHST responses to a panel of 7 recall antigens was conducted. The over-the-counter micronutrient supplement used contained low to moderate doses of 13 vitamins and 9 minerals. Subjects, aged 59-85, where randomly assigned to placebo or micronutrient treatment groups. DHST and circulating concentrations of 9 micronutrients were measured before and after 6 and 12 months of placebo or micronutrient ingestion. For the micronutrient group (n=29), there were statistically significant increases at 6 and/or 12 months in serum concentrations of ascorbate, beta-carotene, folate, vitamin B6, and alpha-tocopherol; these changes were not found in the placebo group (n=27). These data suggest good compliance with the study protocol. DHST responses (induration and the number of positive responses) were significantly increased at 12 months in the micronutrient group but not the placebo group. Most of the increase in DHST responses for male subjects in the micronutrient group occurred by 6 months, but was delayed to the 6-12 month period in the women. The results demonstrate that daily supplementation with low doses of micronutrients can improve DHST responses in older adults and that the timing of this effect differs in men and women. (Supported by grants from Hoffmann-La Roche, Inc)

V 614 L-SELECTIN MEDIATES RECIRCULATION OF NAIVE BUT NOT MEMORY CD4 T CELLS TO LYMPH NODES IN VIVO.

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Extravasation from blood to lymph is necessary for continuous lymphocyte recirculation and presumably for localization of antigen-responsive lymphocytes in lymph nodes draining sites of antigen-exposure. Previous studies have shown that lymphocytes treated with antibody to L-selectin in vitro no longer circulate to lymph nodes in vivo, indicating the dependence upon L-selectin for migration into lymph nodes. Among murine CD4 T cells, naive but not memory cells express L-selectin and appear to recirculate primarily, if not exclusively by way of lymph node high endothelial venules (HEV). In contrast, memory CD4 T cells may utilize alternative adhesion receptors to migrate from the blood to lymph nodes either directly by HEV or alternatively, may first migrate to the tissues by normal or inflamed endothelium then into the lymph nodes by way of the afferent lymph. To further examine the role of L-selectin in the T cell migration, we examined the in vivo effects of antibody to L-selectin (MEL-14) on the development of primary and memory CD4 T cell responses. Systemic exposure to MEL-14 resulted in marked depletion of naive CD4 T cells expressing high levels of CD45RB and low levels of CD44 from lymph nodes but not from spleen. Residual lymph node CD4 T cells were predominately memory phenotype (CD45RB^{lo}, CD44^{hi}). MEL-14 treatment one day prior to subcutaneous immunization with keyhole limpet hemocyanin (KLH) prevented priming of naive CD4 T cells for proliferation and cytokine production (IL-2, IL-4, IFN-γ) in lymph nodes draining the site of injection, but not in the spleen. Primary humoral responses in vivo, as measured by serum IgM and IgG1 antibody specific for KLH, were delayed following MEL-14 treatment by 3 to 5 days. The results suggest that naive cells were not depleted, but rather diverted to other sites where priming occurred. Memory CD4 responses, measured 2 months after priming, were normal in MEL-14 treated mice. In addition, in vivo exposure to MEL-14 did not block the homing of memory CD4 T cells to lymph nodes. The results demonstrate that MEL-14 is exclusively utilized by naive CD4 T cells for recirculation to lymph nodes, and that such access is crucial for the development of local responses. The data support the hypothesis that memory CD4 T cells utilize alternative adhesion molecules to migrate to sites of antigen exposure.

V 615 MODIFICATIONS OF MITOCHONDRIAL MEMBRANE POTENTIAL DURING PROLIFERATION AND APOPTOSIS IN INTACT LYMPHOCYTES BY A NEW CYTOFLUORIMETRIC METHOD, Andrea Cossarizza, Galina Kalashnikova *, Miranda Baccarani Contri, Alberto Masini, Daniela Monti and Claudio Franceschi, Inst. of General Pathology, via Campi 287, 41100 Modena, Italy, and * Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia.

A new method for the cytofluorimetric analysis of mitochondrial membrane potential (MMP) in intact cells has been developed (Cossarizza A. et al., BBRC, in press) by using 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1), a lipophilic cationic probe whose monomer emits at 527 nm after excitation at 490 nm. Depending on the membrane potential, JC-1 is able of forming J-aggregates that are associated with a large shift in emission (590 nm). The color of the dye changes reversibly from green to greenish orange as the mitochondrial membrane becomes more polarized. In two human cell lines (K562 and U937), we have studied by flow cytometry the changes in MMP provoked by the K+ ionophor valinomycin, a drug known to affect mitochondrial membrane potential, while the K+/H+ ionophor nigericin, known to affect intracellular pH but not MMP, was used as control. The incubation with valinomycin for 10 min. at 37°C in a low K+ medium provoked a marked and dose-dependent reduction in JC-1 greenish orange fluorescence, while nigericin had no effect. The same approach was used to analyze MMP variations in human lymphocytes during proliferation and apoptosis. A dramatic MMP decrease was present in preapoptotic and apoptotic cells but not in proliferating cells which presented higher green and orange fluorescences, thus suggesting that mitochondrial alterations play an important role in the onset of apoptosis. On the whole, the data suggest that it is possible to assess the functional status of mitochondria in intact lymphocytes in a variety of physiopathological conditions.

V 616 RECRUITMENT AND APOPTOSIS, NOT LOCAL PROLIFERATION, DETERMINE LUNG LYMPHOCYTE NUMBERS IN THE MURINE RESPONSE TO SHEEP ERYTHROCYTES, Jeffrey L. Curtis, Gerami D. Seitzman, Alicja M. Milik, Sucha Kim, Ted F. Beals, and Patricia J. Scott, Pulmonary Section, VAMC & Departments of Internal Medicine & Pathology, University of Michigan, Ann Arbor, MI 48105

During regional immune responses, net lymphocyte accumulation reflects the balance of recruitment and emigration, proliferation and death. To devise novel immunomodulatory strategies, the relative importance of these competing processes must be determined. Throughout the response of primed mice to intratracheal sheep red blood cells (SRBC), lung CD4+ T cells have a "memory" adhesion molecule phenotype and are 30-67% CD25+, consistent with recent activation [*Chest* 1993; 103:94S]. However, based on the known anti-proliferative effects of surfactant & alveolar macrophages (Mø), we postulate that lung lymphocytes are growth-arrested and hence undergo apoptosis. To test this hypothesis, we measured nucleotide analog uptake (both *in situ* and *in vitro*) and DNA fragmentation. Two weeks after priming, C57BL/6J mice were intratracheally challenged (5 x 10^8 SRBC); from 2-7 days later, tissues were harvested without or with prior BrdU injection (4 mg IP, 1-3 doses). *In situ* labeled bronchoalveolar lavage cells (BALC) and lung sections were stained with anti-BrdU and analyzed by flow cytometry and immunohistochemistry. For *in vitro* labeling, BALC, interstitial lung cells (ILC), or Mø-depleted (G10) ILC were incubated overnight with ^{3}H-TdR, either immediately or after pre-culture with added cytokines. By all these techniques, we found minimal lung lymphocyte proliferation. *In situ* labeling was negligible (< 3 % positive). *In vitro*, BALC labeled minimally when incubated immediately with ^{3}H-TdR (maximal day 4 post challenge < 10,000 cpm, 2 x 10^5 cells). Pre-culturing BALC for 24-48 hours with added IL-2 or IL-4 (10-80 units each), or supernatants of PMA-induced EL-4 cells did not induce further proliferation. ILC and especially Mø-depleted ILC showed lower ^{3}H-TdR uptake than BALC under all conditions. Southern analysis of DNA from freshly-isolated Mø-depleted BALC showed DNA laddering and transmission electron microscopy showed lymphocyte nuclear condensation, indicating apoptosis. Thus, in this model of physiologic pulmonary immune responses most lung lymphocytes, although previously activated, do not proliferate locally and instead undergo apoptosis. Immunologic lung diseases may result from signals (including successful cell cycle progression) that protect recruited lymphocytes from apoptosis.

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V 617 DIFFERENTIAL EFFECT OF TRANSFORMING GROWTH FACTOR-β1 ON ACTIVATION OF HUMAN NAIVE AND MEMORY $CD4^+$ T LYMPHOCYTES

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Transforming Growth Factor-β1 (TGF-β1) can exhibit positive and negative effects on cell growth. To assess the immunomodulatory role of TGF-β1 in relation to the differentiation stage of $CD4^+$ T cells, we evaluated the effect of TGF-β1 on proliferative responses of $CD45RA^+$ (unprimed) and $CD45RO^+$ (primed) $CD4^+$ cells. TGF-β1 was found to suppress outgrowth of peripheral blood $CD4^+CD45RO^+$ cells and, in contrast, to co-stimulate proliferation of $CD4^+CD45RA^+$ cells. Promotion of growth in TCR/CD3 or CD2 stimulated cultures of $CD45RA^+$ cells involved upregulation of CD25 expression and was dependent on the presence of exogenous IL-2 or CD28 mAb; IL-7 driven responses were suppressed by TGF-β1. TGF-β1 also enhanced IL-2 driven *primary* responses in purified cord blood $CD4^+$ T cells up to 7 fold, whereas proliferation was suppressed for about 40% during *secondary* cultures of these cells. Our findings imply TGF-β1 as a bifunctional regulator of $CD4^+$ T-cell growth *in vitro*, with co-stimulatory capacities during $CD45RA^+$ mediated primary responses and growth suppressive effects during $CD45R0^+$ mediated secondary T-cell responses.

V 618 PRESENCE OF IL-4 IS NOT REQUIRED FOR THE INDUCTION OF IL-4 SYNTHESIS IN KLH-PRIMED CD4+ T CELLS. R. H. DeKruyff, Y. Fang, H. Secrist, and D. T. Umetsu. Department of Pediatrics, Stanford University, Stanford, CA

Of critical importance in the development of IL-4 synthesis in CD4+ T cells is the cytokine environment, where the presence of IL-4 has been shown to be required for the development of IL-4 synthesis in naive CD4+ T cells. To determine the role of the cytokine environment in the development of IL-4 synthesis in memory CD4+ T cells, we examined CD4+ T cells taken from lymph nodes of BALB/c mice primed with the antigen KLH and restimulated in vitro with KLH.

Our results with such cells indicated that KLH-primed CD4+ T cells are similar to naive CD4+ T cells in that production of IL-4 required T cell differentiation, which was blocked by irradiation or mitomycin C pretreatment. However, in contrast to naive T cells, KLH-primed CD4+ T cells did not require the presence of IL-4 in order to produce IL-4. Thus, addition of exogenous rIL-4 did not enhance optimal IL-4 production, and culture of KLH primed CD4+ T cells with anti-IL-4 antibody did not ablate IL-4 production.

Both high density (resting) as well as low density (partially activated) KLH-primed T cells could produce IL-4 in the absence of IL-4, supporting the idea that resting memory T cells are qualitatively different from naive resting T cells in their requirement for the induction of IL-4 synthesis. As expected high density KLH-primed CD4+ T cells required more time in vitro before IL-4 synthesis was observed compared to low density T cells. Although IL-4 production by high density T cells did not require the presence of IL-4, the presence of IL-2 was absolutely required, since IL-4 synthesis was ablated in the presence of anti-IL-2 mAb. Addition of exogenous rIL-2 to these cultures also accelerated IL-4 production as measured either by IL-4 mRNA transcripts or IL-4 in supernatants. In contrast, low density T cells produced IL-4 in the presence of any T cell growth factor, either IL-2 or IL-4.

These results indicate that IL-4 synthesis in memory CD4+ T cells differs from that in naive T cells, and suggest that therapies with anti-IL-4 mAb or with soluble IL-4 receptors designed to control IL-4 synthesis in memory T cells will be unsuccessful.

V 619 ROLE OF HUMAN CD38 IN THE INTERACTION OF CD4$^+$CD45RA$^+$ LYMPHOCYTES WITH ENDOTHELIUM

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CD38 is a 46KDa surface glycoprotein displaying several features of co-receptors: it transduces activation signals, it is physically and functionally associated with surface receptors, such as CD3, sIg, and CD16, and it has sequence homologies with the *Aplysia* ADP ribosyl cyclase, which catalyzes production of a potent second messenger in the cytoplasm. CD38 functions are probably not only related to cell activation, since CD38 is highly expressed by mature plasma cells, which are terminally activated cells. In this work, we found that CD38 is expressed by peripheral blood CD4$^+$CD45RA$^+$ (resting/naive) cells, and not by CD4$^+$CD45R0$^+$ (activated/memory) cells. Since naive cells respond poorly *in vitro* to stimuli acting via the CD3/TCR and display unique migration pathways *in vivo*, we investigated the role played by CD38 in their activation and interaction with endothelium. CD38 engagement by mAb did not induce proliferation of naive CD4$^+$ cells by itself and did not potentiate activation induced by other stimuli, but it inhibited their binding to human vein endothelial cells (HUVEC). The same inhibition was detected on the (human x mouse) hybrid cell line CP410.A10, which expresses human CD38, but not on its CD38$^-$ subclone CP14. Anti-CD38 mAb did not inhibit the conventional binding assay between HUVEC and human CD38$^+$ T and B tumor cell lines. However, binding inhibition was apparent when the assay was performed at 4°C on a plate lying on an rocking shelf, conditions that minimized integrin function. These data suggest that CD38 mediates a weak cell binding to endothelium, which is also effective in dynamic conditions. These features are reminiscent of those exerted by selectins, that account for leukocyte rolling on endothelial cells and play an important role in lymphocyte homing. The possibility that inhibition was due to negative signaling by CD38, that inhibits the adhesive function of other molecules seems unlikely, because anti-CD38 mAb inhibited binding under conditions minimizing signal transduction (e.g. low temperature and use of Fab fragments of anti-CD38 mAb).

V 620 T LYMPHOCYTES FROM YOUNG AND AGED MICE PRODUCE A DIFFERENT PATTERN OF CYTOKINES FOLLOWING CD28-MEDIATED COSTIMULATION.

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The induction of proliferation and cytokine production by T cells requires both TCR engagement and costimulation caused by the interaction of accessory molecules present on T cells and APC. The binding of B7 on APC to CD28 on T cells represents an important costimulatory interaction leading to T cell activation. The ability of T cells to respond to a number of stimuli is altered in healthy, aged individuals. We have previously reported a general decrease in CD28-induced proliferation in T cells isolated from aged mice. In this study, resting T cells were isolated from the spleens of healthy young (2 months of age) and aged (21 months of age) C57BL/6J mice and stimulated with immobilized anti-CD3 ε chain (145-2C11) mAb in the presence or absence of soluble anti-CD28 (37.51) mAb. The levels of IL-2 and IFN-γ in culture supernatants were measured. Following 24 hours of culture, there was no difference in the levels of IL-2 produced by T cells isolated from young and aged mice when stimulated with anti-CD3 ε chain mAb alone. The addition of anti-CD28 mAb to these cultures resulted in a significant increase ($p<0.05$) in IL-2 production by T cells isolated from both young and aged mice; however, IL-2 production by T cells isolated from young mice was significantly greater ($p<0.05$). Conversely, T cells isolated from aged mice produced significantly more ($p<0.05$) IFN-γ than T cells isolated from young mice when stimulated with anti-CD3 ε chain mAb alone. The addition of anti-CD28 mAb to these cultures resulted in significantly enhanced ($p<0.05$) production of IFN-γ by T cells isolated from aged mice, but not by T cells isolated from young mice. These differences in cytokine production were also observed at the mRNA level. The results suggest that T cells from aged mice have a different cytokine response to costimulation mediated by CD28 than T cells from young mice. These differences may contribute to altered T cell responses associated with aging. Supported in part by NIH AG10207.

V 621 MEMORY CD4$^+$ T CELL ACTIVATION IS INHIBITED BY MHC-CLASS II-MEDIATED CD4 CROSSLINKING IN THE ABSENCE OF CO-STIMULATORY ACTIVITY,

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Previous studies have established the relative facility of memory CD4$^+$ T cell activation through TCR/CD3 stimulation as compared to naive CD4$^+$ T cell activation, which requires more extensive TCR/CD3 crosslinking and antigen-presenting cell (APC)-derived costimulatory activity. In studies comparing activation requirements of mouse memory and naive CD4$^+$ T cells, we observed a paradoxical finding that memory CD4$^+$ T cells (CD45RB^{lo}) are hyporesponsive to crosslinking of TCR/CD3 by soluble anti-CD3 presented by T-depleted splenocytes -- a stimulus that fully activates naive CD4$^+$ T cells (CD45RB^{hi}). Extensive characterization of this phenonmenon revealed that MHC class II on the APC surface inhibits memory cell activation. Thus, in the presence of anti-CD3, FcγR$^+$MHC class II$^+$ APC provided as T-depleted splenocytes or FcγR$^+$/ MHC class II$^+$ L cell transfectants failed to activate CD45RB^{lo} cells, whereas FcγR$^+$MHC class II$^-$ APC provided as T-depleted splenocytes from MHC class II knockout mice (RHAβo/o), or FcγR$^+$/MHC class II$^-$ L cell transfectants vigorously stimulated memory cells to proliferate and produce IL-4 and IFN-γ. The differential negative effect induced by class II$^+$ APC on CD45RB^{lo} CD4$^+$ T cells but not CD45RB^{hi} CD4$^+$ T cells, could be reproduced by CD4 ligation in the presence of anti-CD3 + class II$^-$ APC , establishing that negative signals are specifically transferred through CD4 on CD45RB^{lo} cells. To determine whether the negative effect exerted by MHC class II+ APC was dominant or irreversible, we endeavored to rescue memory cell function by adding costimulatory agents. We found that the lack of memory cell stimulation observed with anti-CD3 + MHC class II$^+$ APC could be overcome either in the presence of IL-2, antibodies directed against the co-stimulatory receptor, CD28, or LPS-activated APC. Memory cell hyporesponsiveness, as presented here, suggests that memory cells are more stringently regulated than their naive counterparts, most likely to bar spurious activation of these potent immune effectors in the absence of cognate antigen.

V 622 REGULATION OF PERIPHERAL TOLERANCE AND IMMUNITY IN TCR TRANSGENIC MICE

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The ability of peptidic antigen to induce a variety of functionally distinct peripheral immune responses, depending on the dose and route of administration, suggests that factors other than TCR binding to peptide-MHC complexes are crucial in determining the outcome of an encounter with antigen in the periphery. We have set up a TCR transgenic model in which the frequency of T cells reponding to peptide or protein antigen is artificially high, thus making it possible to follow the earliest events in the primary immune response at both a phenotypic and functional level. Our data suggest that tolerogenic encounters with antigen are characterised by an extremely short-lived early response, involving secretion of IL2, IL3 and IFNγ but not IL4, followed by rapid proliferation and disappearance of the activated cells from the peripheral lymphoid compartment. The total number of antigen-reactive T cells of both naive and "memory" phenotype is markedly decreased several weeks after antigen administration, leading to tolerance at the level of the whole animal.

Immunogenic responses, such as those resulting from subcutaneous administration of antigen, are characterised by far more prolonged production of IL2, IL3, IL4 and IFNγ, the Th1/Th2 balance being affected by the dose of antigen and initial degree of T cell activation. Higher doses of antigen bias the response in favour of IL4 production whilst depressing IL2 and IFNγ production, consistent with a dominant role for IL4 positive feedback in control of Th1/Th2 ratios. Although the total numbers of antigen-specific T cells return to baseline in the weeks following immunisation, there is a marked shift in the ratio of naive:memory cells, consistent with the generation of T cell memory *in vivo*. Thus generation of memory in this model is the result of phenotypic shift rather than an increase in precursor frequency.

We are determining whether APC type or activation state is crucial in regulating these immune responses and, in particular, which APC is responsible for peripheral deletion. Other investigators have suggested that naive B cells in particular are tolerogenic for naive T cells. We have therefore set up a model of B cell antigen presentation using a hybrid antigen (hen egg lysozyme linked to cytochrome C) to allow specific presentation by immunoglobulin transgenic B cells to TCR transgenic T cells.

V 623 THE LYMPHOCYTE-SPECIFIC G -PROTEIN-COUPLED RECEPTOR BLR1 DEFINES A SUBPOPULATION OF T MEMORY HELPER CELLS

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The G-protein-coupled receptor BLR1 which shows significant relationship to receptors for chemokines (MIP-1α, IL-8) and neuropeptides has been the first member of this gene family with a lymphocyte specific expression pattern.

A high affinity monoclonal antibody was raised against this receptor and used to investigate expression of BLR1 on lymphatic cells.

Analysis of peripheral blood lymphocytes by three colour flow cytometry demonstrated that BLR1 expression is limited to B cells and to subpopulations of $CD4^+$ (14%) and $CD8^+$ (2%) lymphocytes. Interestingly, studies of T cells originating from secondary lymphatic tissue (spleen or tonsil) revealed that BLR1 is present on the majority (65-80%) of $CD4^+$ cells. To investigate the onset of BLR1 expression during T cell development thymocytes were investigated. 8% of the $CD4^+CD8^-$ cells and 1% of the $CD4^-CD8^+$ cells coexpressed BLR1, but immature $CD4^+CD8^+$ cells do not show any detectable expression levels of this receptor. Analysis of cord blood lymphocytes, which are considered to be naive, laid open that < 1% of both $CD4^+$- and $CD8^+$ cells were associated with BLR1.

In addition, more than 95% of T cells expressing BLR1 were positive for CD45R0 but less than 12% were associated with the CD45RA isoform. Interesstingly, $BLR1^+$ cells independently of their origin did express high levels of CD44 but low levels of L-selectin.

Taken together our results strongly suggests that the G-protein-coupled receptor BLR1 is a marker for memory T helper cells which being involved in cell migration, cell activation and T cell dependent B cell help.

V 624 TISSUE DISTRIBUTION AND PHENOTYPE OF LONG-TERM $CD4^+$ MEMORY T CELLS, Thomas Forsthuber[*§], Luis Soares[*], Hal Sternberg[‡], Alexander Miller[*], Eli E. Sercarz[*] and Paul V. Lehmann[*§¶] [*]*Department of Microbiology and Molecular Genetics, University of California at Los Angeles, CA 90024 USA,* [‡]*Bio Time, Inc. Berkeley, CA 94710, USA.* [§]*Present address: Department of Pathology, Case Western Reserve University, Cleveland Ohio 44106.*

Activated T cells upregulate CD44 (Pgp-1) and downregulate L-selectin (MEL-14), two of the molecules that are thought to guide lymphocyte trafficking. Also, activated T cells are known to home to non-lymphoid tissues rather than lymph nodes (LN). Here we have tested whether long term $CD4^+$ memory T cells retain these characteristics of activated lymphocytes *in vivo* or whether they revert to a resting state in which they reacquire the phenotype (L-Selectin$^+$CD44^{low}) and the LN seeking homing pattern of naive lymphocytes. Up to 4 months after immunization with the prototype antigen hen egg lysozyme (HEL), HEL-specific T cells were detected in 14-19-fold higher frequencies in non-lymphoid tissues (lungs, peritoneal cavity) and the spleen than in LN, consistent with a disseminated, non-LN seeking migration by memory cells. Furthermore, such long-term $CD4^+$ memory T cells did not express L-selectin, the "LN homing receptor" {4}. $CD4^+$L-selectin$^-$ memory cells occurred in a CD44^{high} and a CD44^{low} subpopulation, the former being dominant in the spleen, the latter prevailing in LN. The differential distribution of naive ($CD4^+$L-selectin$^+$) and memory ($CD4^+$L-Selectin$^-$) lymphocytes as well as that of the memory subpopulations ($CD4^+$L-selectin$^-$ CD44^{high} and $CD4^+$L-selectin$^-$CD44^{low}) was also confirmed by flow cytometry analysis. Our data provide both functional and phenotypic evidence for differential distribution of naive and memory $CD4^+$ cells in the mouse. Additionally, the data suggest that the majority of long term $CD4^+$ memory cells, the CD44^{high} subpopulation, is tissue resident and non-circulating, and only a minor proportion is recirculating: the L-selectin$^-$CD44^{low} subset.

V 625 T CELL REPERTOIRE AND FUNCTIONALITY IN HEALTHY CENTENARIANS: STUDIES AT A CELLULAR AND MOLECULAR LEVEL, Claudio Franceschi, Daniela Barbieri, Daniela Monti, Umberto Fagiolo *, Paolo Sansoni **, Giovannella Baggio *, Sabrina Donazzan *, Sonia Quaratino #, Leonarda Troiano, Miriam Capri, Paolo Negro, Marco Londei # and Andrea Cossarizza, Inst. of General Pathology, via Campi 287, 41100 Modena, Italy; Inst. of Internal Medicine, * Univ. of Padova and ** Univ. of Parma, Italy; # Kennedy Inst. of Rheumatology , London, UK.

The study of T cell repertoire has a great importance both from the physiology and pathology of the immune system, as precious informations can be useful for understanding the basis of the susceptibility to infectious and autoimmune diseases, among others. By using a large panel of mAbs to different Vß families (studied on CD4+ and CD8+ T lymphocytes), molecular biology techniques (RT-PCR with 22 primers and relative controls) and stimulation with superantigens (*S. aureus* enterotoxins), we have analyzed the Vß-T cell repertoire in peripheral blood lymphocytes (PBL) of 12 healthy centenarians and of a group of donors 19-50 years old. PBL capability to proliferate after different stimuli (PHA, anti-CD3 etc.) has also been evaluated by ^{3}H-TdR incorporation and cytofluorimetric analysis (short pulses with BrdU and staining with propidium iodide and FITC-conjugated anti-BrdU mAbs). The main results can be summarized as follows: i) centenarians do not present gross alterations of their Vß-T cell repertoire, which is intact both from a phenotypic and functional point of view; ii) in comparison with controls, no significative differences were observed as far as the distribution of the different Vß families among CD4+ and CD8+ T cells was concerned; iii) PBL proliferative capability was well preserved, but cells had a different kinetics of growth, with a delay of 48 hrs. in comparison with younger donors. In conclusion, our data suggest that centenarians have a very well preserved immune system, which likely plays a fundamental role in reaching with success such an advanced age.

V 626 CHARACTERISATION OF A NOVEL HUMAN ENDOGENOUS RETROVIRUS-LIKE ELEMENT IN ACTIVATED T-CELLS, Colm A Kelleher , David A Wilkinson*, Dixie L Mager*, and Erwin W. Gelfand, Department of Pediatrics, National Jewish Center for Immunology, 1400 Jackson St., Denver, CO 80206, *Terry Fox Laboratory, 601 West 10th, Vancouver V6G IL3.

Only ten percent of the human genome encodes protein. The remaining 90% comprises many families of repetitive elements, including several families which share high homology with retroviruses. The RTVL-H family of endogenous retrovirus like elements is the largest human family known, there are 1000 full length elements and a further 1000 solitary LTRs per cell. Using an RTVL-H LTR probe we have detected the presence of a 6kb transcript by Northern analysis which is highly induced in normal peripheral T cells after treatment with phytohemagglutinin (PHA). This 6 kb RTVL-H containing transcript is only seen in cells of the hemopoietic lineage. We also detected it in primary myelocytic and lymphocytic leukemia cells, but not in any leukemic cell lines. The expression is increased within 3-4 hours after treatment with PHA or with phorbol ester, reaches a maximum after 8 hours and then declines to low levels within 24 hours after treatment. Its expression is inhibited totally by Cycloheximide and by the immunosuppressant Cyclosporin A. Using probes specific for the U3 and U5 regions of the RTVL-H LTR, in combination with internal RTVL-H probes, we showed that the 6 kb transcript is polyadenylated from an RTVL-H LTR, but that the majority of the mRNA comprises non RTVL-H sequence. We constructed a cDNA library from T cells which had been treated with PHA for 8 hours and isolated a 1.7kb clone using the RTVL-H probe. The insert, by sequence analysis, contains a novel array of retroposon elements, two endogenous retroviral LTRs and a minisatellite like sequence. Because of its high expression level, its sharply defined transcription kinetics and its novel composition, we hypothesise that the transcript may play a biological role during T cell activation.

V 627 NUCLEAR PROTEINS BINDING TO THE P-ELEMENT OF THE HUMAN INTERLEUKIN-4 GENE PROMOTER IN PRIMED/MEMORY AND UNPRIMED/ANTIGENICALLY NAIVE T CELLS, David B. Lewis and Susan Bort, Departments of Pediatrics and Immunology, University of Washington, Seattle, WA 98195.

The P-element of the human IL-4 gene, located at -50 to -64 from the cap site, appears to play an important role in transcription of the IL-4 gene in T cells. We have previously shown that only about 3-5% of polyclonally activated adult T cells express IL-4 mRNA, and that these cells are contained within the putative memory/*in vivo*-primed, $CD45R0^{hi}$ subset. IL-4 mRNA expression following activation is low in freshly-isolated adult T cells and is undetectable in antigenically-naive ("unprimed") neonatal T cells. However, priming *in vitro* of these cell types with Con A and exogenous IL-2 markedly increases their capacity to express IL-4 mRNA upon reactivation. To determine if the expression of nuclear proteins binding to the P-element correlated with an increased capacity for IL-4 mRNA expression by T cells, nuclear extracts were prepared from T cells after polyclonal activation for 2 hrs and used in electrophoretic mobility shift assays (EMSA) with a human IL-4 gene P-element oligonucleotide. A specific EMSA complex was found using nuclear extracts from activated *in vitro*-primed adult T cells. This complex and IL-4 mRNA levels were absent or barely detectable in unstimulated primed T cells or when these cells were preincubated with cyclosporin or cycloheximide prior to activation. Only low amounts of the complex were found using extracts from activated freshly-isolated adult T cells. In extracts from activated freshly-isolated neonatal T cells a specific EMSA complex was observed with a markedly faster mobility than that observed in *in vitro*-primed adult T cells. UV-crosslinking analysis showed that the major protein binding to the P-element from extracts of *in vitro*-primed adult T cells was larger than that found in neonatal T cell extracts, suggesting that they were different proteins. Thus, we found a striking correlation between the abundance of nuclear proteins that bound to the P-element *in vitro* and the amount of IL-4 gene expression by normal human T cells. Further characterization of these proteins may provide insight into how IL-4 gene expression is upregulated by T cell priming *in vitro* and *in vivo*.

V 628 PARALLEL DEVELOPMENT OF PRIMARY AND 'MEMORY' T AND B CELL RESPONSES *IN VIVO*

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Abstract

The mounting of most immune responses involves a complex interplay between B and T lymphocytes and antigen presenting cells. Studying the response of specific lymphocytes *in vivo* has been particularly difficult due to the very small number of cells with specificity for any one antigen, even in repeatedly immunized animals. Using six-parameter flow cytometry we have been able to identify and characterize a population of helper T cells in draining lymph nodes that become activated and expand exponentially in response to antigen, based on their usage of a specific TCR $V\alpha V\beta$ pair. We also simultaneously quantitate the antigen-dependent development of antibody-secreting and memory B cells as an estimate of effective T cell help *in vivo*. Similar analyses of the secondary response revealed that despite there being no overall increase in responsive cell number, the emergence of an antigen-activated helper T cell population was significantly accelerated, reaching its peak only 4 days after injection. Antibody-secreting cells emerge more rapidly and to a greater extent following secondary challenge while further memory B cell development is ongoing with similar kinetics to the primary response. These data provide direct evidence for the existence of a distinct memory T cell compartment and outline the dynamics of the primary and memory B cell response to the same antigen.

V 629 FUNCTIONAL DISTURBANCE OF NAIVE T CELLS, BEING CONCERNED WITH IL-4 DEPENDENT DEVELOPMENT OF IL-4 PRODUCING T CELLS, IN THE ALLERGIC PATIENT WITH ELEVATED IgE,
Hiroshi Ochi, Toshio Tanaka, Yoshinori Katada, Tetsuji Naka, Masakazu Aitani, Shoji Hashimoto, Masaki Suemura, Tadamitsu Kishimoto, Department of Internal Medicine III, Osaka University Medical School, Osaka, Japan

It has been clearly shown that IL-4 is a essential factor for induction of Th2 in mouse. So, We have analyzed the effect of IL-4 on the development of IL-4 producing T cells in human and compared between T cells from normal individuals and from allergic patients with elevated IgE. T cells isolated from PBMC were stimulated with soluble anti-CD3 mAb and APC in the presence of IL-4 or neutralizing anti-IL-4 mAb. After 7 days, cultured cells were restimulated and total cellular RNA's were isolated. When T cells from normal individuals were examined, high expression of IL-4 mRNA was detected by PCR in response to IL-4. In contrast, if anti-IL-4 mAb was present during the first stimulation, little IL-4 mRNA expression was detectable. On the contrary, in T cells from allergic patients with elevated IgE, almost a similar level of IL-4 mRNA expression was observed irrespective of the existence of IL-4 or anti-IL-4 mAb. To understand these results more clearly, we isolated $CD45RA^+$ naive T cells and $CD45RO^+$ memory T cells and analyzed the IL-4 mRNA induction by the same method. In naive T cells from normal individuals, high expression of IL-4 mRNA was similarly observed in response to IL-4 and if anti-IL-4 mAb was added, little IL-4 mRNA expression was detectable. On the contrary, in naive T cells from allergic patients with elevated IgE, IL-4 failed to augment the IL-4 mRNA expression. When we analyzed memory T cells, high expression of IL-4 mRNA was observed, but not influenced by the addition of IL-4 or anti-IL-4 mAb in both normal individuals and patients. The evidence that IL-4 induces differentiation of naive T cells into IL-4 producing T cells by the stimulation of T cell receptor engagement from normal individuals but not from allergic patients with elevated IgE indicates that there exists functional disturbance, being concerned with the IL-4 dependent development of IL-4 producing T cells, in the peripheral blood naive T cells from allergic patients with elevated IgE.

V 630 THE PRIMARY AND SECONDARY IGM RESPONSES TO FLUORESCEIN, Judith Owen, Harjeet van der Keyl, Christopher Hsu, Aneesh Tolat, Ida Fox and Audrey Park, Department of Biology, Haverford College, Haverford, PA 19041.

The object of our ongoing work is to compare fluorescein-specific IgM antibodies utilized by primary and secondary B cells. Previous work from this laboratory demonstrated that hybridoma cultures from mice immunized just once prior to fusion were less stable than were their secondary counterparts. We showed that this instability can be overcome by their growth in interleukin-containing supernatants. More recently, we have shown that primary hybridomas are also unstable with respect to the secretion of antigen-specific IgM antibodies, a finding currently under exploration.

We have also sequenced 32 heavy and 7 light chains and developed assignments for V_H gene use for each of the heavy chains. A high percentage of fluorescein-specific antibodies utilize genes derived from the 7183 and J558 gene families, in both primary and secondary antibody-producing hybridomas. However, expression of $Q52V_H$ gene segments appears to be limited to primary fluorescein-specific B cells. All four J_H segments are used, but J_H1 is used at a lower frequency than the other three. Analysis of pairs of hybridomas demonstrates that the same variable region can be used with two different J regions and the same V_H:J_H pair has been used with two different D_H regions to generate two distinct fluorescein-specific antibodies.

We conclude that, in spite of previous observations by other laboratories of an idiotypically-diverse, fluorescein-specific antibody repertoire, our data imply the use of a relatively limited set of V_H region gene segments in the construction of IgM anti-fluorescein antibodies.

V 631 CD28 Co-stimulation Enables CD4+ CD45RA+ (Virgin) T cells to respond to Recall Antigen

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The association of CD45RA and CD45RO molecules with virgin and memory T cells respectively was based on the observations that CD45RO+ T cells respond to recall antigen in vitro, while CD45RA+ T cells do not; and also that CD45RA+ T cells differentiate into CD45RO+ cells following stimulation. However, CD45RA+ T cells are hyporesponsive to many stimuli in vitro. Potent accessory cells, such as dendritic cells, are required for activating CD4+ CD45RA+ T cells, but purification of such cells is difficult. We have used anti-CD28 antibody to overcome this problem, when using peripheral blood adherent cells for antigen presentation. CD4+ CD45RA+ and CD4+ CD45RO+ T cells were stimulated with tetanus toxoid (10μg/ml) in the presence or absence of CD28 antibody. CD28 co-stimulation did not increase the proliferation of CD4+ CD45RO+ T cell subset to tetanus toxoid. However a potent increase in the response to tetanus toxoid was observed in CD4+ CD45RA+ T cells when CD28 antibody was present. This effect was dose dependent and optimal at 100 ng/ml CD28 antibody. Subjects whose CD45RO+ T cells were unresponsive to tetanus toxoid in vitro, showed a raised response in both CD4+ T cell subsets, when co-stimulated with CD28 antibody. These results suggest that much of the difference between recall antigen responses in CD45RA+ and CD45RO+ T cells may reflect an inadequate in vitro signal, rather than a difference in precursor frequencies of cells specific for the target antigen.

V 632 CD45RB^{high} CD4$^+$ T CELLS INDUCE A PATHOGENIC TH1 RESPONSE IN THE COLON WHEN TRANSFERRED TO C. B-17 *scid* MICE WHICH IS PREVENTED BY CO-TRANSFER OF THE CD45RB^{low} POPULATION, Fiona Powrie, Michael Leach and Robert L. Coffman, DNAX Research Institute of Molecular and Cellular Biology Inc., 901 California Ave, Palo Alto, CA 94304.

CD4$^+$ T cells in the mouse can be subdivided into two fractions based on the level of expression of the CD45RB determinant. Data to be presented here indicates that regulatory interactions occur between these two subsets which are important in the prevention of pathogenic cell mediated immune responses against immunological self antigens. Injection of the CD45RB^{high} CD4$^+$ T cell population into C. B-17 *scid* mice led to the development of a progressive wasting disease with severe mononuclear cell infiltrates into the colon. In contrast, animals restored with the reciprocal CD45RB^{low} subset or with unfractionated CD4$^+$ T cells did not develop the wasting or colitis. Importantly, co-transfer of the CD45RB^{low} population with the CD45RB^{high} population prevented the wasting disease and colitis. Colitis appeared to be due to the development of a pathogenic Th1 response as the inflamed colons contained elevated levels of IFN-γ and TNF-α mRNA and the disease was inhibited by administration of anti-IFN-γ mAb. These data indicate that CD4$^+$ T cells from normal mice have the capacity to induce a lethal inflammatory response in the colon but that under normal circumstances this response is inhibited by a phenotypically distinct subpopulation of CD4$^+$ T cells. The role of the Th2 cytokines, IL-4 and IL-10, in the suppression of colitis will also be discussed.

V 633 THE PROGRESSIVE DIFFERENTIATION OF PRIMED T CELLS. Mike Salmon, Darrell Pilling, Nicola J Borthwick, Nick Viner, George Janossy, Paul A Bacon, Arne N Akbar. Department of Rheumatology, The Medical School, Birmingham University, Birmingham B15 2TT, UK Department of Clinical Immunology, Royal Free Hospital, Pond St, London UK

Recent studies have suggested that T cell memory for recall antigens resides in clones of primed T cells with a short inter-mitotic half life. In humans such cells express an isoform of the leukocyte common antigen termed CD45RO. Nevertheless, little is known of the fate of these primed T cells after initial activation, since no markers are available to distinguish recently primed cells from long established clones. We have studied the relationship between expression of all CD45 isoforms in peripheral blood by flow cytometry and computer modelling, and also the change in isoform expression during long term in vitro culture. We identified a spectrum of primed CD4$^+$ T cells characterised by an inverse relationship between the expression of two CD45 epitopes: CD45RB and CD45RO. We show that primed CD4$^+$ T cells progress through many cycles of division from a CD45RB^{bright}O^{dull} to a CD45RB^{dull}O^{bright} state, resulting in a highly skewed distribution of TCR variable region usage within this particular population. The progressive differentiation defined by the shift from CD45RB^{bright} to CD45RB^{dull} is paralleled by the gradual loss of bcl-2 and gain of Fas expression, two features associated with an increased propensity for apoptosis. At the same time, the highly differentiated CD45RB^{dull} cells selectively lose the capacity to synthesise IL-2, a cytokine which is particularly effective in preventing T cell apoptosis, although they produce high levels of IL-4. The inability to produce adequate levels of IL-2 leads to the apoptosis of primed CD45RB^{dull} cells, when they are stimulated in the absence of exogenous IL-2. These observations show the crucial dependence of highly differentiated T cells on the availability of exogenous IL-2, and suggest both a major constraint for the persistence of T cell memory, and an important mechanism contributing to the maintenance of T cell homeostasis *in vivo*.

V 634 DETERMINANTS OF SELECTIVE INTERFERON-γ EXPRESSION IN T CELLS. CB. Wilson, LA Penix, WM Weaver, J Osborn and HA Young. Departments of Pediatrics and Immunology, Univ of Washington, Seattle, WA 98195 and Lab of Experimental Immunology, NCI, FCRDC, Frederick, MD 21702.

Interferon-γ (IFN-γ) is restricted in expression to primed or memory T cells and to the TH1 subset of CD4$^+$ T cells. This pattern differs from other TH1 cytokines, such as IL-2, which are expressed in virgin T cells. To explore the basis for this we have first analyzed the 5' flank of the human IFN-γ gene, using β-galactosidase reporter constructs that were transfected into Jurkat T cells. Constructs containing 538 bp of 5' flank gave optimal expression, comparable to constructs driven by the IL-2 promoter. Expression nearly as great was observed with 108 bp of 5' flank. Expression of these constructs mirrored the endogenous gene - both PMA and Ionomycin were required for induction, which cyclosporin A blocked, and expression was not observed in macrophage cell lines. Internal deletions that removed the region between -108 and -40 bp abrogated activity. Within the region between -108 and -40, deletion of either of two elements, which are conserved in the mouse and rat genes, reduced expression by ~70%. In mice containing genomic IFN-γ transgenes with amounts of 5' flank similar to the human -538 constructs, expression of the transgene paralleled and was similar to that of the endogenous IFN-γ gene. Using gel mobility shift assays, multiple specific complexes were observed with the two conserved regulatory elements defined in the transfection analysis. The distal element binds GATA-3 and other proteins which are competed for by an AP-1 oligonucleotide. However, the motif in this element shares closer homology with one in the GM-CSF and MIP-1 genes, than with prototypical AP-1. The proximal element shares homology with the NF-IL2A element of the IL-2 promoter. Proteins binding to this element are competed for by NF-IL2A and AP-1 oligonucleotides; unlike NF-IL2A, the proximal element of the IFN-γ gene does not bind Oct-1. The proximal element of the IFN-γ gene contains a CpG dinucleotide, which is methylated in TH2 T cell lines but not in TH1 T cell lines, and methylation of this element inhibited binding of factors from T cell extracts. However, differences in the abundance of proteins binding to this element were also observed between T cells that do or do not express IFN-γ. These results suggest that differences in the regulatory elements between the IFN-γ gene and their methylation, and in the abundance of proteins binding to these regulatory elements, contribute to the unique pattern of expression of this lymphokine.

V 635 TGFβ HELPS PREVENT APOPTOSIS OF CD4 EFFECTORS AND HELPS DRIVE THEM TO MEMORY CELLS Xiaohong Zhang, Lizzie Giangreco, Beth Broome, and Susan L. Swain. Department of Biology and the Cancer Center, University of California, San Diego, La Jolla, CA92093

The external signals that influence the fate of Th effectors, either keeping them in an activated state, leading them to the quiescent state or inducing or allowing their death are unclear. We showed previously that, in contrast to IL4 and IFNγ, TGFβ caused Th precursors to develop into effector populations with a more memory phenotype (CD45RB low, CD44 high).

To further study the effect of TGFβ on Th development, we generated effectors in vitro by stimulation of naive CD4 T cells from transgenic mice. We then determined the effects of TGFβ and IL2 on effectors restimulated with antigen (Ag)/ APC or APC alone.

When effectors were stimulated with Ag/APC, the combination of TGFβ and IL2 promoted increased expressing *bcl-2* protein, decreased DNA fragmentation, and a much greater cell recovery. Effctors remained highly activated and retained their patern of cytokine secretion. When effectors were cultured without Ag (APC only), TGFβ and IL2 had similar effects on increasing *bcl-2* expression and decreasing apoptosis, but TGFβ alone promoted development of a more resting/memory phenotype (CD45RB low and IL2R low) and the population cultured without Ag also became higher producers of IL2. The results suggested that availability of Ag and cytokines IL2 and TGFβ may help to determine the fate of effector populations.

Microenvironments for Lymphocyte Development

V 636 **DEVELOPMENTAL REQUIREMENTS FOR THYMIC POSITIVE SELECTION IN VITRO,** Graham Anderson, Nel C. Moore, John J. T. Owen and Eric J. Jenkinson, Department of Anatomy, Medical School, University of Birmingham, United Kingdom.

During T-cell development in the thymus, immature $CD4^+CD8^+$ thymocytes are selected for maturation on the basis of the specificity of the αβTCR they express. The cellular and molecular requirements of this process - termed positive selection - are unclear. To address this issue, we have established an *in vitro* reaggregate culture system in which positive selection of $CD4^+CD8^+$ thymocytes takes place in the presence of a single stromal cell type - MHC class II^+ thymic epithelium. Thymocyte maturation in reaggregate cultures shares many features in common with positive selection *in vivo*, such as downregulation of CD4 or CD8, upregulation of αβTCR, transient expression of CD69, expression of genes encoding IL-2 and IL-4 and the acquisition of functional competence. We have further utilised this system to investigate the cellular requirements for positive selection by reaggregating $CD4^+CD8^+TCR^-$ thymocytes with defined stromal cells. Of the stromal cells tested, which include MHC class II^+ thymic dendritic cells, and MHC class II^+ salivary and gut epithelial cells, we find that the ability to provide positive selection signals is a property unique to MHC class II^+ thymic epithelium. Moreover, metabolically inactive ECDI-fixed thymic epithelial cells are capable of supporting positive selection. Thus, while other studies suggest that various cell types can provide MHC-peptide complexes for positive selection, these data are the first evidence that the additional molecules mediating positive selection are ligands uniquely expressed on the surface membrane of thymic epithelial cells.

V 637 LYMPHOSTROMAL INTERACTIONS ARE ESSENTIAL FOR THYMIC EPITHELIAL AND T CELL DEVELOPMENT. Richard.L. Boyd, Peter Mombaerts*, Susumu Tonegawa*, Hanspeter Pircher#, Robson MacDonald+ and Thomas Hunig**. Dept. Pathology and Immunology, Monash University, Victoria; *Dept. Biology, Massachusetts Institute of Technology; #Dept. Pathology, University of Zurich; +Ludwig Inst. Cancer Research, Lausanne; **Dept. Virology and Immunobiology, University of Wurzburg.

The identity of cells mediating thymic selection events was investigated by co-culturing non-selected immature (CD3loCD4+CD8+) normal or αβ-TCR transgenic embryonic thymocytes from mice with a non-selecting MHC background, with freshly purified thymic stromal cell subsets from selecting or non-selecting MHC-bearing mice. When incubated alone the precursors remained as homogeneous CD3loCD4+CD8+ cells or died. Thymic nurse cells (TNC) preferentially induced CD3hiCD4+CD8- cells including those restricted to MHC class I, and to a lesser extent CD3hiCD4-CD8+ cells; this also involved induction of CD69. Usage of the TCR transgenic mice demonstrated an inherent bias of thymocytes to the CD4 expression pathway. Accordingly TNC-enclosed lymphocytes injected into syngeneic SCID mice reconstituted the spleen with αβTCR+CD4+ CD8- cells and limited CD3+CD4-CD8+ cells. The role of T cells in shaping the thymic microenvironment was examined in a series of TCR gene-deleted mice: $\alpha^{-/-}$ (CD4+CD8+ αβ-TCR thymocytes, normal γδ TCR cells); $\beta^{-/-}$ (no αβ-TCR lineage cells, normal γδ-cells); δ-/- (normal αβ-TCR cells, no γδ-TCR cells); βδ-/- (no mature αβ- or γδ-TCR cells); Rag-1-/- mice transgenic or not for TCR β -chain. The initial development of the thymic epithelium was not dependent on T cells (post CD3-CD4-CD8-), however, the maturation, organisation and growth of the medulla requires either αβ- or γδ-TCR mature T cells. With excessive development of CD3-CD4+CD8+ cells in the absence of mature T cells, the medullary rudimentary pockets are greatly reduced. Similarly, the lack of CD4+CD8+ cells results in a poorly defined cortex. Clearly thymopoiesis is an integrated process involving complex bi-directional interactions between developing thymocytes and the stroma.

V 638 THE MAJORITY OF $CD8^-CD4^+$ THYMOCYTES REQUIRE THE THYMUS FOR FINAL MATURATION, Rubendra Dyall and Janko Nikolic-Zugic, Immunology Program, Sloan-Kettering Institute, New York, NY 10021.

Upon successful positive selection, DP thymocytes gradually downregulate either CD4 or CD8 to become SP ($CD4^+CD8^-$ or $CD4^-CD8^+$). Using this gradual downregulation, we have isolated two subsets of $CD4^+$ SP on the basis of their expression of CD8; a $CD8^{lo}CD4^+TcR^{hi}$ subset which is unable to (i) induce GvHd, (ii) populate peripheral organs upon IV injection in syngeneic hosts, and (iii) resist apoptosis upon TcR crosslinking with immobilized antibodies. In these respects the $CD8^{lo}CD4^+$ TcR^{hi} differs from the $CD8^-CD4^+TcR^{hi}$ subset which behaves like its peripheral counterpart. However, $CD8^{lo}CD4^{hi}$ cells do yield long-lived immunocompetent peripheral progeny when allowed to develop in an adoptive thymus. These results indicate the requirement of a thymic environment for final maturation of the majority of $CD4^+$ thymocytes.

V 639 IN VITRO POSITIVE SELECTION OF THYMOCYTES BY DISPERSED POPULATION OF THYMIC EPITHELIAL CELLS, Bettina Ernst, Charles D. Surh, and Jonathan Sprent, Dept. of Immunology, The Scripps Research Institute, La Jolla, CA 92037

We have reproduced the observation of Jenkinson et al (J. Exp. Med. 1992. 176:845) that culturing $TCR^-CD4^+CD8^+$ thymocytes with dispersed populations of thymic epithelial cells (TEC) leads to positive selection: the T cells upregulate TCR expression and switch to $CD4^+CD8^-$ and $CD4^-CD8^+$ cells. With this system we have defined the surface markers on cells undergoing positive selection. We have also found that the level of TCR expression on the selected cells is under the control of steroids. The turnover of cells undergoing selection and the cell types required for selection are currently under investigation.

V 640 LINEAGE POENTIAL OF LYMPHOID PRECURSOR CELLS FROM NORMAL AND IL-7 TRANSGENIC MICE

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An important and as yet controversial issue is the existence of a stem cell committed to lymphocyte development but restricted in alternative haemopoietic options. Bi-potent stem cells capable of macrophage and B lineage differentiation have recently been described as a rare $AA4.1^+B220^-$ population in the embryonic liver of mice. These cells do not fully reconstitute irradiated recipients being unable to generate additional myeloid lineages . Their potential for T lymphocyte development has not been reported and is the subject of this study. We are currently investigating the T-cell potential of these cells in reconstitution assays using lymphoid depleted *in vitro* thymic organ cultures. Since such populations are normally extremely infrequent and require extensive purification we have, in addition, (i) developed a novel bone marrow derived stromal cell line which permits the expansion $AA4.1^+B220^-$ dual-potent cells *in vitro* and (ii) analysed the properties of a phenotypically identical immature population of cells which is abundant in healthy and tumour tissues from IL-7 transgenic mice. Results from these three distinct lines of investigation will be presented.

V 641 **THYMOCYTES AND MACROPHAGES ARE TARGETS OF HIV-1 INFECTION IN THE NEONATAL THYMUS**, Glen N. Gaulton, Ronald Collman, Douglas P. Clark, Elizabeth M. Bunting and Michael Rosenzweig, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Pediatric AIDS is a devastating disease that is increasing exponentially worldwide. The importance of *in-vivo* thymic infection with HIV-1 in pediatric AIDS is exacerbated by the fact that the thymus is the site of T lymphocyte ontogeny during fetal and neonatal development. Analysis of thymic infection by HIV-1 was conducted on both fresh thymic tissue isolated from infected neonates, and human thymic organ culture infected *ex-vivo*. The sites of viral replication were first examined via *in-situ* hybridization for the presence and distribution of viral RNA. Viral RNA was detected in focal cortical caps of the thymus in both thymocytes and macrophages. Infected tissue was also subjected to fluorescent antibody staining for HIV-1 viral proteins, which were again found to be present in thymocytes and cortical macrophages. Neonatal thymic organ culture was next used as a model for thymic infection, to further characterize the thymotropism of various HIV-1 isolates. Infection of thymic organ culture explants reflected the macrophage tropism of the particular HIV-1 isolate. Strains of HIV-1 that are lymphocyte tropic replicated poorly, or were unable to cause infection of the thymic explants. In contrast, macrophage tropic strains showed a high level of HIV replication and induced profound thymocyte mortality. Thymic macrophages were isolated from normal neonatal thymus and shown to be capable of supporting *ex-vivo* HIV-1 infection with minimal cytopathology. These studies identify thymic macrophages and immature thymocytes as primary targets of HIV-1 infection. The role of HIV-1 infection of thymocytes and thymic macrophages in the pathogenesis of pediatric AIDS may be linked to disruption of the normal developmental sequence of thymocyte maturation.

V 642 T CELLS IN THE SMALL INTESTINE AND THE THYMUS ARE INDEPENDENTLY SEEDED FROM PRECURSORS LOCATED IN THE SPLEEN, *Mawieh Hamad and John R. Klein, Department of Biological Science, University of Tulsa, Tulsa, OK 74104.*

To study the early intermediate developmental stages of thymus-independent intestinal intraepithelial lymphocytes (IEL), and to compare that to T development within the thymus, we have used immature T cell precursors obtained from the spleen of donor athymic mice to repopulate the thymus, spleen and gut lymphoid compartment. In Thy-1 congenic mice, T cell repopulation of those compartments from donor precursors occurred first in the gut, then the thymus, and several weeks later in the spleen, indicating that murine IEL and thymocytes are derived from a similar though possibly distinct prethymic T cell pool within the spleen. Reconstituted mice were healthy for at least 60 days post-cell transfer and all major phenotypic subsets of the gut and thymus were generated, including TCR-$\alpha\beta$, TCR-$\gamma\delta$, and CD8α/α or CD8α/β IEL, as well double-negative, single-positive, and double-positive T cell subsets within the thymus. The phenotype of the precursor cell responsible for repopulation was HSA^+ (possibly Thy-1^{lo}), $CD2^-$, $CD3^-$, $CD4^-$, $CD5^-$, $CD8^-$, $CD25^-$, $CD44^-$, $Ly6^-$, TSA^-, TCR^-. Repopulation could be achieved with spleen cells from euthymic mice but was considerably more efficient, kinetically, using spleen cells from athymic mice, indicating that the spleen of athymic mice is enriched for T cell precursors. However, lymphoid repopulation was achieved in splenectomized mice reconstituted with bone marrow, indicating that the spleen is not the only site for intermediate T cell differentiation. T cell repopulation of irradiated mice did not occur using either adult thymocytes, lymph node lymphocytes, or IEL from euthymic mice (mice died 8-10 days post-irradiation and cell transfer), demonstrating that repopulation was not merely due to clonal expansion of mature or developing T cells. Repopulation of the gut and thymus proceeded normally in irradiated splenectomized mice injected with spleen-derived T cell precursors, implying that precursors present within the spleen home directly to final sites of terminal differentiation within those tissues. These findings locate a pathway for pre-thymic and pre-intestinal T cell development which now is amenable to further studies those two diverging T cell populations. (Supported by grant DK35566 from the NIH and by a grant from the Mervin Bovaird Center for Studies in Molecular Biology.)

V 643 EXTRATHYMIC DEVELOPMENT OF CD4 POSITIVE LYMPHOID CELLS IN MOUSE EMBRYOS.
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Using rat monoclonal antibodies directed against surface antigens of fetal mouse thymocytes different aggregates of "lymphoid cells" could be detected in cryosections of fetuses as early as GD 16. Several such aggregates are gut-associated, others are found lining the aorta and the mesenterial arteries, or „node-like" at branching points of peripheral arteries.

8-12 such lymphoid cell aggregates are detected within the lamina propria of the duodenum, jejunum and ileum; these aggregates increase in size from ca 50 µ (diameter) at GD 16 to 200-300 µ at birth. All the lymphoid cells are - in addition to their staining by mab Th V-7/5 - Thy-1-positive (30H12) and weakly CD25-positive (PC61); the majority of the cells are CD4-positive (H 129). Many dentritic cells (ThV-8/4-positive)are found in these areas. Many cells are MHC class II-positive (P7/7).Till GD 18 all the cells in these areas are CD3-(T200A5)- and TCR-$\gamma\delta$–(GL3)– negative; at birth a few CD3- and $\gamma\delta$–positive cells are seen. TCR-$\alpha\beta$ positive (A57-597) and sIgM-positive (b.7.6) do not appear before 2 days p.p.
There is evidence that Peyer's patches will develope in the areas of these prenatal "lymphoid cell cluster".- Similar "lymphoid aggregates" are seen prenatally also in fetuses of nu/nu mice and in SCID mice. In SCID mice these gut-associated cell aggregates seem to persist throughout life and increasing in number.

"Node-like clusters of lymphoid cells" are also seen at the branching of large cervical or inguinal arteries. Again most of these cells are "early thymocyte-marker"-positive, CD3- and TCR-negative; Anti-Thy 1 stains these cells and a capsula around the cells. As seen already in the gut-associated areas, many cells are CD4-positive and/or MHC class II-positive. Similar clusters are also seen in nu/nu fetuses and in SCID fetuses. Their anatomical location would suggest that postnatally - probably by immigration of a new and more mature wave of lymphocytes - lymph nodes will develope from these early clusters.

V 644 IMMUNOHISTOCHEMICAL LOCALIZATION OF PEPTIDERGIC NERVE FIBERS AND NEUROPEPTIDE RECEPTORS IN PEYER'S PATCHES OF THE CAT ILEUM.
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The localization of peptidergic nerves and receptors for neuropeptides in Peyer's patches of the cat ileum was determined immunohistochemically using biotinylated rabbit antibodies and the streptavidin-biotin (SAB) method. Numerous vasoactive intestinal polypeptide (VIP)-, calcitonin gene-related peptide (CGRP)- and substance P (SP)-immunoreactive nerve fibers were distributed throughout the lamina propria of the crypts and villi.
Immunoreactive nerve fibers also ran around the margin of Peyer's patch lymphoid follicles, but only a few such fibers were seen in the subepithelial layer of follicles. These fibers occasionally extended into the margin of follicles. Compared with crypts and villi, Peyer's patches were sparsely innervated. Nerve fibers were found mainly around lymphatics and high endothelial venules (HEV) at the edges of follicles. Specific receptors for VIP were present in the crypts and T-cells particularly in the margins of follicles and in interfollicular areas. Receptor binding sites for VIP were numerous on these cells, within or around HEV and lymphatic vessels. VIP staining was also noted in the vicinity of on "Pan B cells" but was sparse and confined to interfollicular regions. By contrast, specific receptor binding sites for SP were idntified on T- and "Pan B cells" but only at the margins of follicles. VIP-and SP-receptors were absent from B subset.

An important finding of this study is the demonstration that there are specific receptors for VIP which may modulalte the homing of some type of T-cells from the blood into Peyer's patch and / or vice versa. It is postulated that this effect may be mediated by T-cell specific binding of regional neuropeptides, that results in their increased adherence to the HEV of these lymphoid follicles.

V 645 A THYMIC STROMAL MOLECULE INVOLVED IN T CELL ACTIVATION, David J. Izon, Lori A. Jones, Elizabeth E. Eynon and Ada M. Kruisbeek, Division of Immunology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Activation of mature T and B cells is a prerequisite for immune effector function. This activation is primarily induced by the interaction of antigen with its specific receptor; T cell receptor or immunoglobulin respectively. However, other co-stimulatory molecules also play a significant role in this activation process. One of the first such molecules to be described in the T cell system was CD2 which in conjunction with sub-optimal doses of anti-CD3 could co-stimulate T cell proliferation. Other molecules have subsequently been found to have similar actions: Thy-1, LFA-1, CD28 and Ly6. In B cells similar molecules have also been found; CD19 and CD45. In some cases a lack of this second signal may lead to anergy or a block in the activation process eg. anti-B7 or CTLA-4

More recently, novel co-stimulatory/activation molecules have been described. These include CD69, CD27, CD30, 4-1BB, CD40 and its ligand gp 39. These molecules have special interest as they are induced early after activation and one of them, CD40, is also expressed on thymic epithelium.

Here we describe a mAb, MTS 23, originally classified as detecting a subpopulation of medullary epithelium. This mAb was extensively studied for its expression and function in the immune system as it also detected an antigen expressed on both splenic and thymic dendritic cells, B cells and transformed foetal thymocytes. It was therefore hypothesised that it may play a role in lymphoid activation.

Functional studies revealed that MTS 23 inhibited the response of T cells to anti-CD3 and antigen specific stimulation. Immuno-precipitation of a cell line expressing the antigen detected by MTS 23 (23A) demonstrated that it was a single chain molecule of approximately 120kD. Given the tissue distribution, apparent molecular weight and functional attributes of 23A it is postulated that it belongs to a group of molecules associated with T cell activation. It appears that 23A may be a novel adhesion or co-stimulatory molecule

V 646 MULTIPLE RESTRICTION ENZYME SITES AS A MUTABLE SUBSTRATE FOR SOMATIC HYPERMUTATION OF IMMUNOGLOBULIN GENES,
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One of the mechanisms of generating diversity in the antigen binding pocket of immunoglobulin (Ig) genes is somatic hypermutation. Current work to determine the presence or absence of mutations in rearranged Ig genes or transgenes requires that the gene be cloned and sequenced to detect mutations. An emphasis of our lab has been to find a mutable substrate in which mutations are detected without sequencing. A 105 base fragment was created that contains alternating EcoRV and PvuII restriction enzyme sites. This fragment was cloned into the variable region of a kappa light chain transgene that has been shown previously to be a target for somatic hypermutation. Mice containing this transgene (EPS) were immunized and hybridomas were made from splenic B cells. DNA from the hybridomas is amplified by PCR using primers specific to the variable region flanking the restriction site fragment. The amplified product is then digested with EcoRV or PvuII and subsequently run on a high percentage acrylamide gel. In the absence of mutations a ladder of small fragments appears. With a mutation in one of the restriction sites, the enzyme no longer cuts and a larger fragment appears on the gel. The restriction enzyme sites account for approximately 73% of the fragment. Currently we are assessing the level of sensitivity of this assay system and confirming possible mutations.
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V 647 REQUIREMENT FOR THE THYMUS FOR INTESTINAL INTRAEPITHELIAL LYMPHOCYTE DEVELOPMENT,
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Intraepithelial lymphocytes (IEL) of the mouse small intestine have been suggested to be of extrathymic origin. However, results from studies of nude mice do not agree with those from thymectomized irradiated bone marrow chimeras (ATXBM). Nude mice contain low numbers of Tcr$\gamma\delta$ IEL and few Tcr$\alpha\beta$ IEL, while ATXBM mice are reported to contain a normal complement of Tcr$\alpha\beta$ and Tcr$\gamma\delta$ IEL. To determine the reason for this discrepancy we analyzed IEL from neonatally thymectomized (nTx) mice. IEL from nTx mice contained few Tcr$\gamma\delta$ IEL indicating that these cells either originated in the postnatal thymus or that thymic-derived factors or cells were necessary for efficient $\gamma\delta$ IEL production. Tcr$\alpha\beta$ IEL from nTx mice were composed of a minor component of apparently mature IEL and a large subset of $CD8^{low}$ Tcr^{low} cells. Moreover, a subset of $CD8^{+}$ Tcr^{-} IEL were present in nTx intestine. These results suggest that Tcr$\alpha\beta$ IEL precursors migrate to the gut prior to birth where they mature under the influence of the thymus. To determine if IEL originate in the thymus, fetal and neonatal thymus was grafted onto nTx mice. Fetal thymus grafts produced all Tcr$\alpha\beta$ subsets, including the $CD8\beta^{-}$ subset, but did not produce Tcr$\gamma\delta$ IEL. However, neonatal thymus grafts generated Tcr$\gamma\delta$ as well as Tcr$\alpha\beta$ IEL. Thus, at least some IEL, regardless of Tcr expression, undergo a novel two-stage process of development: one step occurring within the thymus and the second occurring outside the thymus but under the influence of thymic factors.

V 648 LYMPHOID DEVELOPMENT IN MICE LACKING IL-7 RECEPTOR
Eugene Maraskovsky, Jacques J. Peschon, Philip J. Morrissey, Fred Ramsdell, Kenneth H. Grabstein, Charlie Maliszewski, Brian Gliniak, Kathy Picha, Douglas E. Williams, Carol B. Ware, Jeff D. Meyer, Steven F. Ziegler and Barry L. Davidson. Immunex Corporation, Seattle WA 98101 USA
Interleukin 7 (IL-7) is a multifunctional cytokine which acts on T cells, B cell progenitors and macrophages. IL-7 acts through a high affinity receptor (IL-7R) belonging to the haematopoietin receptor family. To further elucidate the function of IL-7 in lymphoid development, we have generated mice deficient in IL-7R (IL-7R-/-) via gene targeting in ES cells. Anatomical analysis of the lymphoid organs revealed a 90% reduction in the cellularity of the lymph nodes, thymus and spleen, with minimal effects on bone marrow cellularity. Defects in the thymic developmental pathways were noted and will be presented. Furthermore, the percentage of T cells in the spleen of the IL-7R-/- mice was reduced by 2-fold, although the ratio of CD4 : CD8 : B220 cells were unaffected. Lymphoid cells from these mice fail to bind IL-7 with high affinity and do not respond to IL-7. There was no significant difference in the levels of CD3, CD4, CD8, CD18 expression in T cells from either IL-7R-/- or normal mice. However, both CD4+ and CD8+ T cells from IL-7R-/- mice, cultured with IL-2 at limiting dilution or at low cell density, were 5-10-fold less responsive to immobilized anti-CD3 alone or in combination with anti-CD18 and anti-CD4 or anti-CD8. CD4+ and CD8+ T cells from the IL-7R-/- mice were also hyporesponsive to stimuli such as PMA and Ionomycin as well as to challenge with allo-antigen. Interestingly, flow cytometric analysis of T cells from the IL-7R-/- mice revealed an activated cell phenotype based on CD44 and CD45Rb expression. Cytokine production of T cell cultures stimulated with anti-receptor antibodies or PMA and Ionomycin was assessed by PCR and/or bioassay. IL-7 was not detected in the culture supernatant in either the IL-7R- or control T cells. Cytolyitc activity of CD8+ T cells was also examined. Analysis of the B cell compartment revealed a defect in the "early pro" B cell stage which normally proliferates in response to IL-7. As a result, subsequent stages of early B cell development were also affected. The findings thus far implicate IL-7 as an important regulator of lymphoid expansion.

V 649 **CHANGES IN T CELL SUBSETS IN EFFERENT LYMPH OF CATTLE DURING *Theileria annulata* INFECTION**
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Theileria annulata is a protozoan parasite which causes a serious lymphoproliferative disease of cattle: Tropical theileriosis in tropical and subtropical countries. The parasite is transmitted by ticks of the genus *Hyalomma* and preferentially invades monocytes/macrophages and B cells. Much of the pathology and lymphoproliferation takes place in the lymph node draining the site of infection. We are studying immune responses to the parasite in the efferent lymph of the draining lymph node in a potentially lethal infection as a model for understanding immunity to intracellular parasites. The parasite induces strong lymphoproliferation and the blasting cells start appearing in the efferent lymph after day 6 of infection. Initially, the blasting cells are CD4+ followed by a strong proliferation of CD8 cells. Most of the blasting cells are IL-2R+ as well at this stage. The resting T cells in the efferent lymph are MHC class II negative, but majority of blasting CD4 and CD8 cells have high MHC class II expression as well. However, the CD8 cells do not kill autologous parasite infected cells. The cells proliferate strongly in response to con A and exogenous IL-2, but don't proliferate in response to irradiated autologous *Theileria* infected cells. These findings are discussed in relation to the progression of the infection. Further studies on the function of the various activated cell populations are in progress.

V 650 POSITIVE SELECTION OF T LYMPHOCYTES ON FIBROBLASTS, Tomasz Pawlowski[#], Jill D. Elliott[#], Dennis Y. Loh[*] and Uwe D. Staerz[#+], [#]Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, Colorado 80206, USA, [*]Departments of Medicine, Genetics, Microbiology and Immunology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri 63110, USA [+]Department of Microbiology/Immunology and The Cancer Center, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, Colorado 80262, USA

Thymocytes are selected for expression of aß-T cell antigen receptors (TCR) that recognize antigen in conjunction with self-MHC molecules. It has been concluded that in the thymus the restriction element is imprinted on radioresistant stromal elements, and on cells of hematopietic origin. In $ß_2$-microglobulin-negative ($ß_2m^-$) mice devoid of mature cytotoxic T lymphocytes (CTL), we find that intrathymic (i.t.) injection of different fibroblasts causes the maturation of $CD4^-CD8^+TCR^{high}$ thymocytes with distinct patterns of TCR $V_ß$-distribution. We show that in TCR transgenic (TCR_{trans}) mice i.t. injection of L-cells expressing the selecting H-$2K^b$ molecule (LK^b cells) reconstitutes the maturation of TCR_{trans}^+ thymocytes, and that in normal B10.BR (H-2^k) mice H-$2K^b$ molecules expressed on LK^b cells leads to the development of T lymphocytes with recognition restricted to H-$2K^b$. Thus, we demonstrate that an class I MHC restriction element can be selected by interaction with fibroblasts, i.e. cells of other than epithelial or hematopoietic origin.

V 651 IDENTIFYING THE CIS TARGETING ELEMENTS OF THE SOMATIC MUTATION MECHANISM OF IMMUNOGLOBULIN GENES. Andrew Peters* and Ursula Storb, Department of Molecular Genetics and Cellular Biology, and *Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637

One of the mechanisms of diversification of the immunoglobulin repertoire is the process of somatic mutation. Somatic mutation is defined as point mutations which accumulate only in the variable region and the surrounding flanks in rearranged immunoglobulin genes at a rate of 10^{-3} base pairs per cell per generation. To learn more about the mechanism of somatic mutation a construct was made to identify the cis targeting sequences of the mutation mechanism. The construct is based upon the rearranged light chain gene kappa 167, which has been shown in this lab to mutate as a transgene. Transgenic mice were made with a test gene containing approximately four kilobases of the upstream region, beginning from the leader-variable region intron and including the leader intron and the promoter, cloned upstream of the constant region. If the cis targeting sequences lie upstream of the variable region then mutations should be targeted to the constant region in this construct. The variable region acts as an internal positive control since it has not been altered. The internal control was included to make sure that the construct could mutate at the site of integration. The transgenes will be screened for mutations using the denaturing gradient gel electrophoresis method (DGGE), and the mutated copies will be cloned and sequenced. Two additional modified constructs were made: in one, adjoining intronic MAR and enhancer were removed and placed downstream of the constant region; in the other, the variable and constant regions were exchanged. Preliminary data suggest the presence of mutations in several hybridomas produced from spleens of these transgenic mice.

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V 652 IN VITRO POSITIVE SELECTION INDUCED BY THYMIC EPITHELIAL CELLS, Ghislaine Poirier and Jonathan Kaye, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

One of the most critical roles of the thymus is to screen $CD4^+8^+$ immature T cells by mediating a differential T cell selection. In this developmental process autoreactive cells are induced to undergo apoptosis (negative selection), while cells ultimately able to recognize foreign peptides bound to self-MHC molecules are stimulated to complete their differentiation (positive selection). A large body of evidence emphasizes a role for cortical epithelial cells in positive selection. However, a few reports suggest that other cell types can also mediate positive selection. Using a culture model of positive selection, represented by the differentiation of $CD4^+8^+$ DPK cells (1), we show that thymic cells of non-hematopoietic origin ($CD45^-$ cells), but not peripheral antigen presenting cells can induce positive selection in the absence of an exogenous source of antigen. This result argues in favor of a specialized function for thymic epithelial (TE) cells in positive selection. In the presence of specific antigen, the differentiation of DPK into $CD4^+8^-$ cells is accompanied by high expression of the activation marker CD69, and IL-2R (IL-2 receptor). In contrast, the differentiation of DPK in the presence of TE cells is accompanied by low expression of CD69, and no expression of IL-2R .This result suggests that the TCR ligand on TE cells is of lower affinity or avidity than that of peptide antigen.

1- Kaye J. and Ellenberger D., Differentiation of an immature T cell line : a model for thymic positive selection, 1992, Cell, **7 1**: 423-435

V 653 REVERSAL OF THYMIC ATROPHY BY CASTRATION

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Commencing at the onset of puberty in the mouse and human there is a dramatic thymic involution that continues with ageing. This thymic atrophy (which is associated with an increase in the incidence of autoimmune diseases,) is characterised by a decrease in thymic mass associated with histological changes. Castration has been shown to reverse these changes, and to prevent thymic atrophy in pre-pubertal mice. A regulatory role of sex hormones on thymic weight has thus been established. To understand the changes occuring during ageing, and the mechanisms involved in thymic regeneration post castration, aged mice (6-12 months) have been castrated and compared to age matched and sham operated animals. Thymus, lymph nodes and spleen were analysed by flow cytometry and immunohistology at 2, 4 and 6 days and 1, 2, 4, 8 and 12 weeks post castration; blood was also examined by flow cytometry. Regeneration begins almost immediately post-operation, the thymus being increased in cellularity within the first 6-9 days post castration and at least as large if not larger than a normal young adult thymus by 2 weeks. A remarkable feature was the synchronous increase in all intrathymic precursors and αβ,γδ-CD4,CD8 defined T cell subsets in conjunction with a homeostatic increase in the thymic stromal elements. Immunohistological staining using a panel of mAbs to the stromal elements revealed major upregulation of antigens secreted by the stromal components of the blood-thymus barrier. In addition, there were medullary epithelial pockets within the cortex of castrate thymuses, and mAb MTS 16 expression has revealed an increase in the thymic extracellular matrix. Currently we are investigating the functional role of the post-castration upregulated stromal molecules in normal thymopoiesis, through addition of the relevant mAbs to FTOC and to castrated mice.

V 654 THE LYMPHOSTROMAL MOLECULE, TSA-1 REGULATES THE DEVELOPMENT OF IMMATURE THYMOCYTES.

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MTS 35, a rat mAb, detects the phosphotidyl inositol membrane linked molecule TSA-1, (Thymic Shared Antigen-1). Cloning and sequencing of TSA-1, has shown it to be a unique marker of immature thymocytes and isolated medullary epithelial cells. It is also expressed on dendritic cells. To examine the functional significance of TSA-1 in thymopoiesis, MTS 35 (as purified Ig) has been added to both normal fetal thymic organ culture (FTOC), and 2-deoxyguanosine FTOC reconstituted with fetal liver cells. In both instances MTS 35 downregulated TSA-1 expression, markedly reduced total cell yield and inhibited CD3, CD4 and CD8-thymocyte differentiation post $CD3^-CD4^-CD8^-$ triple negatives, particularly with regards to the $\alpha\beta$-TcR+ lineage and CD4+ thymocyte subsets. Such inhibition was also seen when day 15 fetal thymocytes were prevented from "spontaneously differentiating" into CD4+CD8+ thymocytes overnight. Examination of triple negative thymocytes with respect to the markers CD44 and CD25, revealed that $CD44^+CD25^-$ and $CD44^-CD25^+$ subsets proportionally increased whereas $CD44^-CD25^-$ decreased. All subsets however decreased in number. In conclusion $CD44^-$ $CD25^-$ cells appear to be inhibited from differentiating, a result which might be due to MTS 35 preventing TcR β rearrangement/ expression. Thus TSA-1 appears to be an essential molecule for immature triple negative thymocyte differentiation

V 655 DISTINCT EXPRESSION OF ENDOGENOUS MHC CLASS II:PEPTIDE COMPLEXES IN DIFFERENT CELL TYPES, Alexander Rudensky, Susan Eastman, Paul DeRoos, Michael Deftos, *Ned Braunstein and #Andy Farr, Department of Immunology and Howard Hughes Medical Institute, and #Department of Biological Structure, University of Washington School of Medicine, Seattle, WA 98195 and *Department of Medicine, College of P&S, Columbia University, New York, NY10032.

Expression of endogenous peptide:MHC class II complexes in B cells, macrophages, dendritic and epithelial cells from spleen and thymus, as well as in various cell lines, including processing deficient mutant cell line T2 transfected with IAb, was studied using monoclonal antibodies recognizing naturally occurring peptide:MHC class II complexes. FACS analysis, immunohistochemical and biochemical studies of complexes formed by IAb and a peptide derived from mouse Ea or human DRa chain (Ea52-68 or DRa52-68), as well as IAb and peptides derived from human and mouse invariant chain (Ii81-104 or Ii85-99) in different cell types revealed significant differences in ability to generate these complexes. Analysis of T2IAb cells demonstrated that approximately 50% of mature surface IAb molecules in this cell line are occupied by Ii-derived peptides. Normal antigen-presenting cells demonstrate a substantially lower level of Ii peptide:IAb expression with higher density of these complexes on macrophages than on B cells. The ratio of total IAb molecules to Ii chain expressed in these cells was not different. The relative level of expression of another complex, Ea peptide:IAb, in macrophages and B cells was comparable. However, we found that the concentration of the this complex per total IAb molecules in thymic epithelial cells is 10-20-fold lower than in bone marrow derived antigen-presenting cells. Our data suggest that, in addition to tissue-specific protein expression, differences in processing machinery in distinct types of antigen-presenting cells are responsible for the differential expression of peptide:MHC class II complexes.

V 656 The effects of viral superantigens on the T cell repertoire using *Mtv*$^-$ and single *Mtv* mice. Mark T. Scherer, John Kappler and Philippa Marrack. Howard Hughes Medical Institute, Denver, CO 80206

Most laboratory strains of mice have between two and eight superantigens encoded within their genomes. These superantigens (vSAG's) are found in the 3' long terminal repeats of endogenous mammary tumor viruses (*Mtv's*). A line of *Mtv* negative mice and several lines of mice containing single *Mtv's* were created by inbreeding (CBA/CaJ x C58/J) mice. This allows the native T cell repertoire of $H\text{-}2^k$ mice to be studied, and allows a detailed study of the effects upon this repertoire of the expression of a single viral superantigen. CD4 and CD8 T cells had quite different repertoires in the Mtv^- mice, presumably due to the effects of positive selection on class I or class II MHC molecules. Nevertheless, CD4 or CD8 T cell repertoires from different *Mtv*$^-$ mice were very similar and reproducible. vSAG3 was found to delete Vβ5 T cells in addition to the Vβ3 T cells previously reported. vSAG8 deleted Vβ7 T cells, in addition to its previously reported specificity for T cells bearing Vβ's 5, 11, and 12. This contrasts with vSAG9, another member of the same, *Mls*^{a} family. vSAGs 14, 17, and 30 have no effect on the T cell repertoire, and are thought not to be expressed. According to the affinity hypothesis of positive selection, as it would be expected that a superantigen which had a high affinity for a certain Vβ would have a low affinity for other, homologous Vβ's, and T cells bearing these Vβ's would be positively selected, and increase in frequency. Little evidence of positive selection by any vSAGs on T cells bearing any Vβ tested was seen, despite an extensive search for such effects, and despite published reports to the contrary. This result argues against a simple version of the affinity hypothesis.

V 657 Thymic Selection in vitro: Demonstration that Epithelial Cell Subsets May Participate in Both Positive and Negative Selection to Superantigens. Anis Sen-Majumdar*, Miriam Lieberman# Gun Hansteen* and Irving L. Weissman#. *Becton Dickinson Immunocytometry Systems, San Jose, CA, and Stanford University School of Medicine, Stanford, CA.

Immature thymocytes lacking CD3, CD4 and CD8 surface expression undergo a series of interactions with non-T cells present within the thymic microenvironment to become phenotypically and functionally mature. This maturational process by which CD3-4-8- thymocytes become CD4+8+ includes cell division; rearrangement of T cell receptor genes; expression of low to medium levels of TCR; and expression of both MHC class-II coreceptor CD4 and MHC class-I coreceptor CD8 molecules. The αβ TCR+CD4+8+ cells that recognize self-MHC molecules are signaled to mature into CD4+ or CD8+ T cells by positive selection, while auto-reactive thymocytes recognizing self-antigen/MHC complex are negatively selected and deleted. We have shown that short-term heterogeneous thymic stromal cultures in vitro permit the maturation of all thymic T cell subsets beginning with CD3-4-8- immature thymocytes . The objective of the current study is to determine whether defined thymic epithelial cell subsets, when added to the culture of purified CD3-4-8- thymocytes on thymic stroma, will induce positive and negative selective events. In the present study, we demonstrate that the genotype of the thymic stroma determines the pattern of positive and negative selection of T cell receptor subsets, irrespective of the strain of origin of the thymocytes. When a thymic stroma (from C57BL/Ka mice) that supports maturation but does not mediate particular positive or negative selection events is used as a baseline culture, the addition of freshly isolated and purified populations of thymic epithelial cells taken from in vivo sources permit positive and negative selective events, depending on the thymic stromal donor genotype. The role of thymic cortical and medullary epithelial cell subsets in inducing positive and negative selection of particular Vβ-TCR bearing thymocytes in the in vitro differentiation model will be discussed.

V 658 HEAT-STABLE ANTIGEN IS AN EARLY DIFFERENTIATION MARKER OF EXTRATHYMIC INTESTINAL INTRAEPITHELIAL LYMPHOCYTES, *Donna Stickney, Mawieh Hamad, and John R. Klein, Department of Biological Science, University of Tulsa, Tulsa, OK 74104.*

Using a system of bone marrow (BM) hematopoietic repopulation of irradiated euthymic mice (*Transplantation 53:868*), we have examined the extrathymic development of murine intestinal intraepithelial lymphocytes (IEL). At a time of active extrathymic development within the gut, prior to development of T cells within the thymus, two distinct lymphoid cell populations were present in the intestine epithelium of reconstituted mice. One population consisted of lymphocytes with phenotypic properties typical of mature IEL which decreased in proportion until day 9 post-BM transfer, then gradually reappeared. Those cells consisted of phenotypic subsets of mature IEL, including both TCR-αß and TCR-γδ cells which expressed CD8α/α or CD8α/ß dimeric molecules. The other population consisted of a transient subset of slightly smaller cells that increased in abundance between days 5 and 14 post-BM transfer, then declined. The latter population was void of common markers of mature T cells and B cells but expressed heat-stable antigen (HSA) and was similar to a very immature T cell precursor within the thymus. Studies using freshly-extracted IEL obtained from non-irradiated adult mice and separated by cell size and density revealed a subset of HSA+ IEL with physical and phenotypic properties similar to the transient IEL in radiation chimeras, demonstrating that those cells constitute a normal subset of murine IEL. In adult mice, those IEL comprised <5% of the total IEL which, upon *in vitro* culture with T cell dependent cytokines, acquired a marker of mature thymus-independent IEL, *i.e.*, the CD8α/α homodimer. It is anticipated that identification of this precursor cell of thymus-independent IEL will greatly facilitate analyses of IEL extrathymic developmental pathways. (Supported by NIH grant DK35566 and by a grant from the Mervin Bovaird Center for Studies in Molecular Biology.)

V 659 UBIQUITOUS EXPRESSION OF THYMOPOIETIN mRNA IN A VARIETY OF TISSUES AND CELL LINES. Theodor L, Gokel H, Trachtenbrot L, Grossman Z, Berger R, Brok-Simoni F, *Zevin- Sonkin D, *Ilan E, *Shoham Y, and Rechavi G. Institute of hematology, The Chaim Sheba Medical Center, Tel-Hashomer and Sackler School of Medicine, Tel-Aviv University, *and Life Sciences Faculty, Bar-Ilan University, Ramat Gan, Israel.

Thymopoietin (TP) is known as a protein exerting immunomodulating properties. In addition, TP was shown to specifically bind to alpha-bungarotoxin type nicotinic acetyl-choline receptor in the brain. The cDNA encoding the murine TP was cloned and sequenced. An ORF of 298 bp was identified. The deduced amino acid sequence revealed a 49 a.a.-long protein with 98% homology and 100% similarity to the bovine TP, previously described by our group. No signal peptide was identified in the cloned cDNA. In situ hybridization revealed TP mRNA expression in the thymus (mainly in the cortex), as well as in other tissues. S1 analysis demonstrated an ubiquitous expression of TP mRNA in all tissues analyzed. TP expression was found in several cell lines including those derived from T and B lymphocytes, as well as lines from thymic and bone marrow stroma and glial cells.

V 660 STEROID PRODUCTION IN THE THYMUS: IMPLICATIONS FOR THYMOCYTE DEVELOPMENT, *Melanie S. Vacchio and Jonathan D. Ashwell, Laboratory of Immune Cell Biology, NCI, National Institutes of Health, Bethesda, MD 20892*

Occupancy of the T cell receptor (TCR) is a potent means of inducing programmed cell death (PCD) in thymocytes. Similarly, glucocorticoids induce thymocyte PCD. Work from our lab and others has demonstrated that glucocorticoids antagonize induction of TCR-mediated PCD in T cell hybridomas and thymocytes *in vitro* . While exposure to either stimulus alone induces PCD, simultaneous stimulation with both allows the cells to survive (mutual antagonism) and has led to the proposal that exposure of thymocytes to both of these stimuli during thymocyte differentiation may be a basis for positive selection. Whereas high avidity TCR/ligand interactions may induce a signal too potent to be antagonized, low-moderate avidity TCR/ligand interactions may transduce a weaker signal that can be antagonzied by glucocorticoid/GR interactions and allow positive selection to occur. For this to be a viable model, there necessarily must be exposure of thymocytes to glucocorticoids during thymocyte development. Because the thymus has been reported to have endocrine properties, we investigated whether the thymus can produce steroids. Immunohistochemical analysis of the thymus revealed that enzymes required for glucocorticoid synthesis are present in a subset of thymic cells resulting in the synthesis of detectable levels of steroids *in vitro*. Furthermore, deprivation of steroids synthesized in fetal thymic organ culture resulted in more efficient TCR-mediated thymocyte deletion. These results demonstrate that physiologically significant levels of steroids are synthesized in the thymus and can participate in determining the fate of thymocytes during selection.

V 661 IN ATHYMIC CHIMERAS THY-1, CD5, TCR, AND CD8 DIMERIC MOLECULES DO NOT DIFFERENTIATE THYMUS-DEPENDENT VERSUS THYMUS-INDEPENDENT LINEAGES OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES, *Jin Wang and John R. Klein, Department of Biological Science, University of Tulsa, Tulsa, OK 74104.*

Several studies have reported that murine intestinal intraepithelial lymphocytes (IEL) can be differentiated according to markers of 'thymus-independent' (Thy-1-, CD5-, CD8α/α+, and TCR-γδ+) and 'thymus-dependent' (Thy-1+, CD5+, CD8α/ß+, TCR-α/ß+) IEL. Yet no studies have examined athymic mice using all markers. In multi-color flow cytometric analyses of IEL from euthymic mice and athymic radiation chimeras, we find only minor differences in the expression of those markers in either animal despite 99% reduction in T cells in the periphery of athymic mice. Thus, Thy-1, CD5, CD8, and TCR do not accurately differentiate developmental lineages of IEL in athymic chimeras. Similar experiments are currently under way in nude and neonatally thymectomized mice.

	Percent Expression	
IEL subset	euthymic	athymic
Thy-1+	57	50
CD8α+, CD8ß+	35	17
CD8α+, CD8α+	59	69
TCRαß+, CD5+	22	16
TCRαß+, CD5-	18	15
TCRγδ+, CD5+	1	0
TCRγδ+, CD5-	33	38

	CD5 Expression (%)	
IEL subset	euthymic	athymic
CD4+, CD8-	89	84
CD4+, CD8+	68	59
CD4-, CD8+	16	16
CD8α+, CD8ß+	62	57
CD8α+, CD8ß-	9	19
Thy-1+, CD4+	54	52
Thy-1+, CD8ß+	65	73
Thy-1-, CD8α+	7	5
Thy-1-, CD8α-	10	19
Thy-1-, CD8ß-	12	8

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Molecular Basis for Co-Stimulation of B and T Cell Subsets

V 700 THE MODULATION OF LOW BCL-2 EXPRESSION AND APOPTOSIS IN ACTIVATED $CD4^+$ T CELLS BY *IL-2* AND/OR CD28 COSTIMULATION

Arne N. Akbar, Wendy Gombert, Margarita Bofill, Fiona Whitelaw, Darrell Pilling, George Janossy and Mike Salmon, Department of Clinical Immunology, The Royal Free Hospital School of Medicine London NW3, U.K.

The bcl-2 gene product can prevent apoptotic death. After activation *in vivo* and *in vitro*, T cells acquire CD45RO reactivity concomittantly with a loss in bcl-2 expression. This predisposes these cells to apoptosis and is one mechanism which enables cellular homeostasis to be achieved after an immune challenge. We found that factors such as IL-2 may keep the activated T cells in cycle, increase their bcl-2 expression and prevent their apoptosis. On the other hand, coculture with fibroblasts prolonged the survival of activated T cells but in a non-cycling state. These may be ways in which specifically primed T cells may persist *in vivo*. We have now investigated the ability of 'fibroblast-rested' $CD4^+$ T cells to be reactivated *in vitro*. These cells are $<1\%$ $Ki67^+$ indicating that they are not cycling. If they are removed from fibroblasts they rapidly apoptose. The addition of IL-2, however, significantly increases their bcl-2 expression, induces a large proportion of the cells to cycle (40% after 48 hours) and prevents apoptosis. CD3 stimulation of these cells in the absence of antigen presenting cells also leads to rapid death. If these cells are costimulated with CD3 and CD28, however, bcl-2 is once again increased, $>70\%$ of the cells are in cycle and apoptosis is largely abrogated. These results suggest that stromal factors may induce activated T cells to enter a resting (non-cycling) but primed (rapid response to IL-2) state for extended periods *in vivo* but these cells may be reactivated by appropriate stimulation. This may be important for the rapid deployment of these cells in response to antigenic rechallenge *in vivo*.

V 701 THE ROLE OF 2D10 MOLECULE IN T CELL ACTIVATION AND ANERGY C. Chen, A. Gault, and N. Nabavi. Department of Inflammation and Autoimmune Diseases, Hoffmann-La Roche Research Center, Nutley, NJ 07110.

Our recent studies have shown that a rat mAb, 2D10, recognizes a 60-KDa protein (2D10-A) on the surface of activated B cells, and 2D10-A delivers a potent costimulatory signal to T cells during auto- and alloantigen presentation. To further examine the role of 2D10-A in T cell activation, purified mouse splenic CD4 T cells were stimulated with anti-CD3 for 48h in the presence of syngeneic resting or LPS-stimulated B cells as the source of costimulatory signals. The activation of CD4 T cells was measured by ^{3}H-TdR uptake of the cells in the last 6h of the culture. The results showed that LPS-stimulated but not resting B cells provided potent costimulatory signals to CD4 T cells primed with anti-CD3. The CD4 T cell proliferation was partially (20-40%) blocked by anti-mB7 or 2D10 mAbs. However, combination of the two mAbs at > 5 μg/ml abrogated T cell proliferation by 95%. Isotype-matched control rat IgG or mAb against B220 did not show any inhibitory effects. Next, CD4 T cells were primed with anti-CD3 in the presence of LPS blasts with or without blocking antibodies for 60h. Viable T cells were then harvested, washed, and re-stimulated with anti-CD3 and fresh LPS-B cells. The results revealed that blocking of B7 and 2D10-A by the specific mAbs, during T cell priming with anti-CD3, suppressed the response of the T cells to the subsequent re-stimulation with anti-CD3 by 95% as compared to the control. Furthermore, the unresponsiveness in T cells was directly correlated with lower levels of IL-2, IL-3, and IFN-gamma mRNA, which were detected in these cells by semi-quantitative RT-PCR. Taken together, our data indicate that B7 and 2D10-A are two major costimulatory molecules for CD4 T cells. Collaboration between the two molecules is required for maximum T cell activation. Blocking of both B7 and 2D10-A pathways induces anergy in CD4 T cells.

V 702 DISTINCT SECOND MESSENGER SYSTEMS INVOLVED IN B7 INDUCTION IN RESTING VERSUS ACTIVATED B CELLS *Mark DeBenedette Noosheen Alaverdi, and Tania H. Watts. Department of Immunology, University of Toronto, Toronto, Ontario M5S 1A8*

B7 is the ligand for the CD28 receptor expressed on T cells. B7 ligation of CD28 generates costimulatory signals that are needed to over come the nonresponsive state induced by TCR crosslinking. We provide evidence to show that both PKC activation and cAMP dependent signaling pathways lead to B7 induction. Whether or not a cell uses one pathway or the other is controlled by the state of activation of the cell. Resting B cells are induced to express B7 in response to PMA/ionomycin and not cAMP. PMA/ionomycin activated murine splenic and human tonsillar B cells are responsive to cAMP treatment leading to enhanced B7 expression. M12 B lymphoma cells behave like activated B cells with respect to B7 induction. We also show that T cell contact dependent B7 induction is dependent on the binding of CD40 ligand on the T cell with CD40 on the B cell. This interaction can be blocked with antibodies to the CD40 ligand which ablates T cell contact mediated B7 induction on B lymphoma cells. This suggests that signals generated through the CD40 receptor account for the induction of B7 on B lymphoma cells. Since dbcAMP treatment of these cells also induces B7, this raises the possibility that the CD40 receptor may have a cAMP component linked to its signaling cascade or that B7 induction on actively cycling cells becomes responsive to cAMP dependent signaling mechansims.

V 703 POSSIBLE USE OF SOLUBLE CO-STIMULATORY MOLECULES TO TRIGGER ANTI TUMOUR IMMUNE RESPONSE IN VIVO, Paolo Dellabona, Federica Cavallo+, Evelina Gatti, Daniele Morpurgo, Matteo Bellone, Guido Forni+ and Giulia Casorati. DIBIT, H.S.Raffaele, Milano, Italy, and +Centro C.N.R. CIOS, Torino, Italy.

Tumour cells are antigenic but not immunogenic possibly because they lack co-stimulatory signals for T-cell activation. We have selected B7 and ICAM-1 as prototypic co-stimulatory molecules, and transfected them in different combinations into 6 poorly immunogenic mouse tumours. In this way, we could define the co-expression of B7 and ICAM-1, and not of each single molecule, as the minimal co-stimulatory signal capable of priming an efficient anti tumour CTL response. In order to by-pass tumour transfection, we have generated soluble, chimeric B7-Ig and ICAM-1-Ig molecules, and we are now trying to target them in vivo onto the tumour cells. Preliminary data show that the chimeric B7-Ig can induce the rejection of the ICAM-1^+ J558L plasmocytoma, together with long lasting memory.

V 704 EITHER B7 OR ICAM-1 CAN PROVIDE COSTIMULATION FOR NAIVE CD4 RESPONSES. Caroline Dubey, Michael Croft and Susan L. Swain, Dept of Biology, University of California, San Diego, La Jolla, CA, 92093.

We have previously shown that naive $CD4^+$ T cells respond to antigen presented only by highly costimulatory antigen presenting cells (APC) such as dendritic cells and activated B cells but not by resting B cells.

In order to study the costimulatory molecules involved in naive T cell activation, we have used Ag-presenting class II positive fibroblast cell lines expressing either ICAM-1 and B7 ($ICAM^+$,$B7^+$), B7 alone ($ICAM^-$,$B7^+$), or ICAM-1 alone ($ICAM^+$, $B7^-$, contaminated with less than 6% $B7^+$ cells). These cell lines do not express other known costimulatory molecules including HSA, CD48 (murine equivalent of LFA-3) or VCAM-1. $CD4^+$ T cells were from mice transgenic for the Vβ3/Vα11 T cell receptor which recognize a peptide of cytochrome c (PCCF) presented by IE^k. Transgene-expressing cells are of naive phenotype and have response characteristics of naive T cells.

Naive T cells were able to produce IL-2 and to proliferate in response to PCCF presented by either $ICAM^+$,$B7^-$ or $ICAM^-$,$B7^+$ cells. However, when ICAM and B7 were both expressed on the cell surface, T cells produced much more IL-2 and proliferated more efficiently. In order to rule out effects of other costimulatory molecules present on the cell surface, CTLA-4Ig which is an efficient inhibitor of the CD28/B7 interaction was added in the culture. In the presence of CTLA-4Ig, $ICAM^-$,$B7^+$ cells could not activate naive T cells, whereas $ICAM^+$,$B7^+$ cells were still competent as APC and resulted in IL-2 production and T cell proliferation at a level comparable to the response obtained with $ICAM^+$,$B7^-$ cells.

These results suggest that:

1) the presence of either ICAM-1 or B7 alone on APC is sufficient to activate naive $CD4^+$ T cells.

2) when ICAM-1 and B7 are both present on APC, optimal IL-2 production and proliferation is obtained.

V 705 IN VIVO ACTIVITY OF MONOCLONAL ANTIBODIES AGAINST MURINE B7 (1G10) AND A SECOND COSTIMULATORY MOLECULE (2D10), Denise A. Faherty, Kenneth Kolinsky, Suzanne Connaughton, and Nasrin Nabavi, Inflammation & Autoimmune Diseases, Hoffmann-La Roche, Nutley, NJ 07110

The monoclonal antibody 2D10 recognizes a novel B cell activation antigen (2D10-A) with T cell costimulatory activity. In this study we compared the in vivo effect of administration of this antibody to the effect of an anti-B7 mAb, 1G10, in humoral and cell-mediated immune responses. Administration of a single dose of 2D10 mAb inhibited both the primary IgM and IgG responses to sheep erythrocytes on days 5 and 14, respectively. Treatment with the 2D10 antibody could be delayed until two days after immunization and still induced greater than 50 percent inhibition. However, administration on day 3 had only a minimal effect. In contrast, QOD administration of an antibody against murine B7 had no reproducible effect on the IgM response on day 5 but consistently induced partial inhibition of the IgG plaques on day 14. These data are consistent with a model in which the 2D10 antigen is involved early during the initiation of the response and the B7 molecule is involved during the maturation and expansion of the response. In contrast to the results seen in the humoral model, the delayed-type hypersensitivity response to sheep erythrocytes appeared to be dependent upon both costimulatory molecules. Administration of a single dose of either antibody inhibited the specific swelling of challenged footpads by approximately 60 percent. Combination therapy with both antibodies did not induce any greater suppression than either antibody alone. These data suggest that both costimulatory molecules, B7 and 2D10-A, are required for this response but that possibly there are other molecules also involved.

V 706 THE INDUCTION OF CYTOLYSIS DOES NOT REQUIRE CD28 COSIGNALLING Fiona A. Harding and James P. Allison GenPharm, International, Mountain View, CA 94043, and Department of Molecular and Cellular Immunology, University of California, Berkeley, CA 94720

The role of CD28-mediated signalling in the *in vitro* induction of cytotoxic T lymphocytes (CTLs) has been investigated. CD28 was found to provide a costimulatory signal necessary for the generation of measurable lytic capacity in allogeneic MHC class I-specific mixed lymphocyte cultures. CD28-mediated cosignalling directly provoked the release of IL-2 from $CD8^+$ precursors, which was found to be a necessary component for the generation of measurable lytic capacity in the cultures. Further investigation revealed that CD28/B7 interactions are not necessary for the induction of lytic activity by naive $CD8^+$ T cells using both antigen-specific and redirected cytotoxicity assays. Antigen-specific naive T cell cytotoxicity was examined using F5 transgenic mice, which express a T cell receptor (TCR) specific for peptide representing amino acids number 366-374 of influenza nucleoprotein presented by D^b. High density $CD8^+$, $CD44^{lo}$, $CD45^{hi}$, IL-$2R^-$, $CD69^-$ T cells were isolated from lymph nodes and spleens of either F5 transgenic or C57Bl/6 mice, then immediately placed into assays with ^{51}Cr labelled targets. Peptide antigen-pulsed or anti-CD3 mAb-redirected targets were specifically lysed by 12 hours regardless of B7 expression. Lytic activity of naive $CD8^+$ T cells was not dependent on IL-2 release, however, B7 expression by antigen-specific target cells did correlate with secretion of IL-2 and γ-IFN. These data suggest that CD28 cosignalling serves to enrich mixed lymphocyte cultures for antigen-specific CTLs by promoting their proliferation, and that naive $CD8^+$ CTL precursors are not dependent on a CD28/B7 interaction for completion of differentiation to lytic competence.

V 707 TRANSGENIC MICE EXPRESSING B7 ON PANCREATIC BETA CELLS EXHIBIT DIABETES SUSCEPTIBILITY DUE TO A BREAKDOWN OF T LYMPHOCYTE UNRESPONSIVENESS, David M. Harlan, Hans Hengartner, Mark L. Huang, Yuan-Hsu Kang, Randall W. Moreadith, Hanspeter Pircher, Gary S. Gray, Pamela S. Ohashi, Gordon Freeman, Carl H. June, and Peter Aichele, Immune Cell Biology Program, Naval Medical Research Institute, Bethesda, MD 20889

In order to study the elements responsible for antigen-specific T cell activation in vivo, we have created transgenic mice (inbred strain Harlan FVB/N) expressing mouse B7, a ligand for the CD28 receptor, on pancreatic beta cells. We followed B7 transgenic mice from birth to 52 weeks of age but observed neither insulitis nor diabetes: moreover, islet architecture was normal. We postulated that the absent immune response was due to immune "indifference", i.e. an inadequate density of endogenous beta cell antigens to provide a signal sufficient to activate beta cell antigen specific T lymphocytes. Immune indifference was tested by crossing B7 transgenic mice with mice that express both LCMV glycoprotein (GP) via MHC class I on pancreatic beta cells, and transgenic T cells specific for the GP in the context of $H\text{-}2D^b$. Several triple transgenic mice were bred and by 14 weeks all (14/14) developed diabetes, one as early as 5 weeks. Pancreatic histopathology from the diabetic triple transgenic mice revealed lymphocytic islet infiltration by B cells and both CD4 and CD8 T cell subsets. The immune destruction within the islets was limited to beta cells, islet alpha cells were left intact. We studied pancreatic histopathology from one triple transgenic mouse killed at four weeks of age (not yet diabetic) and observed profound insulitis, but with some residual beta cell function (cellular staining for insulin). Mice transgenic for any two of the three transgenes studied, i.e. GP plus B7, B7 plus the GP specific T cells, and GP plus the GP specific T cells, never spontaneously developed diabetes. Moreover, pancreatic sections from these double transgenic mice revealed normal islet architecture, normal cellular staining for insulin, and no lymphocytic infiltrates. The present results support the general hypothesis that aberrant expression of B7 (or other related T cell costimulatory pathway activating molecules) may play a pathophysiologic role in autoimmunity.

V 708 IDENTIFICATION OF AN ALTERNATIVE CTLA4 LIGAND THAT IS COSTIMULATORY FOR MURINE T CELL ACTIVATION, K. S. Hathcock, G. Laszlo, H. B. Dickler, J. Bradshaw, P. Linsley, and R. J. Hodes, Experimental Immunology Branch, NCI and NIA, NIH, Bethesda, MD 20892, and Bristol-Meyers Squibb PRI, Seattle, WA 98121. Stimulation of T cells to cytokine production and proliferation generally requires two signals: the first provided by engagement of the TcR by antigen presented in the context of tself-MHC products and a second signal, or costimulus, provided by a separate set of receptor-ligand interactions. Using a rat mAb raised against activated murine B cells, we have identified a cell surface molecule (GL1), that is distinct from B7, and that appears to be the predominant ligand for CTLA4Ig expressed by activated B cells. GL1 protein is expressed at low levels by unstimulated murine B and T cells and its expression is increased with activation. GL1 protein immunoprecipitated from ^{125}I-labelled LPS-activated B cells is 68-100 kDa molecular weight (MW) and treatment with N-glycanase yields a homogeneous band with an apparent MW of 35 kDa. The addition of GL1 mAb inhibits T cell proliferation in vitro to accessory cell (Acc)-dependent stimuli but not to Acc-independent stimuli. GL1 mAb also inhibits the in vivo generation of IgG, but not IgM antibody responses to T-dependent antigen. Thus, the costimulatory pathways involved in T cell activation to Acc-dependent stimuli may be more complex than previously appreciated, with GL1 or B7 on the Acc potentially reacting with CD28 or CTLA4 on the T cell.

V 709 CLONING AND SEQUENCING OF THE RAT B CELL ACTIVATION ANTIGEN B7, M. Jackerott, S. Tullin, L. Hornum and H. Markholst, Hagedorn Research Institute, Gentofte, Denmark

The reduced number and unresponsiveness of T cells (lymphopenia) in the diabetic BB rat may be caused by deficient antigen presenting cells. Since antigen processing and presentation by MHC molecules appear to be normal, we have focused on the accessory molecules involved in the stimulation of T cells. Several cell surface molecules on antigen presenting cells are known to be required for proper stimulation of T cells. One of these, the B cell activation antigen B7, expressed on activated B cells, INF-γ, induced monocytes and dendritic cells, has been shown to produce an important co-stimulatory signal upon interaction with its ligand, CD28, present on T cells. Theoretically, the lymphopenia in the BB rat could be explained by an abnormal B7 function. From a cDNA library of MHC II positive thymic cells the rat homologue of the murine B7 was cloned and sequenced. The predicted protein of 294 amino acids was 76% identical to the murine B7 protein. A high homology was found in the Ig-C and Ig-V domains, while in the transmembrane and cytoplasmatic regions very little homology was found. Preliminary results suggest that the level of B7 transcript in lymphoid tissues does not differ in the BB and Brown Norway rat. Furthermore, we intend to map the B7 gene in the rat.

V 710 THYMIC T CELL ANERGY IN NOD MICE IS REVERSED BY COSTIMULATION VIA CD28. Andrés Jaramillo, Kevin B. Laupland, Bruce M. Gill, Nancy Van Houten*, Ralph C. Budd*, and Terry L. Delovitch. Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada, and *Department of Medicine, Rheumatology and Clinical Immunology Unit, University of Vermont, Burlington, VT 05405, USA.

We previouly found that an anergic response, which is associated with reduced IL-2 and IL-4 secretion after TCR crosslinking, occurs in a large proportion of thymic and peripheral T cells from nonobese diabetic (NOD) mice. Exogenous IL-4 reverses this anergy *in vitro* and prevents the onset of diabetes in NOD mice. We now show that CD4$^+$ thymic T cells from NOD mice express higher levels of CD2 than CD4$^+$ thymic T cells from control BALB/c mice. This subpopulation of NOD CD4$^+$CD2^{high} thymic T cells may mediate this anergy. Levels of CD28 surface expression are comparable on quiescent and activated NOD and BALB/c T cells. Costimulation by anti-CD28 mAb reverses the anergic response of NOD CD4$^+$CD2^{high} and CD8$^+$ T cells but not of CD4$^+$CD2^{low} T cells. Similar results were obtained upon T cell stimulation by exogenous IL-4. Potentiation of IL-2 secretion by CD-28 costimulation is comparable in BALB/c and NOD T cells. In contrast, CD28 costimulation restores IL-4 secretion by NOD T cells to normal but is without effect on IL-4 secretion by BALB/c T cells. Anti-IL-2 mAb significantly blocks costimulation through CD28 in both NOD and BALB/c T cells (48% and 45% inhibition, respectively). In contrast, while anti-IL-4 mAb diminishes CD28 costimulation in NOD T cells (27% inhibition) it does not significantly alter CD28 costimulation in BALB/c T cells. These data demonstrate that (i) NOD thymic T cell anergy is manifested mainly by the CD4$^+$CD2^{high} subset, and (ii) this anergy is overcome by CD28 costimulation and is mediated by an IL-4-dependent increase in IL-2 production.

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V 711 CD28 Associates with the Lipid Kinase, PI 3-Kinase via a Cytoplasmic pYMXM Motif, Kanteti V. S. Prasad[1], Yun-Cai Cai[1], Monika Raab[1], Brian Duckworth[2], Lewis C. Cantley[2], Steven E. Shoelson[3], and Christopher E. Rudd[1], [1]Division of Tumor Immunology, Dana Farber Cancer Institute; [2]Department of Medicine, Beth Israel Hospital; [3]Joslin Diabetes Center; Departments of [1]Pathology, [2]Physiology & [3]Medicine, Harvard University, Boston, MA 02115.

The T-cell antigen CD28 provides a co-stimulatory signal that is required for T-cell proliferation. TcRζ/CD3 engagement without CD28 ligation leads to a state of non-responsiveness/anergy, thereby implicating CD28 in the control of peripheral tolerance to foreign antigens or tumors. A key unresolved question has concerned the mechanism by which CD28 generates intracellular signals. Phosphatidylinositol 3-kinase (PI 3-kinase) is a lipid kinase with SH2 domain(s) that binds to the PDGF-R, an interaction that is essential for signaling by growth factor. In this study, we demonstrate that CD28 binds to PI 3-kinase by means of a pYMXM motif within its cytoplasmic tail. CD28 associated PI 3-kinase was detected by lipid kinase and HPLC analysis as well as by re-constitution experiments using baculoviral expressed p85 subunit of PI 3-kinase. CD28 bound directly to the p85 subunit, without the need for the associated p110 subunit. Site-directed mutagenesis and peptide competition analysis using pYMXM containing peptides showed that PI 3-kinase bound to a pYMXM motif within the CD28 cytoplasmic tail (residues 191-194). Mutation of the Y-191 within the motif resulted in a complete loss of binding, while mutation of M-194 caused partial loss of binding. Binding analysis showed that the N-terminal SH2 domain bound CD28 with an affinity as great as the PDGF-R. In terms of signaling, CD28 ligation induced a dramatic increase in the recruitment and association of PI 3-kinase with the receptor. We previously defined a tyrosine phosphorylation independent interaction of lck and fyn-SH3 domain with PI 3-kinase. CD28 recruits 15 to 30 percent of the intracellular PI 3-kinase, many fold greater than bound to CD4-lck or TcRζ/CD3-fyn. CD28 is likely to employ PI 3-kinase as the second signal leading to T-cell proliferation, an event with implications for anergy and peripheral T-cell tolerance.

V 712 IL-12 IS AN IMPORTANT COSTIMULATOR FOR Ag-SPECIFIC ACTIVATION AND GROWTH OF Th1, BUT NOT Th2, CLONES, Mary K. Kennedy, Kathleen S. Picha, Kurt D. Shanebeck, Dirk M. Anderson, and Kenneth H. Grabstein, Immunex Corporation, Seattle, WA 98101

Ag-specific activation of T cells requires both the engagement of the TcR and the provision of other non-specific costimulatory signals by APC. Previous studies have shown that IL-10 inhibits APC-dependent murine T cell proliferation and Th1 cytokine production by downregulating the costimulatory function of certain accessory cells. IL-10 inhibits numerous aspects of monocyte/macrophage function, including LPS-induced expression of the costimulatory molecule, B7, and production of cytokines such as IL-1, IL-6, TNFα, and IL-12. Using a large panel of Th clones, we examined the role of IL-10 and IL-12 in the Ag-specific proliferation of both Th1 and Th2 clones. Proliferation of Th1, but not Th2, clones in response to Ag presented by syngeneic splenocytes was significantly inhibited by either IL-10 or anti-IL-12. The inhibitory effect of IL-10 on the Ag-specific proliferation of Th1 clones was completely reversed in the presence of $\geq$ 0.3 ng/ml of IL-12. In contrast, the inhibitory effect of IL-10 was not reversed by a variety of other cytokines (IL-2, IL-4, IL-7, IFN-γ, IL-1α, and IL-1β) used at concentrations up to 50 ng/ml. Thus, IL-10 most likely exerts its inhibitory effects on Th1 cell proliferation via downregulation of IL-12 production by the APC. In addition, we show that the ability of Th1 clones to respond to IL-4 is dependent upon a costimulatory signal provided by IL-12. Th1 clones preincubated with anti-CD3 and IL-12 developed competence to respond to IL-4 in a secondary culture, whereas Th1 clones preincubated with anti-CD3 or IL-12 alone did not respond or responded suboptimally to IL-4. Surprisingly, resting Th1 clones, which proliferated weakly or not at all to either IL-4 or IL-12 alone, proliferated vigorously to the combination of IL-4 and IL-12. In contrast, IL-4 and IL-12 did not have a synergistic effect on the proliferation of Th2 clones. The results indicate that IL-12 is an important costimulator for Ag-specific activation and growth of Th1, but not Th2, clones.

V 713 *COSTIMULATION THROUGH CD28 ENHANCES T CELL-DEPENDENT B CELL ACTIVATION VIA A CD40-CD40L INTERACTION*, Stephen J. Klaus*, Lesya Pinchuk*, Hans D. Ochs*, William C. Fanslow#, Richard J. Armitage# and Edward A. Clark*, *University of Washington,#Immunex Corporation, Seattle WA.

Changes in T cell helper function were analysed when anti-CD3 activated T cells were costimulated with monoclonal antibodies to the CD28 receptor (anti-CD28). T cell-dependent B cell growth and differentiation were consistently augmented if anti-CD3 stimulated T cells were simultaneously activated with anti-CD28. Although anti-CD28 enhanced IL-2 and IL-4 production, it did not increase B cell responses solely by augmenting production of soluble lymphokines. Anti-CD28 costimulation also induced increases on T cells of CD40 ligand and membrane TNF-α expression, two molecules known to promote B cell proliferation and Ig secretion. Since anti-CD28 promoted T cell helper functions and expression of CD40L, we examined the dependence for CD40L during T-dependent B cell responses. Although soluble CD40 fusion proteins only partially inhibited T-dependent B cell activation, we found a strict requirement for CD40L expression at initiating B cell responses. Both CD40L expression and T cell help were blocked by cyclosporin A after T cell receptor crosslinking, and unlike IL-2 production, both remained cyclosporin A sensitive during CD28 costimulation. In addition, anti-CD28 could not compensate for the T cell helper deficiency of hyper IgM syndrome patients who lack functional CD40L. Thus, anti-CD28 induced T cell help is delivered via a CD40L-dependent process. The fact that crosslinking CD40 on B cells promotes expression of the B7/BB-1 ligand for CD28 suggest T-B interactions may have a reciprocal amplification mechanism.

V 714 CHARACTERIZATION OF MURINE CTLA4: DISTRIBUTION AND FUNCTION, Matthew F. Krummel and James P. Allison, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The murine CTLA4 gene encodes a predicted membrane bound protein with distinct homology to the CD28 protein. Recently a fusion protein consisting of the CTLA4 extracellular region and Immunoglobulin was demonstrated to bind to the CD28 ligand, B7. We have examined subsets of T cells to study the expression of this gene. Northern analysis shows that the CTLA4 mRNA is present on activated splenic T cells of both CD4 and CD8 subsets. The mRNA increases rapidly in conA activated cells with maximal expression seen at 2 days. We have utilized the fusion protein, activated cells, and CTLA4 transfectants to generate monoclonal antibodies to murine CTLA4. Preliminary results indicate that the protein is virtually absent in unactivated splenic populations. Upon activation, CD25+ cells of both CD4 and CD8 subsets have detectable surface expression of the protein. We have begun analysis to determine the requirements for TCR and CD28 crosslinking in the upregulation of CTLA4. In addition we have used a costimulation assay of purified LN cells using αCD3, αCD28 and αCTLA4 monoclonal antibodies to assess the possible negative and/or positive signalling role of CTLA4.

V 715 ANTIVIRAL IMMUNE RESPONSIVENESS IN INTERLEUKIN-2 AND IN CD28 DEFICIENT MICE, Thomas M. Kündig*, Arda Shahinian#, Klaus Pfeffer#, Rolf M. Zinkernagel$, Ivan Horak+, Tak W. Mak# and Pamela S. Ohashi*, *Ontario Cancer Institute and the #Amgen Institute, 500 Sherbourne Street, Toronto, Ontario, Canada, +Institute for Virology and Immunobiology, University of Würzburg, Germany, $Institute for Experimental Immunology, University of Zürich, Switzerland.

Stimulation of the T cell receptor alone is thought to be insufficient for T cell induction. A second, costimulatory signal must be provided. The interaction of CD28 on the T cell with its ligand B7 on the antigen presenting cell can provide this second signal and enhance interleukin-2 production. Recently, mice lacking the interleukin-2 gene and those lacking the CD28 gene have been created. In both strains of mice T help for B cells was found to be reduced. In contrast, cytotoxic T cell responses against lymphocytic choriomeningitis virus were found to be not impaired. The present study reveals interesting findings by further evaluating cytotoxic T cell responsiveness in other viral systems and focuses on mechanisms that may compensate for the lack of the above costimulatory factors.

V 716 ROLE OF gp39, THE CD40-LIGAND, IN THE GENERATION AND MAINTENANCE OF B-CELL MEMORY. Jon D. Laman, Teresa M. Foy and Randy J. Noelle, Department of Microbiology, Dartmouth Medical School, Lebanon, NH 03756

gp39 is expressed on activated murine CD4-positive cells as a result of activation. Interaction between gp39 and its ligand CD40 on B-cells is required for the generation of antibody responses against thymus dependent antigens. Experiments were performed to investigate whether gp39-CD40 interactions are also involved in the generation and maintenance of B-cell memory.

BALB/c mice were immunized i.p. on day 0 with SRBC and received i.p. injections with a gp39-specific hamster antibody, MR1, on days 0, 2 and 4. Control animals received SRBC and normal hamster immunoglobulin. Serum and spleens were isolated on day 9 and 11. ELISA showed absence of SRBC-specific antibodies in the serum of anti-gp39 treated mice, but not controls, confirming earlier observations. Immunocytochemistry was performed on frozen spleen sections. Staining with PNA, a lectin specifically binding to germinal center B-cells, which are memory B-cells showed large germinal centers in control animals. No germinal centers were found in mice which received anti-gp39, although thread-like structural elements showed PNA-binding. Frozen sections were also stained with an antibody against IgD which is present on follicular resting B-cells and which is lost upon transition to germinal center B-cells expressing IgM or IgG. This staining revealed that half of the follicles in spleens of control animals were undergoing transition to become germinal centers, showing rings of intensely stained IgD-positive cells surrounding large bright patches of IgD-negative cells. In contrast, in anti-gp39 treated animals, only by exception did follicles show a limited degree of transition, with small patches of IgD-negative cells.

Functional data supporting these findings were obtained in the congenic BALB/c-CB17 system. CB17 mice were immunized with TNP-BSA and treated with anti-gp39. After four weeks, B-cells were adoptively transferred to KLH-primed Balb/C. Subsequently, animals were challenged with TNP-KLH, and donor-specific antibodies were detected using TNP-specific ELISA. Treatment with anti-gp39 resulted in a 90% decrease in anti-TNP titer, as compared to the controls. These data demonstrate that the gp39-CD40 interaction is not only critical in the generation of the antibody response, but also in the generation of B-cell memory.

V 717 CD28 SIGNALLING IN THE ACTIVATION OF NAÏVE T CELLS, Olli Lassila and Olli Vainio, Department of Medical Microbiology, University of Turku, 20520 Turku, Finland

T cells need two signals to be stimulated by an antigen, the first signal comes from the binding of antigen receptor and the second is costimulatory. It is clear that classical antigen presenting cells (APCs) such as monocytes and dendritic cells are able to give efficiently both signals for naïve T cell activation whereas capacity of either resting or activated B cells to deliver both signals has been controversial. To address these questions we have constructed avian A⇒B chimaeras containing T cells and APCs of the host 'B' strain and B cells from an allogeneic donor 'A' strain. These tolerant chimeras are not able to mount T-dependent antigen responses indicating that resting B cells *in vivo* are unable to activate resting T cells. The lack of effective T and B cell collaboration in these chimeras might be based on that resting B cells bearing little if any B7 molecules are not able to give costimulatory signal via CD28 to virgin T cells. We attempted to overcome this lack of collaboration by treating these chimeras *in vivo* with anti-CD28 monoclonal antibody during primary immunization. However, no IgG response was observed indicating that resting B cells together with CD28 signalling are not able to trigger resting T cells. If the chimeras were first primed with antigen together with professional APCs from donor strain 'A' normal primary response was obtained. But greatly enhanced secondary response was observed after *in vivo* treatment with anti-CD28. These findings indicate that CD28 signalling is not sufficient to render naïve T cells to respond to antigen presented by B cells.

V 718 T-BAM/CD40-L INDUCES B7/BB1 EXPRESSION ON ANTIGEN PRESENTING CELLS AND CD40+ TUMORS, Seth Lederman, Michael J. Yellin, and Leonard Chess, Department of Medicine, Columbia University, New York, NY 10032.

T-BAM/CD40-L is an activation-induced surface protein on CD4+ T cells that delivers a contact dependent signal for B cell survival and differentiation. The counter-receptor for T-BAM/CD40-L is CD40 which is expressed on B cells, activated macrophages and follicular dendritic cells, certain epithelial cells and a variety of tumors. To investigate the consequences of T-BAM/CD40-L interactions with APC and tumors, T-BAM/CD40-L expressing D1.1 cells were cultured with B cells or B-CLL cells and found to induce expression of B7/BB-1 molecules in a contact-dependent manner that is inhibited by the anti-T-BAM/CD40-L specific mAb 5c8. In addition, expression of T-BAM/CD40-L by transfection into non-lymphoid cells confers them with the ability to induce B7/BB-1 molecules on B cells and B-CLL cells. Further, T-BAM/CD40-L priming of B-CLL tumor cells renders them potent stimulators of allogeneic T cell proliferation. Thus, in addition to stimulating the differentiation of B cells, T-BAM/CD40-L also induces CD40+ B cells and tumor cells to express B7/BB-1 molecules, which provides a costimulus for T cell proliferation and IL-2 responses. These data suggest that T-BAM/CD40-L signals appear to play roles in the induction of cell-mediated immunity. These findings may relate to the phenotype of clinical T-BAM/CD40-L deficiency (hyper-IgM syndrome), which, in addition to defective Ig isotype switching, is associated with infections and tumors suggestive of defective cell-mediated immunity. Together with previous studies, these findings suggest two distinct mechanisms by which tumor cell growth is regulated by T-BAM/CD40-L signals: rescue from apoptosis and targeting for cell mediated responses.

V 719 CD27L (CDw70) PROVIDES A COSTIMULATORY SIGNAL FOR T-CELL ACTIVATION

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Molecules of the TNF-R family have been shown to be essential in the regulation of lymphocyte growth and differentiation. The TNF-R family member CD27 binds to a type II transmembrane molecule belonging to the TNF gene family (CD27L) that, as we recently demonstrated, is identical to the lymphocyte activation antigen CDw70. Using transfected mouse fibroblasts expressing human CD27L, it was shown that interaction of CD27 with its ligand provides a potent second signal for cytokine production, activation-antigen expression and proliferation in unprimed, $CD45RA^+$ and to a lesser extent in primed, $CD45R0^+$ peripheral blood T cells. In contrast to costimulatory signals delivered via the CD28-ligand B7, CD27L was found to induce low IL-2 and IL-4 but relatively high TNF-α secretion. Markedly, proliferation of $CD45RA^+$, but not of $CD45R0^+$ T cells was found to be independent of IL-2. As we demonstrated that anti-B7 and anti-CD27L mAb synergize in the inhibition of alloresponses induced by B-LCL, cooperation of both molecules appears to be essential for optimal T-cell stimulation by certain types of APC. Our results indicate that the interaction between CD27 and its ligand CDw70 might be of particular importance in the recruitment of T cells from the unprimed T cell population. Moreover, as CDw70 expression *in vivo* is confined to activated B and T lymphocytes only a limited set of APC are able to generate this specific second signal for T-cell expansion.

V 720 B7-2, AND NOT B7-1(B7), IS THE PRIMARY CO-STIMULATORY MOLECULE FOR T CELL ACTIVATION. D.J. Lenschow[*], G. Freeman[°], R. Hodes[+], M. Cooke[#], Y. Zeng[@], K. Hathcock[+], G. Gray[$], J.R. Thistlethwaite[@], C. Goodnow[#], L.M. Nadler[°], and J.A. Bluestone[*]. [*]Comm. on Immunology and [@]Dept. of Surgery, Univ. of Chicago, Chicago, IL. [+]Natl. Inst. of Aging, NIH, Bethesda, MD. [°]Dana Farber Cancer Inst., Harvard Medical School, Boston, MA. [#]Howard Hughes Medical Inst., Stanford Univ., Stanford, CA. [$]Repligen Corp., Cambridge, MA.

Our laboratories have recently demonstrated the existence of an additional CD28/CTLA4 ligand, termed B7-2. B7-2 is constitutively expressed on dendritic cells and rapidly upregulated on B cells following B cell activation. B7-2 does not require a T-B cell interaction for its induction, since the stimulation of B cells with either LPS or anti-Ig induced B7-2 on the surface of B cells within 6 hours, and this expression was maintained for at least 72 hours. Furthermore, B cells from transgenic mice expressing the anti-HEL Ig receptor upregulated B7-2 following *in vitro* stimulation with nominal antigen (HEL). Murine B7-1(B7) was not detected following stimulation of Tg B cells with HEL. Finally, B cells from *in vivo* primed normal mice also expressed B7-2 following re-stimulation with antigen, *in vitro*. Functionally, while anti-B7 mAb and hCTLA4Ig were able to inhibit T cell proliferation to antigen expressed on B7-transfected antigen presenting cells (APCs), only hCTLA4Ig and anti-B7-2 mAbs were able to block T cell responses to natural APCs. Moreover, B cells expressing only B7-2 and no detectable B7-1(B7) were able to co-stimulate T cell proliferation and IL-2 production. This response was inhibited by Fab fragments of anti-CD28 mAbs, implying B7-2 co-stimulates T cells by interacting with the CD28 molecule. Further evidence of the predominant co-stimulatory role for B7-2 has come from *in vivo* studies. Allogeneic islet transplants were performed in the presence of hCTLA4Ig, anti-B7, anti-B7-2 or control mAbs. Islet cells grafted into control mice or anti-B7-1 treated mice had a graft survival of 15 days. In contrast, animals treated with hCTLA4Ig or anti-B7-2 mAbs maintained their grafts from between 25 to at least 42 days. These results demonstrate that in this model, B7-2 plays the major role in co-stimulating T cell responses.

V 721 CLONING AND SEQUENCING OF THE RAT B CELL ACTIVATION ANTIGEN B7, M. Jackerott, S. Tullin, L. Hornum and H. Markholst, Hagedorn Research Institute, Gentofte, Denmark

The reduced number and unresponsiveness of T cells (lymphopenia) in the diabetic BB rat may be caused by deficient antigen presenting cells. Since antigen processing and presentation by MHC molecules appear to be normal, we have focused on the accessory molecules involved in the stimulation of T cells. Several cell surface molecules on antigen presenting cells are known to be required for proper stimulation of T cells. One of these, the B cell activation antigen B7, expressed on activated B cells, INF-γ, induced monocytes and dendritic cells, has been shown to produce an important co-stimulatory signal upon interaction with its ligand, CD28, present on T cells. Theoretically, the lymphopenia in the BB rat could be explained by an abnormal B7 function. From a cDNA library of MHC II positive thymic cells the rat homologue of the murine B7 was cloned and sequenced. The predicted protein of 294 amino acids was 76% identical to the murine B7 protein. A high homology was found in the Ig-C and Ig-V domains, while in the transmembrane and cytoplasmatic regions very little homology was found. Preliminary results suggest that the level of B7 transcript in lymphoid tissues does not differ in the BB and Brown Norway rat. Furthermore, we intend to map the B7 gene in the rat.

V 722 CD28 SIGNALING INHIBITS THE ACTIVITY OF A CD3-INDUCIBLE AU SPECIFIC RNA BINDING FACTOR IN A T CELL HYBRIDOMA, Anna Mondino and Marc K. Jenkins, University of Minnesota, Medical Center, Minneapolis, MN, 55455

CD28 costimulation synergizes with TCR signals for the production of interleukin 2 (IL-2) and T cell proliferation. It has been suggested that CD28 costimulation does this by enhancing IL-2 mRNA stability. A TCR-inducible RNA binding factor specific for AU rich elements (ARE) in the 3' untranslated region of lymphokine mRNAs was described in normal T cells. This 32 Kd protein (AU-B) may target lymphokine mRNAs for degradation. We investigated whether CD28 signaling interferes with AU-B activity resulting in increased mRNA stability. A synthetic RNA oligonucleotide spanning the ARE of the 3'-UTR IL-2 mRNA was used in electrophoretic mobility-shift and UV cross-linking experiments. Anti-CD3 stimulation of a T cell hybridoma resulted in the appearance of a protein that bound to the IL-2 RNA oligonucleotide. Surprisingly, this protein had an apparent molecular weight of 100 Kd and thus appeared to be distinct from AU-B. The 100 kd complex was specifically shifted by ARE containing probes, but not by ARE lacking probes. When cytoplasmic extracts of cells stimulated with anti-CD28 antibodies were analyzed, inhibition of the CD3-induced RNA binding activity was detected. Parallel Northern blot analysis revealed that costimulation with anti-CD28 antibodies increased the amount of mRNA for IL-2 in the hybridoma above the level observed with anti-CD3 alone. Our results suggest that CD28 signaling inhibits the formation of an RNA-protein complex that could target IL-2 mRNA to rapid degradation. This would account for the increase in IL2 mRNA accumulation that is observed following CD28 costimulation.

V 723 B7 AND INTERLEUKIN-12 COOPERATE FOR PROLIFERATION AND IFN-γ PRODUCTION BY MOUSE TH1 CLONES THAT ARE UNRESPONSIVE TO B7 COSTIMULATION, Erin E. Murphy[1], Geronimo Terres[1], Steven E. Macatonia[1], Chyi-Song Hsieh[2], Jeanine Mattson[1], Lewis Lanier[1], Maria Wysocka[3], Giorgio Trinchieri[3], Kenneth Murphy[2] and Anne O'Garra[1]. [1]DNAX, Palo Alto, California 94304, [2]Washington Univ. Sch. Med., St. Louis,.MO 63110, [3]The Wistar Institute, Philadelphia, PA 19104.

We have shown that dendritic cells, which constitutively express B7 and are potent stimulators of naive T-cell proliferation, are relatively poor at inducing the proliferation of a panel of murine Th1 clones. Maximal stimulation of Th1 clones was achieved using unseparated splenic APC. We now show that FcR+ L cells transfected with B7 stimulate minimal proliferation of Th1 clones in response to anti-CD3 antibodies, in contrast to induction of significant proliferation of naive T cells. However, addition of IL-12 to cultures of Th1 cells stimulated with anti-CD3 and FcR+ B7 transfectants resulted in very pronounced increase in proliferation and IFN-γ production. Exogenous IL-12 did not affect the B7-induced proliferation of naive T cells. Thus, whereas costimulatory signals delivered via B7-CD28 interaction are sufficient to induce significant proliferation of naive T cells activated through occupancy of the TCR, Th1 T cell clones require cooperative costimulation by B7 and IL-12. This costimulation for proliferation and IFN-γ production was inhibited using chimeric soluble CTLA4-Ig and anti-IL-12 antibodies. Furthermore, the significant antigen specific proliferation and IFN-γ production by Th1 clones observed using splenic APC was almost completely abrogated using CTLA4-Ig and anti-IL-12 antibodies. Thus two costimulatory signals, B7 and IL-12, account for the ability of splenic APC to induce maximal stimulation of Th1 clones. IL-10 down-regulates the expression of IL-12 by IFN-γ-stimulated macrophages and this may account for the ability of IL-10 to inhibit APC function of splenic and macrophage APC for the induction of Th1 cell proliferation and IFN-γ production. Indeed we show that IL-12 can overcome the inhibitory effect of IL-10 for the APC-dependent induction of proliferation and IFN-γ production by Th1 clones. Thus proliferation of terminally-differentiated Th1 clones, in contrast to naive T cells, requires stimulation via membrane-bound B7 and a cytokine, IL-12. These signals may result in the activation of unresponsive T cells during an inflammatory response. IL-10 by its role in regulating such innate inflammatory responses may thus help to maintain these T cells in an unresponsive state.

V 724 DEVELOPMENT OF A SYSTEM OF EXPRESSION CLONING FOR MOLECULES COSTIMULATORY FOR T CELLS. N.Murray, D.Simmons, A.J.McMichael* and W.F.Bodmer, Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, U.K.and *Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, U.K.

Using B7 (BB1) as a model, we have established a system to identify plasmids expressing cDNA encoding molecules costimulatory for T cells, in the presence of other plasmids encoding other non stimulatory molecules. We constructed Cos cells stably expressing CD32, an Ig-Fc receptor, and these cells are transfected with plasmid DNA by a DEAE Dextran transient transfection method. Two days after, the cells are dispensed into 96 well plates and, in the presence of anti CD3, mixed with human peripheral blood T cells prepared by E-rosetting. After a further 3 days the non adherent cells are transferred to a second 96 well plate and pulsed overnight with tritiated thymidine; the cells are harvested the next day and tritium incorporation measured. Using this technique we can detect B7 expressing plasmid diluted into plasmid containing other cDNAs, at dilutions where B7 antibody staining can no longer be detected in the Cos cell population. We propose to develop this technique further, and to use it to look for novel cDNAs in libraries from cells with known antigen presentation function.

V 725 RMA-S expresses a novel costimulatory molecule. John D. Nieland and Ada M. Kruisbeek, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Activation of antigen specific T-cells requires more than signalling through the TCR/CD3 complex. A second or costimulatory signal is also required. Triggering through the T-cell receptor (TCR) without simultaneous costimulation induces non-responsiveness in T-cells under certain conditions. The best known receptor-ligand interaction through which second signals can be transduced is formed between CD28 on T-cells and B7 on APC's. Nevertheless, some B7-negative cell lines appear capable of providing second signals under certain conditions. For instance, the peptide transporter defective T-lymphoma RMA-S does not express B7, but can effectively induce peptide specific CTL responses in vitro, once loaded with an exogenous source of antigenic peptide. Such responses cannot be blocked by anti-B7 reagents (i.e. anti B7 or CTLA4-Ig), suggesting that B7 independent costimulatory pathways may exist.
This notion was further investigated in various models. First, RMA-S can support clonal expansion of purified CD4 or CD8 T-cells from unprimed mice activated with immobilised anti-CD3. In fact, also the parental RMA cell line, as well as at least one other T-cell lymphoma (i.e., EL4), can aid in such anti-CD3-induced clonal expansion in a dose dependent fashion. This expansion is accompanied with IL2 production. Secondly, also costimulation of antigen-specific T-cell proliferation of both class I and class II restricted T-cell clones can be provided by B7-negative T-lymphoma cells: non-responsiveness induced by pre incubation of T-cells with ECDI fixed APC plus antigen can be rescued by RMA-S or RMA. The costimulatory signals induced by T-lymphoma lines are destroyed by ECDI fixation, in analogy to the CD28-B7 dependent pathway.
The results thus far indicate a novel pathway able to costimulate T-cell responses. Characterisation of the molecules involved is in progress.

V 726 **CD28 SIGNAL IN T LYMPHOCYTES IS TRANSDUCED BY SPHINGOMYELIN-CERAMIDE SIGNALING PATHWAY**

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Although antigen receptor occupancy with a peptide in association with major histocompatibility complex antigen initiates T cell activation, a second co-stimulatory signal is necessary the optimal proliferation and lymphokine secretion. CD28 is a homodimeric membrane glycoprotein expressed on most of the T cells and the signal generated by the interaction with its natural ligand, B7, has been implicated as a co-stimulatory signal. While the CD28 signal in concord with the TCR signal induces proliferation and lymphokine secretion by T cells, a notable activation event is nonexistent by the CD28 signal alone and very little is known about CD28 signaling pathway. In this presentation we would like to report that CD28 ligation activates sphingomyelinase thereby triggering the sphingomyelin (SM) signaling pathway. Activation of this pathway resulted in the induction of ceramide driven kinases specific for serine and threonine. Importantly the sphingomyelin-ceramide signaling pathway is also known to couple to receptors for interleukin 1 and tumor necrosis factor which are co-stimulatory to T cell activation. The data demonstrate the pivotal role of SM signaling pathway to conduct co-stimulatory signal in T cells.

V 727 A TUMOR-DERIVED PROTEIN WHICH PROVIDES T-CELL COSTIMULATION THROUGH ACCESSORY CELL ACTIVATION. T. J. Powell, Randy Schreck, Miloe McCall, Harald App, Axel Ullrich‡, and Laura Shawver. SUGEN Inc., Redwood City, CA; ‡Max Planck Institute für Biochemie, Department of Molecular Biology, Martinsried, Germany.

Proteins produced and secreted by tumor cells may be involved in regulation of the immune response to the tumor. One example of such a protein is immunoregulin, a 90kD glycoprotein which was originally identified by reactivity with a monoclonal antibody raised against conditioned medium of human mammary tumor cells. This protein enhances the generation of natural killer (NK) and lymphokine activated killer (LAK) activity in PBL from apparently healthy donors. To elucidate the mechanism of action of immunoregulin, we have examined its effect on cytokine production by mitogen-stimulated PBL. Addition of immunoregulin to human PBL stimulated with the T-cell mitogen Concanavalin A caused an increase in the levels of IL-2 produced in a 48 hour culture. This increased response was dependent on the presence of ConA, and was most apparent at suboptimal doses of the mitogen. Under the same conditions, production of the accessory cell-derived cytokines IL-1 and IL-6 was also increased. In contrast to the IL-2 response, immunoregulin alone (i.e., without ConA) was sufficient to stimulate production of IL-6 by PBL, suggesting that the effect of the molecule is at the level of the accessory cell rather than the T-cell. Nylon wool-enriched T-cells did not produce lymphokines in response to ConA, as expected for this accessory cell-dependent mitogen. The addition of immunoregulin failed to restore the ability of the T-cells to respond to ConA, providing further evidence that immunoregulin does not act directly on T-cells. We propose a model in which immunoregulin activates accessory cells, which indirectly leads to T-cell activation, resulting in increased production of IL-2 and other T-cell-derived cytokines. The result of this cascade is enhanced cell-mediated immunity, such as NK and LAK, which may lead to rejection of tumor cells or virus-infected cells in vivo.

V 728 INTERACTION OF CD40 LIGAND WITH A B CELL LYMPHOMA INHIBITS DIFFERENTIATION AND IMMUNOGLOBULIN SECRETION, Troy D. Randall, Andrew Heath, Frances E. Lund, Maureen Howard, and Irving Weissman, Dept of Pathology, Stanford University, Stanford CA 94305 and DNAX Research Institute, Palo Alto CA 94304

The interaction of CD40 on B cells and its ligand on T cells is one of the most important B cell/T cell interactions. Signaling through CD40 has been shown to be vital for immunoglobulin class switching as well as for B cell proliferation. It has also been shown to be critical in germinal centers where signaling through CD40 can inhibit apoptosis of germinal center B cells. It has been assumed therefore that since the CD40/ligand interaction is such a large component of T cell help that it would also augment the differentiation of B cells into antibody secreting cells. Here we show that the interaction of a B cell lymphoma, CH12, with L cells expressing the CD40 ligand inhibits the differentiative response. CH12 cells can be induced to secrete IgM with a variety of stimuli such as LPS, IL-5 or IL-6. However, when CH12 cells were co-cultured with lymphokines and CD40 ligand expressing L cells ($CD40^{lig}$ L cells), antibody secretion was decreased 20 fold. Antibody secretion could not be recovered by the addition of anti-μ or IL-4. The CH12 cells were fully viable after culture on either L cells or $CD40^{lig}$ L cells. No class switching was detectable at the level of secreted antibody. The block in IgM secretion can be fully explained by a decrease in immunoglobulin transcription since mRNA levels were decreased in cells cultured on $CD40^{lig}$ L cells about 20 fold as well. Curiously, antibodies to CD40 do not have the same effect as $CD40^{lig}$ L cells. CD40 antibodies that have profound effects on the proliferation of normal B cells did not affect secretion of IgM from CH12 cells. These antibodies could induce a modest proliferative response, similar to that observed with the transfected L cells. Similar results were obtained when the antibodies were presented to the B cells in a crosslinked form on beads or on Fc receptor expressing L cells. These results suggest that either the CD40/ligand interaction is fundamentally different than the stimulation with antibodies or that CD40 ligand can interact with a different molecule on the surface of B cells. Furthermore, the fact that Ig secretion is inhibited by CD40 suggests that although CD40 may be instrumental in B cell activation and in the initial proliferative phases of the B cell response, other interactions are required for the final differentiation of B cells.

V 729 SIGNALLING VIA CD28 INVOLVES ASSOCIATION WITH AND ACTIVATION OF PHOSPHOINOSITIDE 3-KINASE.David M. Sansom*, Christine E. Edmead*, Nick D. Hall*, John Westwick, and Stephen G. Ward. School of Pharmacy and Pharmacology, University of Bath, Claverton Down, and *Bath Institute for Rheumatic Diseases, Trim Bridge, Bath, UK.

Understanding of the signals required for the activation of T cells is of critical importance in immunology and the study of autoimmune disease. Current models suggest that both engagement of the TCR and the delivery of costimulatory signals are required for activation. In the absence of such costimulatory signals, clonal anergy or apoptosis may result. It has emerged that T cell proliferation is highly costimulation-dependent and evidence suggests that the T cell molecule CD28 transduces such a signal.

We have investigated the ability of various transfectants expressing B7/BB1 and LFA-3, to provide costimulatory signals for resting T cells, previously activated human T cell blasts and CD28 positive Jurkat cell lines. Using resting T cells purified from peripheral blood we have demonstrated that neither B7 nor LFA-3 alone is capable of inducing proliferation. However in combination both ligands provide a potent synergy which results in T cell proliferation. Interestingly this synergy is dependent on both ligands being present on the same cell and cannot be achieved when the ligands are provided by separate transfectants. In contrast, B7 alone is capable of providing signals to previously activated T cell blasts which further enhances proliferation. These data suggests that signalling via CD28 might be controlled by interactions with other signalling molecules.

Given these data we have begun to relate the signalling ability of CD28 to the activity of Phosphoinositide 3-Kinase(PI3K) which we have previously shown to be activated by CD28. In the T cell line Jurkat, B7 alone is capable of stimulating PI3K activity, an effect which is enhanced by the presence of LFA-3. In addition data from immunoprecipitates of CD28 demonstrate a direct association with PI3K during B7 stimulated CD28 engagement suggesting a model of CD28 signalling which requires phosphorylation of the CD28 cytoplasmic domain. Thus the requirement for CD2/CD28 co-association in our system may reflect an interaction between a CD2-associated kinase and the CD28 cytoplasmic domain.

V 730 Differential costimulatory effects of anti CD28 on $CD4^+$ versus $CD8^+$ cells from IL-2 deficient mice. A. Schimpl, I. Berberich, K. Erb, S. Krämer, I. Horak and T. Hünig. Institute for Virology and Immunobiology, University of Würzburg, D 97078, Germany.

Ligation of CD28 on T cells provides an important costimulus for T cell activation initiated via the CD3 complex. Part of the costimulatory effect is thought to be mediated through enhanced transcription of IL-2 and an increase in IL-2 mRNA stability. To assess the contribution of IL-2 to costimulation via CD28, unseparated or purified $CD4^+$ and $CD8^+$ T cells from Il-2 deficient mice (-/-) were cultured in the presence or absence of ligands for CD28 and several submitogenic stimuli. $CD4^+$(-/-) cells were costimulated by anti CD28 mAB or $B7^+$ cells as efficiently as were cells from wildtype controls. Neither anti IL-4 nor soluble IL-4 receptor inhibited costimulation by anti CD28, suggesting that lymphokines other than IL-4 or direct cell/cell signalling mediate the effect. $CD8^+$ (-/-) cells on the other hand failed to respond both with respect to proliferation and induction of cytotoxicity. Both defects were overcome by the addition IL-2. In both $CD4^+$ and $CD8^+$ cells from IL-2 deficient mice anti CD28 failed to boost CD25 expression beyond that obtained with anti CD3 alone, while cells from wildtype controls reponded with a dramatic increase in IL-2R.

The data suggest that upregulation of IL-2 may be an important aspect in costimualtion of $CD8^+$ cells by CD28 ligation but only one of several mechanisms operative in $CD4^+$ cells.

V 731 THE ROLE OF CD40-LIGAND IN B CELL *c-myc* RNA INDUCTION AND PROLIFERATION. Carol E. Schrader, Bryan C. Bordeaux, and David C. Parker. Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01655

CD40-Ligand is an important component of the membrane-bound help activity that is expressed on the surface of activated TH cells. We sought to determine whether some or all of the effects we observed with activated TH cells were attributable to CD40-L expression. We measured the induction of *c-myc* RNA in small resting B cells at 2hr as an assay for the receipt of very early helper signals, and B cell proliferation at 72hr as an assay for late helper signals. We have found that both soluble CD40 (CD40Ig) and anti-CD40L antibody (MR-1) can inhibit *c-myc* induction in small B cells when T cells preactivated on anti-CD3 are used. These reagents do not inhibit antigen-dependent induction of *c-myc* by resting T cells. We obtained similar results when we measured the inhibition of B cell proliferation. We are now using soluble CD40-Ligand to assay direct effects on B cell *c-myc* RNA. In addition, when we added cyclosporin A at different times during a 12hr activation of TH cells on anti-CD3, and then measured CD40L expression and the ability to induce B cell proliferation, we found these two parameters to correlate perfectly. We plan to use this system to compare CD40-L expression and the ability to induce *c-myc* RNA. Titration experiments involving stably transfected, antigen-specific T hybridomas that express varying levels of CD40-L are also in progress.

V 732 DIFFERENTIAL T CELL COSTIMULATORY REQUIREMENTS IN CD28-DEFICIENT MICE, Arda Shahinian, Klaus Pfeffer, Kelvin P. Lee†, Thomas M. Kündig, Kenji Kishihara, Andrew Wakeham, Kazuhiro Kawai, Pamela S. Ohashi, Craig B. Thompson†, Tak W. Mak, Amgen Institute, Department of Medical Biophysics and Immunology, Ontario Cancer Institute, University of Toronto, Toronto, Ontario M4X 1K9, Canada. †Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109.

T cell receptor stimulation without costimulation is insufficient for the induction of an optimal immune response. It is thought that engagement of the CD28 molecule with its ligand B7 provides an essential costimulatory signal without which full activation of T cells cannot occur. A mouse strain with a defective CD28 gene was established. Development of T and B cells in the CD28-deficient mice appeared normal. However, T lymphocytes derived from CD28-/- mutant mice had impaired responses to lectins. Lectin stimulation did not trigger interleukin-2 (IL-2) production, IL-2 receptor α expression was significantly decreased, and exogenous IL-2 only partially rescued the CD28 defect. Basal immunoglobulin (Ig) concentrations in CD28-deficient mice were about one-fifth of those found in wild-type controls, with low titers of IgG1 and IgG2b but an increase in IgG2a. In addition, activity of T helper cells in CD28-/- mice was reduced and immunoglobulin class switching was diminished after infection with vesicular stomatitis virus. However, cytotoxic T cells could still be induced and the mice showed delayed-type hypersensitivity after infection with lymphocytic choriomeningitis virus. Thus, CD28 is not required for all T cell responses in vivo, suggesting that alternative costimulatory pathways may exist.

V 733 B70 ANTIGEN, A SECOND LIGAND FOR CTLA-4 AND CD28, Chamorro Somoza†, Miyuki Azuma*, Daisuke Ito*, Hideo Yagita*, Ko Okumura*, Joseph H. Phillips†, and Lewis L. Lanier†, †Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304, *Department of Immunology, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113

The binding of B7/BB1 on antigen-presenting cells to CD28 on T cells delivers a costimulatory signal required for efficient activation of T cells. Several experimental data have suggested the existence of a second ligand for CD28 and CTLA-4. We have generated a monoclonal antibody (mAb), IT2, that reacts with a 70 kD glycoprotein (B70) that is another CTLA-4 and CD28 ligand. We have recently cloned the B70 cDNA from a B-lymphoblastoid cell line (B-LCL) library. It encodes a new protein of the Ig superfamily with limited homology to B7. B70 is expressed on resting monocytes and dendritic cells and on activated, but not resting, T, NK, and B lymphocytes. IT2 mAb substantially inhibits the binding of a CTLA4-Ig fusion protein to human EBV transformed B-LCL, and together with anti-B7 mAb completely blocks CTLA-4 binding. Further, IT2 mAb efficiently inhibits primary allogeneic mixed lymphocyte responses, demonstrating the functional importance of B70. The results of further studies of the functional aspects of B70/CD28 interaction will be discussed.

V 734 IL-4 TREATMENT OF SMALL SPLENIC B CELLS INDUCES COSTIMULATORY ACTIVITY. EXPRESSION AND FUNCTION OF COSTIMULATORY MOLECULES B7-1 AND B7-2. Risa M. Stack*#, Cindy L. Jellis$, Gary S. Gray$, Deborah J. Lenschow, Jeffrey, A. Bluestone#, and Frank W. Fitch#. The Committee on Developmental Biology*, The Ben May Insitute and the Committee on Immunology#, University of Chicago, Chicago, IL, 60637, Repligen Corporation$, Cambridge, MA 02139.

IL-4 has been shown to be involved in the early stages of B cell maturation. Changes induced by IL-4 include cell enlargement, increased viability, and increased MHC class II expression. However IL-4 alone does not induce B cell activation as defined by proliferation, lymphokine production, or immunoglobin class switching. In this study, we demonstrate that preincubation with IL-4 enhances the ability of murine small splenic B cells, normally poor stimulators of murine Th1 clones, to stimulate lymphokine production and proliferation by Th1 clones. Moreover, small resting B cells induce anergy, while IL-4-treated B cells do not. IL-4-treated B cells were found to express both B7 (B7-1) and a second ligand for CTLA4Ig (B7-2). Although IL-4 induces both B7-1 and B7-2, the kinetics of expression of these molecules is different: B7-2 is detected by 12 hours, wheras B7-1 is not significantly expressed until 48 hours. However, only CTLA4Ig fully blocks IL-4 induced costimulatory activity; a mAb to B7 does not. Thus, these results suggest that IL-4 may function indirectly as a costimulatory factor by inducing costimulatory molecules on resting B cells. Additionally, these findings support the hypothesis that an alternative ligand for CD28 and CTLA4 may be important in providing costimulation.

V 735 **Functional Involvement of c-Rel in the Activation of the IL-2 Promoter by CD28 Costimulation.**

Tse-Hua Tan[1], *Nancy Rice*[2], *Richard Bryan*[1], *Robert Rich*[1], *Yongqin Li*[1], *Gyorgyi Horvath*[1] *and Jenn-Haung Lai*[1], [1]*Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030;* [2]*National Cancer Institute-FCRDC, Frederick, Maryland 21702.*

The induction of lymphokine and IL-2Rα gene expression by CD28 costimulation is mediated by both transcriptional and posttranscriptional mechanisms. We have chosen to study the mechanisms underlying the CD28-mediated activation of IL-2 gene transcription. Previously, a conserved sequence motif present in several lymphokine gene promoters was identified as the CD28 response element (CD28RE), and an induced protein complex (CD28RC) was previously identified in human T cells activated by CD28 costimulation. We previously showed that the proto-oncogene product c-Rel binds to CD28RE and activates the CD28RE-driven CAT expression in transient transfection assays (PNAS 90:1696-1700, 1993). c-Rel belongs to the Rel/NF-κB family of transcription factors that share a highly conserved domain (Rel homology domain) of about 300 amino acids. The c-Rel protein binds to the κB or κB-like enhancer elements and control the transcription of several cellular genes including IL-2Rα (Mol. Cell. Biol. 12:4067-4075, 1992) and INF-γ (PNAS 89:1740-1744, 1992).

In the current studies, we further studied the role of c-Rel in CD28-mediated signal transduction in human peripheral blood T cells. We found that soluble anti-CD28 mAb, which triggered CD28 signalling, enhanced and accelerated the translocation of cytoplasmic c-Rel into the nucleus. Surprisingly, the translocation induced by anti-CD28 plus PMA was not sensitive to tyrosine kinase inhibition, even though T cell proliferation induced by anti-CD28 plus PMA was sensitive. This implies that CD28 signalling is at least partially independent of tyrosine kinase. In addition, CD28 signalling enhanced phosphorylation of nuclear c-Rel. Currently, we are investigating the functional interaction of c-Rel (in combination with other class of transcription factors) with CD28 signalling. Our results strongly suggest that c-Rel may play a crucial role in mediating CD28 signal transduction (Supported by NIH grant GM-49875).

V 736 B CELL APOPTOSIS INDUCED BY ANTIGEN RECEPTOR CROSSLINKING IS BLOCKED BY T CELL SIGNAL THROUGH CD40,
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Recent studies on autoantibody-transgenic mice have clearly demonstrated that self-reactive B cells are eliminated upon interaction with membrane-bound self-antigens in the periphery as well as in the bone marrow, suggesting that both immature and mature B cells are eliminated by multimerization of surface immunoglobulins (sIg). Activation of mature B cells by antigens may thus require a second signal that inhibits sIg-mediated apoptosis. Such a second signal is likely to be provided by T helper cells, since B cell tolerance is more easily induced in the absence of T helper cells. To assess the molecular nature of the signal that inhibits sIg-mediated apoptosis, we employed anti-IgM-induced apoptotic death of WEHI-231 B lymphoma cells as a model system. We found that the signal for abrogating sIg-mediated apoptosis is generated by association of the CD40L molecule on T cells with the CD40 molecule on WEHI-231 cells. The presence of T cell help through CD40 may thus determine whether B cells are killed or activated upon interaction with antigens.

V 737 MESSENGER RNA STABILIZATION IS THE MAJOR MOLECULAR MECHANISM OF CD28 COSTIMULATION OF IL-2 PRODUCTION IN MOUSE T CELL CLONES.
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We have quantitatively assessed the effects of CD28 costimulation on IL-2 mRNA expression at the levels of transcription initiation and mRNA stability in mouse T cell clones. Using crosslinked anti-TCR and soluble anti-CD28 antibody we find that the addition of anti-CD28 antibodies costimulates the cells to make 30- to100-fold more IL-2 than cells triggered through the TCR alone. In this system the accumulation of IL-2 mRNA over the first 6-8 hours closely matches the rate of accumulation of secreted IL-2 protein. Using a quantitative reverse transcriptase-mediated PCR reaction we find a marked difference in the rates of decay of IL-2 mRNA in the presence versus the absence of the CD28 signalling. This difference can explain the majority of the effect of CD28 on IL-2 mRNA accumulation. After 8 hours of stimulation IL-2 mRNA rapidly disappears even in cultures with the anti-CD28 antibody. The mechanism of this decay is unknown. Utilizing transcription reporter assays we do not find a specific effect of CD28 signalling on IL-2 enhancer driven transcription. This is true for either a 353 bp or a 1.8 kb enhancer, over a broad range of kinetics and TCR signal strength and with several TCR signal mimics.

V 738 CHARACTERIZATION OF NUCLEAR PROTEINS THAT BIND TO THE CD28 RESPONSIVE ELEMENT WITHIN THE IL-2 PROMOTER.
Verweij, C.L.[2], Civil, A., Doerre, S.[1], Geerts, M, Breedveld, F.C.[2] and Aarden, L.A. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam; [1]Duke University, Durham NC and [2]University Hospital, Dept. of Rheumatology, Leiden.

Activation of IL-2 gene transcription via the T cell accessory molecule CD28 has been shown to be mediated through a NF-kB like sequence, called CD28RE. Mutation of the CD28RE sequence in the IL-2 promoter abrogates the mitogen induced transcriptional activity. We were able to demonstrate binding of the recombinant NF-kB family members c-rel, p65 and c-rel/p50 heterodimer to CD28RE. In contrast to the HIV-1 NFkB binding sequence the NF-kB p50 homodimer did not recognize CD28RE.

Nuclear expression of CD28RE binding proteins requires mitogenic stimulation of T cells. We characterized the nuclear proteins that constitute the T cell derived CD28RE-protein complex by UV-crosslinking analysis. Two major proteins bands with apparent molecular mass of approximately 35 kD and 70-80 kD were found to bind CD28RE. The nuclear appearance of these proteins is believed to be crucial in the transmission of mitogenic signals that lead to transcriptional activation of the IL-2 gene. The 70-80 kD protein is likely to represent C-rel and/or p65. At present it is not clear whether the 35 kD protein corresponds to a known transcription factor. Further identification of these proteins is in progress.

V 739 CHARACTERIZATION OF THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF CTLA-4 ON MURINE T LYMPHOCYTES,
Theresa L. Walunas, Deborah J. Lenschow, Christina Y. Bakker, Craig B. Thompson and Jeffrey A. Bluestone, Committee on Immunology and the Ben May Institute, University of Chicago, Chicago, IL 60637

CD28 has been defined as a major co-stimulatory molecule on T cells. Another molecule, CTLA-4, is highly homologous to CD28 and found to be expressed at the mRNA level in activated murine T cells. Interestingly, a soluble form of CTLA-4, CTLA4Ig, interacts with high avidity to B7 and the newly described B7-2 molecule. However, the functional role of CTLA-4 expression in immune regulation remains to be clarified. We have developed monoclonal antibodies against murine CTLA-4 in order to elucidate the structural and functional characteristics of the molecule. While CTLA-4 is not expressed on resting $CD4^+$ or $CD8^+$ cells, a variety of stimuli induce CTLA-4 within 48 hours, including anti-CD3 mAb, concanavalin A, staphylococcal enterotoxin B, or co-stimulation with anti-CD28. Biochemical analysis of the molecule on activated T cells demonstrated that CTLA-4 is expressed predominantly as a glycosylated, disulfide-linked dimer of approximately 70 kD, and that the molecule may exist in differentially glycosylated forms which form homodimers.

In a series of experiments, CTLA-4 expression was found to be both TCR/CD3- and CD28-dependent. First, CTLA-4 upregulation following anti-CD3 mAb was inhibited by Fab fragments of anti-CD28 mAb or CTLA4Ig fusion protein. The requirement for co-stimulation could be bypassed by the addition of exogenous IL-2. In addition, T cells from CD28-deficient mice only expressed CTLA-4 following T cell activation with a combination of anti-CD3 and IL-2. These results are consistent with the role of CD28 ligation in IL-2 production. However, the addition of cyclosporin A to cultures containing anti-CD3 mAb also blocked CTLA-4 expression, and this expression could not be reconstituted with IL-2. These results suggest that both TCR signalling and IL-2 production are critical to the expression of CTLA-4. Finally, the functional effects of anti-CTLA-4 mAb on T cells activation will be discussed.

V 740 EXPRESSION OF A NOVEL COSTIMULATORY ACTIVITY ON K46J B LYMPHOMAS. *Tania H. Watts, William F. Wade,* and Mark DeBenedette. Dept of Immunology, University of Toronto, Toronto, Canada M5S 1A8 and *Department of Microbiology, Dartmouth Medical School, Lebanon, NH 03756*

T lymphocyte activation requires 2 signals. Binding of a peptide/MHC complex to the T cell receptor constitutes signal 1 and other costimulatory signals provided by the antigen presenting cell constitute signal 2. CD28 is now widely recognized as a major receptor for delivery of costimulatory signals to T cells. CD28 binds to B7, an Ig supergene family member found on dendritic cells and activated B cells. B7 binds with higher affinity to a second receptor on T cells, CTLA4. A soluble form of CLTA-4, CTLA4Ig, produced by Linsley and collaborators has been used by a number of groups to block the costimulatory activity of B7. We have shown that dibutyryl-cAMP treatment induces B7 expression on M12 B lymphomas or activated B cells from mouse spleen or human tonsil. For resting B cells, PMA and ionomycin but not db-cAMP treatment induces B7. In examining other B lymphomas for B7 inducibility, we found that K46J B lymphoma cells do not express B7 (as measured by CTLA4Ig binding) and are not inducible for B7 by PMA/ionomycin or dibutyryl-cAMP treatment. We report here that despite the apparent absence of B7, the Balb/c lymphoma K46J can costimulate with immobilized anti-CD3 to drive proliferation and IL-2 production by purified Balb/c splenic T cells. This costimulatory activity was not blocked by CTLA4Ig, by anti-ICAM-1, by anti-heat stable antigen or by anti-MHC II. Furthermore, paraformaldehyde fixation of the APC did not block the activity. These data suggest that K46J constitutively expresses an alternate costimulatory molecule, that does not bind to CTLA4.

V 741 CHIMERIC MICE DEFICIENT FOR THE CD40 LIGAND, J. Xu, E. Elliott, J. Elsemore, M. Brown, R. Noelle and R. Flavell, Section of immunobiology, HHMI, Yale Medical School, New Haven, CT 06510; Dept. of Microbiology, Dartmouth Medical School Lebanon, NH 03756.

The CD40 ligand (CD40L) is a type II membrane glycoprotein expressed on activated T cells that induces B cell proliferation, immunoglobulin isotype switch and secretion. The importance of CD40L has been recently underscored by the demonstration that a number of mutations in this molecule are responsible for X-linked hyper-IgM syndrome (HIM) in humans. To study the role of CD40L and to derive an animal model for HIM, we used gene targeting to create a null mutation in the CD40L locus. Homologous recombination events were identified by Southern blot analysis. Three independent male embryonic stem cell lines (from mouse strain 129) bearing the mutation at the CD40L locus were obtained. ES-derived peripheral white blood cells from chimeric mice express no detectable CD40L, whereas control cells express CD40L. The functional consequences of this CD40L defeciency will be discussed.

V 742 CD40 INDUCES T-BAM/CD40-L INTERNALIZATION BY RECEPTOR-MEDIATED ENDOCYTOSIS. Michael J. Yellin, S.M. Fortune, K.C. Sipple, G. Inghirami, Leonard Chess and Seth Lederman. Columbia Univ., N.Y., N.Y. 10032.

T-BAM/CD40-L is an activation-induced, transiently expressed, CD4+ T cell surface molecule that interacts with CD40 and delivers contact-dependent activating signals to B cells. The expression of T-BAM/CD40-L may be tightly regulated because CD40 is constitutively expressed and T-BAM/CD40-L:CD40 interactions lead to unrestricted B cell activation. We report that B cells specifically and rapidly down-regulate surface T-BAM/CD40-L expression on activated CD4+ T cells as determined by FACS analysis utilizing anti-T-BAM/CD40-L mAb 5C8. Furthermore, expression of CD40 by transfection in non-lymphoid cells renders them competent to induce T-BAM/CD40-L down-regulation. CD40 mediated T-BAM/CD40-L down-regulation is specifically inhibited by anti-CD40 mAbs. Pretreatment of activated T-BAM/CD40-L+ CD4+ T cells with cytochalasin B inhibits B cell mediated T-BAM/CD40-L down-regulation as shown by FACS analysis. Pretreatment of activated T-BAM/CD40-L+ CD4+ T cells with chloroquine does not inhibit B cell mediated T-BAM/CD40-L surface down-regulation by FACS but results in the accumulation of T-BAM/CD40-L molecules in cytoplasmic vessicles as shown by immunohistochemistry. Together, these studies strongly suggest that CD40 induces T-BAM/CD40-L down-regulation by receptor-mediated endocytosis. Interestingly, the cytoplasmic domain of T-BAM/CD40-L shares a consensus sequence with several molecules known to be internalized by receptor-mediated endocytosis. CD40 mediated T-BAM/CD40-L internalization may represent a mechanism to limit the inappropriate activation of non-cognate bystander B cells. Supported by grants from the NIH and the Arthritis Foundation.

V 743 THE ROLE OF COSTIMULATORY MOLECULES IN T-CELL ACTIVATION AND IL-2 GENE TRANSCRIPTION, Linda Zuckerman and Jim Miller, Committee on Immunology, University of Chicago, Chicago, IL 60637.

T cells require cognate interaction with antigen presenting cells to stimulate proliferation, cytolysis, and lymphokine secretion. The primary signal to stimulate the T cell results from an interaction between TCR and MHC/peptide complexes. A second interaction, termed costimulation, is generated from the interaction between specific accessory molecules on APCs and their ligands on the T cell. Many accessory molecules have been implicated in providing a costimulatory signal under a variety of conditions. However, it is not known if these interactions are functionally redundent or independent. We are primarily interested in two areas. First, do certain subsets of T cells respond preferentially when supplied with specific costimulatory molecules on the surface of APCs? Secondly, how do specific costimulatory molecules effect subsequent T cell activation and IL-2 gene transcription?

We have generated a model system in which B7-1, B7-2 (alternative ligand), HSA, ICAM, and Ii are expressed with MHC class II on a panel of recipient cells. We examined both CD4+ and CD8+ cells in primary mixed lymphocyte responses. Surprisingly, we observed that some APCs lacking these accessory molecules can still stimulate a dramatic allogeneic response for CD8+ T cells. This observation suggests that certain T cell subsets may not utilize known costimulatory molecules for some types of primary stimulation and/or effector functions. For primary CD4+ responses, however, we have observed a dramatic enhancement of proliferation with the presence of B7 or the condroitin sulfate form of invariant chain, Ii-CS on the APC. In examining the requirements of Th1 T cell clones, we observed that only the presence of B7 on the surface of APCs facilitates proliferation, IL-2 secretion, and protection against anergy. Even though the Th1 clones are LFA-1 and ICAM positive, other accessory molecules tested, HSA, Ii-CS, ICAM, fail to protect against anergy and do not stimulate proliferation or IL-2 secretion. Based on this observation, we are examining B7/CD28 interactions in IL-2 gene transcription at the molecular level. We hope to be able to correlate the presence of B7 on the surface of APCs with the presence of specific transcription factors necessary for IL-2 gene transcription.

B Cell Subsets

V 744 ACQUISITION OF FUNCTION ATTRIBUTES OF GERMINAL CENTER B CELLS, Jeannine M. Durdik, Maureen Entringer, Satyajit Rath, Department of Immunology, University of Colorado School of Medicine, Denver, CO 80262

Germinal centers (GC) are generated in secondary lymphoid tissues during antigenic challenge and are the sites where the T cell dependent response of B lymphocytes matures. This maturation of the B cell response occurs by increasing affinity for antigen, presenting additional processed antigen on a greater number of MHC class II molecules and becoming more receptive to cognate T cell signals by more efficiently coupling accessory molecules to its signaling pathways or using altogether new ones. B cells held in the GC would thus compete with each other to be selected on such criteria, and this selection would be mediated by interactions with antigen, follicular dendritic cells and T cells. The mechanisms that give GC B cells the tools to compete in the selection process are the focus of these studies, namely, the characteristic changes in their phenotype. We have established that treatment *in vitro* with interleukin 4 (IL4) or a Th2 helper line can generate many essential components of the GC phenotype on some normal B cells as well as B cell lines. The minimal requirements for the acquisition of this GC-like phenotype are addressed by determining the stage at which B cells can enter this differentiative pathway, and by examining the molecular mechanisms used in inducing these elements of the GC phenotype.

V 745 INTRODUCTION OF THE BTK GENE INTO XID AND XLA CELLS

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The cytoplasmic tyrosine kinase, Bruton's tyrosine kinase (btk) is expressed at all stages of B cell development and in myeloid cells. Loss of btk kinase activity results in the human immunodeficiency, X-linked agammaglobulinemia (XLA) characterized by a failure to produce B cells. A single point mutation in the unique region of btk is present in mice with X-linked immunodeficiency (xid). Xid mice produce B cells, but they do not respond to a variety of activation signals. These findings suggest a fundamental role for btk at several points in B cell development. Replacement of the defective btk gene in xid and in XLA B lineage cells should reconstitute normal B cell development and activation. We have constructed retroviral packaging lines producing helper free murine btk retrovirus. This virus has been used to introduce btk into xid bone marrow cells prior to transfer into lethally irradiated xid recipients. Btk gene transfer into CFU-s and lymphoid organs of reconstituted mice has been demonstrated. We will also investigate the reconstitution of B cell function in these mice. We have produced human btk retroviral stocks using two different strategies: 1) development of PA317 and GP-env AM12 btk retrovirus producer lines and 2) two plasmid transient transfection of 293-T cells. These viruses have been used to introduce btk into EBV transformed B cell lines from XLA patients and cultured human progenitor B cells.

V 746 SEQUENCE VARIATION AMONG KAPPA CHAIN TRANSCRIPTS FROM A SINGLE B CELL. Prasanna K. Jena, Andrew H. Liu, and Lawrence J. Wysocki, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine and University of Colorado Health Sciences Center, Denver, CO 80206

To explore the somatic hypermutation process, we have isolated and sequenced individual antibody variable region kappa light chain (V_K) mRNAs of a single B cell participating in a defined anti-hapten immune response. Splenic B cells from A/J mice immunized with *p*-azophenylarsonate (Ars) were stained with an antibody directed against the major Ars-associated idiotype and sorted by flow cytometry 13 days after immunization. Single sorted cells were isolated using phase contrast microscopy. To confirm that they were memory progenitors expressing the major idiotype, the chromosomal copies of heavy and light chain V genes from isolated cells were individually amplified by a nested Polymerase Chain Reaction (PCR) and directly sequenced without cloning. From one cell, 37 V_K cDNAs were individually amplified and sequenced without cloning. Sequence analysis revealed 6 single nucleotide substitutions and 1 single base deletion distributed within 4 mRNAs. One of these mRNAs contained two point mutations and a single base deletion. An additional base substitution at position 310 was found to be shared by all the mRNAs sequenced and presumably fixed within the V_K gene of the cell. Most interestingly, 2 of these mRNAs were aberrantly spliced, excising parts of the coding sequences. These aberrant splices apparently occurred at cryptic splice sites, in some cases newly created by mutations. Thus, these mutations are unlikely the result of Taq polymerase or RTase errors through our method, but are rather the result of a biological process then subject to transcriptional splicing in the cell. This interpretation is supported by the low frequency of mutations in mRNAs sequenced from single B-hybridoma cells. These data reveal the presence of somatic mutations in mRNA transcripts. Whether they are specific products of the somatic hypermutation mechanism that operates on V genes is under investigation.

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V 747 CHARACTERIZATION OF THE EXPRESSED ANTIBODY REPERTOIRE OF FACS-SORTED B CELL SUBSETS BY SINGLE CELL PCR, Aaron B. Kantor, Cynthia E. Merrill*, Leonore A. Herzenberg & Jan L. Hillson*, Stanford University and *University. of Washington,

We are analyzing the expressed VH gene repertoire of three murine B cell lineages (see Ann Rev Imm 11: 501): conventional B cells which develop late and are replenished throughout life from progenitors in adult bone marrow; B-1a cells (Ly-1/CD5 B cells), which develop early and maintain their numbers by self-replenishment; and B-1b cells, which share many of the properties of B-1a cells, including self-replenishment and feedback regulation of development, but can also readily develop from progenitors in adult bone marrow. Several studies indicate that the expressed repertoire of mature B-1a and conventional B cells differ, but there is no information on B-1b cells. Earlier methods of repertoire analysis, which include generation of hybridomas, in situ hybridization, and amplification of cDNA from polyclonal cells, are not necessarily representative of the populations studied. We have developed a method for making cDNA from individual, unstimulated, FACS-sorted B cells that permits the recovery and amplification of up to 90% of the cells. B-1a, B-1b and conventional B cells from the adult Balb/c peritoneum were sorted based on size, viability and the expression IgM, IgD, and Ly-1. Initial analysis (>75 transcripts) has focused on the CDR3 region. The distribution of CDR3 lengths are statistically the same for each of the three populations. However, B-1a cells differ from both B-1b and B-2 cells in the use of non-coded N-region nucleotides. There is a significantly larger proportion of B-1a transcripts which have no (or few) N bases at both the V-D and D-J junctions. About 60% of the junctions from B-1a cells have 0 or 1 N nucleotide compared with ~25% for B-1b and conventional B cells. The B-1a cells present in the adult which contain no/few N-region nucleotides most likely arose earliest in ontogeny, when terminal deoxy transferase (TdT) activity is low, and survived via self-replenishment into adulthood. TdT activity, however, does not define B-1a cells. Identification of transcripts with significant amounts of N-region addition, together with our feedback regulation data, suggests that B-1a cells are still being produced through the first weeks of life.

V 748 ANALYSES OF THE CONTRIBUTIONS OF B CELLS TO ABNORMALITIES IN MAIDS. W.K.Kim, Y.Tang, A.Hügin, K.Holmes, H.C.Morse. Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD 20892

MAIDS is a syndrome characterized by severe immunodeficiency and progressive lymphoproliferation. Development of disease requires the presence of $CD4^+$ T cells and B cells and expression of an etiologic replication defective virus, BM5def. The present studies are directed at determining the nature of the B cell requirements for disease. In agreement with earlier studies of μ-suppressed mice, mice that lack all B cells (in this case as a result of a knockout of the IgM gene) do not develop MAIDS and exhibit no T cell abnormalities. Although these animals replicate helper viruses that serve to facilitate cell to cell spread of BM5def in normal mice, expression of BM5def was not detectable by RT-PCR in tissues of the knockout mice infected for up to 14 weeks. This finding suggests that B cells are the primary targets of infection and that virus rapidly spreads from infected B cells to T cells and macrophage populations that were shown to be infected soon after virus inoculation of intact mice. The possibility that the $CD5^+$ B cell subset might contribute disproportionately to the activity of B cells in MAIDS was suggested by the finding that mice with the *xid* mutation develop MAIDS only at late times after infection if inoculated as adults. However, we do not find that $CD5^+$ B cells are expanded in tissues of normal B6 mice with MAIDS and that B1 cells in the peritoneum are actually depleted. Adoptive transfer studies utilizing *xid* and IgM knockout mice are being used to better determine the contributions of B cell subsets to development of this syndrome.

V 749 REGULATION OF APOPTOSIS OF RESTING HUMAN PERIPHERAL BLOOD B LYMPHOCYTES

Jon Lømo, Heidi Kiil Blomhoff, Klaus Beiske, Erlend B. Smeland, Department of Immunology, The Norwegian Radium Hospital, 0310 Oslo, Norway.

The role of physiological cell death (apoptosis) in the regulation of B lymphocyte responses is incompletely understood. We tested whether resting human B cells isolated from peripheral blood undergo apoptosis in vitro. We found that TGFß1 and the cAMP-inducing agent forskolin potently enhanced apoptosis spontaneously occuring in medium, as demonstrated by morphological and biochemical criteria (DNA fragmentation). The effects were dose-dependent with maximal cell death occuring at doses of 10 ng/ml (TGFß1) and 100 micromolar (forskolin). On the other hand, various activation-stimuli including polyclonal anti-IgM antibodies, IL 4, and TPA countered to a variable degree spontaneous apoptosis. Anti-IgM antibodies and IL 4 also consistently inhibited TGFß1- and forskolin-induced death. In conclusion, our studies show that well known modulators of B cell growth also regulate apoptosis of the same cells, and underline the role of apoptosis in determining the outcome of the B lymphocyte response. We are currently investigating the expression of *bcl-2* in this setting, a proto-oncogene that is known to inhibit apoptosis.

V 750 REGULATION OF APOPTOSIS IN RESTING MATURE B LYMPHOCYTES: EXAMINATION OF THE ROLE OF bcl-2 PROTEIN EXPRESSION. Susan A. McCarthy, Christina R. Brock, and Michael S. Mainwaring, Departments of Surgery, and Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15213

Clonal deletion via apoptosis can be induced during at least two stages in B cell development and differentiation: in immature B cells as a mechanism of self-tolerance induction, and in proliferating, Ig-secreting B cells as a mechanism of eliminating cells that have lost antigen-specificity during somatic hypermutation of their surface Ig. In contrast, mature naive B cells do not normally undergo apoptosis upon antigen encounter, but instead generate a primary humoral response. In the present study, we report that in an vitro culture system, resistance to apoptosis in mature naive murine splenic B cells is mediated in part by a protective mechanism that can prevent induction of apoptosis in these cells. Cyclosporin A (CsA), but not FK506, inhibits the protective mechanism and facilitates induction of apoptosis by the calcium ionophore A23187. T helper cell-derived cytokines, particularly IL-4, prevent induction of B cell apoptosis by A23187 plus CsA, suggesting that apoptosis may be activated when B cells are stimulated in the absence of T cell help. We are currently investigating the role of the "anti-apoptotic" protein, bcl-2, in the regulation of B cell apoptosis by A23187, CsA and IL-4. We are using immunofluorescence and flow cytometry to examine bcl-2 protein expression levels in B cells induced to undergo apoptosis by A23187 plus CsA, and in B cells protected from apoptosis by IL-4. Our preliminary results indicate that bcl-2 protein levels are modulated only very modestly in response to these stimuli, suggesting that the regulation of apoptosis in this cell system may reflect a bcl-2-independent apoptosis pathway.

V 751 **ORIGIN OF B LYMPHOCYTE RESPONSE TO THE PHOSPHOCHOLINE OF PROTEUS MORGANII MAY BEGIN WITH LOW AFFINITY RECEPTORS AT HIGH DENSITY.** Steven J. Penner, Julia George, Catherine Browning, Jan Berry, J. Latham Claflin, Dept. of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620.

The first fundamental event in selection of antigen-specific B lymphocytes involves Ag:mIg interactions on the B cell surface. Here we report on calcium responses of B cells expressing Ag receptors which in soluble Ig form have shown undetectable Ag-binding activity. Rearranged VDJ from anti-PC(*Proteus morganii*) antibodies were cloned in heavy chain expression vectors and co-transfected with an identical unmutated light chain vector into the Ig- B lymphoma M12.4, resulting in stable transfectants of mIgM+, mIgD+, and mIgM+D+ phenotypes. One series expresses mIg that in Ab form was of such low affinity that no PC(PM) ELISA binding is detected. A second series expresses receptors identical but for a single Asn to His somatic mutation in CDR2 of VH known to confer Ag binding by soluble Ab. A third and fourth series of mIg+ B cells contain the His mutation plus others with less impact on Ab affinity for Ag. When stimulated with as little as 10e-7 dilution of the PC(PM) immunogen, intracellular calcium fluxes were observed in transfectants with the His mutation. Significantly, slow and weak hapten-inhibitable fluxes were detected in the low affinity series (predicted as the precursor of His-containing clones) but only at much higher [Ag]. In this case, studying interactions with Ag:mIg receptors on the B cell surface may be more revealing about activation or affinity maturation than studies with soluble Ab.

The mIgM+, mIgD+, and mIgM+D+ lines showed qualitatively similar calcium responses to Ag stimulation. Two mIgD+ low affinity B cell lines differing by fourfold in receptor number revealed ability to mobilize calcium only by the line with greater number of receptors. Perhaps the normal function of IgD is to increase total surface Ag receptor density to allow for initiation of B cell clonal selection which can then undergo affinity maturation.

V 752 IMMUNOLOGIC TOLERANCE THROUGH IMPAIRMENT OF GERMINAL CENTERS.
Bali Pulendran, M.Karvelas, A. Light and G.J.V. Nossal.
The Walter and Eliza Hall Institute of Medical Research Post Office The Royal Melbourne Hospital;Victoria 3050; Australia.
This project seeks to explore the molecular and cellular basis of immunologic tolerance in adult mice. Intra-peritoneal injections of soluble form of the antigen (4-hydroxy-3-nitrophenyl) acetyl (NP) conjugated to human serum albumin (HSA), causes a profound tolerance to subsequent immunisation with alum adsorbed NP-HSA .The tolerance occurs even when the soluble antigen is given as late as six days into the response. There is a dramatic and sustained drop in the number of clonable, IgG1 anti-NP-secreting cells in culture.
This drop appears to be mediated through a lack of T cell help.
Phenotypic analysis of the spleens using multi-parameter flow cytometry has shown that NP-specific B cells of germinal center origin (NP+;IgG1+;PNA hi cells) fail to develop in the tolerant mice. In vitro culture of the NP+;PNA hi cells indicate that they are greatly enriched for higher affinity, anti-NP antibody producing cells. However there is little difference in the numbers of PNA lo cells in the immune and tolerant mice.
Preliminary immunohistology shows a significant drop in the number of antigen specific germinal centers in tolerant mice.

V 753 ISOLATION AND CHARACTERIZATION OF M17: A NOVEL GENE EXPRESSED IN GERMINAL CENTERS,
Robert C. Rickert, Thomas Christoph and Klaus Rajewsky, Institute for Genetics, University of Cologne, Weyertal 121, Cologne 50931, Germany
Germinal centers are histologically distinct structures that form within the draining lymphoid tissues following immunization with T cell-dependent antigens (Ag). To approach an understanding of the molecular events which govern B cell differentiation in the germinal center, we sought to isolate germinal center B cell-specific genes using subtractive cDNA libraries prepared from FACS-sorted (CD45R+, IgD-, Thy1.2-) lymph node B cells of immunized mice. A novel gene, designated M17, was isolated from this library and found to be transcribed in spleen and, to a lesser extent, in bone marrow. Strikingly, only PNA+ (germinal center) B cells, but not PNA- splenic B cells expressed M17 mRNA. Germinal center specific expression of M17 was confirmed by staining of histological sections from spleen with an antiserum raised against a GST-M17 fusion protein.

The M17 gene is comprised of four exons spanning 13.2 kb, and encodes a 25 kD cytoplasmic protein of 159 amino acids. Analysis of the primary amino acid sequence revealed a possible lipid binding domain and multiple potential phosphorylation sites, including an antigen receptor signalling motif. To assess the function of this molecule, we have undertaken a gene targeting approach to generate M17 deficient mice. Thus far, mutant embryonic stem cells bearing an inactivated M17 allele have been generated by homologous recombination. Subsequent studies will involve the analysis of B cell differentiation and signalling in derived M17-deficient mice.

V 754 INTERLEUKIN 4-INDUCED MOTILITY AND ADHESION IN MURINE B LYMPHOCYTES, Eva Severinson, Carina Elenström and Edward Davey, Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden
When murine B lymphocytes are cultured together with lipopolysaccharide (LPS), homotypic cell adhesion is activated. This is at least in part due to upregulation of intercellular adhesion molecule (ICAM)-1 on the B cells. ICAM-1 probably interacts with lymphocyte functional antigen (LFA)-1, since adhesion can be blocked by antibodies to either of these structures. The T cell produced cytokine interleukin (IL) 4 also induces homotypic aggregation among B cells, which is independent of LFA-1/ICAM-1. Instead, aggregation occurs via CD2. CD2 is expressed equally in normal and IL 4-activated B cells. The mechanism for its involvement in IL 4-induced adhesion is unknown. When B cells are stimulated by LPS and IL 4 together, they form huge round aggregates. The LPS + IL 4 induced aggregation depends on the above mentioned adhesion molecules, in addition to other, so far unidentified, ligands. Our hypothesis is that the formation of these aggregates resembles that of germinal centers in vivo.
IL 4 induces motility in a subpopulation of B cells as tested by migration into collagen gels. The migration is inhibited by antibodies to β1 integrin, indicating involvement of Very Late Antigens (VLA).
The importance of these findings for in vivo activation of B cells will be discussed.

V 755 Ly1+ B CELLS ARE UNRESPONSIVE TO ANTI-CD38 INDUCED PROLIFERATION. Nanette W. Solvason, Frances Lund and Maureen Howard. DNAX Research Institute. 901 California Ave., Palo Alto, CA. 94304.
CD38 is a 42 kD integral membrane glycoprotein with sequence homology to the *Aplysia* enzyme, ADP-ribosyl cyclase. Agonistic antibodies against mouse CD38 used in combination with Il-4 causes conventional B cells from the spleen of normal mice to proliferate *in vitro*. In contrast, B cells isolated from either the peritoneal cavity of normal mice or spleens of newborn mice, both rich sources of Ly1+ B cells, fail to proliferate in response to anti-CD38 plus Il-4 or anti-CD38 plus a variety of other costimulants. Interestingly, the inability to proliferate in response to anti-CD38 parallels the previously reported unresponsiveness of Ly1+ B cells to stimulation with anti-IgM, while the response to LPS as well as anti-CD40 induced proliferation is intact in Ly1+ B cells. To investigate the mechanism underlying the unresponsiveness of Ly1+ B cells to anti-CD38 stimulation, experiments will be presented on the induction of tyrosine phosphorylation and upregulation of activation antigens after stimulation with anti-CD38 in Ly1+ B cells versus conventional B cells (see Lund, F., this meeting) as well as a survey of CD38 associated cell surface molecules.

V 756 MECHANISMS FOR MAINTENANCE OF LEUKEMIC B CELL SURVIVAL *IN VITRO* : ROLE OF NORMAL T CELLS
Stuart G. Tangye, Kathryn M. Weston and Robert L. Raison
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B cell chronic lymphocytic leukemia (B-CLL) is characterised by the proliferation and accumulation of sIgM+ and/or sIgD+ B cells that fail to progress to the final stages of B cell development. Cells characteristic of the malignant clone resemble a small resting B cell that express some of the morphologic features of mature B cells. Unlike normal B cells, B-CLL cells commonly express the T cell antigen, CD5. Peripheral blood lymphocytes (93% CD5+ B, 6% T) from a patient with B-CLL were found to proliferate and differentiate into Ig-secreting cells following *in vitro* culture in the presence of the tumor promoter PMA and the mitogens, PHA and PWM. Depletion of T cells from the PBL preparations had minimal effect on the inductive ability of PMA. Thus, the PMA-induced differentiation of leukemic B cells is independent of T cells. However, T-depleted preparations (>99% CD5+ B) failed to proliferate and differentiate following mitogenic stimulation. The abrogation of cellular activation of T-depleted preparations was accompanied by a rapid reduction in cellular viability. After 8 days of culture, the viability of unstimulated, T-depleted preparations was <20%. Furthermore, there were no viable cells remaining in the cultures stimulated with PHA and PWM. In contrast, cultures of T-depleted preparations stimulated with PMA contained significant numbers of viable cells throughout the entire culture period. Cell viability in mitogen-stimulated and unstimulated cultures could be restored by supplementing the CD5+ B cells with purified allogeneic T cells. Analysis by immunofluorescence indicated that the enhanced viability was a result of an increase in the number of viable B cells and was not due to long-lived, viable T cells. However, culture supernatants generated from allogeneic T cells, and co-cultures of CD5+ B and allogeneic T cells, preactivated with PHA or PWM were not capable of enhancing the viability of cultured CD5+ B cells to the same extent as intact T cells. Interestingly, supernatants generated from co-cultures of CD5+ B and allogeneic T cells, preactivated with PMA, contained a soluble factor that significantly enhanced the viability of mitogen-stimulated and unstimulated CD5+ B cells in a dose-dependent manner. Thus, depending on the mode of activation, malignant CD5+ B cells appear to require either a cell contact signal delivered from co-cultured T cells or a soluble factor secreted following PMA-stimulation for the maintenance of B cell viability *in vitro*.

V 757 CD5$^+$ B Cells Have Restricted *In Vitro* Differentiation Potential.
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We have assessed the ability of FACS purified peritoneal Ly-1 B cells to differentiate in vitro in response to a number of stimuli in comparison to that of conventional B cells from both peritoneum and spleen. B cells were cultured in limiting dilution experiments in the presence of LPS, IL5, IL4 and 3T3 fibroblasts. The frequency of cells forming IgM and IgG1 secreting clones was measured by ELISA for each cell type. Under these conditions all B cells formed IgM clones at an approximately equal frequency (0.30). Ly-1 B cells, however, were essentially unable to generate IgG1 secreting clones in response to stimulation with mitogen plus IL4. Both conventional B cell types formed IgG1 secreting clones at a frequency of about 50% that of the IgM cloning frequency.

A number of possible explanations have been investigated. We have determined that Ly-1 B cells can respond to IL4 and can proliferate in the presence of IL4. Additionally we have shown that IL4 can induce IgG1 sterile germline transcripts in Ly-1 B cells. Furthermore, we have shown that Ly-1 B cells, like conventional B cells, will switch to IgG1 after stimulation via CD40.

We discuss our results in the context of the Ly-1 B cell compartment being a reservoir of potentially harmfull autoreactivities whose potential to differentiate in response to exogenous signals needs to be strictly controlled.

V 758 IMIQUIMOD INDUCED ACTIVATION OF MOUSE AND HUMAN B LYMPHOCYTES,
Mark A. Tomai, Linda M. Imbertson, Tamara L. Wagner, Michael J. Reiter and Richard L. Miller, Department of Pharmacology, 3M Pharmaceuticals, Maplewood, MN 55144

Imiquimod, previously known as R-837, has been identified as a potent antiviral and antitumor agent in animal models. Much of the biological activity of imiquimod can be attributed to the induction of cytokines including interferon-alpha (IFN), tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-6 and IL-8. The monocyte/macrophage is the predominant cell producing these cytokines in response to imiquimod. This study was undertaken to evaluate the effects of imiquimod and an analog S-27609 on B lymphocytes. We demonstrate that imiquimod and S-27609 are potent inducers of proliferation in cultures of mouse spleen and lymph node cells but not cultures of mouse thymus cells. Proliferative responses were similar to those observed using lipopolysaccharide (LPS) as a stimulus. Unlike LPS, S-27609 was capable of stimulating proliferation in splenic cultures of LPS-hyporesponsive C3H/HeJ mice indicating that the proliferation was not due to LPS contamination. In addition, human peripheral blood mononuclear cells were capable of proliferating in response to imiquimod and S-27609, whereas, LPS was ineffective at inducing proliferation. Flow cytometric analysis indicated that like LPS, S-27609 was stimulating proliferation of B but not T cells. Proliferation of mouse spleen cells was probably due to direct activation of the B cells since nonadherent populations also proliferated in response to S-27609. Further studies indicated that not only was S-27609 stimulating proliferation of B cells but it was also polyclonally activating B cells to produce antibody. Thus, imiquimod and its analog S-27609 not only stimulate monocytes to secrete cytokines but also activate B lymphocytes to divide and produce antibody.

V 759 LIFESPAN OF MURINE B CELLS,
David F. Tough and Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Opinions on the average lifespan of mature B cells vary greatly. In addition, the rate at which newly generated B cells are exported from the bone marrow and incorporated into the peripheral pool is unknown. We have assessed the turnover of murine B cell subsets by examining the surface phenotype of cells incorporating bromodeoxyuridine (BrdU) in vivo. In accordance with previous findings, the turnover of bulk populations of B cells in B6 and BALB/c mice is quite slow with less than 10% of lymph node B cells and 10-20% of splenic B cells incorporating BrdU over a 10 day period. However, we have observed that a minor population of B cells expressing high levels of the heat stable antigen (HSA^{hi}) has a rapid rate of turnover; 40-70% of these cells become BrdU$^+$ in 10 days. In addition, sIgD$^-$ B cells have a higher rate of BrdU incorporation than sIgD$^+$ cells. In order to discriminate between BrdU-labelled mature B cells and B cells that have recently emigrated from the bone marrow, we have studied the turnover of B cells following transfer to SCID mice. We have also examined B cell turnover in nude mice, which lack germinal centres. Data on the turnover of HSA^{hi} and HSA^{lo} B cells in these two situations will be discussed.

V 760 BCMA, PREFERENTIALLY EXPRESSED IN MATURE B CELLS, IS A 23 kDa TRANSMEMBRANE GLYCOPROTEIN OF THE cis COMPARTMENT OF THE GOLGI APPARATUS, Andréas Tsapis, Yacine Laâbi, Marie-Pierre Gras, Gustavo Linares*, Marie-Odile Blondel°, Rosine Tsapis°, INSERM Unité 301 and *Laboratoire de Pharmacologie Experimentale, Institut de Génétique Moléculaire, 75010 Paris, France and °Institut Jacques Monod, 75005 Paris, France

Molecular analysis of a t(4;16) translocation allowed us to isolate a hybrid cDNA composed in its 5' part of three exons of interleukin 2, whereas its 3' part belongs to a novel gene, which is normally expressed in mature B cells. This gene is named BCMA for B Cell MAturation. {Laâbi et al. EMBO J. (1992) 11, 3897-3904}. Stimulation of normal human B cells with Pokeweed mitogen and/or anti-μ antibodies showed that BCMA is expressed only upon differentiation of B cells. RNase protection assays using RNA from human malignant B cell lines, characteristic of different stages of B cell differentiation, showed that the BCMA gene is not expressed in the pro-B stage, while BCMA mRNA is present in the pre-B, B mature and plasma cells. Its amount increases along with the stage of maturation and reaches its maximum in plasma cells. Biochemical studies using rabbit antisera raised against BCMA protein allowed to demonstrate the presence of BCMA protein in mature B cells. Furthermore we demonstrated that BCMA is a glycosylated transmembrane protein, with a lumenal aminoterminus and a cytoplasmic carboxyl terminus. Immunofluorescence studies using either optical and/or confocal microscopic approach showed that the BCMA protein is located in the cis compartment of the Golgi apparatus.

V 761 MEMBRANE IMMUNOGLOBULIN IS ASSOCIATED WITH Ig-α AND Ig-β LIKE PROTEINS ON CHICKEN B CELLS, Olli Vainio, Marko Järveläinen and David Y. Mason, Department of Medical Microbiology, Turku University, 20520 Turku, Finland and Leukemia Research Fund Immunodiagnostics Unit, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom

In both man and mouse the membrane-bound immunoglobulin (Ig) receptor is associated with an invariant heterodimer of glycoproteins, the Ig-α and Ig-β. In the present work we have studied the expression of Ig-α and Ig-β like proteins on chicken B cells by crossreacting mAb. The mAb HM57 and B29/123 used in this study were raised against synthetic peptides, GTYQDVGSLNIADVQ and GEVKWSVGEHPGQE representing conserved amino acids in the cytoplasmic region of Ig-α and Ig-β, respectively. Both mAb stained in flowcytometry more than 90% of bursal B cells after permeabilization with buffered formol acetone. During embryonic development the increase in number of HM57-positive cells correlated well with that of mIg-positive cells. From peripheral blood and spleen HM57 stained about 10% of lymphocytes corresponding to the number of mIg-positive cells. B29/123 did not react well with embryonic bursa, neither with peripheral B cells. Histochemically HM57 stained both cortical and medullary bursa cells, more intensely medullary cells and scattered cells in the spleen that histologically appeared as plasma cells. Both HM57 and B29/123 reacted with permeabilized virally transformed B cell line RP13 but not with several T cell lines, indicating that mIg on RP13 is associated with Ig-α and Ig-β like proteins. Biochemically anti-μ heavy chain specific mAb coprecipitated from digitonin-lysed surface-labeled bursa cells an Ig-associated heterodimer with relative molecular mass M_r of about 44 kDa and 48 kDa.

Taken together our data obtained by crossreactive mAb demonstrate that chicken B cell antigen receptor also consists of mIg associated with a heterodimeric protein complex similar to Ig-α and Ig-β. They also suggest that the gross structure of the B cell receptor in avian and mammalian species has been conserved during the 200 million years of divergent evolution.

V 762 DIFFERENTIAL CONTRIBUTIONS OF CONVENTIONAL B AND B-1 CELLS TO ANTIBODY-SECRETING AND GERMINAL CENTER B CELL POPULATIONS, Sandra M. Wells and Alan M. Stall, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

A significant proportion of IgM and IgA antibody-secreting cells (ASCs) in naive mice have been shown to be B-1 cells. The antibodies secreted by these cells are characterized as being of low affinity and lacking somatic mutations. Primary immunization with T-dependent antigens results in the generation of two functionally distinct populations of B cells, antibody-secreting cells (ASC) and memory cells. High affinity memory cells appear to be generated in the germinal centers (GC) of peripheral lymphoid organs. The respective roles of conventional and B-1 cells in this type of response have not been clearly defined. Using B lineage chimeric mice, in which B-1 and conventional B cells can be easily identified, we examined the relative contributions of these two populations to the ASC and GC B cells found in naive mice; and also in mice following primary immunization with PC-KLH. ASCs and GC B cells were identified by cell surface binding to syndecan and peanut agglutinin (PNA), respectively by immunofluorescent staining of frozen sections and flow cytometry. In naive mice, a significant proportion of IgM ASCs were derived from B-1 cells, however, GC B-1 cells were virtually undetectable. In contrast, although conventional B cells were responsible for a lower proportion of IgM ASCs, virtually all of the GC B cells were derived from this population. Five days following immunization with PC-KLH, the PC-specific ASCs were virtually all T15 idiotype-positive ($T15id^+$) and derived from B-1 cells. Although after 10 to 12 days following immunization well-developed GCs were detected by histology, no T15id staining was present in these GCs. Furthermore, FACS analysis revealed that all of the $T15id^+$ B-1 cells were PNA-negative. These results show that although B-1 cells can produce antibodies in primary TD immune responses, they are not found in germinal centers. This suggests that B-1 and conventional B cells may follow different pathways of differentiation following antigen challenge.

Tolerance and Suppression in the Periphery

V 763 DECREASED GLUTATHIONE IN THOSE HIV INFECTED MAY ACCOUNT FOR DECREASED PROLIFERATIVE RESPONSES AND INCREASED INFLAMMATORY STRESS. Michael T. Anderson, Frank J. T. Staal, Mario Roederer, Carlos Gitler*, Leonore A. Herzenberg and Leonard A. Herzenberg. Dept. Genetics, Stanford Univ, and *Weizmann Institute.

We have shown that glutathione (GSH), the major intracellular antioxidant, is decreased in all T cells from HIV infected individuals. We postulate that this decrease in GSH causes increased susceptibility to oxidants and inflammatory stimulations, and leads to anergy.

As a model for the effects of decreased GSH levels on *in vivo* lymphocyte function, we treat peripheral blood lymphocytes (PBL) and human T cell lines with buthionine sulfoximine (BSO), an inhibitor of GSH synthesis. Treatment with BSO to lower GSH results in increased inflammatory cytokine signaling but decreased T cell receptor (TCR) signaling.

TNF stimulation of the Jurkat cell line leads to tyrosine phosphorylation of many cellular substrates. Pretreatment of Jurkat with BSO markedly increases the levels of TNF-stimulated protein tyrosine phosphorylation. This reflects the redox sensitivity of the tyrosine kinases and phosphatases which control the levels of tyrosine phosphorylation. We also show that NF-kB activation, a major component of inflammatory signaling, requires a tyrosine phosphorylation event. NF-kB activation by PMA, H_2O_2 and TNF is blocked by the tyrosine kinase inhibitor, Herbimycin A. The augmentation of TNF-triggered signals by decreased intracellular GSH may potentiate both NF-kB activation and NF-kB directed gene expression (eg,HIV).

Anti-CD3 stimulation of T cells leads to signal transduction events which include a rise in intracellular calcium. The TCR-stimulated calcium flux is an essential signal for proliferation of lymphocytes and production of IL-2. Treatment of either PBL or Jurkat cells with BSO results in inhibition of the anti-CD3 induced calcium flux. In fact, intracellular GSH levels are inversely correlated with the induced calcium flux; a significant signal inhibition is attained with a loss of 10% of the GSH in PBL.

These results may account for the observations in HIV infected individuals of *increased* inflammatory stress and *decreased* antigen induced proliferation (anergy) of T cells. Thus, GSH replenishment with N-acetyl cysteine (NAC) or oxothiazolidine carboxylic acid (OTC) (both cysteine pro-drugs) may slow the progression of HIV disease and maintain CD4 and/or CD8 T cell function in AIDS.

V 764 CD4+ TS CELLS INHIBIT THE EARLY SIGNAL TRANSDUCTION OF TH CELLS, Yoshihiro Asano and Tomio Tada, Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

The activated CD4 Ts cells could inhibit the increase of intracellular Ca^{2+} of Th clones induced by antigen-pulsed antigen-presenting cells (APC). The identity of MHC restriction specificities was required for the instant suppression of Ca^{2+} influx of Th clones, while a longer period of activation of Ts clones was needed to suppress the reponse of Th clones having different MHC restrictions. A strict selectivity was found in the suppressive activity of Ts clones in that Ts clones could suppress the Ca^{2+} responses of Th1 and Th2 clones but not of other CD4 Ts clones. Ts clones could suppress the activation of Th clones induced only by antigen or anti-TCR antibody but not by Con A. Ts clones released soluble immunosuppressive factors upon stimulation with immobilized anti-CD3 antibody. The factor released from CD4 Ts clones could inhibit the Th cell-dependent antibody formation and the IL-production. The factor could inhibit neither proliferation of Th nor expression of IL2R, CD28 or CD69 of anti-TCR-activated Th cells. The actor is different from any known cytokines including IL10, IFNγ, TNFαβ, and TGFβ. These results indicate that Ts clones inhibit the early signal transduction of Th cells and thus suppressing specific immune responses.

V 765 **THE ROLE OF B CELLS IN LOW ZONE TOLERANCE TO HEN EGG LYSOZYME**

Allison M. Baird and David C. Parker, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01655

We are studying acquired tolerance to foreign protein antigens to gain an understanding of the mechanisms of self tolerance. Our hypothesis is that small resting B cells are the tolerizing antigen presenting cells (APC) in acquired tolerance due to their specific antigen receptors and constitutively high levels of class II MHC expression. In low zone tolerance, C57BL/6 mice are given 10 μg of hen egg lysozyme (HEL) intravenously 3 times a week for 3 weeks and then challenged one week later with 100 μg of HEL emulsified in complete freunds adjuvant. We have found that this protocol induces tolerance in the T cell compartment to HEL as measured by a T cell proliferation assay. Using B cell deficient mice, we have also demonstrated that B cells are not required to prime T cells for a proliferative response to whole HEL. To directly examine the role of the B cell as the tolerizing APC, we are comparing low zone tolerance induction in normal C57BL/6 mice to B cell deficient mice. If we demonstrate a difference in tolerance induction between C57BL/6 mice and B cell deficient mice, we will transfuse B cells into the deficient mice prior to tolerizing and see whether this will restore tolerance.

V 766 ANERGY IN $CD4^-CD8^-$ αβ T-CELLS, Samuel M. Behar, Steve Porcelli, and Michael B. Brenner, Dept. of Rhuem/Immun. Brigham and Women's Hospital, Boston, MA 02115.

Our lab recently demonstrated that the human nonpolymorphic MHC related CD1 proteins can act as antigen presenting molecules for $CD4^-CD8^-$ αβ T-cells (DN T-cells). We have isolated DN T-cells from normal donors that are specific for antigens from mycobacterial species. These T-cells are MHC unrestricted and recognize antigen only when presented by CD1b, one of the four known protein products of the CD1 locus. Human blood monocytes express high levels of CD1a, CD1b and CD1c after treatment with GM-CSF and IL-4. The DN T-cell lines have a strong proliferative response when the cytokine treated monocytes are used as APCs. On the other hand, the DN T-cells fail to proliferate when CD1b transfectants of the lymphoblastoid cell line C1R (C1R.CD1b) are used as APCs, although they do specifically kill antigen pulsed C1R.CD1b targets in a CTL assay. The ability of the T-cells to proliferate only when cytokine treated monocytes are used as APCs, despite being able to specifically kill both cytokine treated monocytes and C1R.CD1b, suggested that the cytokine activated monocytes express a costimulatory factor which the C1R.CD1b cells lack. This hypothesis was confirmed by the demonstrating that the T-cells become anergic when incubated with the C1R.CD1b cells in the presence of antigen, but not when incubated with the C1R.CD1b cells in the absence of antigen. CONCLUSION: We have described human DN T-cells that recognize microbiological antigens when presented by the CD1 family of molecules. Furthermore, these T-cells require a costimulatory molecule that is present on the surface of cytokine treated monocytes but not lymphoblastoid cells lines such as C1R, Daudi, or T2. The T-cells become anergic when their antigen receptors are engaged by CD1 and antigen, in the absence of a required costimulatory signal.

V 767 Peripherally Induced Unresponsiveness to Mls-1^a is True Tolerance

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T cells from TCR Vβ8.1 transgenic mice inoculated as adults with cells bearing the superantigen Mls-1^a exhibit profound unresponsiveness to subsequent restimulation *in vitro*. The mechanism of unresponsiveness involves both anergy and deletion of Mls-1^a reactive CD4$^+$ and CD8$^+$ T cells in the periphery of inoculated mice, which represent the vast majority of T cells in TCR Vβ8.1 transgenic mice. We demonstrate that in addition to unresponsiveness to restimulation with Mls-1^a spleen cells and anti-CD3 antibodies, T cells from Mls-1^a inoculated transgenic mice make a poor response to allogeneic spleen cells. We have therefore used these mice to ask whether peripherally induced unresponsiveness to superantigens has a functional *in vivo* correlate by assessing transplantation tolerance. We show that Mls-1^a inoculated TCR Vβ8.1 transgenic mice reject fully allogeneic skin more slowly than control uninoculated transgenic mice. In the course of these studies, we have discovered that unmanipulated TCR Vβ8.1 transgenic mice have an impairment in the allograft response to minor alloantigenic differences. The results provide insight into the mechanisms by which self tolerance to extrathymic antigens is normally acquired.

V 768 LOSS OF IMMUNOLOGICAL TOLERANCE INDUCED BY THE ISOASPARTYL FORM OF A SELF PEPTIDE, Peter R Blier* and Mark J Mamula§, *Dept of Immunology, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, 06877, and §Section of Rheumatology, Yale University School of Medicine, New Haven, CT 06510

Autoimmune disorders are characterized by the activation of T and B lymphocytes reactive to self antigens to which the individual is usually tolerant. The mechanisms by which tolerance is initially broken in the development of autoimmunity remain incompletely understood. In a model system using induced autoreactivity to murine cytochrome c, activated cross-reactive B cells can resurrect anergic, autoreactive T cells. Either immunization with a foreign cyt c, or with a cryptic cyt c peptide (the C-terminal amino acids 81-104), can cause this loss of tolerance to the intact self protein. In the present study, mice immunized with the murine cyt c peptide 90-104 (α-90-104; ERADLIAYLKKATNE) showed no response by the B or T cell compartments. However, immunization with the isoaspartyl form of this peptide (β-90-104), where the linkage of Asp93 to Leu94 occurs through the β-carboxyl group, resulted in a strong T cell response. These T cells were not cross-reactive with the α-linked isoform. On the other hand, antibodies elicited by immunization with either β-90-104 or cyt c 81-104 did bind both the α- and β-linked forms of 90-104. Isoaspartyl linkages can occur physiologically, through the non-enzymatic cyclization and hydrolysis of asparaginyl (and possibly aspartyl) residues, and are observed in aging or stressed cells. Our finding that isoaspartyl linkages can render self proteins immunogenic and elicit cross-reactive antibodies suggests a novel mechanism by which self antigens could induce autoimmunity in susceptible hosts.

V 769 **CHARACTERIZATION OF THE MOUSE LYMPHOTOXIN-β GENE.** **Jeffrey L. Browning and Pornsri Lawton, Department of Cell Biology, Biogen, Cambridge, MA 02142**

The role of lymphotoxin (TNF-β) is poorly characterized mainly due to the lack of specific mAbs and recombinant protein to study function in the mouse. Recently, our lab defined the gene for a second human LT-like gene (called LT-β) that forms a complex with LT (LT-α) on the cell surface. In view of the lack of progress in the mouse system, we have undertaken a characterization of the mouse LT-β gene. The mouse LT-β protein as inferred from the cDNA sequence shares 72% amino acid sequence identity with the human counterpart. As expected, characterization of a genomic fragment spanning the region 3' of the TNF gene showed that the murine LT-β gene was located in a position in the MHC TNF locus identical to that in the human. Interestingly, due to a mutation in the donor splice site, intron 3 in the mouse is not spliced out in 5 strains of mice examined. An open reading frame is preserved resulting in the insertion of 64 aa's between the transmembrane region and the extracellular receptor binding domain and therefore should yield a functional protein.

V 770 ENTRY OF HIV INTO CD4$^+$ CELLS REQUIRES A T-CELL ACTIVATION ANTIGEN, CD26, C. Callebaut, B. Krust, E. Jacotot and A.G. Hovanessian, Unité de Virologie et Immunologie Cellulaire (UA CNRS 1157), Institut Pasteur, 28, rue du Dr. Roux 75015 Paris France.

HIV infects T lymphocytes, monocytes and macrophages by binding to its principal receptor, the CD4 molecule which is essential for binding HIV particles to target permissive cells but not by itself sufficient for efficient viral entry and infection.

Here we show that the coreceptor which may interact with the third hypervariable domain (the V3 loop) in the surface glycoprotein of HIV, is dipeptidyl peptidase IV (DPP IV) also referred to as the CD26 antigen. This serine protease characterized by a catalytic activity specific to proline residues, cleaves synthetic peptides with motifs GP, RP, KP, AP, EP, DP. In the V3 loop, the RP motif is 94 to 100 % conserved for HIV-1, HIV-2 and related simian isolates whereas the GP motif is more than 90 % conserved among HIV-1 isolates. Consequently, entry of HIV-1 into T lymphoblastoid and monocytoid cell lines, is 80-90 % inhibited, either by a specific monoclonal antibody against DPP-IV or a specific peptide inhibitor of this protease. Other peptides containing the GP, RP or KP motif also inhibited both viral entry and enzyme activity. Finally, coexpression of human CD4 and CD26 in murine NIH 3T3 cells rendered them permissive to infection by HIV-1, thus confirming that CD26 is an essential coreceptor of CD4 for HIV entry. Interestingly, these inhibitory agents that suppressed HIV-1 infection also blocked infection of cells by HIV-2 which is completely unrelated to HIV-1 LAI, indicating that the requirement of DPP IV for viral entry is a general phenomenon for different isolates and types of HIV. These observations provide the basis for developing new, simple but specific potent inhibitors of HIV infection.

V 771 RECOGNITION OF AN IMMUNOGLOBULIN V_H EPITOPE BY INFLUENZA VIRUS SPECIFIC CLASS I MHC-RESTRICTED CYTOLYTIC T LYMPHOCYTES, Wuxiong Cao, Brenda A. Myers-Powell, and Thomas J. Braciale, The Beirne B. Carter Center for Immunology Research and the Departments of Microbiology and Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908

There are two immunogenic sites on the type A influenza A/Japan/57 (H2N2) hemagglutinin (HA) which can be recognized by class I major histocompatibility complex (MHC) H-2K^d-restricted cytolytic T lymphocytes (CTLs). One of these sites encompasses two distinct partially overlapping epitopes, which span HA residues 204-212 and 210-219. During the analysis of the fine specificity of CTL clones directed to the HA 210-219 epitope, we found that one clone 40-2 also recognized myeloma P3x63-Ag8 but not SP2/0. P3x63-Ag8 is derived from MOPC 21 myeloma and they express the same immunoglobulin (Ig) heavy chain variable region (V_H) gene, which is a member of the murine 7183 V_H gene family. Recognition was specific for the endogenously processed MOPC 21 heavy chain in association with the K^d molecules, since SP2/0 is a variant of P3x63-Ag8 which cannot make its own Ig heavy chains. The V_H epitope recognized could be mapped to a ten amino acid peptide spanning MOPC 21 V_H residues 49-58. Crossreactivity for the V_H gene product was also demonstrable in some heterogeneous populations of CTL generated in response to influenza virus infection. These results represent the first demonstration of crossreactivity for an endogenously processed product of a self Ig by CTL directed to a foreign antigen and raise the possibility that Ig V_H expression may regulate the CD8$^+$ T cell response to foreign antigens.

V 772 SOMATIC MUTATIONS IN ANTIBODY VARIABLE REGIONS CREATE IMMUNOGENIC T CELL EPITOPES.

Margaret C. Eyerman and Larry Wysocki. Division of Basic Sciences, Dept. of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine and Dept. of Immunology, University of Colorado Health Sciences Center, Denver CO 80206.

During an immune response, specific antibodies and B cells that are infrequently represented in the immune repertoire become amplified to abundance. Potentially novel T cell epitopes are also created through the physiologic process of somatic hypermutation in antibody genes. This presents a challenge for T cell self tolerance since many potential epitopes are either rare or nonexistent during the maturation of the T cell repertoire in the thymus.

To explore the potential for T cell recognition of antibodies, we have immunized A/J mice with somatically mutated mAbs generated from the same strain. We have produced 10 T cell hybridomas to mAb36-71 and 3 T cell hybridomas to mAb45-49. All of the hybridomas specific for mAb36-71 are restricted to I-A^k, while all of the hybridomas specific for mAb 45-49 are restricted to I-E^k. Processing is required since glutaraldehyde-fixed APC can present tryptic digests of the immunogen but not the native form of the antibody.

We found that each of the T cell hybridomas responded exclusively to somatically mutated portions of either framework or CDR regions. The hybridomas responded to peptides (aa1-18 of the mAb36-71 light chain or to aa42-57 of the mAb45-49 light chain respectively) which each include two somatic mutations but not to the corresponding germline peptides. We therefore hypothesized that the T cell repertoire is tolerant of germline antibody V regions. This is supported by the finding that repeated fusions from mice immunized with an unmutated mAb did not yield immunogen-specific T cell hybridomas.

Our results show that T cells are able to recognize isologous mutated antibody variable regions in a conventional class II MHC-restricted manner. Whether tolerance to autologous somatically mutated V regions is achieved during antigen-driven stages of B cell differentiation is unknown.

This work was supported by an American Heart Association Grant in Aid, Council for Tobacco Research Grant 2419A and by NIH Grant RO1AI33613.

V 773 PRODUCTION OF SOLUBLE α/β T CELL RECEPTORS AS IMMUNOREGULATORY MOLECULES

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Most peripheral T cells utilize a clonotypic α/β T cell receptor to recognize peptides presented in the groove of MHC encoded proteins. Both chains of the receptor possess hydrophobic transmembrane domains and are non-covalently asssociated with a complex of the CD3 and ζ proteins on the T cell membrane. DNP-specific/class I MHC-restricted molecules produced by CD8$^+$ T cells from dinitrobenzene sulfonate-primed mice inhibit the ability of immune T cells to transfer DNP-specific contact sensitivity. A T cell hybridoma, MTs 79.1, constitutively produces a DNP-specific/K^d-restricted suppressor molecule that is a disulfide-linked dimer and is bound by antibodies specific for T cell receptor (TcR) α and β chain determinants. Molecular analyses have indicated that MTs 79.1 utilizes the Vα 14.2 and Vβ8.2 genes to encode the surface TcR. Our previous results have shown that deletion of either the Vα 14 or the Vβ8 gene from MTs 79.1 results in loss of surface receptor expression and the ability to produce the soluble suppressor molecule and that transfection of a cDNA encoding the parental β chain into TcR β chain gene deletion mutants of MTs 79.1 reconstitutes surface TcR expression and the ability to produce the soluble suppressor molecule, supporting our hypothesis that these molecules represent soluble forms of the α/β TcR. To assess the role of the TcR α chain in the composition of these immunoregulatory molecules, we have cloned the MTs 79.1 α chain gene and transfected sense and antisense constructs into α chain gene deletion mutants of MTs 79.1. Transfection of sense contructs into one such mutant restored surface TcR expression and the ability to produce the soluble suppressor molecule. We have identified a subclone of MTs 79.1, 79.1.4, that doesn't produce the soluble suppressor molecule while expressing equivalent levels of surface α/β TcR as that of a subclone, 79.1.8, that continues to produce the soluble molecule. Furthermore, the soluble suppressor molecule is detectable in soluble cell lysates from the 79.1.8 cells but not from the 79.1.4 cells. The soluble molecule is not associated with the CD3 complex and is hydrophilic in nature. Collectively, these results indicate the ability of some T cells, and not others, to produce soluble forms of the α/β TcR. Furthermore, the production of these soluble receptors may involve post-translational modification of the hydrophobic surface form of the receptor.

V 774 INDUCTION AND CHARACTERIZATION OF THE ANERGIC STATE INDUCED IN ALLERGEN-SPECIFIC T CELL CLONES BY INCUBATION WITH SPECIFIC PEPTIDES, Stephan Fasler[1], Gregorio Aversa[1], Patricia V. Schneider[1], Abba Terr[2], Jan E. de Vries[1] and Hans Yssel[1], [1]Human Immunology Department, DNAX Research Institute and [2]Allergy Clinic, Stanford Medical School, Palo Alto, CA 94304.

Cytokines produced by activated CD4$^+$ helper T cells play a major role in the regulation of the IgE response: recombinant (r) IL-4 and rIL-13 have been shown to specifically induce the synthesis of IgE, whereas rIFN-γ, rIFNα and rIL-10 have inhibitory effects on the IL-4/IL-13 induced IgE synthesis. In addition, CD4$^+$ T cells provide a costimulatory signal, in which CD40-CD40L interactions play an important role. We have investigated whether we could modulate the function of allergen-specific CD4$^+$ T cells with respect to their cytokine production profile and their ability to provide B cell help in induction of IgE synthesis. T cell clones, specific for *Der p*I, a major allergen in house dust mite were generated and five T cell activation inducing epitopes on the *Der p*I molecule were mapped, which were all recognized in the context of different MHC Class II molecules. All T cell clones expressed a Th2-like pattern of cytokine production following activation with peptides, representing the specific *Der p*I epitopes. In addition, activated *Der p*I-specific T cell clones could induce polyclonal IgE synthesis by purified B cells, in the absence of exogenous rIL-4 or rIL-13.

Incubation of the *Der p*I-specific T cell clones with supraoptimal concentrations of the relevant *Der p*I-derived peptides induced a state of anergy, during which the cells did no longer respond to antigen-specific stimuli, as measured by cytokine production and proliferation. This anergic state could be maintained for at least 3 weeks. Although anergized T cell clones express CD40L, they were no longer able to provide B cell help in the induction of IgE synthesis, even when rIL-4 was added to the cultures. However, cytokine production, and their IgE inducing capacity was restored by exogenous IL-2. Anergized T cell clones expressed strongly reduced levels of the TCR/CD3 complex, whereas CD25 expression was enhanced. No, or very low Ca^{2+} fluxes could be induced in anergized T cell clones following TCR triggering. These results suggest that down regulation of TCR/CD3 complexes may account for the observed T cell non responsiveness.

V 775 **THE ROLE OF CELLULAR COMPETITION IN THE STRUCTURE AND ORGANIZATION OF THE IMMUNE SYSTEM,** António A. Freitas, Anne-Claire Viale and Alf Grandien, Unité d'Immunobiologie, URA359 CNRS, Institut Pasteur, Institut Pasteur, 25-28 rue du Dr. Roux, 75015 Paris, France.

The role of cellular competition in the organization and structure of the immune system was investigated. Following a competitive repopulation strategy, lethally irradiated mice were reconstituted with bone marrow from either IgH congenic or four different lines of Ig-transgenic mice mixed at variable ratios. Differences in allotypic and/or idiotypic markers allowed the identification of the products of the different donor populations. After reconstitution the number of B cells was identical and independent of the ratio of the injected cells. By comparing the relative representation of the various donor cell lineages among the bone marrow and spleen B cells, the IgM secreting splenocytes and serum IgMs we found that, in chimeras hosting cells from Ig-transgenic mice, it differed among the several compartements diverging from the ratios present in the initial cell inoculum. These results confirm the existence of cellular competition among different B cells. The implications of a cellular competition model of the immune system are discussed.

V 776 TCRα/β MODULATION, APOPTOSIS AND PERIPHERAL T CELL DELETION IN NORMAL AND *bcl-2* TRANSGENIC MICE

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The number of T cells in the peripheral pool is controlled by processes that include output from the thymus, proliferation of T cells in response to antigen stimulation, and apoptosis of post-activation T cells. Introduction of cells that express the superantigen vSAG-7 (mls-1^a) into mice that do not express the superantigen induces a T cell response leading to peripheral tolerance to vSAG-7. During the course of this T cell response , vSAG-7 reactive T cells first undergo proliferation, which is followed by a partial deletion. By a detailed study of the TCR expression on host T cells, we have been able to identify a population of TCRα/β-negative T cells first appearing at the time when vSAG-7 reactive T cells starts to disappear, suggesting the initial "deletion" of vSAG-7 reactive T cells is actually a result of TCRα/β downregulation. TCRα/β-negative cells can be observed in both CD4+ and CD8+ cells, but their prognosis appears different. The CD4+TCRα/β-negative cells are preferentially undergoing apoptosis, suggesting the co-receptors are involved in the signaling leading to apoptosis and clonal deletion. Using a line of *bcl-2* transgenic mice, we have also shown that *bcl-2* has no effect on TCRα/β downregulation, but it inhibits peripheral deletion of vSAG-7 reactive T cells, suggesting the intracellular mechanisms for these two processes are different. Our result also suggests that the mechanism of peripheral T cell deletion driven by antigen is different from that of intrathymic T cell deletion, which has shown by other investigators to be unaffected by *bcl-2* .

V 777 INDUCTION OF NONDELETIONAL TOLERANCE IN TCR TRANSGENIC T CELLS IN VIVO, Elizabeth R. Kearney, Dennis Loh, and Marc K. Jenkins, Departments of Microbiology and Pathology, University of Minnesota, Minneapolis, MN 55455; and Washington University, St. Louis, MO 63110.

Administration of antigen under non-inflammatory conditions (e.g., in incomplete Freund's adjuvant (IFA)) induces a form of tolerance in which T cells fail to respond to priming with antigen in complete Freund's adjuvant (CFA). The mechanism by which these cells are rendered unresponsive is not understood, in part because of the difficulties associated with physically identifying small numbers of antigen-specific T cells in vivo in normal individuals. To address this, T cells from ovalbumin/I-Ad-specific TCR transgenic mice were adoptively transferred into syngeneic normal mice and tracked with a monoclonal antibody (KJ1-26) specific for the transgenic TCR, following priming and tolerizing regimens. Transgenic T cells were transferred such that a low, but detectable level of CD4+ KJ1-26+ cells were found later in the lymph nodes of unprimed normal mice. Priming of these mice with ovalbumin peptide in CFA resulted in increased numbers of KJ1-26+ cells in the draining lymph nodes that were highly responsive to ovalbumin upon restimulation in vitro 7 days later. Three days after priming with peptide in CFA, many of the KJ1-26+ cells were in the S, G2, or M stages of the cell cycle. Tolerance was induced in recipient mice by injecting ovalbumin peptide in IFA eight days prior to priming with ovalbumin peptide in CFA. The number of KJ1-26+ cells in the lymph nodes of the animals injected with peptide in IFA increased marginally after 3 days and fell to a lower level 8 days after injection. Interestingly, at the 3 day time point the KJ1-26+ cells were blasts but were not progressing past the G1 stage of the cell cycle. This population expanded only marginally in vivo following the peptide/CFA injection on day 8 and was unresponsive at the level of T cell proliferation and IL-2 production to in vitro restimulation with antigen 7 days later. Together these results demonstrate that functional unresponsiveness is responsible, at least in part, for this form of tolerance which is induced in vivo by antigen-presentation that does not result in subsequent T cell proliferation.

V 778 CROSSLINKING OF FCγ RECEPTOR TO SURFACE IMMUNOGLOBULIN ON B CELLS CLOSES THE PLASMA MEMBRANE CALCIUM CHANNEL Peter A. Kiener, Bruce M. Rankin, Patrice M. Dubois and Michael L. Diegel. Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Avenue, Seattle, Washington 98121.

Crosslinking of surface Ig (sIg) on B cells with a $F(ab')_2$ anti-sIg fragment gives rise to a prolonged increase in $[Ca^{2+}]_i$. Co-crosslinking sIg with FcγRII gives rise to a much more transient increase. In the murine B cell line, A20, stimulation with $F(ab')_2$ anti-sIg gives rise to the production of high levels of IL-2 while stimulation with intact anti-sIg does not. No differences in the protein tyrosine phosphorylation in response to stimulation of the cells with either Ab was found. Studies carried out in the absence of extracellular calcium or in the presence Ni^{2+}, indicated that both extracellular and intracellular stores of calcium contributed to the overall elevation of $[Ca^{2+}]_i$ induced by $F(ab')_2$ anti-sIg. Furthermore, the prolonged elevation of $[Ca^{2+}]_i$ was maintained by the influx of extracellular calcium. Inhibition of this influx significantly reduced $F(ab')_2$ anti-IgG induced IL-2 production. Under similar conditions, activation of the B cells with intact anti-sIg mobilized calcium from intracellular stores but did not stimulate the influx of extracellular calcium or the production of IL-2. IL-2 production could be restored by the addition of A23187 to the cultures. Activation of the cells in the presence of manganese indicated that $F(ab')_2$ anti-IgG stimulated the opening of the plasma membane calcium channel whereas intact anti-IgG did not. Treatment of cells pre-activated by $F(ab')_2$ anti-IgG with intact anti-sIg rapidly reduced the prolonged elevated levels of $[Ca^{2+}]_i$. Even 2 to 4 hr after stimulation of the cells with $F(ab')_2$ anti-IgG, both intact anti-sIg and EGTA could inhibit IL-2 production. These results indicate that co-crosslinking sIg with FcγRII gives rise to an inhibitory signal in the cell that can both prevent the opening and actively close a plasma membrane calcium channel.

V 779 T HELPER TARGET CELL DNA FRAGMENTATION THROUGH A CD4-POSITIVE T SUPPRESSOR CELL CLONE INDUCING SPECIFIC UNRESPONSIVENESS, Eckehart Kölsch, Christian Becker, Abdo Konur, André Rademaekers, Vitam Kodelja, Andrea Partenheimer and Hans-Gerd Pauels, Institute of Immunology, University of Münster, 48129 Münster, Germany.

The CD4-positive bovine serum albumin (BSA)-specific Ts cell clone BVI/5 from a CBA/J mouse tolerized by low doses of BSA induces specific unresponsiveness in the respective T helper (Th) cell population. Tolerance induction can be measured *in vitro* in proliferation assays using specific Th cell clones or antigen-primed lymphnode cells (LNC) and determined *in vivo* by the failure to produce hapten-specific antibodies. Using the BSA-specific Th cell clone 83/1 as a target one observes in addition ^{51}Cr-release in a 16 h longterm assay but finds no effect in a typical 6 h T cell cytotoxicity test. BVI/5 Ts cells do not produce interleukin-2 but otherwise express a Th1 profile. The suppression of proliferation of 83/1 Th cells is partly due to interferon-γ (IFN-γ). But lysis of 83/1 Th cells as well as suppression of BSA-specific LNC proliferation needs direct cell contact between BVI/5 Ts cells and their targets. Cell lysis and suppression of LNC cannot be simulated neither by IFNγ, the combination of IFN-γ and TNF nor by BVI/5 supernatants. Thus soluble mediators cannot account for specific suppression by BVI/5 Ts cells of *in vitro* responses from LNC and are probably not responsible for the induction of *in vivo* unresponsiveness. Macrophages have been excluded as targets. Instead the data suggest a direct effector/Th target cell interaction. BVI/5 Ts cells induce apoptosis-like DNA fragmentation in cloned BSA-specific 83/1 Th cells and in a fraction of LNC from BSA-primed mice. Apoptosis can also be visualized as chromatin condensation in the LNC population. It can further be demonstrated that BVI/5 Ts cells express perforin and granzyme A upon activation. Thus they are equipped with the effector molecules for target cell destruction. If the BVI/5 Ts cell clone is a representative for regulatory Ts cells inducing specific unresponsiveness in peripheral lymphoid organs, then it is well equipped for this function because of the use of an I-E^k-restricted specific TCR (imposing antigen-specificity) and the endogeneous expression of class II MHC molecules (serving as a focussing device for Th cells). - Supported by DFG through SFB 310.

V 780 ACTIVATION OF FAS EXPRESSION BY HIV IS ASSOCIATED WITH THE SELECTIVE DEPLETION OF CD4+ LYMPHOCYTES, John F. Krowka, Michael S. Ascher and Haynes W. Sheppard, Viral and Rickettsial Disease Laboratory, California Dept. of Health Services, Berkeley, CA 94704

The FAS/APO-1 glycoprotein is an apoptosis-associated transmembrane protein that is a member of the molecular family which includes CD40 and the receptors for tumor necrosis factor and nerve growth factor. The levels of FAS expression on peripheral blood lymphocytes (PBL) from HIV-seronegative (HIV-) and HIV-seropositive (HIV+) subjects in the San Francisco Men's Health Study (SFMHS), a prospective study of gay and bisexual men were analyzed to determine if FAS expression is associated with apoptosis and CD4+ lymphocyte depletion in HIV+ individuals. The percentages of CD3+, CD4+ and CD8+ PBL expressing FAS were significantly higher ($p < 0.0008$) in HIV+ donors (76.6, 86.3, and 63.7 %, respectively) than in HIV- donors (53.8, 63.3, and 30.4 %, respectively). Statistical analysis showed that increased FAS expression was strongly associated with the levels of CD4+ but not with levels of CD8+ PBL in HIV+ subjects. It is possible that FAS-mediated apoptosis is quantitatively or qualitatively different in CD4+ and CD8+ PBL and/or that the replacement rates of CD4+ and CD8+ PBL may be different in HIV+ individuals. Experiments are currently in progress to define the mechanism(s) by which HIV upregulates FAS expression and the role of increased FAS expression in HIV disease progression.

V 781 IN VITRO INDUCTION OF ANERGY IN RAT LYMPHOCYTES, J.C. Lai, B.P. Vistica, R.K. Maturi, B.L. Shirkey, Y. Sasamoto, S.M. Whitcup and I. Gery, Laboratory of Immunology, National Eye Institute, NIH, Bethesda, MD 20892

T-lymphocytes can be rendered anergic if exposed to MHC associated antigenic peptides in the absence of an essential secondary signal. Here we report that rat T cell line lymphocytes become specifically anergic when exposed to antigen in the absence of APC. Line cells specific toward an 11-mer peptide derived from the sequence of the interphotoreceptor retinoid-binding protein were used. Incubation of these T cells with the peptide for 20 hours reduced their response to a subsequent stimulus with the peptide in the presence of APC by approximately 90%. Induction of anergy was accompanied by increased turnover of phosphatidyl inositol, suggesting an active metabolic process. Induction of anergy was also highly specific: alanine-substituted analogs of the native peptide produced anergy only when the analog was recognized by lymphocytes sensitized against the native peptide. Anergy induction was inhibited by antibodies against adhesion molecules (LFA-1 and ICAM-1), indicating the need for cell-cell interaction in the process. Unlike human lymphocytes, (J.M. LaSalle et al., J. Immunol. 151:649, 1993), the rat line cells, however, did not stain with antibodies against MHC class II molecules (OX-3 or OX-6), and these antibodies did not inhibit anergy induction. In contrast, these antibodies stained approximately 30% of spleen cells and strongly inhibited lymphocyte proliferation in the presence of APC. Although they cannot be detected with currently available antibodies, MHC class II molecules on the rat T cells are believed to be involved in anergy induction. We propose that, in a process similar to that in the well established human T-cell system, induction of anergy in the rat system is initiated by antigen presentation on T-lymphocytes that are unable to provide a co-stimulatory signal. This study provides the first demonstration of anergy induction in vitro with non-human lymphocytes.

V 782 SELECTIVE SUPPRESSION OF IgE PRODUCTION BY MHC CLASS-I RESTRICTED CD8$^+$ T CELLS FOLLOWING INHALATION OF SOLUBLE PROTEIN ANTIGENS IN A RODENT MODEL. Christine McMenamin, Carolyn Pimm, Michelle McKellar & Patrick G Holt. Division of Cell Biology, The Western Australian Research Institute for Child Health, Perth, Australia 6008.

The natural immune response to inhaled soluble protein antigens was originally described as a form of immunological tolerance, by analogy with the familiar process of Oral Tolerance to fed antigen. Sensitivity to tolerogenesis via the respiratory tract was shown to be genetically determined (high vs low IgE responders) and was variably selective for the IgE antibody isotype. Thus the tolerant state is more accurately described as Immune Deviation. This state of antigen-specific IgE-hyporesponsiveness can be adoptively transferred by MHC-class I-restricted CD8$^+$ T cells which in the rat express the surface phenotype CD3$^+$, CD4$^-$, CD5$^+$, CD8$^+$ & TcR α$^-$/β$^-$. Kinetic studies on *in vitro* T cell reactivity in aerosol-exposed animals demonstrated biphasic CD4$^+$ Th2 responses which terminated, together with IgE antibody production and coincident with the appearance of MHC class I-restricted OVA-specific INF-γ-producing CD8$^+$ T cells. However, the latter were not autonomous *in vitro* and required a source of exogenous IL2 for initial activation, which could be provided by OVA-specific CD4$^+$ T cells. It is suggested that this form of acquired immune deviation represents a novel control mechanism for protection against development of CD4$^+$-Th2-mediated allergic reactivity to inhaled non-replicating antigens.

V 783 SPLIT TOLERANCE AFTER FEEDING OF HEL TO MICE. Marco Melo, Alex Miller and Eli Sercarz. Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024-1489

In the present study, we administered hen eggwhite lysozyme (HEL) to C3H.HeJ and BALB/c mice by oral feeding. Feeding HEL 7 to 14 days prior to footpad immunization with HEL-CFA suppressed the lymph node T cell proliferative response to HEL about 80%. On the other hand the humoral response in these same animals was not abrogated. IgG1 was the main isotype produced in both groups of animals, and feeding by a single intubation (gavage) with 1 mg of HEL without parenteral immunization) was not enough to produce an antibody response as measured by serum ELISA (total Ig) up to 20 days after feeding. In a second set of experiments the levels of IgG1anti-HEL Ab were significantly increased in the HEL-fed group, 7 days after immunization (either with HEL-CFA or HEL-IFA), but after 11 days there was no significant difference between fed and non-fed groups. In other experiments, two different protocols were followed: **1**)feeding mice by gavage (1 mg per gavage) on days zero, 7 and 20; or **2**) feeding mice for 15 consecutive days either by gavage or by adding HEL to their drinking water (approximately 0.5 mg HEL ingested per day). The lymph node T cell proliferative response of these animals after immunization with HEL-CFA was as before reduced about 80%. In contrast, animals given repeated doses of HEL by gavage or in their drinking water and without any parenteral immunization produced a significant amount of IgG1 Ab.

In the presumed Th1 population, studies performed with peptide-induced oral tolerance in the HEL system have been designed to ask whether dominant determinants induce peripheral anergy or suppression, and these results will be presented.

V 784 APOPTOSIS OF ANERGIC CD4 T CELLS AND THE DOWN REGULATION OF *bcl-2* IN BM5-INDUCED MURINE AIDS. Girija Muralidhar, Elizabeth Broome, Michael Yen and Susan L. Swain, University of California, San Diego, La Jolla, CA, 92093.

The BM5 mixture of replication competent and defective retroviruses causes a dramatic and fatal immunodeficiency disease (MAIDS) in susceptible strains of mice. The disease is characterized by lymphadenopathy and splenomegaly including an early increase in CD4 T cell number, but loss of CD4 T cell function. CD4 T cells from BM5 infected mice fail to proliferate or synthesize cytokines in response to Con A, anti-CD3 with or without PMA, superantigens and antigens *in vitro*. Instead, Anergic CD4 T cells undergo programmed cell death upon *in vitro* restimulation. When CD4 T cells from MAIDS mice encounter TCR mediated signals, apoptosis is induced as early as 4 hrs post stimulation. Within 18 hrs of *in vitro* restimulation, a majority of CD4 T cells from BM5 infected mice undergo programmed cell death in response to Con A and anti-CD3.

To further understand the regulation of apoptotic pathway(s) of anergic CD4 T cells, as opposed to cytokine synthesis pathway(s) of control CD4 T cells, we evaluated the expression of *bcl-2* among the CD4 T cells of uninfected and BM5 infected mice. At 12 weeks of infection, the expression of *bcl-2* was lower among CD4 T cells of BM5 infected mice and further decreased once CD4 T cells were restimulated *in vitro*.

These results suggest that the progression of CD4 T cells to anergic state involves the turn on of a program of activation induced cell death as well as uncoupling of pathways leading to cytokine synthesis.

V 785 **βINTERFERON ENHANCES ORAL TOLERANCE TO MYELIN PROTEINS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS,** Patricia A. Nelson, Ahmad Al-Sabbagh, and Howard L. Weiner*, AutoImmune Inc., Lexington, MA and the *Center for Neurologic Disease, Brigham and Women's Hospital, Boston, MA

Oral administration of myelin antigens has been shown to reduce the incidence and severity of experimental autoimmune encephalomyelitis (EAE) in rats and mice and to decrease the frequency of MBP-reactive cells and the incidence of attacks in some patients with multiple sclerosis. Oral tolerance has been shown to be mediated by Th_2 type regulatory cells that secrete TGF_β and IL4/IL10. Adjuvants and cytokines may modulate oral tolerance. Thus, LPS enhances oral tolerance and γIFN has been shown to abrogate oral tolerance. The addition of βIFN to the experimental regimen in the animal models, either injected or orally, enhances the suppressive effects of oral myelin basic protein (MBP) and proteolipid protein (PLP). Myelin antigens were fed in amounts sub optimal to suppress EAE. SJL mice were fed three times with 0.25 mg of MBP or PLP with and without concomitant IP (69,000 units) or oral (5000 units) of βIFN and immunized with bovine PLP in complete Freund's adjuvant on days 0 & 7. Lewis rats were fed twice with 1 mg MBP with or without concomitant IP (150,000 units α/β IFN). In animals either fed or injected with interferon, there was a decrease in disease severity as compared to animals fed MBP alone or given interferon alone. In a representative experiment in Lewis rats, data for fed animals follow (Disease Scale 0-5):

Mean Maximum Disease Score

PBS	α/β IFN	MBP	α/β IFN + MBP	mock IFN	mock IFN + MBP
3.0	2.4	2.0	0.6	2.6	2.4

These studies are further enhanced by the in vitro production of cytokines by lymphocytes from fed animals in response to specific antigen in culture, showing that the synergistic effect may be related to enhanced production of TGFβ and IL4/IL10. βIFN thus acts as a synergist to enhance oral tolerance to EAE. These observations have important implications in view of the use of βIFN for the treatment of early relapsing remitting multiple sclerosis and the potential combined use of these immunomodulatory therapies to treat MS.

V 786 LOSS OF IL-12 RESPONSIVENESS IN ANERGIC T LYMPHOCYTES. **Helen Quill, Avinash Bhandoola, Giorgio Trinchieri, Joyce Haluskey and David Peritt.** Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, and the Wistar Institute, Philadelphia, PA 19104

The cytokine, IL-12, stimulates proliferation and IFN_γ production in activated T cells, and plays an important role in the preferential development of Th1-type responses. Furthermore, murine Th1, but not Th2 clones proliferate in response to IL-12 following activation. We considered the possibility that production of IL-12 during infection *in vivo* might be a mechanism to reverse tolerance and promote autoimmunity. Although tolerant T cells, anergic for IL-2 production, cannot undergo autocrine-mediated proliferation, they may still be subject to IL-12-mediated expansion. This question was first addressed using an *in vitro* system to induce anergy. Anergic murine Th1 clones were then restimulated with Ag:APC and tested for responses to murine rIL-12. In comparison with control cells, anergic Th1 cells were highly resistant for proliferation to IL-12, demonstrating a novel defect in anergic Th1 clones. To extend these findings to T cells tolerized *in vivo*, anergy was induced in peripheral $CD4^+$ cells of mls-1^b, Vβ8.1 TCR transgenic mice by the i.v. injection of mls-1^a spleen cells. T cells isolated at two weeks post-injection were anergic for IL-2 production when restimulated with mls-1^a *in vitro*, as expected, and were also found to be resistant for proliferation in response to IL-12, as seen with the Th1 clones. Thus, anergy induction *in vitro* or *in vivo* prevents both autocrine-mediated proliferation and proliferation to the cytokine, IL-12, suggesting that tolerance is maintained in $CD4^+$ cells by severely restricting clonal expansion *in vivo*, even when IL-12 is present.

V 787 PERIPHERAL TOLERANCE IN AUTOIMMUNE PRONE MICE, Cynthia F. Rubio, David Nemazee, University of Colorado Health Sciences Center, 4200 E. 9th Ave, Denver, Co, 80220 National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver Co 80206

Systemic Lupus Erythematosus (SLE) is a protoytypic immune complex, or antibody mediated disease with multiple autoantibodies to nuclear antigens. Since there is clearly a defect in tolerance to self antigens in patients and murine models of SLE we have examined genetic crosses between immunoglobulin transgenic murine lines and autoimmune prone mice for evidence of defects in central or peripheral deletion in the presence of membrane bound antigen. Using mice transgenic for IgM and IgD anti-MHC class I genes (3-83 transgenic line) which recognizes H-2K molecules of all allelic form tested except for d and f, and extensively backcrossing these mice to autoimmune prone MRL/lpr (H-2Kk/k) and NZB/W f1 (H-2Kd/z) we found complete bone marrow deletion of autoreactive transgenic B lymphocytes similar to immunologically normal mice. We have now extended our observations examining genetic crosses between mice which contain the same immunoglobulin transgene as above and a transgene for the MHC class I molecle H-Kb under control of the metallothionein promoter, which exhibits expression primarily in the liver (double transgenics), and autoimmune prone mice which do not express the antigen, (MRL/lpr (H-2Kd/d) and NZB (H-2Kd/d)), to reveal any defects in peripheral tolerance . Our initial results are again similar to immunologically normal mice in which a large population of immunoglobulin transgenic B lymphocytes are present in the bone marrow, a smaller percentage in the spleen and a complete absence of transgenic B lymphocytes in the lymph nodes of these animals. These data suggest that when a B lymphocyte encounters membrane antigen in the periphery there is clonal elimination of self-reactive B lymphocytes even in lupus-prone mice. We are currently examining more extensive backcrosses of our "double transgenics" and autoimmune prone mice, as well as examining the mechanisms of peripheral tolerance.

V 788 SUPPRESSION OF NORMAL ACTIVATION RESPONSES IN SPLENOCYTES FROM MICE BEARING ASCITIC TUMORS. Melanie Ruzek and Ambika Mathur. Departments of Microbiology, Oral Science and Pediatrics, University of Minnesota, Minneapolis, MN 55455.

We have examined the expression of various cell surface markers and cytokine production from splenocytes of mice bearing i.p. ascitic B cell hybridomas compared to normal littermate controls. We find significant loss of expression of the IgE Fc receptor (CD23) on B cells from tumor-bearing mice. In mice bearing IgE secreting tumors, however, this decrease is not observed to the same extent, which confirms that the presence of IgE may stabilize the CD23 molecule on B cells (Lee, W.T. et al, J. Immunol, 1987). Upon *in vitro* stimulation with ConA, splenocytes from mice with tumors display a much smaller increase in IL-2 receptor expression compared to controls. *In vitro* stimulation of the tumor-bearing mouse splenocytes with ConA results in significantly decreased production of IL-2 and IFNγ compared to normal mice. In contrast, IL-4 production does not appear to differ, which suggests that the tumors may suppress general Th1-like responses, but do not affect Th2-like responses. Preliminary results have also demonstrated that the decreases in cytokine production are apparent within four days following tumor injection, even though the mice do not succumb to the tumor until eight to eleven days following tumor injection. CD23 expression on B cells , however, is not affected at early time points, but is only observed at later stages of tumor growth. These results suggest that ascitic B cell tumors interact with the host immune system in a manner that renders splenocytes unresponsive to normal activation signals.

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V 789 IMMUNOGENICITY OF SELF PEPTIDES NATURALLY PRESENTED BY MHC CLASS II MOLECULES Bhagirath Singh, Beverley Rider, Qiang Yu and Ester Fraga, Department of Microbiology & Immunology, London, Ontario and Department of Immunology, University of Alberta, Edmonton, Alberta, CANADA

It has recently been shown that peptides eluted from MHC Class II molecules are predominantly fragments of self MHC and non-MHC proteins. These naturally processed self peptides are expected to be present in ontogeny. Therefore immune responses to these peptides in syngeneic host may be of physiological importance in modulating self-reactivity particularly in autoimmune diseases. According to our current understanding T cells reactive to such self antigens are expected to be deleted or clonally anergized in ontogeny. We have therefore investigated immunogenicity of a number of self peptides in mice that express MHC Class II molecules from which these peptides were eluted. Mice were immunized with appropriate synthetic peptides. Antibody and T cell proliferative responses were measured. We were surprised to find that many of these self MHC and non-MHC peptides were highly immunogenic in normal mice. T cells from such mice were restricted to syngeneic MHC Class II and were blocked by anti-CD4 antibody. Antibody response was detected to these peptides only at a high antigen dose. T cells reactive to these self peptides generally produce IFN-γ but not IL-4 therefore suggesting that are of TH_1 phenotype. Despite the presence of such cells in the peptide primed mice, we could not detect any response to these peptides in T cells derived from unprimed normal mice. These result suggest that potential auto-reactive T cells are not clonally deleted but tolerance to auto-antigens is maintained by other mechanisms *in vivo*.

V 790 FUNCTIONAL SPLIT TOLERANCE AMONG Th1 CELLS AS A CONSEQUENCE OF NEONATAL PEPTIDE TREATMENT IN NORMAL AND AUTOIMMUNE MICE, Ram Raj Singh, Eli E. Sercarz, Bevra H. Hahn, Departments of Medicine/Rheumatology, and Microbiology and Molecular Genetics, UCLA, Los Angeles, CA 90024.

Simultaneous study of the T-cell proliferative (Tp) and T-helper (Th) responses to peptides in neonatal tolerance has not been reported. To address this, and its possible implications in antibody-mediated autoimmunity, we induced tolerance to a foreign (HEL 106-116) and a self Ig-derived peptide (A6H 58-69) in a lupus-prone autoimmune strain (NZBxNZW F1 [BWF1]), in H-2-matched normal mice (BALB/cxNZW F1) as well as normal BALB/c mice. We demonstrate that administration of peptides in a "tolerogenic" regimen itself induces strong IgG peptide-specific antibody response. Neonatally tolerized mice of all 3 strains, on subsequent immunization displayed a peptide-specific Tp unresponsiveness in vitro, but an increase in serum peptide-reactive IgG antibodies. Intriguingly, tolerance in Th1 cells (defined by decreased Tp and IL-2 and IFN-γ production) did not correlate with the in vivo anti-peptide antibody response of Th1-dependent IgG isotypes: IgG2a, IgG2b and IgG3 peptide-specific antibodies were markedly elevated. Resistance of Th2 cells to tolerance was also evident; IL-4 and IL-5 production were increased or unaffected, and IgG1 anti-peptide antibodies were made. Thus, we suggest that in neonates, the peptide tolerogen itself induces Th responses in vivo. Th1 cells undergo a form of functional split tolerance, in which Tp and cytokine production are suppressed, but Th function for IgG antibody responses is primed.

Autoimmune BWF1 mice do not appear to have a defect in neonatal tolerance induction to either foreign or self-peptides. BWF1 mice neonatally tolerized with A6H 58-69 had increased IgG anti-dsDNA production and an exacerbation of lupus nephritis. Thus, antibody-mediated autoimmunity in lupus-susceptible strains may actually reflect a split tolerance to autoantigenic determinants of pathogenic relevance.

V 791 INDUCTION AND CHARACTERIZATION OF ANERGY IN T CELLS OF THE TH2 PHENOTYPE, Joanne Sloan-Lancaster and Paul M. Allen, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

In this report we demonstrate clonal anergy in Th2 cells as a consequence of incomplete T cell activation by two different methods. 2.102, a Th2 clone, recognizes the murine hemoglobin β chain peptide, Hbβ(64-76), in the context of I-E^k. Presentation of this peptide by chemically fixed spleen cell APC does not stimulate clonal expansion of 2.102, while activating production of the cytokines IL-4 and IL-3. More interesting, and in contrast to the observations of others, this interaction causes a profound inability to proliferate upon restimulation of the Th2 cells with antigen and functional APC. This unresponsive state is long-lasting, tested up to 13 days after the initial stimulation with fixed APC and antigen. In contrast, T cells which had been preincubated with untreated spleen cells and antigen proliferated as well as freshly isolated T cells at this timepoint. The induction of this Th2 cell anergy can be inhibited by cyclosporine A, implicating involvement of a calcineurin-dependent pathway. However, addition of exogenous IL-1, IL-2 or IL-4 with peptide and fixed APC do not prevent 2.102 from subsequently becoming anergic. We further demonstrate that stimulation of the 2.102 cells with live APC and altered peptide ligand, which activates cytokine production but not proliferation, also results in an anergic phenotype upon restimulation with the immunogenic peptide. This extends our earlier observations with Th1 clones to also include Th2 clones. In summary, we demonstrate that Th2 clones can be made anergic in vitro by prior stimulation with immunogenic peptide and chemically fixed APC. Further, these Th2 cells can also be induced into this unresponsive state by partial activation with altered peptide ligand and live APC.

V 792 **POST-BONE MARROW TRANSPLANT T CELLS: ACTIVATED BUT RENDERED ANERGIC? Jan Storek, Robert P. Witherspoon, and Rainer Storb, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.**

Immunodeficiency secondary to B and T cell defects is a major problem in long-term bone marrow transplant (BMT) survivors. To better understand the mechanism(s) of T cell deficiency we assessed the phenotype of circulating T cells in 10 patients at 1 year and 4 patients at 5 years post-BMT and in 8 adult and 7 cord blood controls by 3-color flow cytometry. (See the following table for the results.)

		Normal Adults	Cord Blood	1 yr post-BMT	5 yr post-BMT
CD4$^+$ T cells	%CD11a^{high}	26±9*	7±6	54±25	29±13
	%CD29^{high}	52±6	13±6	79±13	56±15
	%CD28$^+$	98±2	100±0	71±31	93±10
CD8$^+$ T cells	%CD11a^{high}	40±11	12±8	85±14	44±32
	%CD29^{high}	44±8	9±4	83±15	66±4
	%CD28$^+$	80±11	95±2	32±19	68±20

* Mean ± 1 Standard Deviation

The abundance of T cells expressing high levels of activation antigens (CD11a, CD29) with the diminished occurrence of CD28$^+$ T cells and the historical data showing that post-BMT T cells do not readily repond to antigens and polyclonal stimuli suggest the T cells had been activated but subsequently rendered anergic. (CD28 is downregulated on T cells rendered anergic). Cord blood T cells expressed only low levels of activation antigens probably because, in contrast to the post-BMT patients, fetuses are not exposed to multiple infectious agents.

V 793 INDUCTION OF NONRESPONSIVENESS IN FRESHLY PREPARED T CELLS. Sarah E. Townsend and James P. Allison. Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley CA 94720.

Previous work suggests that manipulation of T cell receptor and costimulatory signals can induce a state of nonresponsiveness in T cell clones, which have been subjected to extensive *in vitro* selection. In order to study the relevance of this *in vitro* observation to T cell function *in vivo*, we have developed systems to study the induction of nonresponsiveness in freshly prepared T cells. Using mice transgenic for an MHC Class I-restricted TcR (F5) and mice transgenic for an MHC class II-restricted TcR (AND) as sources of cells, we have used defined antigen presenting cells and specific blocking reagents to study the role of the CD28/B7 interaction in primary responses *in vitro*, as well as in secondary and tertiary restimulations. In some experiments, after primary stimulation under defined conditions *in vitro*, cells were parked in athymic nude mice before testing responsiveness to secondary stimulation. Preliminary results suggest that non-responsiveness cannot be induced in naive MHC class II-restricted T cells, but may be induced in MHC class I-restricted T cells under some conditions. Non-responsiveness may be inducible in primed cells, but the requirements for this induction are substantially different from the requirements for the induction of non-responsiveness in T cell clones. The mechanism of tolerance induction *in vivo* is also being studied by injecting tolerizing doses of peptide +/- anti-CD28 antibody into these transgenic mice, or into mice in which smaller numbers of transgenic T cells had been parked.

V 794 IDENTIFICATION OF A CARBOHYDRATE EPITOPE EXPRESSED ON A LIMITED SUBSET OF ACTIVATED REGULATORY MURINE T SUPPRESSOR CELLS, David R. Webb, Wayne Godfrey#, John Magnani, Satoshi Fukuse, Bruce H. Devens, Gloria Semenuk, Edgar Engleman#, Carl W. Pierce*, and Judith A. Kapp**, Syntex Discovery Research, Palo Alto, CA 94304, # Department of Medicine, Stanford University School of Medicine, Stanford CA 94305, *Harrington Cancer Center, Amarillo, TX 79106, **Department of Pathology, Emory University, Atlanta, GA 30322

The monoclonal antibody 984D4.6 (mcAb 984) is capable of recognizing a very limited subset of murine T-cells. It has been determined that mcAb 984 will bind to T-cell lines of the antigen-specific suppressor cell type, selected T-cell leukemias, and WEHI-3. It will not bind to T-helper cell clones, B-cell lines, cytolytic T-cell clones, or monocytic cell lines. Studies demonstrated that the epitope recognized by mcAb 984 contained sialic acid (neuriminidase sensitive) and protein (pronase sensitive). Further studies showed that the 984 epitope was variably sensitive to several inhibitors of glycosylation and/or protein synthesis, sensitive to periodate oxidation, and stable to heat, leading to the conclusion that it consisted of a carbohydrate linked to a polypeptide. It was determined that the only carbohydrate structure recognized by mcAb 984 was NeuNAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc also known as LS Tetrasaccharide-C, originally identified in human human milk. Analysis of structures related to LST-C indicates that mcAB 984 recognizes NeuNAcα2-6Galβ1-4GlcNAc. We term this carbohydrate, 984. By differential extraction using the detergent, CHAPS and SDS-PAGE and Western blotting it was determined that the cell surface protein that bears the 984 epitope is 200 kD. Differences in the sensitivity to a variety of enzymes and inhibitors of glycosylation and/or protein synthesis indicate that the 200 kD protein is not CD45, Class I or CD43. The nature of the 200 kD protein remains to be determined. The expression of 984 in the mouse is extremely limited and it is therefore a useful reagent for detecting a unique subpopulation of activated regulatory murine T-suppressor cells and can clearly differentiate these cells from T helper cells and cytotoxic T lymphocytes.

T Cell Development

V 795 HLA-DR AND H-2E TRANSGENES DIFFERENTIALLY MEDIATE T CELL RECEPTOR-SPECIFIC POSITIVE SELECTION

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The use of HLA transgenic mice in models of immunity and disease assumes that human MHC molecules are able to contribute toward the positive selection of the mouse T cell receptor repertoire. As an initial step towards analysis of this question we compared the relative ability of DRα/Eβ or Eα/Eβ complexes to induce T cell receptor (TcR) positive selection in H-2*Ea* and HLA-*DRA* transgenic mice lacking endogenous Eα. We and others have previously reported Mtv-mediated negative selection in single chain HLA class II transgenic mice expressing hybrid mouse/human class II heterodimers. In these studies the mixed heterodimers largely mimic *bona fide* H-2E pairs. This lack of fastidiousness is perhaps not surprising when one considers that superantigen binding shows little MHC class II allele preference in the mouse and appears not to involve the peptide-binding groove. By this token one might predict that positive selection, which is thought to involve presentation of peptide and where polymorphic residues in the peptide binding groove are consequently likely to be more critical, would be less faithfully reproduced in HLA class II transgenic mice. The results show that, like Eα/Eβ, hybrid DRα/Eβ complexes can mediate positive selection of $V\beta2^+$, $V\beta6^+$, and $V\beta10^+$ cells. However differences were found between the effects of the two transgenes. Thus, while $V\beta6^+$ cells were efficiently selected in both H-2*Ea* and *DRA* transgenic mice, positive selection of $V\beta10^+$ cells was less apparent in the *DRA* transgenic mice. Variation between *Ea* and *DRA* transgenic mice is consistent with the notion that this process is dependent on differential binding of endogenous peptides to the Eα/Eβ and DRα/Eβ complexes. Furthermore, contrary to expectations, in neither set of mice was positive selection limited solely to the $CD4^+$ subset. Thus, examples were found in which Vβ specific positive selection was confined to either the $CD4^+$ or $CD8^+$ subsets, and others in which both subpopulations were concomitantly increased. In the case of Vβ2 positive selection, H-2*Ea* transgenic mice showed expansion of these cells in both $CD4^+$ and $CD8^+$ subpopulations while in *DRA* transgenic mice this occurred predominantly in the $CD8^+$ subpopulation.

V 796 TRANSCRIPTION OF TCR GENES IN ABSENCE OF REARRANGEMENT, Serge Candéias and Uwe D. Staerz, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

The T cell precursor differentiation is a highly regulated phenomenon that can be monitored by the appearence of numerous cell surface molecules, among which is the antigenic T cell receptor (TCR). However, before being expressed, the TCR genes have to be rearranged by a site-specific recombinase, in order to bring together the different segments participating in the formation of the exons coding for the TCR α and β chains variable regions. This gene assembly is itself highly regulated, both temporally and in a tissue-specific manner. Different experiments have suggested that transcription of unrearranged genes increases their likelihood to be rearranged.

To determine whether such a relation between transcription and rearrangement exists *in vivo* during the development of T cell precursors, we studied by PCR the germline transcription of different TCR gene segments during the murine ontogeny. The results presented here show that the germline transcription of TCR Vβ genes is restricted to cells of the T lymphocyte lineage. It is an early event during the T cell development, as it happens in the fetal thymus prior to the TCR β genes rearrangement. It is also transiently detected in the fetal liver, indicating that it may be one of the earliest events of T cell precursors differentiation.

V 797 CORECEPTORS AND POSITIVE SELECTION,

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Immature T cells face a crucial decision of whether to become a CD4 helper or CD8 cytotoxic lymphocyte. The mechanisms involved in implementing this decision remain unclear. Two models have been elaborated: one based on instruction, the other on selection. Our studies of MHC class I-, class II-, and double-deficient mice provided data supporting the selective model. There exists a significant population of $CD4^+CD8^{lo}TCR^{hi}$ cells in class II-deficient mice that is intermediate in maturity between $CD4^+CD8^+$ and end-stage $CD4^+CD8^-$ thymocytes and is selected on class I molecules. These findings were consistent with a selective model that entails two TCR-MHC molecule engagements: the first provokes a stochastic down-modulation of either CD4 or CD8 and a degree of differentiation; the second, requiring participation of the appropriate coreceptor, permits end-stage differentiation. It is now evident that both engagements require the appropriate coreceptor. Analyses of class II and CD8 double-deficient mice reveal that the generation of the $CD4^+CD8^{lo}$ intermediate population is obstructed in the absence of CD8. If this intermediate population is forced to express CD8 (using transgene-encoded CD8α and β chains), end-stage differentiation occurs and mature $CD4^+(CD8tg^+)$ cells emerge into the periphery. The ability of these peripheral $CD4^+(CD8tg^+)$ cells to respond to class I-restricted antigens and their helper versus effector phenotype will be discussed.

V 798 EXAMINATION OF THE RELATIONSHIP BETWEEN POSITIVE SELECTION AND AND DIFFERENTIATION DURING THYMOCYTE DEVELOPMENT, Craig B. Davis and Dan R. Littman, Department of Microbiology and Immunology, UCSF, San Francisco, CA 94143-0414.

The antigen specificity and function of T cells expressing the αβ- antigen receptor (TCR-αβ) can be generally assigned based on the expression of the coreceptors CD4 and CD8. $CD4^+$ T cells recognize peptide in the context of class II MHC molecules and generally display the helper phenotype, whereas $CD8^+$ T cells recognize peptide plus class I MHC molecules and display a cytotoxic phenotype. In order to understand how peptide/MHC specificity is linked to T cell function, we are examining the properties of T cells isolated from transgenic mice in which class II - specific thymocytes can differentiate into either $CD4^+$ or $CD8^+$ mature T cells. The phenotype of these cells after activation by various combinations of peptide and MHC or by crosslinking of the TCR with CD4 or CD8 will be presented.

V 799 EXAMINATION OF LINEAGE COMMITMENT DURING POSITIVE SELECTION

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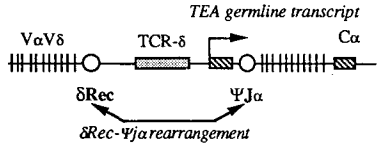
Experiments in transgenic mice have demonstrated that thymocyte differentiation to the $CD4^+$ helper or $CD8^+$ cytotoxic lineages is ultimately dependent upon the specificity of the TCR for class II or class I MHC molecules, respectively. There are currently two models proposed to explain the mechanism of this lineage committment. The instructive model proposes the double positive thymocyte, at the moment of TCR engagement with MHC molecules in the thymus, receives a signal that instructs the cell to abrogate expression of either the CD4 or CD8 coreceptor. Alternatively, the stochastic/selection model contends that downregulation of either CD4 or CD8 occurs randomly; independent of TCR specificity. Using the phenotypically immature $CD4^+8^+$ T cell line DPK(1), we have established an *in vitro* model of positive selection. This cell line, derived from a class II restricted TCR transgenic mouse, differentiates into $CD4^+8^-$ cells in response to a peptide derived from pigeon cytochrome c and $I\text{-}E^k$ MHC molecules. In order to further understand the basis of lineage committment, we have transfected a variant of this cell line that lacks expression of the transgene- encoded α chain, with a Vα2/ Vβ5.2 TCR specific for an ovalbumin peptide and the K^b class I MHC molecule. Initial results suggest that upon activation by ovalbumin and K^b-bearing antigen presenting cells the transfectants differentiate into $CD4^+8^-$ single positive cells and not $CD8^+4^-$ cells. Since DPK cells can differentiate in the thymus in the absence of antigen, we are currently testing the response of the transfectant *in vivo* . These results would appear to contradict either an instructive model or a strict stochastic/selection model. Rather, we suggest thymocytes have already determined their lineage fate at some time prior to TCR dependent selection .

1. Kaye and Ellenberger, 1992, Cell 71:423-435.

V 800 MULTIPLE DNA - BINDING SITES ARE INVOLVED IN THE REGULATION OF THE TCR-Jα (TEA) GERMLINE TRANSCRIPT.

Jean-Pierre de Villartay, Régina de Chasseval, and Christophe Klein. INSERM U132 Hospital Necker, Paris, France.

The TCR-α and TCR-δ loci are located on the same chromosome, but are independently rearranged and expressed during T cell ontogeny. The δRec-ΨJα rearrangement, which is preceded by the germline transcription of the ΨJα region (TEA transcript), leads to the site-specific deletion of the TCR-δ locus.



The TEA transcript may be responsible for the opening of the chromatin in the Jα cluster. We analyzed the regulation of the TEA transcript as a model for the regulation of DNA accessibility in this region. Several motifs are present in the TEA promoter which are conserved between man and mouse. These include TCF1, Ets1, and GATA3 putative binding sites as well as other yet non-discribed sequences. Gel-shift experiments using nuclear extracts from the TEA-expressing pre-T cell P30 demonstrated the interaction of DNA-binding proteins to these sites. The use of recombinant proteins demonstrated the binding of TCF1 (and Sox4) and GATA3 to their cognate target but the absence of binding of either Ets1 or Elf1. Functional analysis of the TEA promoter showed a tissue-specificity for the α/β-expressing Jurkat T cell and not for the γ/δ-expressing Peer T cell line. Introduction of mutations within the DNA-binding sites defined several positive regulatory elements needed for promoter activity. Altogether these results suggest that the TEA upstream sequences could participate in the regulation of the Jα-cluster accessibility to the recombinase machinery. One means could be the binding of one or several regulatory proteins (transcription factors), leading to the TEA transcription.

V 801 T-CELL RECEPTOR β-CHAIN REARRANGEMENT IN IMMATURE THYMOCYTE SUBSETS , Erastus C. Dudley*, Howard Petrie^, and Adrian Hayday*, *Department of Biology, Yale University, New Haven, CT, 06511 and ^Memorial Sloan-Kettering Cancer Center, New York, NY, 10021

Observations that the majority of TcR β-chains are productively rearranged in TcR α-/- mice and also in double negative and double positive thymocytes of CD3 ζ -/- mice indicate a critical role for β in development. Questions of interest include when is β rearranged? and when is productively rearranged β needed? Using an approach that we have recently developed, we have initiated an analysis of DN subsets, using CD44 and CD25 to distinguish stages of development. Analysis of the rearrangement status of β was conducted for several populations. Data demonstrating the stages of rearrangement and requirement for productively rearranged β will be presented, as will a model for the developmental role of β.

V 802 DIFFERENTIAL EFFECTS OF A POINT MUTATION IN THE α3 DOMAIN OF THE D^b MOLECULE ON POSITIVE SELECTION AND PEPTIDE BINDING. Jan P. Dutz, Soo-Jeet.Teh, Hung-Sia Teh, UBC Department of Microbiology and Immunology, Vancouver, BC, Canada.

A point mutation in the α3 domain of the D^b class I major histocompatibility (MHC) molecule (227 asp ↔ lys) is known to abrogate CD8 binding. Using mice doubly transgenic for the HY-T cell receptor (TCR) and for a mutated D^b molecule (D^b lys), we have previously shown that coordinate binding of the HY-TCR and CD8 to the same MHC molecule bearing a positive selecting ligand is required for the efficient positive selection of this TCR. Recently, a model of positive selection has been proposed whereby $CD4^+CD8^+$ thymocytes are induced to express high levels of TCR through a TCR-MHC interaction that is followed by a stochastic down-regulation of either CD4 or CD8, and the rescue of only thymocytes with appropriately matched TCRs and coreceptors. Using the doubly transgenic mice for the HY-TCR and D^b lys or D^b wild type (wt) on an $H2^d$ background, we now show that TCR^{hi} expression in $CD4^+CD8^+$ HY-TCR^+ thymocytes is induced only in mice expressing a D^b molecule which allows a coordinate TCR and CD8 interaction with the MHC (D^b wt). Thus, for the HY-TCR, the CD8 coreceptor is required for the initial TCR upregulation in $CD4^+CD8^+$ thymocytes. We further show that the mutation in the D^b molecule which eliminates CD8 binding is unlikely to alter the ability of this MHC to bind antigenic peptides as acid extracts from male spleen cells and affinity-purified MHC molecules of both the wild type D^b and the D^b lys but not $H2^d$ contain peptidic material recognised by a male-specific cytotoxic T cell clone (PM-1) when added to RMAS cells.

V 803 OVEREXPRESSION OF ACTIVATED p56^{lck} IMPAIRS POSITIVE BUT NOT NEGATIVE SELECTION OF CD4^{-}8^{+} THYMOCYTES, Per-Olof Ericsson and Hung-Sia Teh, Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada V6T 1Z3

Previous studies in our laboratory have shown that the positive and negative selection of CD4^{-}8^{+} thymocytes expressing a TCR specific for the male (H-Y) antigen presented by the H-2D^{b} class I molecule is influenced by the amount of the protein tyrosine kinase, p56^{lck}, that is associated with the CD8 coreceptor molecule. To further evaluate the effect of p56^{lck} on the positive and negative selection of CD4^{-}8^{+} T cells we mated transgenic mice that express this TCR with transgenic mice that constitutively overexpress an activated form of p56^{lck} (Y505 → F505; Abraham et al, J. Exp. Med. 173, 1421, 1991). In doubly transgenic female mice, positive selection of the transgenic H-Y TCR was impaired. Impairment of positive selection was associated with a seven-fold decrease in the H-Y TCR level in doubly transgenic mice. However, the level of the transgenic TCR expressed by CD4^{+}8^{+} doubly transgenic mice was still 2 to 3-fold higher than that of a normal H-2^{b} mouse. Thymocytes from doubly transgenic mice were also less efficient in mobilizing intracellular calcium following TCR cross-linking when compared to single TCR transgenic mice. The reduction in TCR levels was also observed in doubly transgenic mice of the H-2^{d} haplotype, thus indicating that this effect of the *lck* transgene was independent of TCR-ligand interactions. However, negative selection still occurs efficiently in doubly transgenic male H-2^{b} mice. We also found that the expression of TCR can be partially restored by culturing doubly transgenic thymocytes with a PTK inhibitor, Herbimycin A. The level of the heat stable antigen on thymocytes was unaffected by treatment with Herbimycin A.

V 804 ROLE OF ENHANCER SEQUENCES IN THE CONTROL OF T CELL RECEPTOR α/β GENE REARRANGEMENTS, Pierre D. Ferrier, Myriam Capone, Françoise Watrin, Corinne Fernex, Branka Horvat, and Gaëlle Bouvier, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, case 906, Marseille, 13288, France.

Recombination within T-cell receptor (TCR) variable region genes (V, D and J) is involved in the generation of the receptor diversity during T-cell differentiation. We have studied the role of TCRα (Eα) and TCRβ (Eβ) trancriptional enhancer elements in the regulation of V(D)J recombination. Lines of transgenic mice were produced containing a TCRβ gene mini-locus comprised of germ line V, D, and J gene segments associated to either Eα or Eβ. Analysis of transgene rearrangements in these mice demonstrated that rearrangements of Eβ- versus Eα-containing transgenes began at different stages of T cell differentiation in embryonic and adult thymus, with a pattern superimposable onto TCRβ or TCRα gene expression, respectively. These results strongly suggested that sequences within Eβ and Eα are involved in the control of the early rearrangement events at the TCRβ and α gene loci and are responsible for the ordered expression of the TCRβ and α genes during β/α T cell differentiation. In addition, Eα- and Eβ-containing transgenic miniloci seem to be susceptible to the control mechanisms active for allelic exclusion in T cells. To further verify the role of enhancers in V(D)J recombination, homologous recombination technology has been applied to specifically mutate these sequences within the endogenous loci. Analysis of mice deprived of TCR β/α gene enhancers will additionally test the importance of these regulatory elements in the control of V(D)J recombination and T-cell differentiation.

V 805 The TCR+CD4-CD8- Thymocytes In MBP-Specific TCR-Transgenic Mice Are Not Susceptible To Clonal Deletion But Respond In A Dose-Dependent Manner To MBP, Joan Goverman, Stephen Gomez and Ramlah Ringold, Department of Molecular Biotech., Univ. Of Washington School of Medicine, Seattle, WA 98195.

We have analyzed the T-cell subset populations in thymuses of transgenic mice expressing rearranged genes encoding a TCR specific for myelin basic protein. The levels of expression of CD4 and CD8 on these thymocytes indicate preferential maturation of CD4+ thymocytes, a pattern indicative of positive selection. Most thymocytes express high levels of TCR. We used both fetal thymic organ culture and thymocyte suspension culture of the transgenic thymocytes to examine the susceptibility of these cells to clonal deletion following exposure to MBP peptide in vitro. As expected, the CD4+CD8+ population decreased with increasing concentration of antigen, indicating that clonal deletion occurs at this stage of development. In contrast, the CD4+CD8- thymocytes appeared to be stimulated by antigen to proliferate. The number of T cell blasts in this population and the expression the early activation marker, CD69, increased with antigen concentration. The CD4-CD8- population in these transgenic mice is elevated with respect to nontransgenic animals and most of these CD4-CD8- thymocytes express the transgenic TCR. Nevertheless, the CD4-CD8- thymocytes were not susceptible to deletion, as has been previously observed in other transgenic systems. Interestingly, this population also appeared to be stimulated by antigen in a dose-dependent fashion analogous to the CD4+CD8- thymocytes. A significant blasting population appeared in the CD4-CD8- subset that also expressed CD69. Despite this ability to respond to antigen, all of the CD4-CD8- thymocytes were HSA+, generally a marker for immature T cells that do respond to antigen. These thymocytes may represent a novel population of thymocytes that arises from premature expression of the α-chain transgene.

V 806 ANALYSIS OF HOMOZYGOUS MUTANT CHIMERIC MICE GENERATED FROM EMBRYONIC STEM CELLS LACKING *CSK* Jane A. Gross, Mark Appleby, Jill Thomas, Xuan Qian, Masato Okada, and Roger M. Perlmutter, Department of Immunology, University of Washington, Seattle, WA 98195

The activity of most *src*-family protein-tyrosine kinases is critically dependent on phosphorylation of a regulatory tyrosine residue located at the carboxy-terminus; phosphorylation of this site results in 10- to 20-fold reductions in catalytic activity. A unique protein tyrosine kinase, encoded by the *csk* gene, has been identified which can mediate this phosphorylation event. The pattern of csk expression in mice corresponds to that of *src*-family tyrosine kinases in general; it is expressed at high levels in the fetal brain and in other fetal tissues, but has restricted expression in the adult where it is found at high levels in cells of hematopoetic origin. Homozygous mutant embryos lacking the csk gene fail to develop past embryonic day 9.5-10.5, and exhibit a complex phenotype including neural tube defects. In order to further evaluate the function of *csk* in the developing mouse and specifically its role in the hematopoetic cell compartment, we have produced homozygous *csk*^{null} embryonic stem cells. Analysis of chimeric mice made using the *csk*^{null} ES cells reveals that *csk* expression is not essential for development of most cell lineages, but may be required for normal lymphopoiesis.

V 807 DEVELOPMENTAL REGULATION OF TCR-CD3-DEPENDENT Ca^{2+} RESPONSES IN INDIVIDUAL NORMAL AND $pp59^{fyn}$-DEFICIENT T LYMPHOCYTES, Karen E. Hedin and David E. Clapham, Department of Pharmacology, Mayo Foundation, Rochester, MN 55905.
Stimulation of the T lymphocyte antigen receptor complex (TCR-CD3) elevates $[Ca^{2+}]_i$, an important step in the immune activation of mature T cells. Paradoxically, TCR-CD3 signaling also involving $[Ca^{2+}]_i$ regulates the selection and death of immature thymocytes. We asked whether different types of TCR-mediated $[Ca^{2+}]_i$ signals could explain the different responses of mature and immature T cells. Using a digital fluorescence imaging system, we measured $[Ca^{2+}]_i$ of individual CD4 and CD8 thymic and splenic cells following application of CD3-ϵ mAb. An unexpected result was that the peak $[Ca^{2+}]_i$ responses of normal murine splenic T cells were strikingly more synchronized than those of thymocytes. This variable temporal coupling did not depend on $pp59^{fyn}$, shown by examination of cells from $pp59^{fyn}$-deficient mice (Appleby *et al.*, 1992, *Cell* 70:751). Instead, $pp59^{fyn}$ deficiency affected the amplitude and probability of CD3-mediated $[Ca^{2+}]_i$ responses of CD4+CD8+ and CD4+CD8- thymocytes. Herbimycin A and pervanadate application showed that immature cells contain additional tyrosine kinases involved in $[Ca^{2+}]_i$ regulation. In addition, the average peak $[Ca^{2+}]_i$ attained by CD3-responsive cells increased with maturity. These results demonstrate that the coupling of TCR-CD3 to $[Ca^{2+}]_i$ signals changes dramatically during development. In particular, the timing of peak $[Ca^{2+}]_i$ relative to other TCR-CD3-derived signals, such as those involving *ras*, PI3 kinase, and tyrosine kinases, might explain the different effects of TCR-CD3 ligation during T cell development.

V 808 TCR ANTAGONISTS INDUCE POSITIVE SELECTION Kristin A. Hogquist, Stephen C. Jameson, Frank R. Carbone, and Michael J. Bevan, Department of Immunology, HHMI, University of Washington, Seattle, WA 98103
Positive selection of T lymphocytes occurs when an immature T cell interacts with a complex of self peptide plus MHC on the surface of a thymic epithelial cell. We have used organ culture of fetal thymic lobes from β_2M KO mice to study the critical role of peptides in this process. In these mice, CD8+ T cells are not positively selected in the thymus. This process can be restored by the addition of class I binding peptides along with β_2M to organ cultures. We have now applied this approach to TCR transgenic mice.

Mice were generated which have a transgenic TCR isolated from a CD8+ cytotoxic T cell specific for the peptide ovalbulmin 257-264 in the context of Kb. This receptor is positively selected on an H-2b background (Kb specifically) as demonstrated by the skewing of thymocytes and peripheral T cells to the CD8 lineage. On an H-2b β_2M KO background, the receptor is not selected as indicated by the absence of TCRhi CD4-CD8+ T cells and the resultant inabiltiy of these cells to respond to antigen. However, when fetal thymic lobes from such mice were cultured with various MHC binding peptides plus exogenous β_2M, several peptides with the ability to induce the appearance of TCRhi CD4-CD8+ T cells were identified. These cells have the same phenotype as mature CD8+ T cells. More importantly, treatment of the lobes with these peptides restores the ability of the β_2M KO thymocytes to respond to antigen.

Only certain Kb binding peptides were capable of mediating this selection. Two naturally occuring self-peptides had no effect on differentiation. Additionally, a peptide composed of only serine residues at the TCR contact sites had no effect. Those peptides with the ability to induce positive selection were all variants of the antigenic peptide and were identified as TCR antagonist peptides for this receptor. One peptide tested, E1, induced positive selection on the β_2M KO background, but negative selection on the wild-type H-2b background. These results argue that the process of positive selection is highly specific, and support an efficacy model of T cell differentiation.

V 809 TOWARD A MODEL OF POSITIVE SELECTION OF T CELLS ON SINGLE MHC/PEPTIDE COMPLEX.
Leszek Ignatowicz, John Kappler and Philippa Marrack. Howard Hughes Medical Institute, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine,Denver, Colorado, 80206. The repertoire of T cells is mainly shaped during their development in the thymus. It is theory that during the process of positive selection T cells bearing T cell receptors (TCRs) that recognize with low affinity a complex of MHC/self peptides are selected for further development. It has been difficult to test this idea, however, since many self peptides are associated with MHC in the thymus, and it is not known which peptides are involved in positive selection of a particular T cell receptor. To circumvent this problem we are trying to create thymuses in which a particular self MHC protein is saturated with a single peptide. Many of the T cells produced by this thymus should have been selected by this combination of MHC/self peptide.

V 810 TCR ANTAGONISTS AFFECT MULTIPLE STAGES OF CTL ACTIVATION AND DIFFERENTIATION, Stephen C. Jameson, Kristin A. Hogquist, Frank R. Carbone*, and Michael J. Bevan, HHMI, Dept. Immunology, University of Washington, Seattle, WA 98195 and *Dept. Pathology and Immunology Monash Medical School Victoria 3181, Australia.

We previously showed that variants of an antigenic peptide can act as clone specific TCR antagonists for cytotoxic T cells (CTL), inhibiting T cell activation in response to antigen/ MHC (J. Exp. Med. **177**:1541). Further experiments were performed using a TCR transgenic mouse bearing a receptor from an OVA/Kb specific CTL. Variants at several residues in the OVA peptide acted as antagonists for this receptor. Most were substitutions at TCR contacts, but a few were variants in regions of the peptide buried in the MHC groove, suggesting an effect on TCR binding to MHC contact residues. The response of unprimed TCR transgenic T cells to stimulators pre-pulsed with OVA peptide is effectively blocked by antagonist peptides, indicating that naive cells as well as long term lines and clones are susceptible to this form of inhibition. Furthermore, antagonists blocked CTL mediated lysis not only of OVA peptide coated target cells but also of cells expressing OVA as an endogenous antigen. This implies that antagonists can inhibit T cell responses to naturally presented antigens.

Our data indicate that mature T cells fail to respond directly to these peptides. In contrast, we have evidence that immature T cells do respond to antagonists: Using fetal thymic organ culture, we have demonstrated that certain antagonist peptides induce positive selection of TCR transgenic T cells. The effects of these antagonists on various aspects of thymocyte activation is described.

V 811 IDENTIFICATION OF A NOVEL, CYCLOSPORIN-SENSITIVE MOLECULE IN THE THYMUS, Marina Katerelos, Nella Fisicaro, Evelyn Salvaris, James H. Williams, David A. Power and Martin J. Pearse, Department of Clinical Immunology, St. Vincent's Hospital, Victoria, 3065, Australia.

Cyclosporin A (CsA) inhibits the transcription of lymphocyte activation genes such as IL-2, IL-3, IL-4 and γIFN in mature T cells. It also disrupts intrathymic T cell differentiation by preventing both positive and negative selection. The effects of CsA on thymocyte differentiation are not reversed by IL-2 and IL-4, suggesting that as yet unidentified molecules are involved in thymocyte selection. In an attempt to identify these CsA-sensitive molecules, RNA from normal murine thymus and CsA-treated thymus has been used to generate a subtracted cDNA library. Of the several subtracted clones identified, one clone, #19 has its expression down regulated approximately 5-fold in CsA-treated thymus RNA compared with normal thymus RNA.

Clone #19 has been fully sequenced and is homologous to a partially sequenced molecule whose expression is regulated during murine brain development. The complete cDNA sequence of #19 is 700 bp in length and contains a single long open reading frame. Translation of this sequence predicts a protein of 133 amino acids with a pI of 9.9. The predicted amino acid sequence contains a novel lysine-rich domain (KKLNKNKKLVKKLAKK) which may have nucleotide binding properties. Preliminary studies have shown that #19 mRNA is expressed more abundantly in lymphoid than in non-lymphoid tissues. #19 mRNA was also abundant in a variety of murine haematopoietic cell lines, with the exception of P815 (mast cell line). Polyclonal and monoclonal anti-peptide antibodies have been generated and are currently being used in Western blots to determine the physio-chemical properties of the protein.

V 812 CYTOKINE PRODUCING POTENTIAL OF CD25+ CD44+ CD3- CD4- CD8- PRE-T CELLS, Gregory S. Kelner, Jacqueline Kennedy, Sarah Kleyensteuber, and Albert Zlotnik. DNAX Research Institute of Cellular and Molecular Biology. Palo Alto, CA. 94304.

We have recently characterized the developmental pathway of of early CD3-CD4-CD8- (triple negative; TN) thymocytes, based on the expression of CD44 and CD25. These studies have established that T cell receptor beta and gamma chain genes are rearranged as CD25+T N thymocytes downregulate CD44. This observation suggests that CD44+CD25+TN thymocytes represent pre-T cells and are targets for signals that induce TCR gene rearrangement. Also, we have observed that these cells produce high titers of Interleukin-2 upon activation with calcium ionophore, phorbol ester and Interleukin-1. A cDNA library has been generated from activated CD44+25+ TN cells, and was probed for the presence of cytokines. Interleukin-2, Granulocyte Macrophage-Colony Stimulating Factor, Interferon-gamma, and the P40 chain of Interleukin-12 were detected. Other clones did not hybridize to cDNA probes of known cytokines, and are currently under further analysis. These results indicate that pre-T cells have significant cytokine producing potential.

V 813 CHARACTERIZATION OF MOUSE αβT CELL RECEPTOR+ CD4-CD8- THYMOCYTES.

Jacqueline Kennedy, Andrew G. D. Bean, Dale I. Godfrey, Leopoldo Santos-Argumedo, Maureen C. Howard and Albert Zlotnik. The Department of Immunology, DNAX Research Institute, PaloAlto,CA 94304

The αβTCR+ CD4-CD8-(DN) thymocytes represent an independent T cell lineage and are characterized by the overexpression of Vβ8.2 and the production of large amounts of IL4, IFN-γ and TNFα upon stimulation with anti-CD3. These thymocytes also express CD38, a marker which in the mouse thymus is mainly associated with αβTCR+ DN thymocyte subsets. IL4 production does not correlate with Vβ8.2 expression but does correlate with CD38 expression since only the CD38+ αβTCR+ DN thymocytes produce IL4. To further characterize anti-CD3 stimulated αβTCR+ DN thymocytes for other cytokines and gene products they express, two cDNA libraries were constructed: one from mRNA of stimulated αβTCR+ DN thymocytes and one from mRNA of unstimulated αβTCR+ DN thymocytes. A cDNA library subtraction procedure was used to generate a library enriched for factors that are specifically produced by activated αβTCR+ DN thymocytes. Northern, PCR and DNA sequencing techniques can be used to identify these factors.

V 814 THYMIC DEVELOPMENT OF αβ LINEAGE THYMOCYTES THAT EXPRESS A γδ T CELL RECEPTOR, Gilbert J. Kersh and Stephen M. Hedrick, Department of Biology and the Cancer Center, University of California, San Diego, La Jolla, CA 92098

Cells entering the thymus from the bone marrow have the potential to become γδ or αβ lineage cells. At some uncharacterized point in development a pre-T cell will decide to enter either the γδ or αβ lineage. This lineage decision is mediated by an unknown mechanism. In order to explore the role of the T cell receptor in this lineage determination, we have constructed mice that utilize rearranged gamma and delta T cell receptor genes. The transgenes are made so that expression of the rearranged gamma and delta genes occurs in the αβ T cell lineage. These mice have mostly CD4 and CD8 double positive cells in the thymus that express low levels of the transgenic γδ receptor. The rearrangement status of the endogenous delta locus in these mice indicates that these cells are in the αβ lineage. The thymocytes seem unable to undergo positive selection, but do have small populations of thymocytes that show indications of being committed to a CD4 or CD8 single positive lineage. Therefore, these mice demonstrate that a γδ heterodimer can substitute for the αβ heterodimer in all the steps of lineage determination, but cannot mediate positive selection.

V 815 CELL SURFACE ANTIGEN, IMT-1, EXPRESSED ON THYMOCYTES DIFFERENTIATING FROM CD4-8- TO CD4+8+. Hiroyuki Kishi, Dong-Ming Su and Takeshi Watanabe, Medical Institute of Bioregulation, Kyushu University, Fukuoka, 812 Japan.

A new monoclonal antibody, IMT-1, which recognize a cell surface antigen of 35-38kd on immature thymocytes has been recently established. The IMT-1 antigen is expressed on a subset of CD4-8-, CD4-8+ and CD4+8+ adult thymocytes, but is not expressed on CD4+8- thymocytes or peripheral T lymphocytes. The IMT-1 antigen is expressed on CD3- but not on CD3hi thymocytes, indicating that IMT-1 expression on thymocytes denotes immaturity. In scid mice differentiation of thymocytes is arrested at the CD4-8- stage because TCR gene rearrangement is defective. Although the IMT-1 antigen is absent from scid thymocytes, the IMT-1 antigen is expressed on the thymocytes of "leaky" scid mice in which a CD4+8+ population is generated in the thymus. During the development of normal fetal thymocytes, day 14.5 to15.5 fetal thymocytes, which are CD4-8-, do not express IMT-1. However, day 16.5 fetal thymocytes, which are beginning to differentiate into CD4+8+ thymocytes, express the IMT-1 antigen. In subsets of CD4-8- thymocytes, IMT-1 is not expressed on CD44hi or HSA-populations but is expressed on HSA+, CD44-/lo, CD25+ or CD25- populations. Taken together these results indicate that IMT-1 is expressed on immature thymocytes highlighting the latter intermediate stage of differentiation between CD4-8- to CD4+8+ maturing thymocytes.

V 816 A TCR V_β DECAMER-BINDING COMPLEX THAT APPEARS TO PLAY A ROLE IN REGULATING TCR REARRANGEMENT Ellen Kraig, LiMin Yang, E. Randall Lanier and Joan Goverman, Univ of Texas Health Science Center, San Antonio, TX 78284-7762 and Univ of Washington, Seattle, WA 98195.

Using extracts prepared from murine thymus, we have identified a tissue-specific protein complex (designated T2) that binds to the decamer, a conserved promoter element in TCR V_β genes. T2 has been implicated in the initiation of TCR gene rearrangement: (1) The T2 decamer binding proteins are developmentally regulated and are absent in SCID mice; (2) The activity of T2 is diminished by the presence of functionally rearranged TCR α and β transgenes and (3) The T2 activity is up-regulated in thymocytes by crosslinking CD4 and CD8 surface proteins. The T2-decamer binding factors require phosphorylation for activity; at least one of the components is related to CREB and can be competed with the negative regulatory protein, CREB-2.

In order to clone the genes that encode T2 binding proteins, a human thymus cDNA expression library was screened with the decamer insert. Of the 5 unique cDNAs obtained, 3 were members of the CREB family and 2 were unique. The CREB-like cDNAs were expressed ubiquitously; of these, one was the negative regulator CREB2, one was the positive transcriptional factor CRE-BP1, and the other was a novel differentially processed form of CRE-BP1.

Also, we have used competitive mobility shift analysis to map the *cis* element required *in vivo* for T2 binding; Dr. Dennis Loh provided us with oligonucleotides that contained the decamer and approximately 30 base pairs of 5' and 3' flanking information. In addition to the wild-type template, he provided several in which 10 base pairs, either the decamer or one of the flanking regions, had been mutated. The wild-type template was able to compete for T2 binding, but mutants lacking the decamer sequence were not. Interestingly, mutants altered in the flanking regions were also less effective competitors suggesting a role for DNA secondary structure, possibly involving the 5' and 3' sequences.

V 817 LINEAGE-SPECIFIC REGULATION OF TCR α/δ GENE REARRANGEMENT BY TCR α AND TCR δ ENHANCERS, Michael S. Krangel and Pilar Lauzurica, Department of Immunology, Duke University Medical Center, Durham, NC 27710

We established a transgenic mouse model to study V-D-J rearrangement of a human TCR δ gene minilocus. The minilocus is composed of V, D, J and C segments, but cannot encode a functional TCR protein. We first studied minilocus rearrangement under the control of E_δ within the J-C intron. Rearrangement occurs stepwise, first V to D, and then V-D to J. Minilocus V-D rearrangement mimics a unique feature of the endogenous TCR δ locus. V-D-J rearrangement is T cell-specific, but occurs equivalently in $\alpha\beta$ and $\gamma\delta$ T cells. We deleted E_δ from the minilocus and found that although V-D rearrangement occurs normally, V-D to J rearrangement is specifically impaired. Thus, E_δ controls access to J segments, but does not control access to V and D segments. The minilocus is apparently divided into two regulatory domains, only one of which is controlled by E_δ. Additional elements must control V and D segment accessibility. We propose that an insulator is located between D and J segments, and demarcates the boundary between regulatory domains. In the endogenous TCR δ locus, this may represent the 5' end of the chromatin domain that is opened by E_δ during T cell development. We have now substituted E_α in place of E_δ in the minilocus. Under the control of E_α, minilocus V-D rearrangement remains enhancer-independent and is equivalent in $\alpha\beta$ and $\gamma\delta$ T cells. However, minilocus V-D-J rearrangement occurs specifically in $\alpha\beta$ T cells. Thus, E_δ is activated and directs TCR gene rearrangement in the precursors of both $\alpha\beta$ and $\gamma\delta$ T cells, whereas E_α is activated and directs TCR gene rearrangement only in the precursors of $\alpha\beta$ T cells. E_α therefore responds to an important lineage commitment signal that controls TCR gene rearrangement in developing thymocytes. E_δ and E_α may also be responsible for temporal control of TCR gene rearrangement during ontogeny. Minilocus V-D-J rearrangement is detected in day 14.5 fetal thymocytes under the control of E_δ. The timecourse of minilocus rearrangement under the control of E_α is currently under investigation.

V 818 CELL CYCLE CONTROL OF V(D)J RECOMBINATION ACTIVATOR PROTEIN RAG-2. Weei-Chin Lin and Stephen Desiderio, Department of Molecular Biology and Genetics and Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The antigen receptor genes of B and T cells are assembled during lymphocyte development by a series of site-specific DNA rearrangements; the products of the recombination activator genes RAG-1 and RAG-2 are essential for this process. We have observed an association between phosphorylation of RAG-2 by cyclin-dependent kinase(s) and degradation of RAG-2 protein (Science 260:953-959). This, and the observation that rearrangement of immunoglobulin and T cell receptor genes occurs in expanding populations of lymphoid progenitor cells, suggested that expression of RAG-2 is regulated in the cell cycle. To test this, we analyzed expression of RAG proteins as a function of cell cycle stage in lymphoid cells or in transfected fibroblasts. In a B progenitor cell line and in normal thymocytes, expression of RAG-2 protein is restricted to the G0/G1 phases of the cell cycle, despite the presence of RAG-2 RNA at all cell cycle phases. In contrast, levels of RAG-1 protein show little or no variation during the cell cycle. Expression of RAG-2 protein in transfected fibroblasts showed similar cell cycle dependence; as observed in lymphoid cells, RAG-1 protein did not vary appreciably during cell cycle. These observations imply that V(D)J recombination is restricted to the G0/G1 phases of the cell cycle by posttranscriptional control of RAG-2 expression; we suggest that failure to restrict recombination within the cell cycle may result in inappropriate and deleterious rearrangements. The mechanism responsible for posttranscriptional control of RAG-2 expression and the biological significance of this control are now under study.

V 819 THYMIC EDUCATION IN HUMAN AFTER BONE MARROW TRANSPLANTATION.

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The T cell repertoire is considered to be mainly controlled by the thymus. The process of thymic education is so far not well characterized in man, due to the outbred nature of the human population and the obvious impossibility to perform experiments as described in animal models. An unique opportunity to study this process is provided by Bone Marrow Transplantation (BMT). We have analyzed the variable region distribution in 7 patients and their relative HLA matched siblings marrow donor before BMT treatment. We then followed the reconstitution of the T cell repertoire in the recipients 30, 60 and 180 days after BMT. A panel of 14 anti TCR monoclonal antibodies was used to define the distribution in different subpopulations by 4 four colours FACS analysis.In the majority of the cases the repertoire profile, in CD4+ T cells, was highly comparable between recipient and HLA matched sibling donor prior to BMT. However discrete but significant differences were observed for some variable regions.
After BMT we focused our attention on the distribution of the variable regions which differed between donor and recipient prior treatment, to monitor whether a donor or recipient profile, was observed in the reconstituted recipient. In most, but not all, of the cases a donor's imprinting persisted during reconstitution. Thus our results provides direct evidence that the process of thymic selection in man is taking place in man.

V 820 ζ- OVEREXPRESSION IN EARLY THYMOCYTES ACTIVATES A TCR-INDEPENDENT SIGNALING RESPONSE THAT REGULATES RAG-1 AND RAG-2 EXPRESSION AND αβ T CELL DEVELOPMENT.

Paul E. Love*[1], Elizabeth W. Shores[2], Eric J. Lee[1],Alexander Grinberg[1], Terry I. Munitz[2], Heiner Westphal[1] and Alfred Singer.[2] [1]Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, and [2]Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

The ζ-family dimers (ζ, η and FcεRIγ) are a group of structurally and functionally related proteins that are expressed in developing thymocytes and play a central role both in receptor surface expression and in signal transduction. Conserved sequences (termed Tyrosine-based activation motifs; TAMs) within the cytoplasmic domains of these proteins are required for signal transducing activity. To examine the role of TAM - mediated signaling in thymocyte development we generated transgenic mice that expressed: (*i*) full length (FL) ζ-chain , (*ii*) η-chain, a naturally occurring variant of ζ derived from alternative splicing, or (*iii*) truncated ζ-chain (CT108), all under the control of the human CD2 promoter and regulatory elements. The transgene encoded proteins therefore contained 3, 2 or 1 TAM functional elements, respectively. Overexpression of FL ζ-chain, but not η or CT108, in fetal $CD4^-CD8^-TCR^-$ thymocytes activated a signaling pathway that terminated RAG-1 and RAG-2 expression, prevented productive rearrangement of the TCRα and TCRβ genes and blocked entry of thymocytes into the CD4/CD8 developmental pathway. These results identify an early signaling pathway in precursor TCR^- thymocytes that can regulate RAG-1 and RAG-2 expression and is differentially responsive to individual members of the ζ-family dimers.

V 821 DEVELOPMENT OF T CELLS IN MICE WITH A DISRUPTED CD3 ζ/η OR CD3ε GENE .Marie Malissen and Bernard Malissen Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, 13288 Marseille cedex9, France.

The development of αβ T cells is driven by the interplay of a small set of surface molecules among which the TCR-CD3 complex play a determining role. Recently, several groups, including ours have shown that the intracytoplasmic tails of the CD3 subunits may be organized in at least two autonomous transduction modules made of γδε and ζ chains, respectively. To study the in vivo function of CD3 ζ and CD3 ε we inactivated the corresponding genes using the technique of homologous recombination in embryonic stem cells. The analysis of thymocyte populations revealed that the products of the CD3-ζ/η gene appear needed for the efficient generation and/or survival of $CD4^+CD8^+$ thymocytes. Despite the near total absence of mature single positive thymocytes, the $\zeta/\eta^{-/-}$ lymph nodes were unexpectedly found to contain $CD3^-$ $CD44^+$ single positive T cells. In contrast to the situation observed in the thymus, the thymo-independent gut intraepithelial lymphocytes present in $\zeta/\eta^{-/-}$ mice do express TCR complexes on their surface and these are associated with FcεRIγ homodimers. These results establish an essential role for the CD3-ζ/η gene products during T cell differentiation and further emphasize the difference between conventional T cells and thymus-independent gut intraepithelial lymphocytes. We will present results on the complementation of the homozygous $\zeta/\eta^{-/-}$mutant with various CD3-ζ constructs containing point mutation in the different transduction motifs and their effect on thymocyte development. The analysis of thymocyte development in CD3-$\varepsilon^{-/-}$ mice will also be presented.

V 822 GENERATION OF MATURE T CELL POPULATIONS IN THE THYMUS: CD4 OR CD8 DOWN-REGULATION OCCURS AT DIFFERENT STAGES OF THYMOCYTE DIFFERENTIATION.

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We have studied the differentiation and repertoire selection during the maturation of $CD4^+CD8^+$ (DP) thymocytes into $CD4^+CD8^-$(CD4SP) and $CD8^+CD4^-$ (CD8SP) T cells, in normal mice, mice transgenic for αβ-TcRs restricted by either class I or class II MHC, and in mice deficient in class I or class II MHC expression. Our data suggest that CD4 and CD8 mature T cells derive from different pathways of T cell differentiation in the thymus. Thus, interaction of DP with MHC Class II leads to the immediate down-regulation of CD8, which occurs simultaneously with an increase in TcR expression; $DPTcR^{lo}HSA^{hi}$ thymocytes mature into a $CD4^+CD8^{lo}$ $TcR^{hi}HSA^{hi}$ intermediate population. This cell population generates CD4SP thymocytes, the majority of which are still HSA^{hi}. In contrast, interaction with MHC Class I induces the up-regulation of TcR, which precedes the down-regulation of CD4; $DPTcR^{lo}$ generate $DPTcR^{hi}$ thymocytes, the majority of which are the committed precursors of CD8SP cells. Further differentiation results in CD4 down-regulation and the transition from $DPTcR^{hi}$ into $CD8^+CD4^{lo}$ $TcR^{hi}HSA^{lo}$ and $CD8SPTcR^{hi}HSA^-$ T cells. Since down-regulation of CD4 and CD8 occurs at different stages of thymocyte differentiation, our results do not support a stochastic/ selective model of lineage commitment in the thymus.

V 823 HOW EFFICIENT IS POSITIVE SELECTION?

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Thymocyte maturation depends on interactions with thymic stromal elements expressing appropriate MHC molecules. Mutant mouse strains lacking MHC class I or class II expression fail to generate normal CD8 or CD4 T cell populations, respectively, and provide model systems for reconstitution experiments. Aiming to better define the requirements for T cell maturation, we have constructed in vitro chimeras between normal and MHC deficient thymi. Unexpectedly in the light of previous data, the generation of phenotypically mature single positive thymocytes was proportional to the fraction of wild type (i.e. MHC expressing) stroma over a wide range of chimerism. Similar results were obtained for the development of TCR transgenic thymocytes in graded chimeras expressing selecting and non-selecting MHC alleles, suggesting that in positive selection, thymocytes may not distinguish between the complete lack of class II and the presence of a 'wrong' class II allele. In contrast to negative selection, therefore, positive selection is inefficient when only a minority of the relevant stromal cells express selecting ligands. T cell maturation may be limited not only by the candidate TCR repertoire available for selection, but also by the abundance of competent stromal cells. These findings are best explained if positive selection involves a rate-limiting step at which each thymocyte interacts with only one stromal cell niche.

V 824 INDUCTION OF RECOMBINATION ACTIVATION GENE(RAG) TRANSCRIPTION IN HUMAN LYMPHOID PROGENITOR CELL LINE BY RECOMBINANT CYTOKINES AND STROMAL LINES, Atsushi Muraguchi, Hiromi Tagoh, Hideyuki Kurioka, and Hiroshi Isshiki, Department of Immunology, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Regulation of commitment to lymphoid cells from lymphoid progenitors remains poorly understood. The earliest event that occur in lymphocyte development is V-D-J gene rearrangement of Ig or TCR genes. Recently lymphoid specific genes named RAG-1 and RAG-2 were identifed as playing a crucial role in the recombination process. We have studied the effect of various recombinant cytokines and the bone marrow-derived stromal cells on the expression of RAG-1 mRNA in lymphoid progenitor-like cell lines which had been established from human fetal liver at 8 weeks of gestation. These lines retained Ig and TCR genes in their germ line configuration and expressed a T cell marker(CD2) as well as a B cell marker(CD19). These lines did not express the detectable RAG mRNA when analyzed a PCR-based assay or Northern blot method. The induction of RAG mRNA expression, however, was observed in these cells when co-cultured with PA-6, a mouse bone marrow stromal cell line, in the presence of the recombinant cytokines (a mixture of SCF, GM-CSF, IL-3, IL-4, IL-6, and IL-7). The kinetic study revealed that the RAG expression was induced at 12 hr after the initiation of co-culture. Before the expression of RAG transcripts, the progenitor cells were engulfed and laid beneath the stromal cells. Co-culture of the progenitors with a human bone-marrow derived cell line (RASV 5-5), which sustains the growth of the murine B progenitors, also induced the RAG mRNA expression in these cells. Furthermore, we obtained the 5' flanking region and characterized the cis elements of the human RAG-1 gene in order to clarify the mechanism of its lymphoid specific expression. This system will be useful for the delineation of potential regulatory factors or signals from stromal cells acting on RAG transcription as well as the further understanding the control and regulation of the early steps in human lymphocyte development.

V 825 FAS EXPRESSION DURING FETAL AND ADULT THYMOCYTE DEVELOPMENT, J. Nikolic-Zugic[1], J. Drappa[2], E. Lacy[3], K. Elkon[2] and S. Andjelic[1], Immunology[1] and Molecular Biology[3] Programs, Sloan-Kettering Institute and The Hospital for Special Surgery[2], New York, NY 10021.

The Fas gene encodes a 48 Kd surface protein capable of transducing apoptotic signals upon ligation with mAbs. The natural ligand for Fas is not known. Defective transcription of Fas was found to be the molecular explanation of the lpr defect (an SLE-like syndrome in mice). To investigate the role of Fas during T cell development, we analyzed its expression in fetal and adult thymocyte subsets. Fas is not expressed on fetal nor adult $CD8^-CD4^-$ (double negative, DN) T cell precursors. DN cells that express Fas appeared to be either non-T cells or $\alpha\beta^+$ "mature" DN cells. The first subset that may express low levels of Fas is also the first to express low levels of CD8, CD4 and the TcR molecules (triple low, TL). These cells are the immediate precursors of $CD8^+$ $CD4^+$ (double positive, DP), the cell type that expresses the highest levels of Fas. This expression develops late in the fetal ontogeny since we found only 25% Fas cells among Fd (fetal day) 16 DP thymocytes. By Fd 17 the proportion of Fas^+ cells is enlarged such that 80% of DP cells are Fas^+. The adult pattern of Fas expression is only achieved after birth. After reaching the peak of its expression on DP cells, Fas is gradually downregulated on both fetal and adult $CD8^+$ or $CD4^+$ (single positive, SP) thymocytes in parallel to their maturation. Resting peripheral T cells express no or very little of Fas; it is thus possible that Fas downregulation is completed as the cells exit the thymus. Strong Fas expression thus characterizes cells subject to intrathymic selection, but the function of Fas in the thymus is unclear at present. Another difference between fetal and adult thymocytes concerns the Fas expression on $\gamma\delta^+$ DN cells. Namely, up to 90% of these cells in the fetal thymus are Fas^+, in contrast with very few adult $\gamma\delta^+$ Fas^+ cells (7%). The functional significance of this observation is not clear, but it is possible that a particular wave of fetal $\gamma\delta$ cells has a unique property of high Fas expression.

V 826 THYMIC DEVELOPMENT IN HUMAN CD4 TRANSGENIC MICE: POSITIVE SELECTION OCCURS FOLLOWING COMMITMENT TO THE CD8 LINEAGE. R. Kaufman Paterson*, D. Kurahara*, L. C. Burkly@, A. Dunlap*, D. Kioussis#, C. Mamalaki#, R. A. Flavell ** and T. Helman Finkel*.*Dept. Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO. @Biogen Inc. Cambridge, MA. **Howard Hughes Medical Center, Yale University School of Medicine, New Haven CT. #Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London, England.

In thymus, T cells are selected by their ability to recognize polymorphic major histocompatibility complex (MHC) molecules. The CD4 and CD8 coreceptors bind to MHC class II or class I, respectively, and are required for maturation of T cells to either lineage. We have studied the selection process in mice transgenic (TG) for human CD4 (huCD4). huCD4 was functionally expressed on thymocytes and mature T cells in TG mice; crosslinking of huCD4 signalled an increase in intracytoplasmic calcium and huCD4 mediated positive selection when murine CD4 (mCD4) was blocked by specific antibody (mAb).

A central question of T cell development is whether positive selection of cells specific for self-MHC occurs at a double positive or single positive stage of differentiation. We directly addressed this question by crossing huCD4 TG mice with MHC Class I-negative (CI^-) mice. If single positive cells are targets for positive selection, maturation of $mCD8^+4^-$ T cells would take place in the absence of Class I, due to interaction of Class II with the constitutively expressed huCD4. Indeed, huCD4 mediated maturation of $mCD8^+$ T cells in CI^- mice. In lymph nodes of CI^- mice, $mCD8^+$ cells were only $0.2 \pm 0.04\%$ but increased to $2.5 \pm 0.8\%$ ($p<.01$) in huCD4 TG x CI^- mice. When mCD4 was blocked by mAb, $mCD8^+$ cells increased from $0.7 \pm 0.2\%$ for CI^- mice to $17.5 \pm 2.7\%$ for huCD4 TG x CI^- mice ($p<.001$). $mCD8^+$ T cells in Class I^+ mice were $35.1 \pm 3.9\%$. An increase in $mCD8^+4^-$ mature thymocytes was also detected: increasing from $0.3 \pm 0.1\%$ in CI^- mice or $0.5 \pm 0.1\%$,in anti-mCD4 mAb treated-CI^- mice, to $0.7 \pm 0.2\%$ in huCD4 TG x CI^- mice ($p<.05$) and to $2.4 \pm 0.7\%$ in anti-mCD4 mAb-treated huCD4 TG x CI^- mice ($p<.02$). The proportion of $V\beta14^+$ T cells among $huCD4^+mCD8^+$ mature T cells, generated in huCD4 TG x CI^- mice, was increased ($9.0 \pm 0.7\%$) above that of control $mCD8^+$ T cells ($2.9 \pm 0.4\%$) and almost reached the levels found in $mCD4^+$ T cells ($11.7 \pm 1.3\%$), further indicating that huCD4 had mediated the positive selection of immature, lineage-committed ($mCD8^+4^-$) thymocytes via interaction with Class II. These data suggest that positive selection of $CD8^+$ T cells can occur at a late, single positive stage of thymocyte differentiation.

V 827 CLASS II MHC-RESTRICTED HELPER T CELLS IN CD4-DEFICIENT MICE, A. Rahemtulla, T. Kündig, A. Narendran, M. Julius, C. Paige, R. Zinkernagel, T. Mak. Nuffield Department of Medicine, University of Oxford, Oxford, OX3 9DU. Ontario Cancer Institute,Department of Medical Biophysics and Immunology, 500 Sherbourne Street, Toronto, Ont. M4X 1K9 Institute of Pathology, University of Zurich, Sternwartstrasse 2, 8091 Zurich, Switzerland.

We have studied T cell development and helper-function in CD4-deficient mice. These mice have a normal number of T cells and B cells. The $CD8^+$ T cells in the periphery have expanded to apparently occupy the compartment previously occupied by the $CD4^+$ T cells but they also possess a significant population of $CD4^-8^-TcR_{\alpha\beta}^+$ T cells. These cells are present at a low level in the thymus and lymph nodes of normal mice but the significance of these cells in immune development and function iis not known. Surprisingly, mice lacking CD4 show *in vivo* immunoglobulin isotype class switching from IgM to IgG in response to vesicular stomatitis virus. In this study we have depleted various sub-population of T cells *in vivo* and shown that the population of $CD4^-8^-TcR_{\alpha\beta}^+$ T cells is responsible for providing "help" in the antibody response of CD4-deficient mice to vesicular stomatitis virus infection. We have used the antigen-specific proliferation assay and blocking studies with class I and II MHC specific purified antibodies to show that these cells are class II-MHC restricted in responses against the T cell-dependent antigen keyhole-limpet haemocyanin (KLH). These results indicate that CD4 is not absolutely necessary for positive selection or effector function of class II MHC-restricted helperTcells.

V 828 DEREGULATION OF IL-2Rα EXPRESSION IN IMMATURE $CD4^+8^+$ AND $CD4^-8^+$ N-METHYL-N-NITROSOUREA INDUCED THYMIC LYMPHOMAS. E. R. Richie, D. Klug, D. Walker and M. Holloway. University of Texas M.D. Anderson Cancer Ctr., Science Park-Research Division, Smithville, TX 78957

IL-2Rα (CD25) expression is tightly regulated on thymocyte subsets during T-cell differentiation. Although approximately 50% of precursor $CD4^-8^-$ thymocytes express IL-2Rα polypeptide chains, IL-2Rα expression is down-regulated during subsequent stages of intrathymic differentiation. Thus, previous studies have reported that IL-2Rα expression does not occur on immature T cells including the immature $CD4^-8^+$ subset and the major cortical $CD4^+8^+$ thymocyte subset. Murine thymic lymphomas induced by the carcinogen N-methyl-N-nitrosourea (MNU) correspond phenotypically to immature $CD4^+8^+$ and $CD4^-8^+$ thymocyte subsets. We recently found that the majority of these lymphomas express IL-2Rα as detected by indirect immunofluorescence and immunoprecipitation analyses. In addition, Northern blot analysis demonstrated the presence of IL-2Rα mRNA in $IL\text{-}2R^+$, but not in $IL\text{-}2R^-$ lymphomas. The functional activity of the cell surface IL-2Rs was demonstrated on several lymphomas by IL-2 mediated stimulation of ^{3}H-thymidine uptake in short term culture. Electrophoretic mobility shift assays using nuclear extracts from $IL\text{-}2R^+$ and $IL\text{-}2R^-$ lymphomas were carried out as a first step in investigating transcriptional conrol of IL-2Rα gene expression. The pattern of NF-κB binding proteins in $IL\text{-}2R^+$ and $IL\text{-}2R^-$ lymphomas suggests that this family of transcriptional activators is not solely responsible for deregulated transcription of the IL-2Rα gene in these lymphomas. Functional assays are underway to extend these findings.

V 829 DEVELOPMENT AND PROLIFERATION OF LYMPHOCYTES IN MICE DEFICIENT FOR BOTH INTERLEUKIN-2 AND 4, Benjamin Sadlack, Ralf Kühn+, Hubert Schorle, Klaus Rajewsky+, Werner Müller+ and Ivan Horak. Institute of Virology and Immunobiology, University of Würzburg, Würzburg, Germany. +Institute of Genetics, University of Cologne, Cologne , Germany.

Mouse mutants deficient for IL-2 (1) and IL-4 (2) were recently derived. Despite of some predictions that both IL-2 and IL-4 are indispensable for development of mature thymocytes, thymus and T-cell subsets develop normaly in both types of deficient mice. Furthermore, young $IL\text{-}2^{-/-}$ mice generated only partially reduced in vivo CTL responses and helper dependent and independent T-cell responses when challenged with various viruses (3). The observed high levels of IgG1 and IgE isotypes in sera of $IL\text{-}2^{-/-}$ animals indicated a preferential use of the IL-4-directed switch and suggested that the lack of IL-2 might be compensated by IL-4. We report that in mice made deficient for both IL-2 and IL-4 all major T-cell subsets and B-cells were normal, indicating that IL-2 and IL-4 are not essential for development of the immune system. Paradoxically, proliferation of T-cells was increased in both $IL\text{-}2^{-/-}$ and in $IL\text{-}2^{-/-}$ $IL\text{-}4^{-/-}$ mice. The deregulation of the immune system manifests later with complex pathological changes very similar to that described for $IL\text{-}2^{-/-}$ mice (4). The polyclonal activation and proliferation of T- and B-cells is an early indicator of this process that triggers the autoimmune disease.

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V 830 EFFECTS OF THE *fas* GENE ON THE DEVELOPMENT AND FUNCTION OF $CD4^+$ T CELLS EXPRESSING A SINGLE TRANSGENIC ANTIGEN RECEPTOR, Gary G. Singer, Ann Marshak-Rothstein and Abul K. Abbas, Department of Pathology, Harvard Medical School and Brigham & Women's Hospital, Boston, MA 02115, and Department of Microbiology, Boston University School of Medicine, Boston, MA 02108

In order to analyze the role of *fas* gene defects in the development and functions of antigen-specific T cells, we have bred a transgenic T cell receptor (TCR) specific for pigeon cytochrome c + $I\text{-}E^k$ into *fas*-defective MRL/*lpr* mice and their normal MRL-+/+ counterparts. $CD4^+$ T cells develop normally in MRL/*lpr* mice. TCR-transgene-expressing *lpr* cells are resistant to high-dose suppression by antigen and to DNA fragmentation (apoptosis) induced by anti-TCR antibody. Transgene-expressing *lpr* cells do not display an abnormality in their requirement for or responsiveness to costimulation. High doses of cytochrome c (81-104) peptide administered systemically cause comparable deletion of thymic T cells in TCR transgene-expressing *lpr* and +/+ mice, but mature peripheral T cells in transgene-expressing *lpr* mice appear relatively resistant to antigen-induced deletion. Finally the expression of the transgene prevents the development of autoimmunity in MRL/*lpr* mice. Our results indicate that the *fas* gene plays a role in peripheral but not central (thymic) tolerance. We postulate that this gene is involved in peripheral tolerance caused by high dose antigen-induced clonal deletion, but may not play a role in clonal anergy caused by antigen recognition without costimulation. Thus, these two mechanisms of tolerance may differ in operative mechanisms, and their failure may result in distinct types of autoimmune diseases.

V 831 TCR SIGNALS INDUCE UP-REGULATION OF CD5 EXPRESSION ON DEVELOPING THYMOCYTES. Yousuke Takahama#*, Michael Sheard#*, Harumi Suzuki# and Alfred Singer#. #Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892; *Syntex Institute of Immunology, Niihari, 300-41, Japan.

In the present study, we have attempted to understand the basis for the coordinated increases between TCR and CD5 expression during thymocyte development. We have found that TCR signals to developing T cells including $CD4^+CD8^+$ thymocytes up-regulate the cell surface expression of CD5. Such an increase in CD5 expression was regulated at a transcriptional level. Interestingly, $CD4^+CD8^+$ thymocytes from TCR$\alpha\beta$-transgenic mice undergoing positive selection expressed higher CD5 levels than non-selecting $CD4^+CD8^+$ thymocytes expressing the same transgenic TCR. These results indicate that the up-regulation of CD5 expression levels during thymocyte development can result from the TCR signals that induce clonal selection.

V 832 EXTRATHYMIC DIFFERENTIATION OF A T CELL BEARING AN INVARIANT Vα14Jα281 TCR

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We found that a homogenous $CD4^-/CD8^-$ T cell bearing invariant TCR encoded by Vα14Jα281 with a one-base N-region is highly dominated in the periphery (2-3% in spleen). Surprisingly the high expression of the homogenous Vα14 TCR is a general phenomenon in all laboratory strains irrespective of MHC haplotypes and in some wild mouse subspecies. The majority of $V\alpha14^+$ TCR are associated with Jα other than Jα281 at the neonatal stage and then the frequency of invariant Vα14Jα281 TCR expression increases with time and reached a maximum at around 5-8 weeks after birth. The dominant expression of Vα14Jα281 TCR is found both in euthymic and athymic mice. These results indicate that homogenous Vα14Jα281 T cells are positively selected in the periphery without thymic influence and that their VJ junction is important for the positive selection. We also demonstrate that $V\alpha14^+$ TCR gene rearrangements take place in extrathymic sites, such as bone marrow, liver, and intestine, since frequent nonproductive Vα14 TCR products and Vα14-Jα281 gene mediated signal sequences of the circular DNA are detected as a result of TCR rearrangements in extrathymic tissues rather than the thymus, indicating the extrathymic development of Vα14Jα281 T cells. No circular DNA generated by Vα1.1-Jα281 which is known to be thymus-dependent was detected in extrathymic tissues of athymic mice. Moreover, the decrease in the invariant Vα14 TCR expression was tightly correlated with the development of autoimmune diseases. This suggests the crucial role of the invariant Vα14Jα281 T cells in the regulation of anti-self responses.

V 833 ANALYSIS OF NF-ATc EXPRESSION DURING THYMIC MATURATION AND SELECTION EVENTS.

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NF-AT, a T cell specific transcription factor complex, is critical for IL-2 gene expression during T cell activation. The NF-AT complex contains an inducable nuclear component (which can be provided by various fos/jun family members) and a pre-existing cytoplasmic component, NF-ATc, which translocates to the nucleus and is transcriptionally further upregulated in response to T cell stimulation. Translocation is regulated by the phosphatase calcineurin, and can be inhibited by blocking calcineurin's activity with Cyclosporin A or FK506; which also prevents the increase of NF-ATc transcripts. Cyclosporin A and FK506 have also been reported to inhibit the induction of T cell anergy *in-vitro*, and to impair both positive and negative thymic selection *in-vivo* and in fetal thymic organ cultures, although not negative selection in more recently developed *in-vitro* thymocyte culture systems. These observations implicate a role for NF-ATc in the regulation of T cell development, and we present data describing the constitutive and induced pattern of expression of NF-ATc through major hematapoietic lineages and thymic subsets in the mouse; as well as changes in it's expression levels and subcellular localization during thymic selection.

V 834 POSITIVE SELECTION OF $CD8^+$ T CELLS IN VIVO IN ABSENCE OF A FUNCTIONAL TAP COMPLEX,

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MHC class I molecules present short peptides, mainly derived from cytosolic proteins, to $CD8^+$ T cells. The heterodimeric TAP complex (Transporters associated with Antigen Presentation) translocates these peptides (or precursors thereof) from the cytosol to the ER. These peptides are essential for the proper assembly of class I complexes, and only correctly folded complexes are efficiently transported to the cell surface. In cells and mice mutant for one or both of the TAP subunits, most of the class I molecules are arrested in the ER due to the lack of stabilizing peptides, and cell surface expression of class I molecules is therefore markedly reduced. The low level of class I expression in TAP1 mutant mice impairs positive selection of $CD8^+$ T cells and the number of $CD8^+$ T cells in the peripheral lymphoid organs is reduced by ~20 fold.
We crossed TAP1 mutant mice with mice double-transgenic for either HLA-A2 and human(h)β2m or HLA-B27 and hβ2m. The number of $CD8^+$T cells in the TAP1 mutant/HLAhβ2m Tg mice is ~6 fold higher than in TAP1 mutant mice. While human MHC class I complexes are hardly (HLA-A2), or not (HLA-B27), detected at the cell surface, cell surface expression of the murine class I complexes (K^b and D^b) in both types of mice is ~5 fold higher than in TAP1 mutant mice. The murine class I heavy chains preferentially associate with hβ2m, and these complexes are transported more efficiently to the cell surface.
We propose that this increase in class I expression is responsible for the partial rescue of the $CD8^+$ T cell pool. We are currently characterizing the functional properties and specificity of this population of T cells.

V 835 DETECTION OF A NOVEL SURFACE MOLECULE ON IMMATURE THYMOCYTES AND THEIR PROLIFERATION INHIBITION BY A MONOCLONAL ANTIBODY SPECIFIC TO THE MOLECULE, Hiroshi YAMAMOTO, Hiroo TAMURA, Hiroyuki KUZUHARA and Tamotsu TAKEUCHI, Department of Immunology, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan.

We previously described that the nude mouse-derived splenic T cell clone N-9F exhibits a proliferative response when cultured on thymic stromal cells (TSC). This N-9F proliferation is mediated by direct cell-to-cell interactions between T and TSC. A thymic epithelial cell clone, SL10.3, also supports N-9F growth. In order to identify the molecule involved in T cell development in the thymus, we established monoclonal antibodies (MoAb) specific to the N-9F clone. Among these MoAb, one, QR6.6, was found to inhibit the N-9F proliferative response on SL10.3. QR6.6 positive cells were detected in thymus but not in other lymphoid organs such as bone marrow, lymph nodes or spleen. QR6.6 positive cells accounted for 3-5% of the cells in adult thymuses with higher percentages found in neonatal (10-20%) and fetal thymuses (70% at E17 and 10-20% at E15). The positive cells were primarily $CD4^-8^-$ thymocytes in fetuses and $CD4^-8^-$ to $CD4^+8^+$ thymocytes in adults. The QR6.6 MoAb precipitates a 100kD molecule from the N-9F clone. The addition of the MoAb to fetal thymus organ culture reduces the recovery of cells at day 4. It was also found that the MoAb inhibits fetal thymocyte proliferation on the SL10.3 thymic epithelial cell line. These results suggest that the 100kD molecule detected by the QR6.6 MoAb may play a crucial role in the early stage of thymocyte development.

V 836 THE CD4-CLASS II MHC INTERACTION: WHAT IS THE ROLE OF CO-RECEPTOR DURING POSITIVE SELECTION?, Deborah L. Yelon and Leslie J. Berg, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

During the process of positive selection, developing thymocytes mature into $CD4^+$ or $CD8^+$ cells based on the MHC recognition specificities of their TCRs. Although several lines of evidence suggest that co-receptor-MHC interactions are required for maturation, the precise roles of CD4 and CD8 in the lineage instruction of thymocytes are still unclear. We are currently investigating the importance of the CD4-class II MHC interaction during the development of thymocytes expressing the class II-restricted 2B4 TCR. We have tested a panel of mutant class II molecules for their ability to bind CD4. Our results suggest the existence of a novel CD4 binding site in the β1 domain of $I\text{-}E^k$. Mutations in this site do not appear to alter the ability of $I\text{-}E^k$ to bind peptides or to engage the 2B4 TCR. We are currently creating a line of transgenic mice expressing this mutant class II molecule; the degree to which development can proceed in these mice will clarify the role of CD4 during thymic maturation.

V 837 TIMING OF TCR β GENE REARRANGEMENT DURING T CELL DEVELOPMENT IN THE ADULT MOUSE THYMUS: c-kit EXPRESSION MARKS PRE-T CELLS WHICH HAVE NOT YET REARRANGED THEIR TCR GENES, Albert Zlotnik, Jacqueline Kennedy, and Dale I.Godfrey, Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304

The generally accepted pathway of early T cell development is as follows: $CD4^{low}CD3^-CD8^-CD44^+CD25^-$ [earliest thymocytes believed to be arrivals from the bone marrow: (abbreviated $CD4^{lo}$)] $\rightarrow CD3^-CD4^-CD8^-$ (Triple negative;TN)$CD44^+CD25^- \rightarrow CD44^+CD25^+TN \rightarrow CD44^-CD25^+TN \rightarrow CD44^-CD25^-$"TN"$\rightarrow$ $CD4^+CD8^+$ thymocytes. Previous studies have established that the three earliest populations express c-kit. These include the $CD4^{lo}$, some of the $CD44^+CD25^-$TN and all the $CD44^+CD25^+$TN. We have now studied the TCR β and γ gene configuration of these subsets. While the $CD4^{lo}$ have their TCR genes in germline configuration, the $CD44^+CD25^+$TN also exhibit a germline pattern while the $CD44^-CD25^+$TN have rearranged TCR β and γ gene loci. This was inconsistent with previous reports where TCR β gene rearrangements were found in $CD44^+CD25^-$TN. Consequently, we separated $CD44^+CD25^-$TN by c-kit expression and analyzed their TCR genes. $CD44^+CD25^-ckit^+$TN exhibit a germline pattern at their TCR β and γ genes while $CD44^+CD25^-ckit^-$TN have these loci rearranged. The $c\text{-}kit^+$ cells in these populations have T cell precursor activity. The $CD44^+CD25^-ckit^+$TN population also expresses low levels of CD4, making these cells indistinguishable from the $CD4^{lo}$ earliest precursors. Taken together, these results support the following revised pathway: $CD4^{lo}ckit^+ \rightarrow CD44^+CD25^+ckit^+TN \rightarrow CD44^-CD25^+ckit^{low}TN \rightarrow CD44^-CD25^-ckit^-$"TN"$\rightarrow$ $CD4^+CD8^+$ thymocytes. Thus, c-kit expression defines early pre-T cells that have not yet rearranged their TCR genes. TCR β and γ gene rearrangements occur as $CD44^+CD25^+ckit^+$TN downregulate CD44 and c-kit. These results imply that the latest point in the pathway where the $\gamma\delta$ T cells can split (from the main $\alpha\beta$ pathway) is the $CD44^-CD25^+ckit^-$TN stage.