ANTIGEN PRESENTATION FUNCTIONS OF THE MHC

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Poster Sessions

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Class I Structure and Function

O 001 CHARACTERIZATION OF COMPLEXES FORMED BY CLASS I MHC (HLA-A2) AND AN HIV PEPTIDE (IV9) ACTIVE AT SUB-PICOMOLAR CONCENTRATIONS, Theodore J. Tsomides and Herman N. Eisen, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

$CD8^+$ cytotoxic T lymphocytes (CTL) recognize cell surface complexes formed by class I MHC glycoproteins and antigenic peptides of either endogenous or exogenous origin. We have identified a peptide nonamer (termed IV9) derived from the human immunodeficiency virus that is over a million-fold more active (at sub-picomolar concentrations) than peptide analogues longer or shorter by 1-3 amino acid residues when recognized by an HLA-A2-restricted CTL clone. This optimal peptide conforms to an independently-derived consensus motif for endogenous A2-binding peptides. To understand the dynamics of binding between HLA-A2 and IV9, we carried out studies both *in vitro* using purified molecules and with $HLA\text{-}A2^+$ cells. IV9 does not detectably bind to isolated HLA-A2 as measured by equilibrium dialysis or gel filtration, but it binds specifically to HLA-A2 on intact cells and can be recovered from affinity-purified complexes in unaltered form. Less than 1% of cell surface HLA-A2 binds IV9, consistent with the idea that most cell surface class I MHC molecules harbor tightly-bound endogenous peptides. In further support of this view, our measured dissociation rate constant for isolated IV9/HLA-A2 complexes at 37°C is between $3x10^{-7}$ and $1x10^{-6}$ sec^{-1}, corresponding to a half-time of 200-600 hours. Thus binding between IV9 and HLA-A2 requires cell-surface, presumably peptide-free HLA-A2 molecules, and results in a relatively small number of exceedingly stable complexes (<1000/cell) for efficient CTL recognition.

Other peptides have been screened for binding to HLA-A2 by their capacity to inhibit binding by stoichiometrically-iodinated IV9 as an indicator peptide. HIV-derived nonamer sequences predicted from the independently-derived consensus motif were shown to bind HLA-A2 to varying degrees; whether any of these products arises from cellular processing in virally-infected $HLA\text{-}A2^+$ cells is under investigation. Longer peptides, containing the IV9 sequence extended by 1-3 residues in either direction, also inhibit IV9 binding to HLA-A2 and possess biological activity. However, at least one such longer peptide, a 12-mer, tested for direct binding to HLA-A2 on intact cells does not exhibit such binding in unaltered form, raising the possibility that the 12-mer undergoes processing to IV9. The mechanism of activity of longer peptides such as the 12-mer is presently under investigation.

O 002 CHARACTERISTICS OF ENDOGENOUSLY PROCESSED PEPTIDES BOUND TO CLASS I MHC MOLECULES, Stanley G. Nathenson[1], Grada van Bleek[1], Monica Imarai[1], Ken-ichiro Shibata[2], Sebastian Joyce[1], Rui Sun[1], Weiguo Zhang[1], Earl Goyarts[1], Gilbert Kepecs[1], Kiyotaka Kuzushima[1], and Edith Palmieri[1], [1]Albert Einstein College of Medicine, NY 10461, [2]Hokkaido University, Sapporo, Japan.

We have developed an approach to define the precise characteristics of endogenously processed peptides bound in situ to MHC molecules using a model viral antigen system. This approach was also extended to analyze constitutively bound self peptides. In addition, the role of each residue of vesicular stomatitis virus (VSV) N52-59 peptide in interacting with the MHC and/or TCR was determined.

In the viral studies, metabolically radiolabeled VSV infected cells were used as a source of K^b and D^b from which the complexed peptides were released by acid treatment and isolated by gel filtration and HPLC. We established that the major epitope recognized by VSV cytotoxic T cells existed in situ as an octomeric peptide associated with K^b. We next extended the approach to the analysis of endogenous "self" peptides in order to probe the range and characteristics of naturally bound peptides as well as to examine the effect of diversity in the architecture of the MHC antigen binding groove on the set of bound self peptides. We studied K^b, and its paralogous gene product D^b, and two naturally occurring mutants of K^b, K^{bm1} and K^{bm8}. The latter differ from the K^b wild type by small (one to two) localized clustered changes in the antigen binding cleft. We found that naturally processed peptides are approximately 7-10 amino acids long, and that there was a major motif of Y or F residues at positions 3 and 5 in many of the individual peptides bound to K^b. Study of the K^b mutant MHC molecules showed that clustered discrete changes in different regions of the walls of the groove exerted an absolute effect by changing the subsets of "self" peptides bound to these MHC molecules. For K^{bm1}, the binding of the characteristic major set of K^b associated peptides with position 3Y, or both 3Y and 5Y was abrogated; although the molecule still bound several peptides with Y at position 7 that also bind to K^b. Thus, differences in binding selectivity between K^{bm1} and K^b appears to be a major determinant for observed alterations in the in vivo immune responses in the mice bearing these mutations.

The role of individual amino acid residues in the VSV nucleocapsid derived antigenic octapeptide, N52-59 (R-G-Y-V-Y-Q-G-L) in MHC and/or TCR interaction was determined. By using a competition assay to measure the binding of single A substituted peptides, we found that the positions 3Y, 5Y, and 8L in the peptide were K^b contact (anchor) residues. Positions 1R, 2G, 4V, 6Q, 7G, of the peptide were identified as TCR residues by testing the peptide analogues for their capacity to sensitize T Cells. The octomer N52-N59 was the optimal length of the peptide for binding. The absolute requirement for this length together with the intermingled pattern of MHC and TCR contact residues is consistent with the conclusion that the peptide is constrained by the MHC cleft in an extended conformation. Furthermore we found that at position 1, the R side chain functioned as a TCR contact residue whereas the main chain amino group appeared to bind the MHC molecule. Our conclusions fit with the concept that the peptides bound to MHC molecules lie along the entire length of the antigen binding groove in an extended conformation in which a portion of the residues (five in this case) appear to be available for contact with the TCR, whereas other residues (three for this peptide) form anchors for interaction with the MHC molecule.

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The Class I Pathway of Antigen Presentation

O 003 INTRACELLULAR TRANSPORT OF MHC MOLECULES, H.L. Ploegh, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

MHC molecules present short peptides to the antigen-specific receptor on T cells. The two types of MHC molecules, MHC Class I and MHC Class II, sample distinct sets of peptides, that originate from extracellularly added proteins, and from polypeptides synthesized by the antigen presenting cell itself, respectively. This difference is reflected in the ways in which the antigen-presenting cell handles traffic of MHC-molecules.

A combination of biochemical and cytochemical techniques, applied to normal and mutant cell lines of human and mouse origin, was used to study intracellular traffic of MHC molecules.

The intracellular transport routes will be described and discussed in the context of interactions of MHC molecules with peptides. Novel approaches to the study of peptide-MHC interactions will be discussed, with special emphasis on differences between MHC Class I alleles.

O 004 PEPTIDES NATURALLY PRESENTED BY MHC CLASS I MOLECULES. Hans-Georg Rammensee, Kirsten Falk, Olaf Rötzschke, Max-Planck-Institut für Biologie, Abteilung Immungenetik, W-7400 Tübingen, Germany.

The peptides naturally presented by MHC class I molecules can be isolated from whole cells or from purified MHC molecules by acid extraction followed by HPLC separation. Analysis of such peptides representing normal self peptides, viral peptides, minor histocompatibility peptides, and peptides recognized by alloreactive T cells indicated the following: i) Each normal class I-expressing cell simultaneously presents hundreds or thousands of peptides derived from cellular proteins. ii) The peptide content of cells is dependent on the expression of MHC class I genes. For example, the H-2K^b-restricted minor H peptide H-4^b is not detected in H-4^b cells not expressing K^b. iii) Cells may contain peptides that are not dependent on coexpression of MHC molecules. For example, H-4^b cells (irrespective of MHC expression) contain an additional peptide recognized by H-4^b-specific, H-$2K^b$ restricted CTL with low efficiency. Such MHC-independent peptides may be precursors for the peptides finally presented by MHC molecules. iv) Several peptides naturally presented by MHC class I molecules have been identified, for example, ASNENMETM (D^b-restricted) and TYQRTRALV (K^d-restricted) are naturally presented by influenza-infected cells. v) the peptides presented by class I molecules adhere to allele-specific rules or motifs, which require allele-specific lengths (8 residues for K^b, 9 for K^d, D^b, and HLA-A2.1) and correct occupancy of two anchor residues, one of which is always C-terminal and is aliphatic for the alleles mentioned. Based on these data, we propose the following model for peptide processing in the MHC class I pathway. Proteases in various cellular compartments (e.g., cytosol) are degraded by an endopeptidase cutting C-terminal of aliphatic residues. The resulting peptides that share the C-terminus but not the N-terminus with the final product are then translocated to the compartment of MHC class I assembly and bind there to MHC molecules. Binding requires accomodation of side chains of the allele-specific anchor residues into the allele-specific pockets of MHC molecules. Finally, the N-terminal residues of the precursors are trimmed by an hypothetical protease activity. Thus, class I molecules have an instructive as well as a selective role in processing.

Non Classical MHC Antigens

O 005 MOLECULAR DEFINITION OF THE MATERNALLY TRANSMITTED ANTIGEN, MTA. Kirsten Fischer Lindahl, Evan Hermel, Elena Grigorenko, Elsy P. Jones, and Chyung-Ru Wang, Howard Hughes Medical Institute, Departments of Microbiology and Biochemistry and Immunology Graduate Program, University of Texas Southwestern Medical Center, Dallas, TX 75235-9050

Major histocompatibility complexes (MHC) have more class I genes than they need to code for the one to three classical molecules that present antigen to $CD8^+$ cytotoxic T cells (CTL). The excess, non-classical class I genes range from a few in humans to more than sixty in rats; although many of these genes are known to be expressed, their origin and biological role is still unclear. The mouse transplantation antigen, Mta, provided the first example of antigen presentation by a non-classical class I molecule. The antigen is detected on the surface of cells of a variety of tissues by CTL that do not show classical MHC restriction, and it has three components: MTF, HMT and β_2-microglobulin (1). MTF is a peptide derived from the amino-terminus of the mitochondrially encoded (hence maternally transmitted) ND1 protein, and a conservative polymorphism in the sixth position accounts for the four allelic forms of the antigen. Synthetic peptides can mimic MTF and restore surface expression of Mta in the RMA-S cell line, which is deficient in peptide transport (2). HMT is an MHC class I heavy chain, encoded by the *H-2M3* gene, which is located in a region, distal of *Tla*, that contains at least eight other class I genes of unknown function. The extracellular domains of M3 have the same length as classical H-2 and HLA molecules, but the cytoplasmic tail is only eight amino acids long. Residues considered critical for folding and function are conserved in M3, with the exception of a Phe at 171 rather than the consensus Tyr, which is thought to interact with the usual, free amino-group at the end of a bound peptide. *M3* is highly conserved, and three alleles have been sequenced (3). The *b* allele, defined as a null allele that fails to present MTF, differs by only two amino acids in the mature protein; site-directed mutagenesis has shown that the change from Leu to Gln at position 95 abolishes recognition by Mta-specific T cells. Expression of *M3* is detectable as early as day 8 of gestation, it is highest in the thymus, and it can be elevated by γ-interferon. *H-2M3* and an expressed, orthologous rat class I gene are more similar to each other to any class I gene of their own species, suggesting conservation of a specialized function (4).

1. K. Fischer Lindahl *et al.*, Annu. Rev. Immunol. 9:351-372, 1991.
2. E. Hermel *et al.*, Int. Immunol. 3:407-412, 1991.
3. C.-R. Wang *et al.*, Cell 66:335-345, 1991.
4. C.-R. Wang *et al.*, in *NATO Conference on Mhc Evolution*, ed. J. Klein, Springer Verlag, in press.

Accessory Molecules Interacting with MHC

O 006 MECHANISM AND FUNCTIONAL RELEVANCE OF LIGAND BINDING TO SURFACE CLASS I MOLECULES, Ted Hansen, Joseph Smith, Martha Alexander, John Gorka, W.-R. Lie, and Janet Connolly, Washington Univ. Sch. of Med., Depart. of Genetics, St. Louis, Missouri 63110.

To elucidate the structural and functional consequences of the interaction of peptide with class I, we have studied the L^d molecule of the mouse. L^d is distinguished by its readily accessible ligand binding site, suggesting a more limited pool of endogenous peptides bind specifically to L^d (1). Furthermore, a significant proportion of both intracellular and surface L^d molecules can be detected in a partially-denatured, non-peptide associated conformation designated L^dalt (2). Exploiting these unique features of L^d we established binding assays to monitor the intracellular or extracellular interaction of class I with ligand. To study the intracellular interaction of peptide with L^d, cell lysates were used (3). In cell lysates, peptide ligand was found to convert L^dalt molecules to properly folded L^d, establishing their precursor-product relationship. Conversion of L^dalt to L^d affected almost exclusively immature (Endo H^s) class I molecules. Thus, this in vitro antigenic conversion of nascent L^dalt molecules accurately mimics in vivo folding, demonstrating that intristic properties of immature class I molecules or their associated chaperonins are maintained in cell lysates. To study the mechanism and consequences of ligand binding to surface class I molecules, live cells were cultured in media containing known L^d peptide ligands. In comparison with other class I molecules, L^d has a relatively rapid surface turnover and exogenous peptide dramatically prolonged L^d surface half-life (1,3). By contrast L^dalt molecules are stably expressed on the surface and their half-life is unaffected by exogenous peptide. Thus in contrast to immature L^dalt molecules, surface L^dalt molecules appear refractory to peptide binding and consequent folding. To better quantitate the interaction of surface L^d with ligand, live cells were grown with iodinated peptides(2,3). Using this assay, peptide binding to surface L^d was found not to depend on new expression at the cell surface, or β_2m exchange. However, peptide binding to L^d did correlate precisely with the amount of β_2m association implying that predominantly L^d:β_2m heterodimers bind peptide. To determine whether the surface binding of peptide occurs to "empty" versus previously "occupied" L^d molecules, several analytical approaches have been taken. Our data clearly indicate peptide ligands have relatively rapid surface dissociation and that a significant proportion of the peptide binding occurs to previously peptide-occupied L^d molecules. To determine the functional significance of peptide binding at the cell surface, immunogenic peptides were used to induce L^d-restricted CTL. Although peptides have generally not been found to prime CTL, L^d-restricted CTL were readily induced with peptide alone (4). The extraordinary ability to load L^d molecules with peptide allowed us to precisely quantitate the role of determinant density in CTL responses (4). We found that the primary stimulation of peptide-specific CTL requires higher doses of peptide than subsequent target cell sensitizations. Furthermore, peptide-specific clones were found to differ widely in both their determinant density requirement and their CD8 dependency (4). Thus CTL readily discriminate differences in determinant density implying that surface binding of peptide would have a profound effect on the immune system.

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4. Alexander, M., et al., J. Exp. Med. 173:849 (1991).

O 007 LYMPHOCYTES FUNCTIONS AND ONTOGENY IN GENE-TARGETTED MUTANT MICE, Tak W. Mak, Amin Rahemtulla, Marco Schilham, Dow R. Koh, Drew Wakeham, Julia Potter, Kenji Kishihara, Dawn Gray, Christopher Paige, Richard Miller, Wai-Ping Fung-Leung, The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario M4X 1K9

T lymphocytes recognize their antigen peptides and Major Histocompatibility Complex products with the use of their T cell antigen receptors (TcR). In addition to the α and β chains of TcR, the interaction between T cells and their target cells or antigen presenting cells is also assisted by a series of other cell surface polypeptides. Most notable of these are CD4 and CD8, which are selectively expressed on mature helper/inducer and killer/suppressor T cells, respectively. Upon engagement of their ligands, a series signals are being transduced intracytoplasmically via some of these molecules and their associated proteins. Perhaps the most important enzyme in this signal transduction process is the lymphocytes specific tyrosine kinase lck. In an attempt to gain better understanding on the roles of these molecules in T lymphocyte functions and ontogeny, we generated a series of mutant mice with disruptions in these genes. These mutant mice are being analysed in order that we can evaluate the importances of these genes in T cell development.

In addition to studying development, the roles of these molecules in autoimmune diseases, transplant rejection and tumor injection can also be analysed in the appropriate experimental mouse strains carrying mutations of these genes.

O 008 UNUSUAL HLA-B ALLELES IN TRIBES OF SOUTH AMERICAN INDIANS. Peter Parham[1], Monica P. Belich[1], J. Alejandro Madrigal[1], Roberto Luz[2] and Maria Luiza Petzl-Erler[2]. [1]Stanford University School of Medicine, Stanford, CA. 94305, [2]Federal University of Parana, 81531 Curitiba, PR, Brazil.

For over 15 years it has been appreciated that HLA polymorphism in Amerindian tribes is low compared to other populations. At each locus 3-5 antigens predominate and similar groups of antigens are found throughout the Americas. It has been debated as to whether the limited polymorphism is due to founder effects or to selection. We have isolated and characterized class I HLA alleles from two tribes of Brazilian Indians. All of 6 HLA-B alleles isolated gave novel sequences not previously seen in other populations.One of these HLA-B alleles has unusual substitution at position 245 of the alpha 3 domain that is predicted to affect interactions with CD8. In contrast the HLA-A alleles were familiar. These results indicate that the HLA-B locus has undergone a marked evolution in the 15-40,000 years since humans entered America from Asia across the Bering land bridge. Differences in HLA-B polymorphism between the two tribes suggest that although individual tribes have limited polymorphism, the Amerindian population has an extensive and unique class I HLA polymorphism. These results will be discussed in terms of selection for antigen-presenting functions.

Structure and Function of the Invariant Chain

O 009 FUNCTIONAL ROLE OF THE CLASS II-ASSOCIATED INVARIANT CHAIN, Peter Cresswell, Ravi Avva, Carilee Lamb, John Newcomb, Janice Riberdy, and Paul Roche, Howard Hughes Medical Institute and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510.

HLA-DR molecules rapidly associate with the invariant chain upon synthesis in the endoplasmic reticulum. They remain associated during transport through the Golgi apparatus and until the complex reaches an endosomal compartment. Here the invariant chain is proteolytically degraded and the HLA-DR molecules released and expressed on the cell surface. During this process, the invariant chain performs two functions which are essential for proper antigen processing. First, it prevents peptide binding to the nascent DR αβ dimer upon synthesis in the endoplasmic reticulum, and second, it directs the class II complex to acidified endosomes, defined as such by the intracellular co-localization of HLA-DR, invariant chain, and internalized influenza virus. The molecular aspects of the regulation of these processes will be discussed.

O 010 INVARIANT CHAIN: VARIATIONS IN FORM AND FUNCTION

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Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

Invariant chain (Ii) rapidly associates with class II after synthesis in the ER and the complex remains intact throughout intracellular transport. Prior to insertion of class II in the plasma membrane, the majority of Ii is degraded in an endosomal compartment that is thought to represent the antigen processing compartment. To address the role of Ii in class II-restricted antigen presentation events, we have established a series of transfected cells that express class II with and without co-expression of Ii. We have identified three distinct effects of Ii on class II biosynthesis and function, two of which are associated with distinct molecular forms of Ii.

1. Ii can function as a chaperone protein, facilitating class II folding in the ER. Class II that folds in the absence of Ii loses certain monoclonal antibody epitopes, is inefficiently transported to the Golgi, and is more sensitive to Golgi glycosylation events.

2. Expression of Ii enhances the ability of class II-positive cells to stimulate both primary allogeneic and mitogenic T cell responses. This effect can be attributed entirely to the chondroitin sulfate form of Ii (Ii-CS), as transfection of a site specific mutant that eliminates the chondroitin sulfate addition site, but still allows Ii to impart normal chaperone functions, does not allow for primary T cell stimulation. Because of the unique localization of Ii-CS at the cell surface, we propose that this proteoglycan may function as an accessory molecule facilitating T cell adhesion or activation.

3. The alternatively spliced p41 form of Ii can enhance the efficiency of antigen presentation for a subset of antigens. Class II expressed in the absence of Ii or in the presence of the p31 form of Ii present antigen to T cells equally. However, co-expression of p41 allows for an increase in the efficiency of antigen presentation as much as 100-fold, depending on the specificity of the T cell line tested. This effect is independent of the role for Ii in class II folding, as p31 is sufficient to impart the folding effects, without enhancing antigen presentation.

These studies demonstrate that the various molecular forms of Ii can influence unique events in the biosynthesis and immunological recognition of class II molecules. Each of the functions attributed to p31 (class II folding and transport) and p41 (antigen presentation, as well as class II folding and transport) and to Ii-CS (T cell activation) could be independent from one another. Alternatively, the recent observation that Ii-class II complexes may exist as multimers during intracellular transport raises the possibility that the various forms of Ii may collaborate on directing the transport of an entire cohort of class II through appropriate compartments on the way to the cell surface. Experiments are in progress to determine the exact mechanisms whereby the various forms of Ii can modify class II biosynthesis and antigen presentation.

Intracellular Aspects of Class II Restricted Antigen Processing

O 011 INTRACELLULAR TRANSPORT PATHWAYS FOR ANTIGEN PRESENTATION BY CLASS I AND CLASS II HISTOCOMPATIBILITY MOLECULES, F.M. Brodsky, L. Guagliardi, and B. Koppelman, University of California, School of Pharmacy, San Francisco, California, 94143.

Peptides bound to class I histocompatibility molecules are recognized by T cell receptors during the development of an anti-viral immune response. T cells respond to peptides derived from cytoplasmic viral proteins as well as viral membrane proteins, indicating that a pathway exists for the transport of proteins or peptides from the cytosol into the compartment(s) where the MHC class I molecules assemble. To investigate this pathway, we have developed an *in vitro* assay for the transport of peptides into microsomal vesicles. We have observed transport of peptides derived from antigenic HIV gag and influenza B nucleoprotein sequences, but transport of a third randomly selected peptide was not detected, suggesting specificity of the transport process. We were not able to demonstrate ATP-dependence of this peptide transport process using apyrase and an ATPase inhibitor. This result was unexpected in light of the recent identification of MHC-linked genes with homology to "ATP-binding cassette" transporters, that have been proposed to mediate peptide transport.

Class II molecules can present peptides from endocytosed antigen as well as endogenous antigen. To determine whether intracellular trafficking and the associated invariant chain could be responsible for the versatility of peptide binding displayed by Class II histocompatibility molecules, their intracellular location was mapped by immunoelectron microscopy. Class II molecules associated with invariant chain, *en route* to the cell surface were observed to intersect the antigen uptake pathway in both early and late endocytic compartments. Proteolytic enzymes Cathepsin B and Cathepsin D colocalized in these compartments, along with mature Class II molecules. Since very little mature Class II is internalized by these cells, the endosomal mature Class II is probably generated by dissociation of invariant chain, which requires activity of proteases Cathepsin B and D. The presence of mature Class II molecules in both early and late endocytic compartments indicates that dissociation of invariant chain occurs throughout the endocytic pathway. This maturation is most likely accompanied by peptide binding, as associated invariant chain obscures the peptide binding site, suggesting a mechanism by which Class II molecules can bind peptides generated in several different endocytic compartments. Class I molecules were not observed in endocytic compartments, suggesting that a trafficking pathway specific to invariant chain-associated Class II molecules contributes to their antigen presenting function.

O 012 MOLECULAR DISSECTION OF VESICULAR TRANSPORT, James E. Rothman

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Insights into the molecular mechanisms involved in forming and fusing transport vesicles have come from fractionation of cytosol requirements using a cell-free reconstitution of intra-cisternal transport in the Golgi as an assay. Transport between successive cisternal compartments appears to be due to the budding of non-clathrin coated vesicles, which lose their coats upon contacting the target cisterna, and then fuse. A complex of proteins from the cytosol (coatomer) is the likely assembly unit of the coat. An NEM-sensitive fusion protein (NSF) has been purified, that utilizes ATP hydrolysis to help power the fusion of uncoated vesicles with target cisternae. NSF assembles with a series of attachment proteins (SNAPs) and a multi-SNAP receptor on the Golgi surface to create an active fusion complex. This complex disassembles in a reaction also requiring ATP hydrolysis, linked closely to the fusion process.

Superantigens and Others

O 013 T CELL RECOGNITION OF BACTERIAL AND RETROVIRAL SUPERANTIGENS, H. Robson MacDonald, Thomas Herrmann, Gary A. Waanders, Rosemary K. Lees, Selene Baschieri, Alexander Lussow, Werner Held, Alexander Shakhov, and Hans Acha-Orbea, Ludwig Institute for Cancer Research, Ch. des Boveresses 155, 1066 Epalinges, Switzerland.

In contrast to conventional (peptide) antigens, superantigens preferentially activate T cells bearing restricted T cell receptor β-chain variable (TCR Vβ) domains. Two major classes of superantigens have been defined so far 1) the exotoxins of Staphylococcus aureus (and related microbial products) 2) the so-called «Mls» antigens, which are now known to be encoded by an open reading frame (orf) in the 3' LTR of mouse mammary tumor virus (MMTV).

In order to stimulate T cells, superantigens must be presented by cells expressing MHC class II molecules. In the case of bacterial enterotoxins, this requirement reflects high affinity binding of the enterotoxins to the MHC molecules themselves. No direct binding of MMTV orf proteins to MHC class II has yet been described, although it seems probable that this is the case. Once bound to MHC class II, superantigens then presumably interact directly with TCR Vβ. Sequence comparisons and transgenic mouse experiments suggest that the C-terminal amino acids of the MMTV orf protein are important for Vβ specificity. The enterotoxin residues contacting Vβ remain to be defined.

In vivo, T cell recognition of superantigens can lead to either stimulation or non-responsiveness (anergy). We have compared in vivo immune responses to Staphylococcal enterotoxins and MMTV. In both cases, initial clonal expansion of cells bearing TCR Vβ specific for the injected superantigens was followed by cell death and anergy. The latter phenomenon was manifested as a specific lack of proliferation (and interleukin-2 production) in response to the injected superantigen. Molecular aspects of this anergic state are currently under investigation.

O 014 THE T CELL REPERTOIRE OR YOU CAN FIND SUPERANTIGENS UNDER LOTS OF ROCKS,
Philippa Marrack, Xavier Paliard, John Donahue, Jill Callahan, Yongwon Choi, Patrice Hugo, Leszek Ignatowitz, James McCormack, Mark Scherer, Gary Winslow, Brian Kotzin* and John Kappler, HHMI, Department of Medicine, and Department of Pediatrics*, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Superantigens are proteins which bind to Class II MHC and engage T cell receptors primarily by binding to Vβ. These antigens cause deletion of developing thymocytes which bear target Vβs and stimulation and/or death or inactivation of mature T cells. Because superantigens have such potent effects on the immune systems of mouse and man, and because superantigens are widely expressed by the microbial world we have studied various human diseases for signs that superantigens may have played some role in them. Recently, for example, we have shown that a superantigen has probably affected the immune systems of individuals suffering from rheumatoid arthritis. In a search for the origin of this superantigen we are examining common human viruses for evidence that they produce superantigens.

O 015 T CELL RESPONSES TO MLS-ANTIGENS, Susan R. Webb[1], and Jonathan Sprent[1], [1]The Scripps Research Institute, La Jolla.

Mls^a antigens, now known to be encoded by endogenous mtv-7 provirus sequences, are cell-surface superantigens. Whereas conventional antigens are recognized by variable elements of both the α and β chain of the TCR, recognition of superantigens depends primarily on expression of particular TCR Vβ chains, The Vβs associated with recognition of Mls^a antigen are Vβ6, Vβ8.1, Vβ7 and Vβ9. In mouse strains expressing Mls^a, and permissive MHC molecules, T cells bearing these TCR undergo clonal deletion in the thymus.

We previously showed that injection of adult thymectomized Mls^a-negative mouse strains with Mls^a-bearing cells leads to Mls-specific tolerance of the host T cells and disappearance of the majority of Vβ6$^+$ CD4$^+$ T cells. The elimination of Vβ6$^+$ cells is preceded by marked expansion of these cells, which suggests that tolerance of mature T cells can be a consequence of a prior immune response. Both CD8$^+$ T cells and B cells induce Mls^a tolerance of mature T cells and expansion/deletion of Vβ6$^+$ cells. This sequence of events is less marked for Vβ8.1$^+$ T cells than for Vβ6 cells. Why Vβ8.1$^+$ T cells are less susceptible to both expansion and deletion than Vβ6$^+$ cells is unclear. This difference cannot be attributed to unequal precursor frequencies because FACS-purified Vβ8.1$^+$ and Vβ6$^+$ cells give equivalent responses to Mls^a antigen in vitro. The explanation we currently favor is that the eventual deletion of mature T cells responding to Mls^a antigen in vivo is largely a reflection of high avidity binding. Vβ8.1 cells bind less avidly to Mls^a stimulator cells than Vβ6$^+$ cells and are thus less susceptible to elimination. Binding avidity might be affected by the intrinsic affinity of Vβ-Mls^a interactions and also by the number of class II molecules on the APC presenting Mls^a. With regard to this second possibility, it is of interest that the deletion of Vβ6$^+$ cells in strain combinations expressing only I-A molecules (D1.LP → B6) is much less marked than in combinations expressing both I-A and I-E molecules (AKR/J → B10.BR).

In view of the finding that CD8$^+$ T cells are powerful stimulators of anti-Mls^a responses and tolerance in vivo, we are re-examining our earlier failure to detect stimulation by CD8$^+$ cells in vitro. With the addition of PMA and the cytokines IL-1 and IL-6, we have recently found that CD8$^+$ cells can stimulate a weak but significant in vitro anti-Mls^a-MLR. Nonetheless, even with highly purified CD8$^+$ stimulators and CD4$^+$ responders, anti-IA antibodies directed to the stimulator cells block this in vitro response. We are currently exploring the possibility that mouse T cells, like T cells in other species, can express low levels of class II molecules following activation.

The Twilight Zone: Natural Killers and Hybrid Histocompatability

O 016 BIOLOGY AND GENETICS OF MURINE BONE MARROW CELL TRANSPLANTATION, Michael Bennett, Amaha Aberra, Shehla Ansari, Katarina Blomer, Kirsten Fischer Lindahl, Porunelloor Mathew , Colleen O'Brien, Lawrence Yu, Lorraine Flaherty[1], and Vinay Kumar, University of Texas Southwestern Medical Center, Dallas TX, and [1]New York State Department of Health, Albany, NY

Hypothesis: Bone marrow cell(BMC) transplantation includes three 'forces'. Stem cells are rejected by NK and/or $CD8^+$ T cells. Alloreactive T cells of the marrow inhibit rejection. Host T cells reject the marrow T cells. Evidence: 1) NK cells recognize Hemopoietic histocompatibility-1 (Hh-1) antigens on stem cells; NK $5E6^+$ and $10A7^+$ subsets specifically reject $Hh\text{-}1^d$ and $Hh\text{-}1^z$ marrow cells, respectively. SCID mice or purified SCID NK cells devoid of $CD3^+$ cells transferred to irradiated mice deprived of their own NK cells can reject Hh-1 disparate, but not class I disparate, marrow cells specifically. 2) $CD8^+$ T cells recognize class I Ags on stem cells. 3) BMC depleted of T cells, BMC of SCID mice, and liver cells of newborn mice with an immature immune system are hypersensitive to rejection. 4) Alloreactive lymph node T cells are rejected by irradiated mice. To our surprise, this resistance was not mediated by $NK1.1^+$ or $5E6^+$ NK cells. Instead, host T cells are probably the effectors, since athymic nude and SCID demonstrate very weak resistance to the T cell grafts. Marrow T cells may be rejected by similar cells.

Hypothesis: Three types of H-2 MHC genes contribute to the formation of target antigens on stem cells recognized by NK cells. These include Hh-1r and Hh-1s (regulatory and structural) genes which map between H-2S and H-2D, and class I heavy chain genes. Evidence: 1) BMC of D8 mice, which are D^d class I to B6 $H\text{-}2^b$ transgenic mice, lack $Hh\text{-}1^b$, i.e. are not rejected by B6D2F1 hybrids. NK cells of D8 mice reject $H\text{-}2^b$ BMC, supporting the idea that NK cells survey target cells for 'self' class I antigens and reject BMC which fail to express such antigens. 2) However, D8 BMC are rejected by NK cells of irradiated host mice that themselves do not express D^d. This is consistent with the idea that certain NK cells can recognize particular class I antigens. 3) Hh-1 maps at or near H-2D, but BMC from mice of several H-2S/D recombinant strains differ for Hh-1 from both parents. Some, e.g. B10.R106, are Hh-1 null due to gain of Hh-1r genes. Others, e.g. B10.RQB1, have decreased Hh-1 expression due to loss of both Hh-1r and Hh-1s genes. 4) BMC of B6.R4 mice, derived from B6 ($Hh\text{-}1^b$) and RIII ($H\text{-}2^r$, Hh-1 null) parents, share the fate of D8 BMC, as if D^d and D^r express similar determinants. The D^r associated determinant must be down-regulated by genes centromeric of $H\text{-}2D^r$, since $H\text{-}2^r$ BMC are not rejected by B6 or any other strain of mice tested. 5) Somewhat similar findings were made in studies of B10.SP2 (Spretus) mice and their H-2S/D recombinants with $H\text{-}2^s$, suggesting that genes centromeric of H-2D determine whether or not BMC are rejected by allogeneic hosts.

What Do T Cell Bearing Gamma Delta Receptors See?

O 017 RECOGNITION OF HSP PEPTIDES BY γδ T CELLS, Willi K. Born[1], Yang-Xin Fu[1], Harshan Kalataradi[1], Simon Carding[2], Beverly Koller[3], Douglas Young[4], and Rebecca L. O'Brien[1], [1]National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206; [2]University of Pennsylvania School of Medicine, Philadelphia, [3]University of North Carolina, Chapel Hill, and [4]Hammersmith Hospital, London.

Despite many similarities with αβ T cells whose functions center around the defense against pathogens, the biological role of γδ T cells has remained uncertain. As in the case of αβ T cells and B cells, ligand specificities might provide clues to function; however, only few antigens have been identified that stimulate γδ T cells. Presently, the best characterized examples are 60 kD heat shock proteins (HSP-60). We have found that a major subset of murine γδ T cells in thymus and spleen express T cell receptors (TCR) capable of interacting with HSP-60. HSP-60 reactive γδ cells have also been identified in man. γδ TCRs reactive with HSP-60 are heterogeneous but exhibit common features. In mice, all HSP-60 reactive γδ cells reported to date express Vγ1-J4-C4 and a limited number of Vδ genes (frequencies in C57BL/10 mice: Vδ6.3>Vδ6λ12>Vδ4>other). In contrast to HSP-60 reactive αβ T cells, and surprisingly in view of the heterogeneity of involved receptors, ligand specificities of all HSP-60 reactive γδ cells in mice are focused on a single region of HSP-60, within amino acids 180-196 of mycobacterial HSP-60. This selectivity is evident in stimulation experiments with synthetic peptide antigens. In trying to define minimal requirements for antigenic stimulation of γδ T cells, we have analyzed truncated peptides, using a panel of HSP-60 reactive γδ TCR bearing hybridoma clones as responder cells. Reminiscent of the antigenic requirements of MHC class I-restricted αβ T cells, all hybridomas could be stimulated with 9-mer peptides. However, individual cells differed in peptide preferences, suggesting a role for TCR junctional sequences and/or antigen processing requirements. Stimulation with species-specific peptide antigens showed that HSP-60 reactive γδ cells recognize not only mycobacterial but also autologous, yeast and other HSP-60 derived sequences, although preferences of individual clones may vary. Thus, HSP-60 reactive γδ cells may be focused on an antigenic "motif" rather than any particular foreign antigen. If further γδ T cell subsets have similarly focused specificities, the number of such "γδ motifs" could be rather small (10-20). To further define requirements for antigen stimulation, we have selected HSP-60 reactive hybridoma cells lacking MHC class I molecules. Class I-negative clones lost all antigen reactivity but responses could be reconstituted in the presence of antigen presenting cells. The correlation between MHC class I expression and antigen reactivity strongly suggests that HSP-60 reactive γδ cells require antigen presentation via MHC class I or related molecules. It thus should be possible to isolate naturally processed antigens for γδ cells in a fashion similar to that established for αβ T cells, by acid elution, and without prior knowledge of the precise nature of the antigen presenting molecule. In conclusion, our data reveal close similarities in antigen recognition of γδ T cells and MHC class I-restricted αβ T cells. In contrast to αβ T cells, however, γδ T cell specificities appear to be largely a property of entire subsets instead of individual clones. This could imply that γδ cell functions are different from αβ T cell functions. More specifically, γδ cell reactivity may be expected in situations where "teamwork" is more important than exquisite specificity.

O 018 DISTINCTIONS BETWEEN THE SELECTION AND SIGNALING PATHWAYS USED BY γδ AND αβ THYMOCYTES AND T CELLS, Stephen M. Hedrick[1], Alexander Dent[1], Nicki McRoberts[1], Jeffrey Bluestone[2], Faith Wells[3], and Louis A. Matis[3], [1]University of California, San Diego, La Jolla, CA 92093-0063, [2]The Ben May Institute, University of Chicago, Chicago, IL 60637, [3]National Cancer Institute, Frederick, MD 21702.

The problem of antigen recognition in γδ T cells may be determined in part by the selection mechanisms that take place in the thymus. We have developed models of T cell selection using transgenic mice that have incorporated genes encoding either the α- and β-chains or the γ- and δ-chains of an antigen-specific T cell antigen receptor (TCR). These mice serve as models for the study of positive and negative selection mechanisms. Experiments with αβ transgenic mice show that immature αβ thymocytes undergo a rapid active cell death when cultured with antigen presenting cells. This cell death is highly dependent on the activation of PKC, but is not blocked by the addition of cyclosporin A (CsA). The deletion of thymocytes cannot be overcome by the addition of lymphokines such as IL2 and IL7. In contrast, the activation of lymph node T cells requires PKC activation and is highly sensitive to the effects of CsA. By comparison, the mechanism of γδ thymcoyte clonal deletion appears to be quite different. Deletion of thymic γδ T cells can be overcome by the addition of IL2. These thymocytes appear to be selected in a manner similar to peripheral T cells. Antigen induces blast transformation, and the expression of IL2 receptors, and over a period of several days the cells will die in the absence of lymphokines. We conclude that negative selection of γδ thymocytes operates as a form of clonal deletion, but one that is mechanistically distinct from αβ thymocyte deletion.

O 019 POSITIVE AND NEGATIVE SELECTION OF Vδ4⁺ INTESTINAL INTRAEPITHELIAL LYMPHOCYTES, Leo Lefrancois, University of Connecticut School of Medicine, Farmington, CT.

A significant number of intraepithelial lymphocytes (IEL) of the small intestine express the γδ T cell antigen receptor (Tcr). One of the major variable (V) δ regions that is employed by these cells is Vδ4 as determined by a monoclonal antibody specific for this V region. We have previously shown that Vδ4⁺ IEL undergo a selection process in certain strains of mice that results in a substantial increase in Vδ4⁺ IEL. Thus, ~30% of IELs from mice with the major histocompatibility complex (MHC) haplotype H-2^b are Vδ4⁺ while ~60% of IELs from H-2^k mice are Vδ4⁺. Analysis of recombinant inbred (RI) and congenic mouse strains demonstrated that expression of the class II MHC molecule I-$E^{b,s,k}$ resulted in an increase in Vδ4 usage. F_1 analysis suggested that positive selection was responsible for the increase. However, we have now analyzed IEL from H-2^b mice transgenic for I-E, and did not observe an increase in Vδ4⁺ cells. This result suggests that other selecting element(s) are involved in Vδ4 selection. Further evidence for this possibility was obtained from analysis of IEL from AKR/J mice. This mouse strain is the only exception to the rule that high numbers of Vδ4⁺ IELs are present in H-2^k mice. Thus, AKR/J IEL contain ~25% Vδ4⁺ cells. While IELs from B6.AKR mice were $V\delta4^{high}$, IELs from both (AKR/JxC57BL/6J)F_1 or (AKR/JxC3H)F_1 mice were of the $V\delta4^{low}$ phenotype. These results indicated that negative selection of Vδ4⁺ IELs was occurring in AKR/J mice. Moreover, the negative selection element was dominant over the positive selection element that is expressed in C3H mice. Preliminary analysis of RI strains derived from AKR/J suggest a complex selection process with perhaps multiple selecting elements. It is possible that viral superantigens similar to those involved in Vβ selection are involved in selection of γδ T cells as well. Our present efforts are concentrated on identifying the selecting antigen(s).

Class I MHC

O 100 INTRACELLULAR TRANSPORT OF CLASS I ALLELES IN HUMAN AND MOUSE MUTANT CELL LINES, Karen S. Anderson, Jeff Alexander, and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

The intracellular transport and stability of MHC class I glycoproteins is intimately associated with the availability of endogenous peptides in the secretory pathway. Two antigen processing mutant cell lines, the mouse cell line RMA-S and the human cell line T2, fail to process and present whole antigen to T cells and have low cell surface expression of class I heterodimers. It has been previously shown that transfected human alleles in T2 (except A2) are not expressed on the cell surface, while mouse alleles (except K^k) are expressed. To examine the corresponding transport phenotype of RMA-S, the human and mouse alleles A3, B27, and K^k were transfected into RMA-S with human ß-2 microglobulin and the cell surface expression of these alleles was examined. At 37°C, these alleles are barely detectable on the cell surface of RMA-S, but their expression is dramatically increased at 26°C, which presumably stabilizes "empty" heterodimers at the cell surface. In contrast , expression of these alleles in T2 is not improved upon incubation at 26°C. These results suggest that the mechanisms of transport of class I heterodimers in these cell lines may be different.

O 101 IDENTIFICATION OF ENDOGENOUS PEPTIDES ASSOCIATED WITH HLA-A2 IN THE PROCESSING MUTANT CELL LINE T2, Matthew J. Androlewicz, Maria L. Wei and Peter Cresswell, Howard Hughes Medical Institute and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

The human antigen processing mutant cell line T2 possesses a homozygous deletion of the entire HLA class II coding region, and a hemizygous deletion of the HLA class I coding region. As a result, T2 expresses no class II antigens, and expresses limited amounts of class I antigens. HLA-B5 is not expressed on the cell surface; however, HLA-A2 is expressed at ~30% the level of the parental cell line. This defect in class I processing may result from a lack of peptide transport into the ER due to the codeletion of the Peptide Supply Factor genes (RING genes). To investigate this possibility, T2 cells were metabolically-labeled, class I antigens purified by affinity chromatography, and the associated peptides isolated and analyzed by reverse-phase HPLC. We found that while neither class I antigen possessed a full complement of peptide as indicated by control cells, A2 consistently contained three peptides. Sequence analysis of the peptides associated with A2 revealed that they possessed characteristics consistent with the known allele-specific peptide motif for A2. Furthermore, two of the peptides (an 11-mer and a 9-mer) were derived from the leader sequence of the 30 kDa γ-interferon inducible protein, IP-30, described by Luster, et al. (J. Biol. Chem. 263:12036, 1988). These data suggest that lack of peptide transport results in impaired class I expression in T2, and that endogenous peptides located within the ER can still bind to class I antigens and allow for the partial expression of class I genes in the absence of the Peptide Supply Factor genes.

O 102 AN EVALUATION OF THE ROLE OF MHC-BOUND PEPTIDES IN THE RECOGNITION OF HLA-A2.1 BY ALLOREACTIVE T CELLS. Linda D. Barber and Peter Parham. Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.

Although it is well established that T cells recognize foreign antigen as peptides associated with self MHC molecules, the nature of the ligands recognized by alloreactive T cells remains poorly defined. In this study, the possible requirement of specific MHC-bound peptides is evaluated by assessing the extent to which alloreactive T cells exhibit specificity for both the allogeneic class I molecule and bound peptide. Bound peptides will be eluted from the HLA-A2.1 molecules purified from EBV-transformed B cells and these peptides will be fractionated by reverse-phase HPLC. The ability of each individual peptide fraction to sensitize the human mutant B cell line LCL 721.174 for lysis by a panel of anti-HLA-A2.1 alloreactive T cell clones will then be assessed. This cell line expresses HLA-A2.1 molecules which are devoid of peptides, and therefore it offers a mechanism by which all HLA-A2.1 molecules present at the cell surface can be loaded with a single specific peptide sequence. Consequently, the use of peptide-pulsed 721.174 as target cells will enable an assessment of the ability of alloreactive T cells to discriminate among the diverse range of endogenous peptides isolated from class I molecules. This will serve to demonstrate whether alloreactive T cells recognize the allogeneic MHC molecule *per se* or exhibit specificity for both the class I molecule and the bound peptide. If the latter is found to be the case, it will lend support to the proposal that the high frequency of alloreactive T cells responsible for graft rejection represents the sum of numerous T cell clones specific for a diverse range of peptides presented in the context of allogeneic MHC molecules. If peptides bound by allogeneic MHC molecules are found to contribute to the ligand recognized by alloreactive T cells, an attempt will be made to identify the proteins from which these peptides are derived. This will be achieved by sequencing the peptides and comparing them to proteins found in sequence databases. The results from this study should increase our understanding of the molecular basis for allorecognition and so facilitate the development of a rational basis for controlling the immunological responses to tissue allografts.

O 103 EFFECT OF HCMV INFECTION ON MHC CLASS I PROTEINS.

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Human cytomegalovirus (HCMV) has been reported to downregulate MHC class I expression without affecting the level of heavy chain mRNA in infected cells. This virus expresses a glycoprotein with sequence similarity (20%) to class I heavy chain, which is coprecipitable with β_2m when both are expressed in vaccinia virus. Therefore, it has been suggested that HCMV interferes with class I expression by competing with the heavy chain for β_2m binding. If true, this should result in a surplus of nonassembled heavy chains in HCMV infected cells.

Contrary to this expectation, we observed a drastically reduced level of free heavy chains in HCMV infected cells. The levels of free β_2m were comparable to those in uninfected cells. We could not detect viral proteins coprecipitated with either β_2m or heavy chains.

RNA blot analysis and in vitro translation of poly-A RNA showed equivalent levels of translatable class I mRNA in HCMV infected cells and control cells, suggesting a posttranslational effect of the virus. Pulse chase analysis of HCMV infected cells showed that free heavy chains as well as class I complexes could be detected after a short pulse. However, these proteins have a very short half life when compared to the situation in uninfected control cells.

Our results suggest a new mechanism by which a virus downregulates class I proteins to escape immune surveillance, namely by disturbing an early step in the assembly of class I complexes. Adenovirus, the only other virus for which a posttranslational regulation of class I expression has been described, acts in a completely different way: the viral protein E19 contains an ER-retention signal and strongly binds to class I complexes, thereby preventing their transport to the cell surface. By coinfection with HCMV and adenovirus we could show that the HCMV effect is dominant over that of Adenovirus.

O 104 DEFECTIVE ASSEMBLY OF MHC CLASS I MOLECULES IN EMBRYO-DERIVED CELL LINES, Elizabeth K. Bikoff and Elizabeth J. Robertson, Department of Obstetrics, Gynecology, and Reproductive Sciences, Mount Sinai School of Medicine, New York, NY 10029 and Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY 10032.

The absence of expression of class I transplantation antigens at early stages of development might be essential to allow the fetus to escape recognition by maternal lymphocytes. In addition to the MHC class I H chain and $ß_2$-microglobulin ($ß_2$m), antigenic peptide is an essential structural component of the class I molecule. Indeed peptide transporter molecules and possibly components of a proteolytic complex appear to be necessary for MHC class I assembly and stability at the cell surface. We recently described an embryonic cell line (EE2H3) showing a defect in MHC class I assembly. The phenotype exhibited by EE2H3 closely resembled that described for somatic cell mutants RMA-S and .174/T2. By contrast, EE2H3 was established from primary cultures of mouse embryo cells without immunoselection, and might therefore represent a normal embryonic cell type. To strengthen the argument that transporter function is developmentally regulated, the present study examined additional H-2D^d-transfected embryo-derived cell lines. To insure high levels of expression of H-2D^d and $ß_2$m protein, these genes were introduced under control of the human ß actin promoter. We found that transformants expressed abundant levels of H-2D^d H chains and $ß_2$m protein, but only small amounts of H-2D^d surface protein. Surface expression was rescued in the presence of an appropriate antigenic peptide or by treatment with interferon. Consistent with this, HAM1 mRNA was not constitutively expressed, but was inducible by interferon treatment, or during differentiation in vitro. There was a good correlation between expression of surface H-2D^d molecules and expression of the HAM1 transporter. Tolerance of the fetal allograft may thus in part be controlled at the level of peptide dependent MHC class I assembly.

O 105 CHARACTERIZATION OF PROTEINS ASSOCIATED WITH MHC CLASS I MOLECULES IN CELLS EXPRESSING THE ADENOVIRUS PROTEIN E3/19K

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The early non structural glycoprotein E3/19K of adenoviruses (Ad) binds to human class I histocompatibility antigens (human MHC or HLA) in the rough ER interfering with transport of MHC antigens to the cell surface. In immunoprecipitates from human cells that upon transfection express a murine MHC molecule and the E3/19K protein we find not only the MHC heavy chain, $β_2$ -microglobulin and E3/19K but also two additional proteins with apparent molecular weights of 100kD and 110kD, repectively. These proteins, called p100 and p110, were characterized biochemically. Peptide mapping studies demonstrate that p100 and p110 are distinct molecules and not higher molecular weight forms of heavy chain, $β_2$ -microglobulin and E3/19K. They also suggest that p100 and p110 have a similar if not identical protein backbone. Both proteins contain N-linked carbohydrates and intramolecular disulfide bonds. From all the data we envisage both being transmembrane proteins with probably several transmembrane domains. To investigate their function we follow two directions: First, p100/110 could be chaperones that promote correct folding of MHC antigens. Alternatively, p100/110 may be involved in proteolytic processing of antigens or in peptide binding to MHC. Preliminary data support the latter view.

O 106 **Binding of Synthetic Petide Antigens to Purified Antibody-Bound Class I H-2 Molecules.** Deborah N. Burshtyn and Brian H. Barber., Dept. of Immunology, Univ. of Toronto, Toronto, Canada M5S 1A8

In order to better understand the formation of class I MHC - restricted cytotoxic T-lymphocyte target structures on virus-infected cells, we have developed an *in vitro*, solid-phase method to assay the interaction of defined synthetic peptides with purified class I MHC molecules. Class I H-2 molecules, isolated from whole cell detergent lysates by adsorption to monoclonal antibodies bound to agarose beads, were assayed for the ability to bind radioiodinated synthetic peptide antigens while still bound to the immunoadsorbent beads. High-occupancy, allele-specific binding was observed for defined influenza nucleoprotein peptide antigens such as Y366-374 with H-2D^b isolated from the mutant cell line RMA-S. RMA-S is believed to contain a high proportion of empty class I molecules due to a defect in transport of peptides from the cytoplasm to the ER. However, comparable levels of peptide binding were also observed for H-2D^b isolated from non-mutant cell lines such as RMA. It is noteworthy that a similar amount of peptide binding was observed when the D^b molecules were bound to either an antibody specific for a β2m-independent epitope in the α3 domain (28-14-8S), or to an antibody specific for a β2m-dependent determinant in α1 (B22-249). Utilizing the α3-reactive monoclonal to isolate free D^b heavy chains from the β2m-negative cell line R1E.D^b, it was possible, by the addition of purified human β2m, to create complexes that bound peptide and therefore presumably homogeneous. Consistent with these findings, the addition of purified human β2m to RMA and RMA-S derived D^b molecules held through the α3 domain, increased the level of peptide binding observed. In contrast, peptide binding to the α1-bound population of molecules was not influenced by the addition of human β2m. Collectively, these results suggest that this immunoadsorbent bead binding system will provide a useful approach to the analysis of kinetic, thermodynamic and conformational parameters governing the interaction of peptide antigens and purified class I MHC molecules.

O 107 **ROLE OF HLA-B27 POCKETS IN VIRAL ANTIGEN PRESENTATION.** Beatriz M. Carreno, Christine C. Winter, Joel D. Taurog*, Ted H. Hansen+ and William E. Biddison. Mol. Immunol. Sec., NeuroImmunology Branch, NINDS, Bethesda, MD, 20892; *Dept. Internal Medicine, Univ. of Texas, Southwestern Med. Ctr., Dallas, TX, 75235; +Genetics Dept., Washington University, St. Louis, MO, 63110.

The three dimensional structure of HLA-B27 has revealed 6 depressions or pockets (A through F) extending from the peptide binding groove. Amino acid side chains of endogenous peptides resident in the groove have been shown to interact with these pockets. To determine the role these structures play in viral antigen presentation, a series of HLA-B27 mutants with single amino acid substitutions in pockets B through F have been generated. HLA-B27 amino acid residues at these single positions were replaced by those found in HLA-B37, a molecule shown by our group to bind a spectrum of peptides different from those bound by HLA-B27. The effect these mutations have in Influenza A NP 383-391 presentation to specific CTL lines was assessed by exogenous (peptide feeding) and endogenous (viral infection) loading of peptide. Feeding experiments over a wide range of peptide concentrations, showed that all HLA-B27 mutant molecules present the NP peptide as well as, or better than, wild type HLA-B27. Conversely, some mutants in pockets B and F failed to present endogenously loaded antigen following infection with Influenza A virus. Results indicate these mutations have a differential effect when antigen is loaded exogenously versus endogenously. Initial studies suggest that mutations in pockets B and F affect the rate with which viral peptide antigen dissociates from HLA-B27.

O 108 ISOLATION OF CLASS I PEPTIDES REQUIRED FOR RECOGNITION BY ALLOREACTIVE AND TUMOR-SPECIFIC CYTOTOXIC T CELLS, Suchismita Chattopadhyay, Timothy Burke and Linda Sherman, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Major histocompatibility complex (MHC) class I molecules contain endogenous peptide antigen within their antigen-binding groove, produced by the processing of normally expressed cellular proteins. Studies from this laboratory have demonstrated that alloreactive cytotoxic T lymphocytes (CTL) have specificity for both Class I MHC molecules and endogenous peptides. The vigorous response of CTLs to allogeneic tissue is due to the additive effect of individual T cells responding to complexes formed between the allogeneic MHC molecule and one of the numerous self peptides derived from intracellular proteins.

We have extracted peptides directly from class I molecules and demonstrated their ability to reconstitute allorecognition of "empty" class I molecules. This same method is now being used to isolate and identify peptides that may be recognized as tumor-specific antigens.

O 109 PEPTIDE BINDING TO HLA-A2 FROM THE MUTANT HUMAN CELL LINE, T2, Roman M. Chicz, Robert G. Urban, William S. Lane, and Jack L. Strominger, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

Peptide binding to class I HLA molecules in the endoplasmic reticulum is required for stability of the heavy chain/β_2-microglobulin complex. As the result, free heavy chain and β_2-microglobulin accumulate internally in mutants which cannot transport peptides into the ER and do not normally reach the cell surface. However, the mutant T2 (a T-cell/B-cell hybridoma) has been reported to express empty HLA-A2 molecules on the cell surface; the HLA-B locus alleles of this cell line remain in the cytoplasm. FACS analysis indicated that the surface expression of HLA-A2 on T2 cells was about 1/3 that of EBV transformed human B-cell lines, confirming previous reports. HLA-A2 was immunoaffinity purified from a lysate of T2 cells and reacted identically to HLA-A2 purified from several B-cell lines by ELISA using PA2.1, a HLA-A2 specific mAb. Peptide binding to the HLA-A2 purified from T2 cells, measured using ^{125}I labelled synthetic peptides, was low and identical to normal HLA-A2, suggesting that the HLA-A2 molecules isolated from T2 cells were not empty. Moreover, acid extraction of the HLA-A2 from T2 cells yielded peptides which were purified and separated by HPLC. Unique peptides were identified, as compared to previous reports of peptides bound to HLA-A2, and are presently being compared to peptides isolated from HLA-A2 purified from normal B lymphoblastoid cell lines. The nature of the peptides will be reported.

O 110 PEPTIDES DERIVED FROM HLA-B LOCUS SEQUENCES BLOCK HLA CLASS I DIRECTED IMMUNE FUNCTIONS, Carol Clayberger, Shu-chen Lyu, and Alan M. Krensky, Departments of Cardiothoracic Surgery and Pediatrics, Stanford University, Stanford, CA 94305

The alpha helices of the alpha 1 and 2 domains of HLA molecules are highly polymorphic regions which comprise part of the peptide binding site and are thought to interact directly with T cells. To investigate possible effects of these residues on T cell functions, we prepared synthetic peptides corresponding to residues 60-84 or 145-169 of a panel of HLA molecules. These peptides were then tested for effects on class I and II restricted responses. Most of the peptides had no demonstrable effects. However, peptides corresponding to residues 60-84 of HLA-B2702, Bw46, and Bw62 were found to block the differentiation of cytotoxic T lymphocytes (CTL) from pre-CTL, as measured by limiting dilution analysis. Surprisingly, this inhibition was not allele specific, in that these peptides blocked the differentiation of CTL from all donors tested against any HLA specificty examined. The peptides were not toxic to cells, since they had no measurable effects on class II restricted responses such as mixed lymphocyte or mitogen induced proliferation. The peptide derived from residues 60-84 of HLA-B2702 was also found to block lysis by mature CTL, and once again this effect was not restricted to HLA-B27 specific CTL. An analogous peptide corresponding to residues 60-84 of the related HLA-B2705 allele differs in only three amino acids from the HLA-B2702 peptide and it did not exhibit any of the inhibitory properties of the HLA-B2702 peptide. Efforts are currently underway to identify the cell surface ligands for these peptides and to elucidate their modes of action.

O 111 **Peptide Binding Capabilities of HLA-B27 Folded from *E. coli* Inclusion Bodies,** Edward J. Collins and Don C. Wiley, HHMI, Harvard University, Cambridge, MA 02138

HLA-B27 is a class I MHC molecule that has been crystallized and the X-ray structure determined (Madden *et al.* Nature (1991) **353** 321-325). As is the case with other HLA molecules, HLA-B27 has a variety of peptides in the peptide binding groove making the evaluation of peptide binding difficult. We would like to produce a complex of HLA-B27 with a single peptide bound. Eleven endogenous peptides sequences have been determined for peptides eluted from papain cleaved detergent solubilized HLA-B27 (Jardetzky *et al.* Nature (1991) **353** 326-329). Three of these peptides have been synthesized and shown to induce reconstitution of denatured lymphocyte HLA-B27 as seen by native isoelectric focusing. The extracellular domain of HLA-B27 has been produced in *E. coli* as inclusion bodies (Parker *et al.* (1991) Molecular Immunology, in press). Once solubilized in GnHCl, it can be properly folded in the presence of an appropriate peptide and β2m. The conditions that fold HLA-B27 from *E. coli* appear to vary depending on the length and sequence of the peptide. At this time three peptides have been shown to induce folding. Folding occurs slowly (greater than 9 days to generate 2% productive folding in one case) and optimal conditions vary with pH. A complex of HLA-B27, β2m and one peptide has been isolated and crystallization trials are under way.

O 112 ISOLATION AND CHARACTERIZATION OF SELF PEPTIDES ELUTED FROM ENGINEERED SOLUBLE MAJOR HISTOCOMPATIBILITY ANTIGENS H-2L^d AND H-2D^d. M. Corr, L. Boyd, and D.H. Margulies. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.
In this study we have isolated and characterized endogenous peptides from two MHC class I molecules, H-2L^d and H-2D^d. Genes encoding soluble analogues of these were transfected into L cells or were introduced into moth ovary cells by infection with recombinant baculovirus. The MHC molecules were purified from supernatants by immunoaffinity chromatography. Peptides that copurified with these class I molecules were eluted by acid treatment, purified by reverse phase HPLC, and individual peptide fractions were sequenced. These peptides, were eight to ten amino acids in length and reveal several sequence motifs. The elution profile of the peptides from H-2D^d molecules produced in murine fibroblasts had many more peaks than those from the molecules produced in insect cells. Thus, the spectrum of self peptides that copurify with MHC class I molecules differs not only due to the particular class I molecule, but also depending on the cell type producing that molecule.

O 113 A SELF PEPTIDE SEQUENCE IS CONVERTED INTO A H-2Kd-RESTRICTED NEOANTIGEN BY A Tyr SUBSTITUTION, Christian Drouet[1,2], Frank Healy[2], Pedro Romero[2], Jean Gagnon[3] and Janet L. Maryanski[2], INSERM U238, DBMS/ICH, CENG, 38041 Grenoble France[1], Ludwig Institute for Cancer Research, Lausanne branch, 1066 Epalinges Switzerland[2] and DBMS/BS, CENG, 38041 Grenoble France[3].

T cell antigenic epitopes can be mimicked by synthetic peptides. Using a functional competition assay, we have recently defined a binding motif that is common to antigenic peptides recognized in the context of the same MHC class I molecule, H-2 K^d (J. Exp. Med. 174 : 603-612, 1991). A key feature of the binding motif appears to be a tyrosine residue in the second position with respect to the NH2 terminal end of the peptide. We now show that it is possible to convert a self peptide sequence (P91A$^+$, ISTQNRRALDL) into an immunogenic one by replacement of the second residue (serine) with tyrosine. As predicted, immunization of H-2^d mice with this mutated-self sequence elicits a peptide-specific cytolytic T lymphocyte response that is H-2K^d-restricted.

O 114 **MHC class I specific and restricted recognition of ß$_2$-microglobulin deficient cells by CD8$^+$ cytotoxic T-lymphocytes**

Rickard Glas*, Lars Franksson*, Claes Öhlén*, Petter Höglund*, Beverly Koller^, Hans-Gustaf Ljunggren* and Klas Kärre* *Department of Tumorbiology, Karolinska Institute, Sweden and ^Department of Pathology, University of North Carolina, Chapel Hill, USA.

Cytotoxic T cells recognize MHC class I molecules composed of HLA/H-2 heavy chains, ß$_2$-microglobulin (ß$_2$m) and peptide antigens. All three components of the complex are considered important for recognition; cells deficient in ß$_2$m, as for example observed in many types of tumors, do not export class I molecules efficiently and fail to express them in a form that is recognized by conventional CTL. We now report that inoculation of mice with live allogeneic ß$_2$m deficient lymphoma cells results in tumor rejection as well as CD8$^+$/TcR alpha-beta$^+$ cells capable of killing ß$_2$m deficient targets in a H-2 specific CD8/MHC class I dependent manner. The killing did not require serum as a source of external ß$_2$m in the target cell culture or assay medium. CTL capable of lysing ß$_2$m$^-$ cells could be generated across a major as well as a minor histocompatibility barrier. CTL in both responses killed ß$_2$m deficient cells, but only the allo H-2 specific ß$_2$m independent CTL were able to kill a antigen processing deficient target. The results show that, contrary to the prevailing dogma, heavy chains in ß$_2$m deficient cells can act as antigens or antigen presenting molecules to CTL. The findings have implications for the understanding of MHC class I heavy chain folding and transport, and are of practical importance in studies of responses against engineered ß$_2$m deficient grafts and escape mutants in tumor immunology.

O 115 MHC CLASS I-RESTRICTED PRESENTATION OF tum$^-$ P91A ANTIGEN FROM CHIMERIC PROTEINS WITH DIFFERENT SUBCELLULAR LOCALIZATION Danièle GODELAINE, Aline VAN PEL* and Henri BEAUFAY International Institute of Cellular and Molecular Pathology and *Ludwig Institute for Cancer Research (Brussels Branch), B-1200 Brussels, Belgium.
The question we have addressed is whether presentation of an MHC class I-restricted antigen to T lymphocytes depends on the subcellular localization of the precursor protein. The protein P91Ap, from which the tum$^-$ rejection antigen P91A is processed (Lurquin et al. (1989) Cell 58:293-303), is normally localized to the cytosolic compartment of P91 cells (derived from mouse P815 cells by mutagen treatment). In contrast, rat liver esterase (genetic nomenclature ES-10) is a resident protein of the endoplasmic reticulum (ER) lumen because it bears an ER retention signal (HVEL) at its carboxyterminus. Mutating HVEL-COOH into HVER-COOH results in esterase secretion (Robbi and Beaufay (1991) J. Biol. Chem., in press). We have introduced a double-stranded oligonucleotide encoding the antigenic sequence of P91A (amino acid residues 189-204 of P91Ap) into the cDNA of ES-10 esterase ending with either HVEL or HVER. These constructs were first transiently expressed in COS cells ; using an anti-esterase serum, we have verified that, depending on the carboxyterminal end, the chimeric protein is retained in the ER, or secreted into the medium. The constructs were then stably transfected into mouse P1.HTR cells, a highly transfectable P815 cell line. In both cases, transfectants were almost as efficiently lyzed by specific anti-P91A CTL as they were after transfection with the genuine P91A cDNA. These results indicate that the P91A antigen can be correctly produced and presented to T cells, being expressed in the context of an ER-resident, or a secreted protein. They also support the idea that an antigen processing pathway exists in the ER.

O 116 SINGLE AMINO ACID RESIDUE CHANGES OUTSIDE OF THE PEPTIDE BINDING SITE IN CLASS I MHC MOLECULES STIMULATE A RESPONSE TO SELF-PEPTIDES, Andres G. Grandea III and Michael J. Bevan, Department of Immunology, Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195.

Single substitutions were made in the protein sequence of the class I molecule, H-2K^b, at positions 65 and 69. The changes were predicted to interact with the T cell receptor and not with peptide presented by K^b; these predictions were supported by some of our data. Additionally, the mutant molecules stimulated a strong alloreactive response in mice bearing wild-type K^b. Alloreactive T cell clones recognize the mutation in combination with specific self-peptides presented by the mutant molecule. These self-peptides can be bound by wild-type K^b. Current studies are focused on the nature of these allostimulatory self-peptides, and on the role that these mutant class I molecules can play in positive selection.

O 117 **INFECTIOUS VIRUS IS REQUIRED FOR PRESENTATION OF OVERLAPPING CLASS I AND CLASS II MHC RESTRICTED T CELL DETERMINANTS,** C.J. Hackett, M. Wysocka, L. Otvos,Jr., J.W. Yewdell*, J.R. Bennink*, and L.C. Eisenlohr*. The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104, and * NIH, NIAID/LVD, Bethesda, MD 20892

Amino acids 74-88 of the influenza virus PR8 (H1N1) neuraminidase (NA) molecule were found to contain one class I MHC-restricted (Dd) CTL epitope and two distinct class II-restric.. (I-Ed) determinants. Residues 79-88 are necessary for recognition by all T cells, and suffice for recognition by one of the class II restricted T hybridoma clones. This demonstrates extensive overlap of the epitopes, although determining the precise boundaries of the sites requires further analysis. Infectious virus was found to be required for presentation of the NA 74-88 region to both class I and class II restricted T cells. Endocytosed UV-inactivated virus was not recognized by either category of T cell, showing that virus in endosomes is not presented. The class II restricted T cells recognized UV virus introduced into the cytosol of APC by viral fusion, ruling out UV damage to the epitope as being causal in the requirement for infectious virus. This suggests that NA 74-88 has a uniform requirement for processing in the cytosol for presentation with either class I or class II. Antigen in the cytosol therefore can have access to class II as well as class I MHC molecules.

O 118 PROTECTIVE CD8 T CELL RESPONSE TO A SECRETED PROTEIN OF L. MONOCYTOGENES. John T. Harty and Michael J. Bevan Howard Hughes Medical Institute and Department of Immunology, University of Washington, Seattle WA.98195

Acquired immunity to the facultative intracellular bacterium L. monocytogenes can result from CD8$^+$ T cell responses although acute, sublethal infection can be cleared in a T cell independent manner through NK cell secretion of γIFN and activation of macrophages. Generation of protective CD8$^+$ T cells requires that L. monocytogenes secrete listeriolysin O (LLO), a major virulence factor which mediates escape of the organism from the endocytic pathway into the cytoplasm of the infected cell. LLO has been found to contain a major class I MHC restricted epitope detectable by CTL lysis of LLO expressing transfectants and the naturally processed epitope has been identified as a nonamer. CD 8$^+$ T cell lines generated from infected mice and stimulated in vitro with the LLO transfectant exhibit specificity for the same LLO epitope as that identified by a fraction of T cells stimulated by infected macrophages. In addition, adoptive transfer of LLO transfectant stimulated CD 8$^+$ T cells confers significant protection against lethal L. monocytogenes infection suggesting that CD8$^+$ T cell responses to a single secreted protein and likely a single nonamer epitope in that protein may be sufficient to mediate protection against intracellular bacteria. The role of CTL generated γIFN in protection is under investigation.

O 119 SEQUENCE AND FUNCTIONAL CHARACTERIZATION OF PEPTIDES ASSOCIATED WITH THE CLASS I MHC MOLECULE HLA-A2.1, R. A. Henderson, D. F. Hunt, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A. L. Cox, E. Apella, and V. H. Engelhard, Depts. of Microbiology and Chemistry, University of Virginia, Charlottesville, Va. 22901 and Lab. of Cell Biology, NCI, NIH, Bethesda, MD, 20892.

A complex mixture of peptide fragments derived from viral or normal cellular proteins is displayed on the surface of nucleated mammalian cells in association with major histocompatability complex class I molecules. We have used microcapillary HPLC in conjunction with electrospray ionization/tandem mass spectrometry to achieve high resolution separation of the mixture extracted from the human class I molecule, HLA-A2.1, and to determine the primary structure of peptides isolated at the subpicomole level. All peptides were present only in extracts of HLA-A2.1$^+$ cells, and synthetic peptides based on the sequences were able to compete with antigenic viral peptide for binding to this molecule. Of the 200 peptides detected, 10% were estimated to be present at the level of 150-600 fmol per 10^8 cells while 90% were present at 30-150 fmol. Sequences of some of these peptides were identified in normal cellular proteins with intracellular locations, providing evidence that such proteins are processed and presented in the same way as viral proteins. All of the peptide sequences were distinct from those previously reported for another human class I molecule, HLA-B27. All peptides are 9 residues long, contain Leu/Ile at position 2, hydrophilic residues at positions 1 and 8, and a hydrophobic alkyl side chain, usually Val or Leu/Ile, at position 9. These features define a unique structural motif for peptides bound to HLA-A2.1, and by comparison with the peptides bound to HLA-B27, emphasize the specificity with which different class I molecules bind peptides. The mass spectrometric technique reported provides a unique, powerful, and generally applicable approach to the analysis of peptides bound to molecules of the major histocompatibility complex.

O 120 CLASS I MODIFYING (*cim*) GENE AFFECTING ANTIGEN PRESENTATION BY A COMMON HLA B MOLECULE Ann Hill and Andrew McMichael. Molecular Immunology Group, Institute of Molecular Medicine, Oxford, OX39DU, U.K.

Pulse chase studies of HLA B51 revealed that this molecule is inefficiently assembled, with a significant portion of heavy chain still present in the endoplasmic reticulum three hours after synthesis. Because of the effect of the rat *cim* gene on assembly rate, we wondered whether HLA B51 might be particularly sensitive to a similar effect in man. Allo-reactive B51 specific T cell clones were raised which recognized only approximately one third of B51-positive B cell lines. Each group contained lines of known normal sequence B51. A family with paternal and maternal haplotypes each expressing A2, B51 but differing in the class II region was studied. All 3 individuals with the maternal haplotype were recognized, including one with both maternal and paternal B51 containing haployptes. 3 individuals bearing only the paternal haplotype were not recognized. The effect is unlikely to be due to recognition of peptide derived from a polymporhic protein bound to B51 as a similar phenomenon was seen with antigen specific T cells. EBV-specific B51-restricted T cell clones also distinguished classes of B51; in this case, only a subset of the lines which had not been recognized by the allo-reactive clone was seen. Assembly efficiency in each group and genetic linkage are being investigated.

O 121 THE RMA-S ANTIGEN PRESENTATION DEFECT IS LEAKY. Nancy A. Hosken[1] and Michael J. Bevan[2], University of California at San Diego, La Jolla, CA, 92037[1] and Howard Hughes Medical Institute and Department of Immunology, University of Washington, Seattle, WA, 98105[1,2]

The RMA-S cell line is defective in the ability to present endogenously synthesized antigens to Class I MHC-restricted CTL. This defect has been attributed to the inability of RMA-S to deliver antigenic peptides derived from antigens in the cytosol into the endoplasmic reticulum (ER) where they can associate with Class I MHC molecules. We have found that RMA-S can present at least one endogenous antigen, vesicular stomatitis virus nucleoprotein (VSV-N), to Class I MHC-restricted CTL. Mutant RMA-S and wild-type RMA cells are recognized and lysed by VSV-N-specific CTL both when infected with VSV or transfected with the VSV nucleoprotein gene. In both cases the wild-type RMA and mutant RMA-S cells synthesize similar amounts of the VSV-N protein as measured by SDS-PAGE. The natural antigenic VSV nucleoprotein peptides purified from either RMA or RMA-S cell lysates are indistinguishable when analyzed by HPLC. We have also mapped the genetic defect responsible for the RMA-S phenotype to the murine chromosome 17. Mapping of the RMA-S defect was performed by fusion of RMA-S with BW/Lyt2.4 cells and isolation of 40 fusion clones. All fusion clones had higher levels of cell surface Class I MHC expression than RMA-S. Selection of a single fusion clone (RxB.6) with allo(anti-H-2^k)-CTL resulted in loss of the BW/Lyt2.4 derived chromosome 17 in 3/3 selected cell populations as determined by Southern blot analysis. These CTL-selected cell populations also exhibited the RMA-S Class I MHC expression and antigen presentation phenotype. Chromosome 17 encodes the murine Class I MHC genes as well as two genes, HAM-1, and -2, with homology to the ATP-dependent transporter superfamily. Our data suggest that the system that delivers antigenic peptides from the cytosol to the ER in RMA-S may still be present and retain partial function.

O 122 A CHIMERIC CLASS I GLYCOPROTEIN REVEALS THAT PEPTIDE ANTIGEN IS A MAJOR COMPONET OF ALLOREACTIVITY, Henry D. Hunt and Larry R. Pease, USDA, ARS, Avian Disease and Oncology Laboratory, East Lansing, MI 48823, and Department of Immunology, Mayo Clinic, Rochester, MN 55905

A chimeric class I glycoprotein was created to investigate the functional contribution of the α helices and the β pleated sheets in forming the antigen recognition site (ARS) of antigen presenting molecules. This novel class I glycoprotein was generated by replacing the DNA sequences encoding the α helices of the L^d gene with the corresponding sequences from the K^b gene. Serologic analysis of transfected L-cells expressing the chimeric molecule reveal that the engineered class I glycoprotein retains two conformational epitopes associated with the α helices of K^b (K10.56 and 28-13-3). These results demonstrate that the α helices of K^b can associate with the β pleated sheets of Ld to form a stable structure, which is expressed on the cell surface. To address the role of the α helices of the ARS in determining T cell cross reactivity, alloreactive CTL were used to analyze L-cells expressing the chimeric construct. CTL raised against K^b or L^d as alloantigens were incapable of lysing L-cells expressing the chimeric construct. This indicates that alloreactive CTL cannot recognize structures determined by the α helices or by the β sheets of the ARS. In contrast to the lack of cross reactivity observed among anti-K^b or anti-L^d CTL, bulk and cloned alloreactive CTL that were generated against the mutant K^b glycoprotein K^{bm8} reacted strongly with the chimeric glycoprotein. In addition to the K^b α helices, the K^{bm8} ARS shares the B pocket with the chimeric glycoprotein. These results identify pocket B as an important anchor site for self peptides and provides a molecular explanation for alloreactive cross reactions between K^{bm8} and the chimeric glycoprotein.

O 123 STRUCTURAL FEATURES OF THE MURINE T CELL ANTIGEN RECEPTOR RECOGNIZING THE VESICULAR STOMATITIS VIRUS (VSV) ANTIGENIC OCTAPEPTIDE IN THE CONTEXT OF THE H-2K^b MOLECULE. **Monica Imarai and Stanley G. Nathenson. Albert Einstein College of Medicine. Bronx, New York 10461.**

Murine Cytotoxic T Lymphocytes (CTL) respond against vesicular stomatitis viral infection recognizing the antigenic octapeptide (N52-59) from the N protein of the virus presented by the H-2K^b molecule. We have studied the structural features of the T cell receptor α and ß chains from a number of VSV specific CTL clones using direct PCR sequencing. Preliminary data show a restricted usage of Vß13 gene in over 50% of the clones. Thus far, the sequences in the junctional regions, which have been postulated to be responsable for the recognition of the peptide, do not show any apparent selection for any amino acid residue. Using MHC and peptide variants, we are presently classifying each CTL clone for its unique recognition pattern of residues in the peptide and the MHC molecule in order to correlate with the usage of Vα and Vß gene segments and amino acid sequences in the junctional regions of the T cell receptor proteins. The results of such correlation as interpreted by three dimensional modeling will be reported.

O 124 EXPRESSION OF EMPTY MHC CLASS I IN INSECT CELLS Michael R. Jackson, Elizabeth Song, Young Yang and Per A. Peterson. Department of Immunology, The Scripps Research Foundation, La Jolla, CA 92037.

As insects do not have a conventional immune system they provide an ideal cell in which to determine wether MHC dedicated proteins have been developed for the assembly and transport of these proteins and also for the production and loading of antigenic peptides. We have co-expressed cDNAs encoding mouse MHC heavy chains (K^b, L^d or D^b) and mouse or human β2 microglobulin in drosophila cells and studied the transport rates of the different molecules as single chains and heterodimers. We find that heterodimers are efficiently assembled however in contrast to mammalian cells, $D^b/\beta2$ is much more rapidly transported than $L^d/\beta2$ and slightly more rapidly than $K^b/\beta2$. In addition all heavy chains are transported to the cell surface where they can reassociate with β2 microglobulin from the culture media. The heterodimers generated in this system have the properties of "empty" or peptide free MHC class I molecules recently described in mutant mammalian cell line RMAS. These empty class I molecules are expressed at high levels on the cell surface in cells grown at 27°C. We have studied the thermostability of the different class I molecules in Triton X100 lysates and at the cell surface by FACS. We find that $D^b/m\beta2$ is more stably than Kb/mb2 which is more stable than $L^d/m\beta2$. Co-transfection with human β2 produces significantly more stable heterodimers, the order β2 thermostabilization is human > bovine > murine. In addition the thermostability is greatly affected by bound peptide, the degree of stabilization depends on the peptide sequence and length. For murine Class I molecules only those binding peptides of 8 or 9 amino acids are sufficiently thermostable to survive at 37°C for more than a few hours.

O 125 ISOLATION AND CHARACTERIZATION OF TUMOR-SPECIFIC ANTIGENS RECOGNIZED BY T CELLS.

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Recent evidence suggests that T cells can recognize tumor antigens which probably derive from naturally occurring cellular products. These antigens could potentially be products of mutated oncogenes, antioncogenes or other mutated proteins. Alternatively they could be reexpressed fetal proteins to which the immune system is not completely tolerant. In order to characterize these antigens we first produced CTL lines and clones specific for murine tumors that were CD8+ and MHC class I-restricted. This was done by first immunizing mice with tumor cells engineered by retroviral transduction to secrete lymphokines such as IL-4. This strategy attracts antigen presenting cells to the tumor and results in efficient priming of tumor-specific T cells in vivo. Clones can then be produced in vitro against tumors previously considered to be nonimmunogenic. We have acid extracted peptides from a murine colon tumor and a melanoma and used these to sensitize autologous cells for lysis by the tumor-specific CTL clones. The peptides were furthur fractionated on reversed phase HPLC and fractions were tested by the same sensitization technique. These peptides are currently being isolated for sequence analysis as a general method to characterized tumor-specific T cell antigens.

O 126 A Murine Cell Variant Differentially Presents Antigens Derived After VSV or Influenza Infection

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Biotechnology Laboratory and the Departments of Medical Genetics, Microbiology and Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Viral antigens require internal processing by antigen presenting cells. Murine variants which apparently lack the ability to present foreign antigens have been described. In the RMA-S cell line, the ability to present foreign antigens after infection with influenza virus is apparently lost in comparison to the wild-type cell line RMA. We find that RMA-S cells are able to present Vesicular Stomatitis Virus (VSV) antigens at a level approaching that of the RMA cell line. We conclude that the whereas the genetic mutation in RMA-S cell dramatically affects the presentation of influenza antigens, the presentation of VSV antigens is relatively unaffected. It is therefore likely that the replication cycle and mode of assembly of the virus can control its ability to be efficiently processed into antigenic peptides.

O 127 FINE PEPTIDE SPECIFICITY OF CYTOTOXIC T LYMPHOCYTES DIRECTED AGAINST ADENO-VIRUS-INDUCED TUMORS AND PEPTIDE-MHC BINDING,

W. Martin Kast, Remco M.P. Brandt, Jan Wouter Drijfhout and Cornelis J.M. Melief, Department of Immunohematology and Blood Bank, University Hospital Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands

A peptide encoded by the adenovirus type 5 early region 1 (Ad5E1) is the target structure for H-2 D^b-restricted cytotoxic T lymphocytes (CTL) that are capable of tumor eradication in vivo. With the use of a complete set of peptides, generated by the "pepscan" (pin)method, in which each individual aminoacid (aa) was replaced by 19 different aa, we analyzed to what extent these replacement mutant peptides were still recognized by an Ad5E1-specific CTL clone and which of the replacement mutant peptides were still binding to the H-$2D^b$ molecule. Binding was analyzed with RMA-S cells that express largely empty and unstable MHC-class I molecules which are stabilized by peptide binding. A peptide of 10 aa turned out to be optimal for MHC binding and T cell recognition. Areas of the peptide primarily involved in binding to MHC or in T cell recognition are delineated. Unexpected results in respect to possible or non-possible binding and recognition of the replacement mutant peptides were checked by generating peptides with the "tea bag" method. Furthermore a 3D nuclear magnetic resonance study was performed on this 10 mer peptide. All information was fed into a computer modelling system and the best possible fit of the peptide in the groove was calculated and will be shown.

O 128 NOVEL GENES IN THE CLASS II REGION OF THE HUMAN MHC, Adrian P Kelly, Stephen H Powis, Richard Glynne, Ian Mockridge, Isabel Hansen, Lesley-Anne Kerr and John Trowsdale, Human Immunogenetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

We have isolated a number of novel cDNA transcripts (RING genes), which map to the class II region of the human MHC. Functionally these fall into three broad categories, those involved in aspects of antigen processing/presentation, those encoding new HLA class II-like genes, and those with no apparent MHC association. Antigen processing involves the generation of peptides from cytosolic proteins and their transport into the endoplasmic reticulum where they participate in HLA-class I assembly. We have identified two genes in the MHC class II region, *RING4* and *RING11*, which are believed to encode the peptide transport proteins. Closely associated with the transporters are two proteasome-related genes, *RING10* and *RING12*. The proteasome, a large complex of subunits with multiple proteolytic activities, may be involved in the generation of antigenic peptide. *RING12, 4, 10* and *11* form a tightly linked cluster of interferon inducible genes, within the MHC, with an essential role in antigen processing. Centromeric of *RING12* lie two genes, *RING6* and 7, that encode new members of the Ig gene family (*HLA-DMA* and *HLA-DMB*). Both have diverged from class I and class II sequences long before the duplications that gave rise to the different class II loci. These genes have class II X and Y box promoter elements and are gamma interferon inducible. Amino acid analysis of membrane proximal domains shows *RING7* to be equidistant between class I and class II. It is proposed that these genes encode a new MHC heterodimer displaying an antigen binding cleft with four potential disulphide bonds. *RING1* and *RING3* are two genes of unknown function. The former encodes a novel zinc finger motif (RING finger) and the latter is a homologue of the Drosophilia female sterile homeotic (fsh) gene. We present data on sequence, expression and genomic organisation of genes within the class II region of the MHC.

O 129 MOLECULAR CHARACTERIZATION OF ALLOGENEIC CLASS I MOLECULE - T CELL RECEPTOR INTERACTIONS. Catherine C. Killion, Pei-Jia Chen, Katherine Louie, Denise McKinney, and Minnie McMillan. Norris Cancer Center, USC School of Medicine, Los Angeles, CA 90033.

In order to understand further the molecular basis of alloreactivity we are presently correlating TCR (T cell receptor) structure with the allorecognition of class I molecules plus associated peptides. We have therefore generated a panel of T cell hybridomas from a Balb/c-H-2-dm2 (D^d) anti-Balb/c (D^d L^d) mixed lymphocyte culture. We have analysed these hybridomas for the production of IL-2 in response to a panel of cells expressing hybrid D^d-L^d class I molecules or L^d-specific sequences (encoded by mini-genes) in the context of class I. We have found that the hybridomas recognize different regions of the L^d molecule.

We have also determined the amino acid sequences of the TCR β-chains from a number of the hybridomas. Our data indicate that although the hybridomas differ in specificity for class I and peptide, the Vβ gene usage is surprisingly limited given the high frequency of alloreactive T cells in the immune repertoire. We are presently investigating the α-chain diversity or lack thereof. Our results will be discussed in the context of the Bjorkman-Davis model for TCR structure.

O 130 DIRECT BINDING OF PEPTIDES TO MHC CLASS I MOLECULES ON THE SURFACE OF LIVING TARGET CELLS. Ulrich Kubitscheck, Raphael Levi, Robert J. Horwitz, Ruth Arnon and Israel Pecht, *Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.*

Antigenic peptides are recognized by the T cell receptor in their bound state to major histocompatibility complex gene products (MHC class I and II proteins). However, no direct observation of peptide binding to MHC class I molecules on living cells has so far been attained. Here we report on experiments where this process has been monitored directly. A synthetic peptide comprising the sequence 147-158 of the nucleoprotein from the influenza A virus (NP147-158) and its analogue lacking the arginine at position 156 (NPR^-) are shown to bind to the H-$2K^d$ on living P815 mastocytoma cells. The peptides, biotinylated at their N-termini, were incubated with cells at 4°C, and the binding was monitored via reaction with a streptavidin-phycoerythrin conjugate (SA-PE) by PE fluorescence in a flow cytometer. Binding was specific as demonstrated by competition with the unmodified peptides and could be mapped to the K^d molecule by inhibition of the PE fluorescence signal following coincubation with the K^d-specific mAb 20-8-4. We estimate that 0.5 - 1% of the MHC class I molecules present on the cell surface can bind the externally added peptide (a total of 400-1600 per cell). The results of the direct binding measurements are compared to those obtained by CTL killing experiments.

O 131 GORILLA CLASS I MHC ALLELES: COMPARISON TO HUMAN AND CHIMPANZEE CLASS I, David A. Lawlor[1], Ean Warren, Patricia Taylor and Peter Parham, Department of Immunology, U.T. M.D. Anderson Cancer Center, Houston, TX 77030[1] and Departments of Cell Biology, and Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5400

Fourteen gorilla class I MHC alleles have been isolated, sequenced and compared to their counterparts in humans and chimpanzees. Gorilla homologues of HLA-A, B and C were readily identified and 4 Gogo-A, 4 Gogo-B and 5 Gogo-C alleles defined. In addition an unusual Gogo class I gene with features in common with HLA-A and its related pseudogene, HLA-H, is described. None of the gorilla alleles is identical or even closely related to known class I alleles and each encodes a unique antigen recognition site. However, the majority of polymorphic substitutions and sequence motifs of gorilla class I alleles are shared with the human or chimpanzee systems. In particular, elements shared with HLA-A2 and HLA-B27 are found in Gogo-A and -B alleles. Diversity at the Gogo-B locus is less than at the Gogo-A locus, a trend the opposite of that seen for HLA-A and B. The Gogo-C locus also appears to have limited polymorphism compared to Gogo-A. Two basic Gogo-C motifs were found and they segregate with distinctive sets of HLA-C alleles. HLA-A alleles are divided into 5 families derived from 2 ancient lineages. All chimpanzee A alleles derive from one of these lineages and all gorilla alleles derive from the other. Unlike chimpanzee Patr-A alleles, the Gogo-A alleles do not clearly partition with one of the HLA-A families but have similarities with two. Overall, gorilla class I diversity appears from this sampling to show more distinctions from class I HLA than found for chimpanzee class I.

O 132 THE CONFORMATION OF THE L^d-LIKE MOLECULE, L^{w16}, IS INFLUENCED BY L^d-RESTRICTED PEPTIDES

David R. Lee, Usha Tummuru, and Beth L. Rengers, Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65203

The recently deduced amino acid sequence of the mouse MHC class I molecule, L^{w16}, indicated that it is a member of the L^d family. Both the mAbs 30-5-7 and 28-14-8 which recognize the $\alpha 2$ and $\alpha 3$ domains of L^d, respectively, also recognize L^{w16} based on flow cytometry analyses of an L^{w16} gene L cell transfectant ($L.L^{w16}$) and of splenocytes from the B10.GAA37 mouse strain. These anlayses reveal a higher level of staining using the mAb 28-14-8 ($\alpha 3$) as compared to that observed with 30-5-7 ($\alpha 2$). Similar results are observed in lysates from $L.L^{w16}$ cells and B10.GAA37 splenocytes biosynthetically labeled with ^{35}S-met and -cys. Using excess amounts of each mAb, more L^{w16} was immunoprecipitated with 28-14-8 ($\alpha 3$) than with 30-5-7 ($\alpha 2$). Furthermore, sequential immunoprecipitation analyses revealed two serological forms of L^{w16}: one 30-5-7^+ 28-14-8^+ and the other 30-5-7^- 28-14-8^+. In contrast to previous studies of the L^d molecule in which two similar serological forms had been observed, the 30-5-7^- 28-14-8^+ L^{w16} molecules are associated with β_2-m and are predominately mature endo H^R forms that are also found on the cell surface, consistent with the above flow cytometry analyses. Incubation of $L.L^{w16}$ cells with two distinct L^d-restricted antigenic peptides increased the surface levels of 30-5-7^+ L^{w16} molecules; furthermore, the number of 30-5-7^+ L^{w16} molecules could be increased in lysates by incubation with the same L^d-restricted peptides. Together, these studies suggest that L^{w16} can bind and present two L^d-restricted peptides. Furthermore, they suggest that the binding of some self-peptides by L^{w16} induces a 30-5-7^- serological form, whereas the binding of other self-peptides and L^d-restricted peptides by L^{w16} induces a 30-5-7^+ conformation. (supported by NIH grants AI31129, AI0726, and GM0839601, and by a University of Missouri Weldon Springs Award)

O 133 TRANSPORT OF PEPTIDES ACROSS THE ER MEMBRANE AND ASSEMBLY OF MHC CLASS I ANTIGENS TRANSLATED IN VITRO. Frédéric Lévy, Reinhard Gabathuler and Sune Kvist, Ludwig Institute for Cancer Research, Box 60202, S-104 01 Stockholm, Sweden.

Peptides have been shown to be an important component in the assembly of MHC class I heavy chain with β2-microglobulin. We have used an in vitro system in which exogenously added mRNA encoding HLA-B27 is translated in a rabbit reticulocyte lysate supplemented with human microsomal membranes containing endogenously β2-microglobulin. This allowed us to analyze the early events in the assembly of MHC class I antigens. By using microsomes prepared from Daudi cells we find a strict requirement of β2-microglobulin for detection of peptide interaction with the MHC class I heavy chain. The use of a biotinylated peptide specific for HLA-B27 made it possible to analyze the transport of peptides across the endoplasmic reticulum (ER) membrane. So far the mechanism by which peptides are transported from the cytosol into the ER has remained unclear. Here we could show that the transport of peptide across the ER membrane is a rapid process that does not require ATP, whereas the assembly process does. In addition, Ca^{2+}-ions seem to be important. In microsomes prepared from Raji and T1 cells, the peptide induced assembly is similar, whereas assembly in T2 microsomes is 10-fold lower. The inefficient assembly in T2 microsomes is not due to impaired transport of peptides across the membrane, as no difference was found compared to microsomes isolated from T1 cells.

O 134 THE ADENOVIRUS E3/19K PROTEIN BLOCKS THE PHOSPHORYLATION OF MHC MOLECULES, Cyprien Lomas, Roger Lippé and Wilfred A. Jefferies, Biotechnology Laboratory and the Depts of Microbiology, Zoology, and Medical Genetics, University of British Columbia, B.C., CANADA V6T 1Z3

Major histocompatibility complex (MHC) molecules are receptors which function in the presentation of foreign antigens to cytolytic T cells (CTLs). MHC molecules undergo post translational modifications including glycosylation and oligomerization. MHC proteins bind to antigenic peptides and present them to the CTLs at the cell surface. Each of these steps may regulate the surface expression of MHC. For instance, removal of the site(s) of phosphorylation of MHC has an effect on the recycling of MHC from the plasma membrane. At present it is unclear whether phosphorylation also regulates the egress of MHC molecules from the endoplasmic reticulum to the cell surface or has an effect on the function of MHC molecules. We have studied the effect of Adenovirus on the expression of MHC. Adenovirus is a double stranded DNA virus which has developed the ability to hinder the recognition of adenovirally infected cells by CTLs by blocking the egress of MHC molecules to the plasma membrane. This effect has been mapped to a viral protein called E3/19K which retains the MHC in the endoplasmic reticulum (ER) before MHC leaves the ER. We have investigated the phosphorylation of MHC class I protein in cells transfected with E3/19K or the mutant protein M621, which lacks the ER retention signal. Our results indicated that in cells transfected with E3/19K, but not the M621 mutant protein, phosphorylation of MHC molecules is blocked. These data provide evidence that the phosphorylation of MHC class I molecules takes place at or near the plasma membrane.

O 135 Heterologous β_2-Microglobulin Exchange into H-2D^b on Intact Cells Occurs via a Free Heavy Chain Intermediate. Mark A. Luscher, Barbara Newton, and Brian H. Barber. Dept. of Immunology, University of Toronto, Toronto, Canada M5S 1A8.

The kinetics of β_2-microglobulin (β_2m) exchange into class I MHC molecules on intact cells have been examined in an effort to improve our understanding of the structural dynamics of the exchange process. The monoclonal antibody W6/32, which recognizes a monomorphic determinant of class I HLA molecules, cross-reacts with a conformational determinant created by the association of bovine or human (but not murine) β_2m with the H-2D^b heavy chain. Thus, using flow cytometry to monitor the appearance of W6/32 reactivity following the addition of bovine or human β_2m to H-2D^b-expressing cells (*e.g.* RMA or EL4), it is possible to determine the kinetic properties of β_2m exchange into H-2D^b. In this way, we have established that the rate of heterologous β_2m exchange into H-2D^b is independent of the β_2m concentration from 0.5-50μg/ml of human β_2m (or 5-50% fetal calf serum). The data are consistent with a mechanism involving the appearance or creation at the cell surface of a free H-2D^b heavy chain intermediate, as opposed to a direct displacement of endogenous β_2m from the H-2D^b heavy chain by the incoming exchange partner. Further, we have determined that the rate of β_2m exchange is not altered by incubation of the cells with the high-affinity H-2D^b-specific influenza nucleoprotein peptide 366-374 (0.5-50μg/ml). The results allow us to place constraints on the various models advanced to account for the dynamics of H-2D^b heavy chain, β_2m, and peptide interactions.

O 136 HLA-B7 IS DETECTED ON T2 CELLS IN AN ALTERED CONFORMATION C.T. Lutz, K.D. Smith & J. McCutcheon, Dept. Pathology, U. of Iowa, Iowa City, IA 52242

The T2 cell line is deficient in peptide antigen processing, resulting in low cell surface expression of most endogenous and transfected HLA class I molecules. However, T2 expresses moderate levels of HLA-A2 and many mouse H-2 molecules. To investigate the expression of HLA-B7 in the absence of normal antigen processing, we studied HLA-B7 transfected T2 (T2/B7) and 721.221 (.221/B7), a control cell line capable of processing peptide antigens. Consistent with the results of others, anti-HLA-B7 mAb BB7.1 detects HLA-B7 on T2/B7 at 5% the level detected on .221/B7. However, six other mAbs (MB40.2, ME1, MB40.3, BB7.6, SFR8-B6 and 126.39) detect HLA-B7 on T2/B7 at 22-95% the level detected on .221/B7. We also tested T2 transfected with HLA-B7 variant Q32E. Residue 32 contacts β 2m; also, H-2 molecules with Glu32 are expressed at high levels on T2. We hypothesized that the Q32E substitution may increase the affinity of HLA-B7 heavy chain for β 2m and affect HLA-B7 surface expression. When expressed in 721.221, the Q32E substitution (.221/Q32E) causes a 2-4 fold increase in antibody binding. When expressed in T2, however, the Q32E substitution (T2/Q32E) abrogates MB40.2 binding. We also studied how alloreactive anti-HLA-B7 CTL lines and clones recognize HLA-B7 expressed on T2. Alloreactive CTL lines readily lyse both .221/B7 and T2/B7. Alloreactive CTL clones efficiently lyse .221/B7, but not T2/B7. These results suggests that HLA-B7 is expressed on T2 in an altered conformation that is 1) detected poorly by BB7.1 and CTL clones, and 2) sensitive to an amino acid substitution that is not detected in the presence of normal antigen processing. Altered HLA-B7 conformation on T2 is consistent with empty HLA-B7 molecules or molecules loaded with peptides derived from an alternative source.

DETECTION OF HLA-B7 ON T2 AND 721.221

mAb/CTL	.221/B7	T2/B7	.221/Q32E	T2/Q32E
BB7.1	+++	+	++++	+
MB40.2	+++	++	++++	-
CTL lines	+++	+++	NT	NT
CTL clones	+++	-	+++	NT

O 137 A RECOMBINANT, SOLUBLE SINGLE CHAIN CLASS I MHC MOLECULE THAT RETAINS BIOLOGICAL ACTIVITY DESPITE SEVERAL POINT MUTATIONS. Michael G. Mage*, Steven Kozlowski#, Randall K Ribaudo#, Li Lee*, Maripat Corr#, and David H. Margulies#. *Laboratory of Biochemistry, NCI, and #Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

As an aid in studying the role of heterodimerization in the assembly, transport, and function of MHC class I molecules, we have expressed in mouse L cells a chimeric, single chain, soluble class I MHC molecule (scβD^d) that consists of β2 microglobulin linked at its carboxyl terminus to a peptide spacer linked in turn to the amino terminus of a form of the H-2D^d heavy chain previously engineered for solubility. Western blots of reduced denatured scβD^d, and immunoprecipitation of radiolabelled cell lysates showed a 60 kd band that reacted with antibodies for both β2m and H-2D^d. Nucleotide sequence determination of the recombinant gene encoding this chimeric protein revealed three point mutations in the H-2D^d portion of the construct leading to substitutions of 39D->N, 48R->W, and166E->G, most likely representing artifacts related to the use of PCR in the construction of the recombinant gene. Transfected L cells expressing the scβD^d secrete this molecule at 27°C, but not at 37°C. Despite these mutations, which prevent the formation of salt bridges which are present in the wild type molecule, the affinity purified scβD^d molecules stimulate an alloreactive T cell hybridoma. Further, when exposed to an H-2D^d restricted antigenic peptide ("p18") derived from the HIV-1 gp160 envelope glycoprotein, the scβD^d molecules also stimulate a peptide specific, H-2D^d restricted T cell hybridoma, although not as efficiently as the wild type two chain H-2D^d molecule. Experiments are in progress to determine whether the inefficiency in peptide presentation and the intracellular thermal instability reflect the point mutations or the tethered β2m.

O 138 AN ASSESSMENT OF THE FUNCTIONAL SIGNIFICANCE OF MUTATIONS IN THE POCKETS OF THE PEPTIDE BINDING GROOVE OF HLA-A2.1, Jane A. McCutcheon and Peter Parham, Departments of Cell Biology and Microbiology and Immunology, Stanford School of Medicine, Stanford, CA 94305.

Class I HLA molecules present peptides to T lymphocytes. The structure of the peptide binding groove of HLA-A2.1 shows six "pockets". In addition, a peptide has been modelled into the peptide binding groove of B*2705. Many of the residue differences between HLA alleles ocur at positions forming the pockets. These data suggest that HLA alleles select different peptide repertoires by varying pocket usage. Thus changing the residues forming the pockets should alter the peptide repertoire selected. To test this, we propose to mutate all the residues forming each pocket in A2.1. Each pocket will be tested using three sets of mutations; all residues forming the rim, the body, and rim and body combined. Each set of mutant pockets will be transfected into the HLA-A,B,C$^-$, cell line 721.221. The surface expression for each set of mutant pockets will be measured by FACS analysis. Gamma-interferon and/or room temperature incubation will be used to increase surface expression for those pockets with no or low levels of expression. A panel of FLU-NP peptides will be synthetized to test the ability of each set of mutant molecules to bind peptides in a cell binding assay. We predict that *wild-type* peptide will bind rim mutants but only variant peptides will bind body mutants. After determining which peptides bind mutant A2.1 molecules, these mutants can then be used to examine the interaction between peptide, A2.1, and CTL. One prediction is that both rim and body residues changes will affect CTL recognition. These studies should provide considerable information about the interaction between peptide, HLA molecules and T lymphocytes.

O 139 T CELL AND MONOCLONAL ANTIBODY RECOGNITION OF HYBRID D^d-L^d CLASS I MOLECULES, Denise M. McKinney and Minnie McMillan, Norris Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033.

To identify amino acids critical for peptide binding and for T cell receptor (TCR) recognition and also to map epitopes which interact with L^d and D^d-specific monoclonal antibodies, we have constructed a cassette corresponding to the α-1 and α-2 domains of the L^d class I molecule into which we have introduced D^d-specific codons. One construct was used to generate a gene which encodes a hybrid class I molecule in which lysine at position 31 of L^d is replaced by the D^d -specific amino acid, threonine, L^d (K31T).

We show that the change of this single amino acid in the β-pleated sheet introduces D^d -like properties as demonstrated by both monoclonal antibody and CTL reactivities, while retaining many of its L^d properties. As judged by a panel of L^d and D^d-specific monoclonal antibodies, L^d (K31T) reacts as D^d in the α-1 domain, shows some D^d and all L^d specificities for the α-2 domain, and, as expected, shows L^d specificities for the rest of the molecule. The fact that α-2 domain as well as α-1 domain specificities are affected in the hybrid molecule indicates that local conformation in the class I molecule as well as the primary sequence may be important for monoclonal antibody recognition.

In addition, L^d (K31T) was recognized by both L^d -specific and dm1-specific alloreactive cytotoxic T lymphocytes (CTLs). Thus the mutation of amino acid 31 changes the structure of the molecule so that it is perceived by the TCR as D^d -like, despite the fact that the sequence is primarily that of L^d.

O 140 **IMMUNOGENICITY OF OVERLAPPING T CELL EPITOPES IN INFLUENZA HEMAGGLUTININ**

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Within the hemagglutinin molecule of an H2N2 influenza strain A/Japan/57 there is an immunodominant site for the H-2^d haplotype that maps to residues 202-221. The site is comprised of two distinct but overlapping cytotoxic T lymphocyte (CTL) epitopes encompassing residues 204-213 and 210-219. Each of the epitopes utilizes a tyrosine-linked binding motif to associate with its restricting molecule K^d. Despite the fact that the C-terminal epitope expresses a motif that appears to be of a higher avidity for K^d, as defined by peptide competition studies, it's the N-terminal epitope which is immunodominant in the *in vivo* CTL response. BALB/c mice primed with influenza are able to respond vigorously to the N-terminal epitope whereas no more than 50% of these same animals recognize the C-terminal epitope. Moreover, the C-terminal specific response is consistently weaker than that directed to the N-terminus. Immunization with the isolated epitope expressed in a vaccinia virus vector does not improve the intensity or variability of the C-terminal response; hence the subdominance of the C-terminal specifc immune response may be the result of regulation at the level of the TCR repertoire rather than antigen processing and presentation.

O 141 STIMULATION OF CLASS I-RESTRICTED INFLUENZA-SPECIFIC CYTOTOXIC T CELLS BY DENDRITIC CELLS.

Ruta Nonacs, Cornelia Humborg, and Ralph M. Steinman. Department of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021.

It is known that dendritic cells (DC) are potent stimulators of antiviral T cell responses; however, their mechanism of action has not been analyzed. We have verified that *in vitro* murine dendritic cells are superior to other antigen presenting cells in their capacity to induce an influenza-specific cytotoxic T lymphocyte (CTL) response. This response is focused primarily on the dominant nucleoprotein (NP) peptide and depends upon the participation of CD4+ helper T cells, as well as CD8+ cytotoxic T cells. Virus-infected dendritic cells stimulate a strong CTL response, yet when pulsed with the nucleoprotein peptide, dendritic cells are much less potent. The NP peptide is not recognized by class II-restricted helper T cells, and without the lymphokines these CD4+ helper cells produce, dendritic cells are not able to stinulate a strong CTL response. Dendritic cells exposed to non-infectious forms of influenza, such as UV-inactivated or bromelain-treated virus, are unable to generate class I-restricted CTL but are able to stimulate the CD4+ helper T cells. Dendritic cells, in expressing high levels of both class I and class II MHC molecules, are capable of stimulating both CD4+ helper and CD8+ cytotoxic T cells and thus may play an important role in the generation of an optimal influenza-specific CTL response.

O 142 **IMPACT OF AMINO ACID SEQUENCE DIVERSITY ON ANTIGEN PRESENTATION BY CLASS I MOLECULES,**

L.R. Pease, J.K. Pullen, H.D. Hunt, Z. Cai, E.M. Rohren, and T.J. Yun. Department of Immunology, Mayo Clinic, Rochester, MN 55905

An extensive panel of L cells transfected with variant K^b genes was used to characterize the functional significance of the naturally occurring amino acid diversity found in the antigen binding domains of class I antigen presenting molecules. K^b and K^{bm10} restricted CTL clones, specific for an ovalbumin peptide ($Ova_{258-276}$) were used to assess the impact of amino acid substitutions on antigen presentation. Critical contact points throughout the peptide binding cleft, including residues on both the α1 and α2 helices as well as on the ß sheets were identified. Series of genes representing naturally occurring amino acid substitutions in key regions of the antigen recognition site were prepared to assess the significance of diversity in presentation of the ova peptide. The minimal peptide was defined ($Ova_{257-264}$) to permit a systematic complementation analysis to determine the basis for peptide sequence motifs associated with the observed class I polymorphisms and to examine the fine structure of K^b and K^{bm10} restriction of peptide presentation.

O 143 **CELL SURFACE EXPRESSION OF HLA-B27 IN TRANSGENIC MICE: COMPETITION FOR ß2 MICROGLOBULIN VS. ASSEMBLY REGULATORS.**

Susana Pedrinaci, Cheryl Nickerson-Nutter and Chella S. David. Department of Immunology, Mayo Clinic, Rochester, MN 55905.

We have previously reported that cell surface expression of HLA-B27 in transgenic mice varies in different H-2 haplotypes. There is good expression of B27 in mice homozygous for b, s, f, r, and k, but only marginal expression in haplotypes d,q, and v. Studies with recombinant mice indicated that the low expression of B27 in H-2^d haplotype mapped to the H-2D region. Two H-2D mutants suggested that the low expression may be due to genes mapping between H-2D and H-2L. Immunoprecipitation and endoglycosidase H experiments demonstrated considerable amount of HLA-B27 heavy chains in the low expressing haplotypes. These heavy chains did not associate efficiently with the mouse ß2M and remained in an immature high mannose form suggesting active or passive retention in the premedial-golgi compartment. Stimulation with IFN-γ can rescue expression of B27 and a class I band resistant to Endo-H was seen. Introduction of human ß2 microglobulin gene into the H-2^d/HLA-B27 mice increased the cell surface expression of B27 several fold. This suggested that as the number of class I molecules increase, HLA-B27 cannot compete with endogenous class I molecules for mouse ß2 but can be rescued by human ß2. In order to test this theory, HLA-B27 gene was introduced into an H-2^b mouse expressing an H-$2D^d$ transgene. HLA-B27 expression decreased confirming that B27 cannot compete with D^d. A transgenic mouse expressing a hybrid B27/K^k gene comprising the first two domains of B27 and a third domain of K^k also had a low expression in the H-2^d haplotype negating a role for the α3 domain in the affinity for mouse ß2. In order to determine whether genes between H-2D and H-2L play any role in the assembly and transport of HLA-B27 molecule, the B27 gene was introduced into a wild haplotype strain, B10.GAA37, which expresses the H-2D and H-2L gene products but lacks the chromosomal segment between D and L which has been deleted. The results from these studies will be reported.

O 144 ANTIGEN TRANSPORTER GENES INFLUENCE THE ANTIGENIC CONTENTS OF CLASS I MHC MOLECULES.

Simon J. Powis, Jonathan C. Howard and Geoffrey W. Butcher.
Dept. of Immunology, AFRC Inst. of Animal Physiology and Genetics Research, Babraham, Cambridge, United Kingdom.

Class I major histocompatibility complex molecules encounter, within the environment of the endoplasmic reticulum, peptide antigens which they are destined to transport to the cell surface and present to T lymphocytes. The mechanism by which these endogenously derived antigens gain access to this intracellular membrane bound compartment is now thought to be mediated through the auspices of the two MHC encoded "antigen transporter" genes. These genes bear striking resemblances to sequences of genes with known transporter functions in both prokaryotes and eukaryotes.
We present here evidence that transfection into *in vitro* cell lines of one of the transporter genes derived from the rat directly influences the contents of the antigen binding pocket of class I molecules, and can also alter class I assembly and transport characteristics, including those of the class I deficient mutant cell line RMA-S.
This data indicates that the MHC encoded antigen transporter genes are directly involved in the class I antigen presentation pathway, and also raises the possibility of selective antigen transport across the ER membrane prior to MHC binding.

O 145 *RING4* AND *RING11*, TWO ABC TRANSPORTERS WITHIN THE CLASS II REGION OF THE HUMAN MHC, Stephen H Powis, Adrian Kelly, Ian Mockridge, Lesley-Anne Kerr, Richard Glynne, Stephan Beck and John Trowsdale, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, U.K.

T lymphocytes recognise antigen which is presented bound to class I or class II MHC molecules at the cell surface. In the case of class I, small antigenic peptides are initially bound in the endoplasmic reticulum (ER). However, as most antigens originate in the cytoplasm, a mechanism must exist to transport them across the ER membrane. We have isolated two genes, *RING4* and *RING11*, which share sequence identity with members of the ABC (ATP-binding cassette) transporter superfamily, and are candidates for such peptide transporters. Members of the superfamily include the oligopeptide permease transporters in bacteria and the multidrug resistance and cystic fibrosis gene products in eukaryotes. *RING4* and *RING11* are located within the class II region of the MHC, between the genes *DNA* and *DOB*, and encode proteins of 748 and 686 amino acids, respectively. Both genes are up-regulated by interferon, a property shared with other genes known to be involved in antigen presentation. Comparison with other ABC transporters reveals that both RING4 and RING11 consist of an N-terminus hydrophobic region, with at least 6 transmembrane spanning segments, and a C-terminus ATP-binding cassette region. RING4 and RING11 share 60% a.a. identity over the ABC region and 30% over the hydrophobic region. This compares with 50% a.a. identity between the ABC regions of RING11 and the human multidrug resistance gene product. We propose that RING4 and RING11 form a heterodimer which pumps peptides from the cytoplasm into the ER. As many human diseases, including autoimmune disorders such as coeliac disease and diabetes mellitus, are associated with the MHC, and genes within the region are in linkage disequilibreum, it is of interest to determine whether *RING4* and *RING11* are polymorphic. So far we have identified three alleles of *RING11*, one of which has an additional seventeen amino acids at the C-terminus. All three alleles are frequently represented in a normal, Caucasoid population.

O 146 REACTIVITY OF AN HLA-B*2705-SPECIFIC MONOCLONAL ANTIBODY IS ASSOCIATED WITH A SURFACE CLUSTER OF CATIONIC AMINO ACID RESIDUES, David T.Y. Yu, Xiao-Kuan Cheng, and Richard B. Raybourne, Rheumatology Division, Department of Medicine, UCLA, Los Angeles, CA 90024 and Immunobiology Section, Division of Microbiology, FDA, Laurel, MD 20708

The Ye-2 monoclonal antibody is specific for HLA-B27+ cells. Its epitope was mapped, first with a panel of synthetic peptides which indicated that residues 63-84 of B*2705 were reactive. Scanning with overlapping peptides then localized the reactivity to the R-E-D-L-R region. Replacement sets substituting each of these residues consecutively by 19 amino acids identified the 2 arginines as being critical. That a cluster of cationic residues was responsible was verified by positive antibody reactivity with a homopolymer of arginine, and to a lesser degree of lysine, but not with 12 other amino acids.

To test the native HLA-B27 itself, 2 of the 4 cationic residues in the alpha-1 domain, the R83 and the K70 were changed to G83 and Q70 respectively, using site-directed mutagenesis. The mutated genes were expressed in L cells. The anti-HLA class I W6/32 and the anti-HLA-B27 B27.M2 antibodies reacted positively, but not the Ye-2.

Recognition that there is an accessible cluster of cationic residues in HLA-B27 provides another molecular focus to study antigen presentation as it may relate to the pathogenesis of the HLA-B27-associated arthritis.

O 147 **A NATURALLY PROCESSED OVALBUMIN FRAGMENT IS PREFERENTIALLY RECOGNIZED BY THE H-2K^b-RESTRICTED CYTOTOXIC T CELL CLONE 10BK.1,**

Angelika B. Reske-Kunz*, Martin Staege*, Thomas Dick*, Peter Walden#, and Hans-Georg Rammensee#. *Institute for Immunology, Johannes Gutenberg University, W-6500 Mainz, #Max-Planck-Institute for Biology, W-7400 Tübingen, FRG.

The cytotoxic H-2K^b-restricted T cell clone 10BK.1 specifically reacts to ovalbumin (OVA) by the production of lymphokines (IL-2, IFN-γ, IL-3) and by proliferation in the absence of added antigen-presenting cells, suggesting self-presentation of the antigen by the T clone cells (Proc. Natl. Acad. Sci. USA 86, 2316 (1989)) However, distinct batches of OVA differed in their capacity to activate 10BK.1 cells. Fragmentation of non-stimulatory batches of OVA by CNBr-treatment resulted in stimulatory potential. The relevant T cell epitope was shown to be located on OVA-peptide 258-276. Upon testing several preparations of this peptide we realized that highly purified peptide preparations exhibited low efficiency in stimulating the T cells. This finding suggested that fragments contained within the peptide preparations might represent the active moiety. Because 10BK.1 cells proliferate in response to OVA-transfected E.G7-OVA tumor cells (Moore et al., Cell 54, 777 (1988)) the octapeptide OVA257-264 (SIINFEKL) corresponding to the naturally processed OVA-peptide recovered from H-2K^b molecules of these tumor cells was used to induce T cell proliferation. On a molar basis this octapeptide was several logs more efficient than the 19-mer peptide. Our data suggest that the octapeptide OVA257-264 naturally bound by H-2K^b molecules is recognized most efficiently by the TCR of 10BK.1 cells. Longer peptides with ends jutting out of the binding groove of H-2K^b molecules appear to be recognized with much lower efficiency or not at all. In addition, longer peptides may bind to H-2K^b molecules with much lower affinity than the naturally processed octapeptide.

O 148 THREE HUMAN SMALL CELL LUNG CANCER (SCLC) CELL LINES DO NOT PRESENT ENDOGENOUS ANTIGENS IN A MODEL CHIMERIC SYSTEM, NP Restifo, JW Yewdell, JJ Mulé, SA Rosenberg, and JR Bennink, NCI and NIAID, NIH, Bethesda, MD 20892. We have recently described a murine tumor which may evade recognition by $CD8^+$ T cells by failing to present peptide fragments of endogenous proteins in a class I restricted fashion (*Restifo et al JI 1991 147:1453*). In extending this approach to human tumors, the problem of polymorphism at the class I loci prompted the development of a rapid screening system using a recombinant vaccinia virus expressing the mouse K^d gene (vac-K^d). Vac specific, K^d restricted effector cells generated in BALB/c mice were used to probe vac-K^d infected human tumor lines for their abilities to process and present endogenously generated vac antigens. Validity of chimeric methodology was confirmed by studies with cell lines known to have functioning antigen processing and presentation systems (some human melanoma cell lines) as well as the hybridoma, T2, known to be defective in its ability to process and present endogenous antigens. Using cryopreserved human tumor specimens and previously established human tumor lines, 26 different human samples were studied from a variety of tumor histologies including breast, colon, lung, and melanoma. While tumors varied widely in their abilities to process and present antigen, 3 tumor lines, all SCLC lines, were reproducibly (in 4/4 experiments) found to have near absolute inabilities to process and present vaccinia antigens. Below is a representative 4 hr ^{51}Cr release assay with lung cancer lines showing tumor designation, % specific ^{51}Cr release, and histology at an E:T of 10:1: H146 **5%** (*SCLC*); H1092 **-1%** (*SCLC*); H82 **-2%** (*SCLC*); control lines were: H720 **36%** (*carcinoid*); H1155 **51%** (*neuroendocrine*); and H28 **45%** (*mesothelioma*). Failure to be killed by appropriately directed $CD8^+$ T cells was not due to lack of infection by vac-K^d: Production of vaccinia virus antigens and restricting class I K^d molecules was verified by FACS. These findings support the hypothesis that antigen processing and/or presentation defects may be employed by human tumors to escape recognition by $CD8^+$ T cells.

O 149 **THE ROLE OF ANTIGENIC PEPTIDES AND β2-MICROGLOBULIN ON THE *IN VITRO* TRANSLATION, FOLDING, AND ASSEMBLY OF MURINE CLASS I MHC MOLECULES.** Randall. K. Ribaudo, and David .H. Margulies. Molecular Biology Section, Laboratory of Immunology, NIAID, NIH, Bethesda, Md. 20892

Using an *in vitro* translation/translocation system we have studied the effects of peptides and β2 microglobulin (β-2m) on the early events of folding and assembly of murine class I MHC molecules. cDNAs encoding a variety of murine class I MHC molecules were isolated and cloned into pGEM RNA expression vectors. RNA was then transcribed and capped *in vitro* and used to prime a rabbit reticulocyte lysate *in vitro* translation system supplemented with canine pancreatic microsomes. Both the heavy chain H-$2L^d$ and β–2m are translated, and translocated into the microsomes. Further, adjustment of the redox potential by addition of oxidized glutathione (GSSG) results in the formation of intrachain disulfide bonds which stabilize the native conformation. Addition of an H-$2L^d$ restricted peptide from the cytomegalovirus immediate early p89 protein, pMCMV, promotes the folding of the α1α2 domain and the stable association with β-2m as determined by immunoprecipitation with conformationally sensitive monoclonal antibodies. Analysis of a set of peptides in which alanine was substituted at each position of pMCMV has identified critical residues involved in the folding and assembly of these MHC I complexes.

O 150 LIMITED CROSSREACTIVITY AND SLOW EVOLUTIONARY CHANGE OF MINOR HISTOCOMPATIBILITY ANTIGENS. Derry C. Roopenian, Alan P. Davis and Greg A. Christianson, Larry E. Mobraaten, The Jackson Laboratory, Bar Harbor, ME 04609

Immunological self, as perceived by the T cell repertoire, is determined by the collection of peptides that bind the antigen pocket of MHC proteins during T cell ontogeny. Since minor histocompatibility (H) genes encode 'self' peptides that happen to be polymorphic, and are thus antigenic between genetically dissimilar individuals, they provide a useful approach for exploring basic questions concerning the peptides that define 'immunological self'. This study utilizes the minor H antigen system to address two questions: (1) How much antigenic crossreactivity is there within the universe of self peptides? (2) How much antigenic change of self peptides occurs over evolutionary time? To address these questions, mouse strains representative of distinct species and subspecies were analyzed for expression of a number of defined minor H antigens recognized by cloned class I and class II restricted T cells. Many apparently unrelated strains expressed common minor H antigen epitopes, and there was no correlation between evolutionary distance and antigen expression. The common epitopes could not be attributed to crossreactivity with allogeneic MHC. To determine whether the genes encoding the common epitopes are allelic with the sensitizing minor H antigen or a result of crossreactivity of products of different minor H genes, classical genetic segregation analysis involving both intraspecific and interspecific mouse crosses was carried out. In every case analyzed, the crossreactive epitope was shown to map to the same locus as the sensitizing minor H antigen, suggesting that they are encoded by the same gene. These results lead us to hypothesize: (1) the universe of self peptides recognized as immunological self tends not to overlap antigenically; (2) self peptide epitopes are retained over extended evolutionary periods.

O 151 GENETIC MODULATION OF ANTIGEN PRESENTATION IN A FAMILY WITH HLA-B27

Sarah Rowland-Jones, Laszlo Pazmany,Stephane Huet, Julian Sutton, Ruth Murray, Jill Brooks and Andrew McMichael,Institute of Molecular Medicine, Oxford, OX3 9DU,U.K.

We have studied presentation of three different HLA-B27 -restricted viral epitopes by the lymphoblastoid cell-line of a healthy individual with HLA-B27,together with lines from seven members of his family who share an A3 B27 class I haplotype. These cells were unable to present known peptide epitopes to cytotoxic T cells (CTL), despite adequate expression of HLA-B27 on the cell surface. This B27 molecule was identified as B2702 and no coding changes were found when cDNA coding for B2702 was cloned from the proband's cell line and sequenced. When the cloned DNA was transfected into HLA A and B negative HMy/C1R cells, the B2702 molecules generated in this environment were able to present peptide normally . This suggests that one or more genetic factors are interfering with antigen presentation by otherwise normal B2702 molecules in this family. Studies on antigen presentation by other HLA molecules in this family are underway.Two family members have HLA-A2 and show normal presentation of both added peptide and virally-derived antigen to CTL. However, although presentation of added peptide by HLA-B8 was normal using cells from the proband, there was reduced lysis by B8-restricted influenza-specific CTL of these cells when infected with virus compared with controls. This suggests that effects on antigen presentation may extend to the other HLA- haplotype.

O 152 TEMPERATURE SENSITIVE ASSEMBLY AND TRANSPORT OF CLASS I HLA DIMERS IN CELLS TRANSFECTED WITH MUTANT HLA HEAVY CHAIN GENES, R. Salter, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Class I HLA proteins are heterodimers composed of polymorphic heavy chains and invariant B_2m subunits, which present peptide antigens to T cells of the immune system. It has previously been shown that heavy chains associate within 5-10 min. after synthesis with B_2m, presumably in the ER, and more recently it was hypothesized that peptides generated in the cytosol and transported into the ER lumen bind to heavy chains or class I dimers.

To analyze structural requirements for assembly and transport of class I dimers, I have generated mutant HLA heavy chains by site-directed mutagenesis with substitutions in the interface between the $\alpha 3$ domain and B_2m. Upon transfection, these mutants are not detectable on the cell surface with MAb, and are shown to have reduced affinity for B_2m. Surface expression can be rescued by growing the cells at reduced temperatures, and is dependent on enhanced association with B_2m. Based on the kinetics of induction in the presence of cycloheximide and Brefeldin A, it is concluded that a relatively stable HLA mutant heavy chain pool accumulates in a pre-Golgi compartment before temperature downshift. The defective phenotype can be reversed by a second site mutation in the peptide binding site of the heavy chain at position 97 arg>met. How this substitution allows the double mutant to be transported apparently normally at 37° is being investigated by measuring the affinity for β_2m and peptide binding capacity of the mutant and wild type A2.1 molecules.

O 153 POTENT INHIBITION OF ALLOREACTIVE T CELLS BY DIVALENT, SOLUBLE HIGH AVIDITY CLASS I MHC MOLECULES, Jonathan Schneck, Joseph Dal Porto, Dave T Tuveson, Branimir Catipovic, Steve Kozlowski, Douglas Fearon, The Johns Hopkins University, School of Medicine, Baltimore, MD 21224

The major histocompatibility complex (MHC) class I molecules are integral membrane glycoproteins which present antigenic peptides derived from cellular, tumor, and viral proteins to cytotoxic T cells. MHC-mediated stimulation of T cells is driven by multivalent interactions between MHC molecules on the antigen presenting cells and T cell receptors on the T cells. Genetically engineered or chemically purified soluble monovalent MHC molecules which had previously been used to study T cells did not effectively block T cell responses. Here we describe a novel, genetically engineered divalent class I MHC heavy chain protein which is a potent inhibitor of T cell responses. This chimeric protein, H-$2K^b3$/IgG_1, was generated as a fusion protein with an immunoglobulin heavy chain polypeptide. The chimeric protein has serological and biochemical characteristics of both the MHC and IgG polypeptides. Nanomolar concentrations of H-$2K^b3$/IgG_1 not only inhibited alloreactive anti-H-$2K^b$-specific T cell clones, but also inhibited alloreactive anti-H-$2K^b$-specific primary T cell cultures. The potency and specificity of inhibition of T cell responses make it a potentially valuable reagent in selectively suppressing undesired MHC-specific T cell responses, as seen in autoimmune diseases and transplantation rejection.

O 154 IDENTIFICATION OF HUMAN MINOR HISTOCOMPATIBILITY PEPTIDE PRESENTED BY HLA-B35 MOLECULE, Masayuki Sekimata[1], Peter Griem[2], Kohji Egawa[1], Hans-Georg Rammensee[2] and Masafumi Takiguchi[1], [1]Department of Tumor Biology, Institute of Medical Science, University of Tokyo, Tokyo, JAPAN and [2]Max-Planck-Institute für Biologie, Abteilung Immungenetik, Tübingen, GERMANY

Human minor histocompatibility antigens are believed to induce rejection of the graft in organ transplantation and graft vs. host reactino in bone marrow transplantation. To characterize human minor histocompatibility antigen, we attempted to identify human minor histocompatibility peptides recognized by T cells. By using human minor histocompatibility antigen specific, HLA-B35 restricted CTL clone which was previously generated from a patient who had been grafted with the kidneys from mother and two HLA-identical sisters, naturally occuring human minor histocompatibility peptides were identified from a donor drived B cell line by an acid elution technique. The peptides were also identified from transfectant of HLA-A, B null human B cell line, Hmy2C1R cells expressing HLA-B35 but not from those expressing HLA-B51 or HLA-A11. These results not only demonstrate human minor histocompatibility peptides recognized by T cell but also confirm previous study suggesting that MHC class I molecules themselves determine the peptides which are naturally processed and bound to MHC molecule.

O 155 PRESENTATION OF NATURALLY PROCESSED OVALBUMIN PEPTIDE/K^b COMPLEX TO T-CELLS, Nilabh Shastri and Federico Gonzalez, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The generation of peptide/MHC complexes in antigen presenting cells (APC) was analyzed in the ovalbumin (OVA)/K^b model system. The OVA peptide 257-264 (SIINFEKL) was optimal for stimulating OVA/K^b specific T-cells when added exogenously or when synthesized within K^{b+} APC transfected with cDNA constructs. T-cell stimulating activity of exogenously supplied octapeptide SIINFEKL was about three orders of magnitude higher than either shorter or longer analogs. By contrast, T-cell stimulation by transfected K^{b+} APC presenting endogenously synthesized OVA peptides was remarkably insensitive to size, intracellular location, flanking sequences, or to abundance of the translated gene products. These characteristics of the endogenous presentation mechanism explain why simultaneous display of a large number of distinct peptide/MHC complexes occurs constitutively. Possible mechanisms to account for these findings are discussed.

O 156 DUAL SELF MHC-RESTRICTED CYTOTOXIC T CELL RESPONSE TO A HORSE CYTOCHROME C PEPTIDE. James M. Sheil, Sara E. Shepherd, Todd D. Schell, and Yvonne Paterson[1], Department of Microbiology & Immunology, West Virginia University Health Sciences Center, Morgantown, WV 26506 and [1]Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Cytotoxic T lymphocytes (CTL) were induced in C57BL/6 (B6) and B6.C-H-2^{bm1} (bm1) mice by *in vitro* peptide stimulation with a tryptic digest of horse cytochrome c (cyt c). The peptide specificity for clones from both CTL effector populations is the p40-53 horse cyt c tryptic fragment, and the nonamer p41-49 (Gly-Gln-Ala-Pro-Gly-Phe-Thr-Tyr-Thr) peptide can substitute for this fragment with full antigenic activity. Analysis of CTL reactivity in the presence of tryptic digests of cyt c from horse, bovine, chicken and pigeon species reveals that the B6-derived CTL clones respond to both bovine and horse tryptic digests, but the bm1-derived CTL clones recognize only the horse digest. Within this peptide sequence, both bovine and horse cyt c contain 44-Pro (which is replaced by 44-Glu in chicken and pigeon species), suggesting a primary influence of this residue in recognition by B6-derived CTLs. A second polymorphism involving the substitution of 47-Thr in horse cyt c with a Ser residue in all three bovine, chicken, and pigeon species, indicating a major influence of 47-Thr in recognition by bm1-derived CTLs. Additional analysis of CTL reactivity reveals that the hexamer peptide, p43-48, is the minimal sequence recognized by both B6- and bm1-derived CTL clones. Analysis of CTL reactivity in the presence of the p41-49 peptide on L cell targets transfected with either the K^b, D^b, or K^{bm1} genes reveals that both B6-derived and bm1-derived CTL clones recognize this peptide when presented by either H-2K^b, D^b, or K^{bm1} Class I molecules. Neither B6-derived nor bm1-derived CTLs, however, respond to this peptide when presented by H-2^k orH-2^d targets. Evidence also suggests H-2D^b and H-2K^{bm1} Class I molecules may be related in their ability to present this peptide due to the presence of identical 152-Ala and 156-Tyr residues in the long α-helix region of the α2 domain.

O 157 DIRECT EVIDENCE THAT T LYMPHOCYTES HAVE LOW AFFINITY FOR SELF-MHC, Linda Sherman, Michael Irwin and Sabine Hesse, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Recognition by CTL of highly polymorphic class I MHC molecules involves both the T cell receptor (TCR) and the co-receptor molecule, CD8. It is generally believed that the TCR recognizes antigenic peptides in conjunction with the allele specific portions of the 1, 2 domains of class I whereas the CD8 co-receptor recognizes the more conserved 3 domain of class I. Murine CD8 does not interact with the 3 domains of some xenogeneic class I molecules and this contributes to the low frequency of murine T cells that can respond to the human class I molecule, HLA.A2.1 (A2), as compared with a chimeric molecule, A2/K^b, comprised of the 1 and 2 domains of A2 linked to a murine 3 domain. We have compared the ability of CTL derived from murine transgenic lines that express either an A2 or A2/K^b transgene to utilize these molecules as restriction elements during an influenza virus specific response. CTL from A2 transgenic mice can respond to an A2-restricted viral antigen and such recognition is CD8-independent. Interestingly, even in the absence of antigen these CTL display lysis of A2/K^b targets, however, such lysis is CD8 dependent. These findings indicate the TCRs on A2 restricted CTL have low affinity for A2 per se and that by permitting interaction through CD8 this affinity is increased to a level that permits lysis of A2/K^b bearing cells in the absence of antigen. This represents direct evidence that the receptors on T cells with high affinity for self-MHC plus antigen have low affinity for self-MHC.

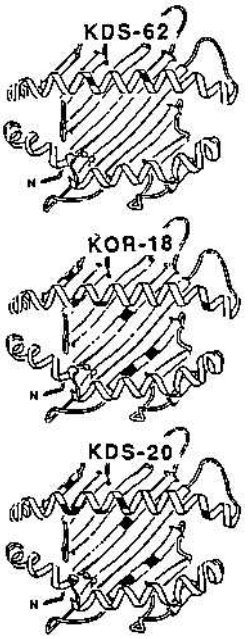
O 158 DISPARATE INTERACTION OF PEPTIDE LIGAND WITH NASCENT VERSUS MATURE CLASS I MHC MOLECULES: COMPARISONS OF PEPTIDE BINDING TO ALTERNATIVE FORMS OF L^d IN CELL LYSATES AND THE CELL SURFACE, Joseph D. Smith,* Wen-Rong Lie,* John Gorka,† Cathy S. Kindle,* Nancy B. Myers,* and Ted H. Hansen*

*Department of Genetics and †Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri, 63110, USA

To determine the mechanism and structural consequences of peptide binding to class I molecules, we have studied the L^d molecule of the mouse. L^d molecules exist in two different antigenic conformations designated L^d and L^dalt. L^dalt molecules are non-ligand associated and show weak if any β_2m association. We find that L^d molecules have a relatively rapid surface turnover compared with other class I molecules and that exogenous peptide dramatically prolongs L^d surface half-life. By contrast L^dalt molecules are stably expressed on the surface and their half-life is unaffected by exogenous peptide. To study the interaction of peptide with surface L^d, live cells were incubated with iodinated peptides and L^d molecules were precipitated from cells precoated with mAb prior to lysis. Using this assay, peptide binding to surface L^d molecules was found not to depend upon exchange with exogenous β_2m, but did correlate with the level of β_2m association. To study the intracellular interaction of peptide with L^d, cell lysates were used. In cell lysates, peptide converts L^dalt molecules to properly folded L^d. This peptide induced folding was almost complete at earlier but not later time points in pulse-chase analyses. Moveover, conversion of L^dalt to L^d was found to affect almost exclusively immature (Endo H^s) class I molecules. Thus intrinsic properties of immature L^dalt molecules or their associated chaperonins are maintained in cell lysates that allow them to undergo de novo folding in vitro. These combined results demonstrate that immature L^dalt molecules are precursors awaiting constituents such as peptide and β_2m that influence folding, whereas surface L^dalt molecules appear refractory to association with peptide, β_2m and consequent folding.

O 159 THREE PATTERNS OF HLA-B7 ALLORECOGNITION BY CTL CLONES K. D. Smith, A. Valenzuela and C. T. Lutz. Dept. of Pathology, U. of Iowa, Iowa City, IA 52242

In recognizing allogeneic MHC, CTL may 1) contact MHC molecules alone with bound peptide being irrelevant, 2) contact peptides alone with MHC molecules determining the peptides presented, or 3) contact both MHC molecules and peptide. To determine which of these mechanisms may be relevant in allorecognition, we are studying how alloreactive CTL recognize HLA-B7. We generated a panel of 32 HLA-B7 variants, 15 with mutations at positions pointing toward the T cell receptor (TCR) and 17 with mutations at positions pointing into the peptide binding groove. Preliminary data, testing 18 of the HLA-B7 variants, divides anti-HLA-B7 alloreactive CTL clones into three groups. One CTL clone (KDS-62) is affected only by HLA-B7 mutations at positions 65 and 72 pointing toward the TCR and not by mutations in the peptide binding groove. A second CTL clone (KOR-18) is affected only by HLA-B7 mutations at positions 9, 45, 63, 66, 80, 114 and 116 pointing into the peptide binding groove. A third CTL clone (KDS-20) is affected by HLA-B7 mutations both at positions 62, 69, and 163, pointing toward the TCR, and at positions 9, 45, 63, 66, 80, 114 and 116 in the groove. These preliminary results support all three of the mechanisms for MHC allo-recognition. Thus, some alloreactive CTL may recognize only foreign MHC molecules, some may recognize only peptide bound to foreign MHC molecules and some may recognize both.



O 160 ASSEMBLY AND INTRACELLULAR TRANSPORT OF MHC CLASS I MOLECULES, **Elizabeth S. Song, Young Yang, Michael R. Jackson, Dennis A. Noe and Per A. Peterson, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037**

The binding of the antigenic peptide to the assembling class I molecule contributes to the stability of the class I complex. In theory, the following intermediates of class I assembly may exist: Free heavy chains, heavy chains associated with beta-2-microglobulin (b2m; empty complex), heavy chains associated with the peptide, and heavy chains associated with both b2m and the peptide (full complex). To be able to examine the putative intermediates in the class I assembly process, the intermediates were quantitated by using a panel of conformation -sensitive and -insensitive antibodies. From these experiments, we have determined the kinetics of the various class I intermediates. The peptide loading appears to occur earlier than b2m binding *in vivo*. A significant portion of the heavy chains which obtains b2m but fails to get peptides, will never get peptides despite their relatively long half-life. These heavy chains can be "rescued" to become full complexes by increasing the peptide supply by gamma-interferon treatment. Thus, the peptide and b2m addition may be temporally and/or spatially restricted within the cell and the availability of peptide is limiting factor in class I assembly. Heavy chains engineered to be retained inside of the endoplasmic reticulum (ER) receive b2m and peptide with the same kinetics as the wildtype molecules, suggesting that assembly and peptide-loading are strictly confined to the ER. However, degradation of the heavy chains appears to occur outside of the ER, possibly in autophagosomes. These and other data have allowed us to describe the formation and the kinetics of the class I assembly process and calculated the kinetic parameters of this process.

O 161 ADENOVIRUS PROTEIN E3/19K RETAINS BOTH EMPTY AND PEPTIDE BOUND CLASS I HLA ANTIGENS IN THE ENDOPLASMIC RETICULUM, Rakesh Srivastava, Michael R. Jackson, Per A. Peterson. Department of Immunology, The Scripps Research Institute, La Jolla, California 92037.

Recent studies on intracellular trafficking of class I MHC antigens have convincingly argued for the existence of cellular mechanisms that prevent the exit of peptide-free, "empty", class I molecules from the endoplasmic reticulum (ER). Cells infected with adenoviruses are thought to evade immune surveillance by preventing the exit of class I molecules from the ER. Specifically, E3/19K protein encoded by adenovirus 2 interacts with those regions of the class I molecules that are also critical for antigen binding, thus implying that it may prevent the exit of class I molecules from ER by directly blocking the acquisition of peptides in the antigen binding groove of the class I molecules. E3/19k may, therefore, represent a viral homologue of the proposed cellular proteins involved in the retention of empty class I molecules in the ER. We provide evidence that the binding of E3/19k precedes the peptide acquisition by class I molecules in the ER and this interaction does, in fact, result in the accumulation of large amounts of empty class I molecules. However, a significant fraction of class I molecules is able to acquire peptides in the presence of E3/19K.

O 162 STRUCTURAL REQUIREMENTS OF PEPTIDE-HLA-A2 INTERACTION, Ursula Utz*, Ken Parker#, John E. Coligan, and William E. Biddison*, *Neuro-immunology Branch, NINDS, NIH, Bethesda, MD 20892, #Biological Resources Branch, NIAID, NIH, Bethesda, MD 20892.

The influence of distinct substructural features within the HLA-A2.1 molecule on the presentation of three different viral peptides was analyzed, in order to determine, whether similar molecular structures would be involved in the interactions with each of the three peptides. HTLV-I Tax 12-19, HCMV gB 619-628, and influenza M1 58-66 are peptides that induce HLA-A2.1-restricted non-crossreactive CTL. A panel of 14 HLA-A2 mutants with single amino acid substitutions within pockets located in the peptide binding site was tested for effects on presentation of the three viral peptides. Ten of the 14 mutants showed concordant effects on the presentation of the peptides. Four mutants affected presentation nega-tively for only one or two of the peptides. Thus, common structural features in HLA-A2 determine the binding and conformation of different peptides. Predictions for requirements for HLA-A2 binding, based on a comparison of the structure of the three peptides and the effects that mutations in A2.1 had on their presentation, lead to the design of potential HLA-A2-binding peptides. One of four tested designer peptides bound to HLA-A2.1 as determined by a cell-free peptide binding assay.

O 163 STRUCTURAL FEATURES REQUIRED FOR THE PRESENTATION OF ANTIGENIC PEPTIDES IN THE CONTEXT OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I MOLECULES. **Grada van Bleek and Stan Nathenson, Albert Einstein College of Medicine, Bronx NY 10461.**

Cytotoxic T Lymphocytes recognize short peptides presented on the cell surface by MHC class I molecules. The peptides are the products of proteolysis of intracellular or foreign proteins. Recently, we developed a procedure to isolate endogenously bound peptides from MHC class I molecules. Analysis of those peptides revealed that they are short: 8-10 amino acids long. Furthermore, an MHC specific binding motif is apparent in the presented petides which is different for different MHC molecules even when only few amino acids that line the antigen binding groove are changed. However, each MHC molecule binds some peptides that do not have the major binding motif. Further, peptide sequences in proteins that have major binding motifs are not found in association with class I molecules on the cell surface. Thus other requirements like non-anchor residues in the peptides or aminoacid stretches around the peptide influence the selective processes witch culminate in cell surface presentation.

O 164 HUMAN CYTOMEGALOVIRUS INFECTION BLOCKS ANTIGEN RECOGNITION BY CD8$^+$ CYTOTOXIC T CELLS.

A P Warren, C Y Wang, F Gotch†, A Persidis,$^+$ W K Magoba*, J G P Sissons$^+$ and L K Borysiewicz, Department of Medicine, UWCM, Cardiff, UK,* Department of Endocrine Immunology, RPMS, London, UK.,$^+$ Department of Medicine, University of Cambridge, UK.,† Department of Molecular Immunology, Institute of Molecular Medicine, Oxford, UK.

Human cytomegalovirus (HCMV) specific CD8$^+$ cytotoxic T cells (CTL) regognise HCMV antigens in association with MHC class I (HLA A and B) on the cell surface. These CTL are an important component of the host protective response against the virus and may serve to maintain the virus/host equilibrium in the persistantly infected host. Furthermore, there appears to be a bias in the HCMV specific CTL repertoire towards non-structural antigen. To investigate a possible role for the virus in selecting CTL specificity, we studied the effect of HCMV infection on antigen presentation function. Surface staining of HCMV infected fibroblasts reveals an increase in ICAM-1 expression at 6h p.i., followed by a 5-10 fold decrease in expression of cell-surface MHC class I. This led us to test whether antigen presentation by HCMV infected cells is impaired. To examine this we challenged HMCV infected fibroblasts to present 'third party' antigens to 'third party antigen' specific CTL. Influenza matrix protein specific CTL lyse MHC matched fibroblasts treated with the synthetic peptide corresponding to the relevant matrix protein sequence. No killing occurs if the fibroblasts are first infected with HCMV. Similarly , infection with recombinant vaccinia virus encoding influenza matrix protein can sensitise fibroblasts to lysis by influenza specific CTL, but this is not seen in cells co-infected with HCMV. This defect in antigen presentation caused by HCMV infection may be linked to the decreased MHC class I levels. Investigations are underway to determine how the decreased expression of class I is mediated.

O 165 PRIMARY *IN VIVO* GENERATION OF A CTL RESPONSE IN RELATION TO *IN VITRO* UP-REGULATION OF MHC-I BY SHORT SYNTHETIC PEPTIDES.

Xianzheng Zhou, Louise Berg, Ussama Mohammed and Mikael Jondal, Department of Immunology, Karolinska Institute, Box 60400, 104 01 Stockholm, Sweden.

Anti-viral cytotoxic T lymphocytes (CTL) recognize short peptides bound to MHC class I molecules (MHC-I) and play a crucial role in the recovery from many viral infections. Such short peptides have an optimal length of 7-10 aa for stable complexing with MHC-I. In the present work we defined optimal conditions for generating a primary *in vivo* CTL response using short synthetic peptides and compared this with their capacity to up-regulate MHC-I expression *in vitro*. For D^b binding peptides we found that the optimal length was important both for the generation of a primary CTL response *in vivo* and for MHC-I up-regulation *in vitro*. The influenza NP366-374 peptide thus generated a stronger *in vivo* CTL response, and was 100-fold better in up-regulating D^b and 10,000-fold better in sensitizing virus-infected or peptide-treated target cells for specific CTL killing than longer peptides. Using L^d binding peptides we obtained a similar correlation between a primary *in vivo* response and *in vitro* up-regulation. K^b binding peptides, however, did not up-regulate K^b molecules, although they induced an *in vivo* CTL response. We conclude that short peptides are strongly immunogenic, given that they have an exact length corresponding to endogenously processed peptides and this immunogenic property is reflected *in vitro* by the capacity of the peptides to enhance MHC-I expression, at least with D^b and L^d alleles. These results suggest that short synthetic peptides could become candidates for anti-viral vaccine developement.

O 166 ENDOGENOUS AND EXOGENOUS LOADING OF HLA-A2 BY PEPTIDE ANALOGS OF THE INFLUENZA VIRUS MATRIX PROTEIN.

H. Zweerink, M. Gammon, K. Parker*, M. Bednarek, W. Biddison*, B. Cunningham, J. Hermes, G. Porter, S. Sauma, S. Tamhankar and A. Williamson. Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065 and *The National Institutes of Health, Bethesda, MD 20892.

The physiologically most relevant association of peptides with MHC class I molecules occurs in the endoplasmic reticulum. In order to define the essential amino acids in the matrix peptide 57-68 that specify peptide binding to HLA-A2 under these conditions and subsequent presentation to T cell receptors we expressed analogs with alanines substituting each of the amino acids in peptides 57-68 (12-mer) and 58-66 (9-mer) in the cytoplasm of HLA-A2 positive cells using a stable mammalian expression vector. Susceptibility of transfected cells to lysis by HLA-A2 restricted bulk and cloned CTL cultures specific for peptide 57-68 and 58-66 was compared with that of cells sensitized with the same synthetic peptide analogs.

Results for endogenous and exogenously supplied peptides were virtually identical. Changing residues 57-59 (K, G and I) or 66-68 (L, T and V) was of little consequence. Changes in residues 60 (L), 61 (G) or 65 (T) led to a significant reduction in lysis, whereas substitutions in residues 62 (F), 63 (V) or 64 (F) resulted in further reduction. Direct binding experiments showed that alanine substitutions in positions 62, 63 and 64 reduced but not eliminated binding whereas substitutions in positions 60 and 61 largely eliminated binding. These and CTL inhibition experiments suggest that residues 62, 63 and 64 interact with the T cell receptor and residues 60 and 61 with the HLA-A2 molecule.

These observations show that the specificity of the interaction between peptides and HLA-A2 molecules in the ER and at the cell surface is virtually the same; and they extend results reported by others who, using synthetic peptide analogs, showed the importance of amino acids in positions 60-65 for HLA-A2 binding and recognition.

Class II MHC

O 200 ANTAGONISM IS DEMONSTRATED AT THE T CELL RECEPTOR LEVEL BY ANTIGEN ANALOG/MHC COMPLEXES, Jeff Alexander,* M.Teresa De Magistris,* Mark Coggeshall,‡ Amnon Altman,‡ Ken Snoke,* John Sidney,* Scott Southwood,* Melissa Wall,* Federico C. A. Gaeta,* Howard M. Grey * and Alex Sette,* *Cytel Corporation, 3525 John Hopkins Ct., San Diego, CA 92121, ‡ La Jolla Institute for Allergy and Immunology, 11149 N. Torrey Pines Rd., La Jolla, CA 92037
The mechanism by which antigen analog/MHC complexes inhibited T cell proliferation was examined. Preliminary experiments demonstrated that nonstimulatory influenza hemagglutinin (HA 307-319) analogs inhibited HA-specific T cell DR1 restricted proliferation more efficiently by two orders of magnitude (down to 60 nM 50% doses) than non-HA peptides that exhibited similar binding affinities for DR1. These HA analogs did not affect stability of the HA/DR1 complexes, did not bind to different subsites on the MHC relative to non-HA peptides, did not induce T cell tolerance, or bind to the TCR in the form of free peptides. A prepulse protocol was also developed to exclude any influence of competion for binding to MHC. Using this protocol, only the HA analogs demonstrated inhibition of T cell proliferation. This inhibition was competitive in nature such that increasing antigen concentration could overcome inhibition. Furthermore, analog/MHC complexes did not trigger early events of T cell proliferation such as Ca^{++} influx or inositol phosphate turnover. It is therefore likely that HA analog/MHC complexes compete with HA antigen/MHC complexes for binding the TCR and thus act as classical receptor antagonists.

O 201 CRYPTICITY OF T CELL DETERMINANTS: RELATIONSHIP TO THE MOLECULAR CONTEXT OF THE ANTIGEN AND TO ITS PROCESSING REQUIREMENTS, Akio Ametani, Alessandro Sette,* and Eli E. Sercarz, Department of Microbiology and Molecular Genetics, University of California, Los Angeles, California 90024 and *Cytel, La Jolla, California 92037
The T cell determinant 87-96 in hen eggwhite lysozyme (HEL) is a cryptic determinant in H-2^a or H-2^k mice, and therefore, specific T cells are not activated towards this determinant after HEL priming. This is the case despite its strong binding to the I-E^k molecule, and the existence of an available T cell repertoire. A series of lysozyme derivatives, fragments and peptides were prepared in order to try to establish the rules of crypticity for this determinant or others. B10.A mice were immunized with each of the derivatives, or they were used for antigen presentation to 87-96-specific T cell hybridomas. In both of these assays, only the smaller peptides (74-96, 81-96 and 85-96) could stimulate the T cell populations. Longer lysozyme derivatives were inactive so that their binding to MHC was directly tested, using a method with E^k molecules solubilized in detergent. Interestingly, although native HEL does not bind under this condition, all other large and small derivatives bind well to E^k, indicating that even a minimal degree of unfolding or fracturing of HEL permits binding to the MHC. Thus, between the binding event and further fragmentation to yield a peptide resembling 74-96, the capacity to serve as a provider of residues 87-96 is lost. Several reasons applicable to different cases can be presented. 1) "T11+" (74-96 :(Cys64-Cys80):62-68) can bind to E^k but does not stimulate T cells, presumably because there is some hindrance to access of the TCR to this peptide in the MHC groove. 2) The longer peptides bind with an upstream or downstream E^k-restricted determinant, which provide a competitive force that diverts the response to these determinants. Otherwise, A^k molecules may also capture the large antigenic peptide and alter the processing of the rest of the determinants, perhaps by enzymatic trimming after binding to the MHC molecule. These results show that crypticity of 87-96 in H-2^a mice is essentially due to the natural processing pattern of HEL molecules in APC. We could also demonstrate that the C-terminal site of determinant 87-96 was easily degradaded by proteolytic attack resulting in losing T cell-inducing ability. The immunogenicity of determinants on a protein molecule is not only a product of its structural features, but also closely relates to the binding affinity and availability of up and downstream determinants restricted to the same or other MHC molecules.

O 202 GRAFT REJECTION IN CLASS II DEFICIENT MICE
Hugh Auchincloss, Jr., Richard Lee, Jay S. Markowitz, Michael J. Grusby, and Laurie H. Glimcher, Dept. Surgery, Mass. General Hospital and Harvard School of Public Health, Boston, MA 02114

Mice lacking expression of MHC class II antigens were generated by targeted disruption of the $A\beta^b$ gene of ES-D3 cells. Mice which are homozygous for this defective gene not only lack class II antigens but also have few $CD4^+$ T cells. These mice do not produce IgG anti-TNP responses.

Transplantation experiments have been performed with the class II deficient mice. Class II deficient skin was rejected rapidly (day 9) by normal recipients with multiple minor histocompatibility antigen differences. Normal skin with major and minor histocompatibility differences was also rejected rapidly (day 13) by class II deficient mice, but normal skin with only minor antigen differences survived about three times longer on mutant mice compared to wildtype littermates. A pig skin xenograft survived about 30 days on a class II deficient mouse although the same mouse rejected a simultaneous whole-MHC disparate allograft on day 13. No anti-donor MLR or CTL activity was detectable in mutant mice after xenogeneic skin grafting. A class II deficient mouse reconstituted with 20 x 10^6 normal B6 spleen cells rejected a minor antigen disparate allograft on day 16 but kept a pig skin xenograft until day 25.

These results are consistent with the interpretation that: 1) neither recipient class II antigen presentation nor $CD4^+$ T cells are required for whole-MHC disparate allograft rejection, 2) recipient class II antigen presentation is not required but $CD4^+$ T cells are important in minor antigen disparate allograft rejection, and 3) both recipient class II antigen presentation and $CD4^+$ T cells are important in xenograft rejection.

O 203 HLA DR-4 RESTRICTED T-LYMPHOCYTES RECOGNISE DECAPEPTIDES REPRESENTING THE NOVEL FUSION REGION OF THE BCR/ABL PROTEIN IN CML. Barrett AJ, Jiang YZ, Kars A, Gordon AA, Datta A, Department of Hematology, RPMS, Hammersmith Hospital, London, United Kingdom.

Two forms of a unique protein P210 formed by the fusion of thr BCR and ABL genes occur in chronic myelogenous leukemia (CML). We obtained synthetic decapeptides representing the novel leukemia-specific peptide sequences spanning the BCR/ABL junction region: b2a2 asn-lys-glu-glu-ala-leu-glu-arg-pro (NP), and P210 b3a2 lys-glu-ser-ser-lys-ala-leu-glu-arg (KR) (Neosystems Ltd) . We used 6 day proliferative assays and T-helper cell precursor frequency (HTLp) to measure response to the peptides at concentrations of 0.1-1.0 μg/ml. We tested 10 normal subjects, and donor lymphocytes given to three patients with CML to treat leukaemic relapse following marrow transplantation. 5/5 HLA DR4 normal subjects showed proliferative responses to both peptides. In contrast 5 others (with DR, 1,2,3,5,6) did not. HTLp frequency response to KR and NP was 3-9 fold higher in the DR4+ donor cells taken from the patients 3-6 months post-transfusion than in untransfused donor cells. These results suggest that there may be HLA restriction to exogenous CML derived antigens and that responses to such antigens occur *in vivo*.

O 204 CLASS II EXPRESSION ALONE IS NOT SUFFICIENT TO STIMULATE ALLOREACTIVE T CELL HYBRIDS, Jerry Bill, Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262. We have previously reported that most but not all (36 of 42 tested) I-A^{bm12} reactive T cell hybrids produce IL-2 when stimulated by L cells expressing the I-A^{bm12} molecule. By introducing B6 genomic DNA into the I-A^{bm12} positive L cell we have obtained a transfectant which stimulates one of the 6 hybrids not responsive to the I-A^{bm12} L cell itself. This transfectant, however, does not stimulate the other 5 non-responsive hybrids, indicating that the corrected defect is unique to the one hybrid and suggesting that the defect is at the level of TCR/MHC interaction rather than the lack of an accessory molecule. One obvious solution to this puzzle would be that the I-A^{bm12} L cell lacks the ability to produce some necessary self peptides either due to lack of substrate or of cell-specific processing/transport machinery. Cloning of the transfected gene is in progress and should clarify this defect and help define the requirements for allostimulation. We are now transfecting I-A^{bm12} into other non-lymphoid murine and xenogenic cell lines including the protease/transport defective T2 cell line in order to define the role of self peptides in Class II allostimulation. Interestingly, CHO-K1 transfected with I-A^{bm12} fails to stimulate at least 5 of the 6 hybrids not stimulated by the I-A^{bm12} L cell and in addition a few other hybrids. Results with the other transfectants now being generated will be presented.

O 205 ANTI-MHC CLASS II MONOCLONAL ANTIBODIES IMMUNOTARGET HIV PEPTIDES IN MICE AND MACAQUES. Judy Caterini, Barry Caplan, Gloria Zobrist, Pele Chong, Heather Boux, and Michel Klein. Connaught Center for Biotechnology Research, Connaught Laboratories Ltd., Toronto, Canada, M2R 3T4. We have investigated whether the immunogenicity of synthetic HIV peptides could be enhanced by "targeting" the peptides to cells which express MHC class II antigens by using class II-specific mAbs. Two HIV peptides, one from the p24 core protein and the other from the gp120 envelope glycoprotein, were used in these studies. Both of these peptides contain well characterized tandem T- and B-cell epitopes. The peptides were conjugated to targeting and control mAbs using a heterobifunctional cross-linking reagent. Consistent levels of peptide were linked to the mAbs resulting in conjugates containing between 4 and 8 moles of peptide per mole of mAb. In mice, conjugates prepared with TIB92 mAb (anti-I-A^k specificity) were immunogenic in I-A^b x I-A^k (C57Bl/6 x C3H/HeJ)F_1 mice, inducing anti-peptide antibody responses that were 100- to 1000- fold higher than the responses observed with peptide alone. Conjugates prepared with an isotype-matched negative control mAb, HB65, were non-immunogenic at the doses tested. The antibodies induced to the p24 peptide recognized intact p24 protein on immunoblots. In macaques, two mAbs that recognize non-polymorphic determinants of human HLA-DR antigens were used to evaluate immunotargeting. FACS analysis demonstrated that these mAbs and their peptide conjugates reacted specifically with 10-15% of macaque PBLs. Immunization with 10 ug doses of p24 peptide conjugated to the targeting mAbs resulted in substantial anti-peptide antibody responses in 1 of the 2 macaques immunized with each of the two mAbs. No responses were observed in the 2 macaques immunized with p24 conjugated to HB65. As observed in mice, the antibodies induced to "targeted" p24 reacted with the intact p24 protein as demonstrated by western blot. In marked contrast to the results obtained with 10 ug doses of targeted p24, 100 ug doses of peptide in aluminum phosphate did not induce detectable anti-peptide, or anti-protein, reactivity. These results clearly establish immunotargeting as a valuable technology for enhancing the immunogenicity of HIV synthetic peptides.

O 206 THE ANTIGEN PRESENTING ACTIVITY OF PLACENTAL MACROPHAGE CELL LINES Ming-der Y. Chang*, Jeff Polard#, Houman Khalili*,Sanna Goyert* and Betty Diamond+ *Department of Medicine, North Shore University Hospital-Cornell University Medical College, 300 Community Drive, Manhasset, N.Y. 11030 #Department of Developmental Biology and Cancer, +Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave. Bronx, N.Y. 10461. Macrophages can readily be found within an animal's placentae. However, they apparently do not initiate T cell response which may lead to rejection of the allograft-fetus. Previous studies attempting to understand how the function of macrophages in the placenta is regulated have been inconclusive due to the inability to isolate a pure population of macrophages. We have generated several macrophage cell lines from murine placentae. Cell lines were generated by either transforming with SV40 DNA, using the calcium phosphate precipitation method, or by spontaneous transformation in the culture. All of the placental macrophage clones show CSF1-dependent growth, have Fc receptor and can perfom Fc-receptor-mediated phagocytosis. They express the Mac-1, the F4/80, the CD14, and can secrete IL-1, tumor necrosis factor upon LPS stimulation. Although all the clones express MHC class II molecules constitutively, some of them can present only enzymatically digested peptide fragments, but not whole protein antigens to T cell hybridomas. This defect is not due to proteolytic enzyme deficiency. One macrophage cell line cannot present neither the whole protein antigens nor antigenic peptides, and this defect cannot be overcome by the addition of co-stimulatory factors.

O 207 ACETYLCHOLINE RECEPTOR-α SUBUNIT T CELL EPITOPE WITHIN 146-162 IS INVOLVED IN THE DEVELOPMENT OF MYASTHENIA GRAVIS IN MICE BEARING I-A^b MOLECULE, Premkumar Christadoss*, Minako Oshima+, M. Zouhair Atassi+ and Mohan Shenoy*, *Department of Microbiology, University of Texas Medical Branch, Galveston, TX 77550, and +Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030. I-Aβ chain region 67-71 has been implicated in the pathogenesis of experimental autoimmune myasthenia gravis (MG), because mutation at this region in bm12 mice prevented them from developing MG following immunization with Torpedo acetylcholine receptor (AChR). This mutation has also altered the T cell recognition profile of AChR-α chain epitopes. AChR-primed B6 T cells respond maximally to Torpedo-AChR-α chain peptides 111-126, 146-162, and 182-198, while bm12 T cells respond to 111-126 and give a markedly lower response to peptide 146-162 and 182-198. Therefore, the epitopes within 146-162 and 182-198 may be involved in the development of MG, if the *in vitro* T cell proliferative response predicts the outcome. In order to test this hypothesis, we neonatally tolerized B6 (I-A^b) mice with Torpedo AChR or peptide 146-162. Neonatal tolerance to AChR or peptide 146-162 reduced the incidence of clinical disease when neonatally tolerized adult animals were subsequently immunized with AChR in CFA. Non-tolerized B6 mice or B6 mice neonatally tolerized with AChR-α subunit peptide 182-198 (human sequence) developed MG as usual. Thus, neonatal tolerance to peptide 146-162 could have caused deletion of specific clone, and/or caused clonal anergy and/or activated specific regulator cells to prevent MG when subsequently immunized with AChR. The data implicates the epitope within 146-162 in MG pathogenesis in B6 mice. Presumably, T cell epitopes within 146-162 stimulate specific helper T cells in B6 which interact with specific B cells to produce specific populations of pathogenic antibodies, the primary culprit in MG end-plate lesion.

O 208 PRESENTATION OF MHC CLASS II DEGRADATION PRODUCTS: RECOGNITION OF NATURALLY PROCESSED CLASS II PEPTIDES BY ALLOREACTIVE T-CELL HYBRIDOMAS.

Elizabeth De Pirro Ward, Jennifer VanderWall and John H. Freed, National Jewish Center for Immunology & Respiratory Medicine, Denver, CO 80206

MHC class II molecules serve as the self recognition structures for the T cell receptor during the presentation of foreign antigens to $CD4^+$ T cells. As part of this process, class II molecules selectively bind peptides resulting from intracellular "processing" of antigen. In order to explore the processing of MHC class II molecules, we have selected a panel of alloreactive T-cell hybridomas based on their ability to respond, as measured by IL-2 production, to spleen cells or B-cell lymphomas but not to $I\text{-}A^k$ positive EL-4 transfectants. Several of the alloreactive T cells will respond to the EL-4 transfectants if a tryptic digest of denatured, affinity-purified $I\text{-}A^k$ is added. These results suggest that these T-cell hybridomas are specific for $I\text{-}A^k$-derived peptides presented by the $I\text{-}A^k$ molecule and that EL-4, as a cell of the T-cell lineage, lacks the ability to process $I\text{-}A^k$ although the $I\text{-}A^k$ expressed on the surface of these cells is capable of presenting the class II peptides. Because the alloreactive T-cell hybridomas are able to respond to cell types that naturally process exogenously added proteins, we propose that these antigen presenting cells are capable of processing and presenting self $I\text{-}A^k$ molecules. Therefore, $I\text{-}A^k$ molecules were affinity purified from these antigen presenting cells and the peptides in the antigen binding groove were isolated using an acid elution protocol. Eluted peptides were fractionated by reverse phase HPLC. We are in the process of characterizing the fractions based on their ability to stimulate the $I\text{-}A^k$ peptide-specific alloreactive T-cell hybridomas.

O 209 POLYMORPHISM OF THE SELF-PEPTIDE SETS DISPLAYED BY HLA-DRw11 FROM DIFFERENT INDIVIDUALS.

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Antigen-presenting cells (APC) constitutively process self-proteins and present them as antigen fragments bound to MHC molecules.

We previously described that some DRw11 B cell lines process and present the tetanus toxin 847-867 T cell determinant (competent B cell line) whereas other DRw11 B cell lines do not (defective B cell line).

We have now biochemically characterized the peptides bound to DRw11. Different reverse phase HPLC elution profiles were observed between the peptides extracted from DRw11 preparations purified from competent and defective B cell lines. Furthermore, we have derived DRw11-restricted allo-reactive T cell clones that distinguish between competent and defective B cell lines. Some of these allo-reactive T cell clones recognize DRw11-transfected murine L cell fibroblasts. These data suggest that these allo-reactive T cell clones are specific for complexes between DRw11 and non-polymorphic peptides which are not generated by the deficient B cell lines.

These observations indicate that antigen processing capabilities of the APC influence the sets of peptides bound to MHC molecules, thereby the shaping of the T cell repertoire.

O 210 Cytoplasmic Domain Heterogeneity and Functions of IgG Fc Receptors in B-lymphocytes,

James R. Drake, Sebastian Amigorena*, Christian Bonnerot*, Karl Matter, Walter Hunziker, Paul Webster, Catherine Sautes*, Wolf H. Fridman*, and Ira Mellman, Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510 and *Laboratoire d'Immunologie Cellulaire et Clinique, INSERM U 255, Institut Curie, 26, rue d'Ulm, 75005, Paris, France.

B-lymphocytes and macrophages express low affinity receptors for the Fc portion of IgG (FcγRII) that differ in the structure of their cytoplasmic domains. Compared to the macrophage receptor (FcγRII-B2), the B-cell isoform (FcγRII-B1) contains a 47 amino acid in frame insertion. By transfecting the two receptor isoforms and various receptor mutants into a FcγR-negative B-cell line, it was found that the FcγRII-B1 insert as well as specific amino acid residues in the cytoplasmic tail of FcγRII-B2 determine the functions of the FcγRII in B-lymphocytes. While amino acids 19 to 31 of the cytoplasmic domain of FcγRII-B2 are critical for coated pit localization and subsequent endocytosis, the 47 amino acid insert in FcγRII-B1 inhibits receptor coated pit localization and endocytosis . Consequently, cells expressing endocytosis positive FcRs, but not those expressing endocytosis negative FcRs, are capable of efficiently processing and presenting antigen-antibody complexes. The region of the cytoplasmic domain required to regulate B-cell activation upon cross-linking to surface immunoglobulin is common to both FcγRII-B1 and FcγRII-B2. Finally, the B1 insert is required for efficient capping of B-cell FcγR-B1, consistent with suggestions that FcγRII-B1, but not FcγRII-B2, associates with the detergent-insoluble cytoskeleton after cross-linking.

O 211 NUCLEAR MAGNETIC RESONANCE STUDIES OF A SOLUBLE CLASS II MHC MOLECULE AND ITS INTERACTIONS WITH PEPTIDES.

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Kazuyasu Sakaguchi, James G. Omichinski & Ettore Appella, Laboratory of Cell Biology, NCI, NIH, Bethesda MD 20892.
J. Jay Boniface & Mark M. Davis, Department of Microbiology and Immunology, Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

Soluble mouse $I\text{-}E^k$ class II molecules have been produced in mammalian cell culture in quantities sufficient to perform a number of structural studies[1]. We will report on our experiments aimed at investigating the nature of the class II MHC-peptide interaction by nuclear magnetic resonance spectroscopy. The ^1H spectrum of the soluble $I\text{-}E^k$ class II molecule shows a generally broad envelope consistent with an estimated molecular mass of 70 kDa. However, a number of resolved resonances are detected in the upfield region of the spectrum. These peaks have been used to monitor the physical state of the MHC molecule over the pH range 4.0-8.0. At pH < 5.0 the ^1H these resolved resonances decrease in intensity and are not immediately returned on neutralising the sample. The spectrum appears to return to its normal form over a period of time on the order of days. These data are consistent with a pH dependent conformational change of the protein that is not reversible on a short timescale. A moth cytochrome *c* peptide (A^{88}-N-E-R-A-D-L-I-A-Y-L-K-Q-A-T-K^{103}), labelled with NMR-active ^{13}C nuclei in the methyl positions of its four alanine residues, has been introduced into the MHC molecule in high yield. Preliminary NMR experiments have been performed to investigate the ^{13}C and ^1H spectra of the bound peptide. The two-dimensional spectrum contains eight well-resolved peaks of varying linewidth and chemical shift, indicating that the peptide is bound in an environment rich in aromatic groups, and that different parts of the peptide experience different regimes of mobility. On lowering the pH of the complex below pH 6.0, new peaks appear in the spectrum consistent with unbound peptide. These peaks do not disappear again on raising the pH to neutral. We hope to perform new experiments to investigate the multiplicity of the bound peptide peaks, and to specifically assign those signals already observed to particular groups in the peptide.

1. Wettstein *et al.*, J. Exp. Med. (1991) *174*, 219-228

O 212 CD4+ T CELL RESPONSES IN VIVO TO ANTIGEN-PULSED PRESENTING CELLS, David D.Duncan, Linda M. Bradley, and Susan L. Swain, Dept. of Biology and UCSD Cancer Center, University of California, San Diego, La Jolla, CA 92093-0063.

CD4+ T cells recognize peptide fragments of antigen in the context of Class II molecules expressed on the surface of antigen presenting cells (APC). Typically an in vivo response requires antigen delivery in adjuvant, although responses have been elicited through delivery on dendritic cells, on liposomes, and with targeted T cell epitopes using anti-Class II antibodies. Here we describe vigorous mouse CD4+ T cell responses elicited by injection of antigen-pulsed B cells or macrophages.

B cells consisted of nonadherant, T cell-depleted splenic white cells which were greater than 95% $sIgM^+$ cells; macrophages (at least 95% $Mac\text{-}1^+$) were derived from bone marrow cultured with CSF-1 followed by IFNγ. APC were cultured overnight with KLH, washed, and injected via the tail vein. To evaluate the priming, splenic CD4+ T cells from APC-injected mice were stimulated in vitro with pulsed APC, and proliferation and lymphokine production were measured.

The responding T cells were naive, since there were no responses in adult thymectomized mice. A short-lived effector population was generated by 3 days and proliferated and produced large quantities of IL2 and IL4, but low levels of IFNγ. The effector response subsided by 14 days. Injection of as few as $1.7x10^4$ APC gave a response, but 5×10^5 injected APC were optimal. B cells and macrophages elicited similar lymphokine profiles differing only in magnitude. A specific memory response was seen 6 or more weeks after priming and consisted of IL2 but little IL4. In contrast to results seen with antigen/adjuvant priming followed by a soluble boost, boosting APC-primed mice with soluble antigen failed to elicit T cells producing large amounts of IL4. This may have been due to a failure to prime B cells with the antigen-pulsed APC: when splenic CD4+ T cells from mice injected 6 weeks earlier with KLH-pulsed APC were injected into NIP-ovalbumen-primed hosts along with soluble NIP-KLH, a vigorous response was observed.

These results indicate that adjuvant-free priming using KLH-pulsed APC give CD4+ T cell responses analogous to responses seen when unprimed mice are challenged with KLH in adjuvant.

O 213 IDENTIFICATION OF AN ANTISENSE TRANSCRIPT WITHIN THE Eß REGION OF THE MHC, Rita Egan, Jeffrey Brockman and Jerold Woodward, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536

Recently our laboratory has detected transcription of the non-coding strand of the Eß gene of the murine Major Histocompatibility complex. The highest level of reverse strand transcription appears to be in a region containing the third exon of the Eß gene as shown by nuclear runoff analysis. Interestingly, the amino acid sequence encoded by the reverse strand of the third exon is highly conserved among murine, bovine, and human homologs of the Eß gene with a termination codon maintained at the start of the exon. This is relevant due to the fact that the third base of the Eß codons are maintained evolutionarly. Other lines of evidence indicate the presence of this transcript. RT-PCR analysis with primers designed to detect antisense transcripts of the Eß third exon shows the presence of this transcript in both Ia+ and Ia- cell lines. Likewise, Northern blots probed with riboprobes specific for antisense transcripts detect an approximate 6 kb transcript present in both total and poly A selected RNA from Ia+ and Ia- cell lines. The fact that this transcript appears to be polyadentylated and the conservation of the reverse strand codons is maintained raises the intriguing possibility that an unidentified functional gene is present in the MHC and is located on the opposite strand of the Eß gene. Further investigation of this possibility is currently underway.

O 214 FINE SPECIFICITY OF CLASS II MHC PRESENTATION OF THE IMMUNO-DOMINANT PEPTIDE OF MYOGLOBIN, Richard D. England and Jay A. Berzofsky, Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892.

We have previously reported on the fine specificity of T-cell clones recognizing the immunodominant 17-amino-acid sequence (102-118) of sperm whale myoglobin. Residues 109 and 116 were found to be critical for the stimulation of all clones. We have now further characterized this fine specificity using 42 serially substituted analogue peptides and two T-cell clones that recognize the 102-118 sequence in conjunction with $I\text{-}A^d$. The fine specificities of the two clones differ significantly despite the fact that both clones recognize the native sequence equally well. Class II MHC binding of all peptides was assessed using competitive inhibition of ovalbumin 323-339 presentation by $I\text{-}A^d$. All nonstimulatory peptides were also tested for functional inhibition of wild-type peptide activity. Two of the analogue peptides are heteroclitic in that they are 1000-fold more potent than the native peptide sequence in stimulating the proliferation of one T-cell clone, though they are inactive with the other clone. This heteroclicity appears to be due to increased T-cell receptor affinity rather than increased class II MHC binding since no change in competitive binding was observed. Computer modelling was used to quantify the experimental results. The mechanism of this unusual heteroclicity for the T-cell receptor rather than the MHC is being investigated.

O 215 EFFECT OF MHC class II MUTATIONS ON MOUSE MAMMARY TUMOR VIRUS ACTIVATION OF T CELLS. Hans Fischer, Philippa Marrack and John Kappler. National Jewish Center for Immuology and Respiratory Medicine. Goodman Bldg 5th floor. 1400 Jackson Str. Denver 80206 CO.

Mutations of the β1 domain of MHC class II was performed using oligonucleotides that were randomly mutated throughout the entire sequence. Restriction sites were introduced to the β1 domain of MHC class II without changing the amino acid sequence by PCR technique. The β1 domain was cut with restriction enzymes and mutated oligonucleotides were ligated representing mutations of approximately 2-3 base pairs throughout the oligonucleotide sequence. The mutated construct was put in a retroviral vector (LXSN) and transfected to the GP+E retrovirus packaging cell line. Maximum amounts of infectios retrovirus was produced after 48 hrs. $I\text{-}E^k$ negative M12.C3 fibroblasts express $I\text{-}E^k$ α chain mRNA but not $I\text{-}E^k$ β chain mRNA and thus do not express MHC class II. M12.C3 cells infected with $I\text{-}E^k$ β gene expressed $I\text{-}E^k$ on the cell surface. After infection, the M12.C3 cells were split and seeded at a clonal cell density in 96 well plates under selection pressure. By this technique it is possible to achieve hundreds of mutations, within a region of the $I\text{-}E^k$ beta 1 domain, in one experiment. The mutated $I\text{-}E^{k+}$ M12.C3 cells will be screened for inhibition of MMTV stimulation of T cell clones as assayed by IL-2 production. Furthermore, binding studies will reveal which amino acids that are important for interaction of these retroviral superantigens with MHC class II on the cell surface.

O 216 POINT MUTATIONS IN POLYMORPHIC REGIONS OF THE I-A MOLECULE REGULATE ITS ABILITY TO BIND AND PRESENT PEPTIDES. John H. Freed, Elizabeth De Pirro Ward, Edward F. Rosloniec and K. Scott Beard, National Jewish Center for Immunology & Respiratory Medicine, Denver, CO 80206

The MHC class II molecule plays a dual role in the presentation of antigen to helper T cells: on the one hand, it must bind peptides produced by the processing of antigen while, on the other hand, it must present residues which can be recognized by the T cell receptor (TCR) during the tripartite interaction of MHC/peptide/TCR. Our laboratory has been studying the effect of substituting residues in the polymorphic regions (PMR) of the class II molecule on its ability to carry out these two functions. We have prepared three different panels of T cell hybridomas, each restricted by the I-A^k molecule and specific for either hen egg lysozyme (HEL) peptides or an α chain peptide (residues 6-20) from I-A^k, or for the I-A^k molecule itself (conventional alloreactive T hybrids). Studies with the HEL specific T hybrids suggested that, while some T cells are sensitive to changes in any PMR, alterations in the third PMR of the α chain or in the second PMR of the β chain were the most disruptive to the function of the molecule. Direct binding experiments using ^{125}I-HEL(46-61) peptide and affinity purified I-A molecules demonstrated that, although the chimeric molecules with alterations in the second PMR of the β chain were severely impaired in their ability to present HEL peptides, these molecules bound HEL(46-61) as efficiently as did wild type I-A^k. In contrast, the chimeric molecule that had *b* allelic residues inserted into the third PMR of the $A\alpha^k$ chain, bound HEL(46-61) 3-4 fold less well than did the wild type. We next explored mutations at individual polymorphic positions within the third PMR of the α chain. Mutations at position #75 (the serologically dominant residue) or position #76 did not alter presentation of HEL peptides or the Aα derived peptide and only slightly affected the stimulation of the alloreactive T hybrids. However, a semi-conservative mutation at position #69 severely impaired the ability of this mutant to present both sets of peptides while a semi-conservative mutation at position #70 prevented presentation of peptides altogether. The alloreactive T cells exhibited a variable response pattern to the mutations suggesting that neither mutation grossly deformed the class II molecule. These combined data will be discussed in terms of the current MHC class II model.

O 217 **EVOLUTIONARY CONSERVATION OF MHC-PEPTIDE INTERACTIONS,** Annemieke Geluk*, Diënne G. Elferink*, Nel Otting#, René R.P. de Vries*, Tom H.M. Ottenhoff* and Ronald E. Bontrop#, * the Department of Immunohematology and Bloodbank, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands and # the Institute for Applied Radiobiology and Immunology TNO, Primate Center, 2288 GJ Rijswijk, The Netherlands.

In order to be recognized by T cells, peptides must be bound by MHC molecules. Peptides bind to highly polymorphic specificity pockets within the MHC encoded peptide binding groove.

Recently we have shown that the hsp65 p3-13, that is exclusively recognized by DR3 restricted T cell clones, binds specifically to HLA-DRw17 molecules in humans.

We have now performed a direct binding assay using chimpanzee and rhesus monkey MHC class II positive EBV-BLCL, and biotinylated hsp65 p3-13. As a control, we used an influenza hemagglutinin peptide, HAp307-319, which shows degenerate binding to HLA-DR molecules. Both peptides bound to the non-human BLCL in a differential way. Binding was inhibited by anti-class II mAbs.

The corresponding nucleotide sequences of the non-human primate MHC have been determined and comparison of these sequences with the known human HLA-DR sequences allowed us to further specify the amino acids that are critical for binding of these peptides.

Furthermore these results indicate the conservation of a peptide binding groove for over 30 million years, which probably accounts for the functional importance of the groove.

O 218 USE OF ENDOSOMAL PATHWAY IN MHC CLASS II PRESENTATION OF ENDOGENOUS ANTIGEN.

Denis Gerlier[1], Patrick Bertolino[1], Frédérique Forquet[1], Véronique Calin-Laurens[1], Martine Humbert[2], Jean Salamero[2], Jean Davoust[2] & Chantal Rabourdin-Combe[1]. [1]Immunobiologie Moléculaire, UMR 49, CNRS-ENS Lyon, Lyon, & [2]CIML, Marseille, France.

To determine the rules governing the access to the cell compartment where the peptide-MHC class II complexes are formed and to identify this compartment, MHC class II presentation of endogenous hen egg lysozyme (HEL) was tested and compared to that of exogenous HEL. B cells and MHC class II expressing mouse L fibroblasts secreting HEL or synthesizing an ER-retained HEL hybrid molecule were isolated after transfection. Secreted endogenous HEL was presented by every MHC class II molecule tested and recognized by every T cell hybridoma isolated on their ability to recognize exogenous HEL. The MHC class II presentation of secreted endogenous HEL was found to be far more efficient than that of exogenous HEL since 100 to 10,000-fold less HEL molecules per APC were required in the surrounding medium to give rise to a similar T cell activation signal. ER-retained HEL was also recognized by MHC class II restricted T cells. Co-culture experiments showed that presentation of endogenous HEL cannot result solely from re-uptake by bystander APC. MHC class II presentation of both secreted and ER-retained HEL was sensitive to chloroquine treatment and dependent on the level of invariant chain expression. Taken together, these results shows that endogenous and exogenous antigen take a similar if not identical processing pathway, and indicate that secreted and ER-retained endogenous HEL can reach and be processed within the endosomal compartment.

O 219 PEPTIDE BINDING, CONFORMATIONAL CHANGES, AND INTRACELLULAR TRANSPORT IN THE MHC CLASS II PRESENTATION PATHWAY, Ronald N. Germain[1], Oddmund Bakke[2], Jack Bennick[3], Laura Hendrix[1], Corine Layet[1], Austin Rinker, Jr.[1], Paola Romagnoli[1], Scheherazade Sadegh-Nasseri[1], and Jon Yewdell[3], [1]Laboratory of Immunology and [3]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892 and [2]Univ. of Oslo, Olso, Norway

The class II major histocompatibility complex (MHC) encoded molecules AαAβ and EαEβ in mice and DRαDRβ, DQαDQβ, and DPαDPβ in humans are peptide binding and transport molecules. They acquire fragments of protein antigens in an intracellular location and display these peptides on the plasma membrane for recognition by αβ-receptor bearing CD4+ T lymphocytes. Recent data demonstrate that class II α and β chains are initially synthesized as unstable "open" dimers associated with invariant chain (Ii) that upon binding of suitable peptides undergo a conformational change to a more stable, compact form. In vitro studies with purified class II molecules indicate that entry into the stable, peptide-associated compact form is dependent on loss of Ii. They also demonstrate that peptide binding and conformational change can be quite rapid under appropriate conditions of pH shift and temperature. Changes in class II binding site flexibility at different pH appear to play a major role in determining the extent and rapidity of stable peptide-class II molecule association. The details of the relationship between class II structure and peptide binding differ in significant ways from those of class I and peptide, with important implications for the biology of antigen presentation in the class II and class I pathways.

The cell biology of the class II pathway has also been investigated. Immunoprecipitation studies with metabolically labelled molecules demonstrate that the appearance of stable compact dimers follows passage of class II through the medial Golgi. Immunofluorescence studies indicate that class II first becomes accessible to peptide in a post-Golgi vesicular compartment to which the Ii-class II complex is targeted by signals in the invariant chain. The presence of Ii in this vesicular compartment may alter the usual flow of membrane and molecules through this site. Following complete Ii dissociation, peptide binding and acquisition of the compact stable conformation can be detected. The precise compartment in which this occurs, the pathway of class II from this site to the plasma membrane, the relationship between peptide binding and transport, and the involvement of multiple regions of interaction between class II and Ii in controlling peptide binding and transport are under investigation.

O 220 PEPTIDE/MHC INTERACTIONS: A COMPARISON OF THE MYELIN BASIC PROTEIN EPITOPE 1-20 AND THE NESTED 1-11 EPITOPE IN THEIR ABILITY TO STIMULATE MBP-SPECIFIC HYBRIDOMAS, Joan M. Goverman and Lee Hood, Division of Biology, Calif. Instit. of Tech., Pasadena, CA 91125

We characterized the amino-terminal epitope of Myelin Basic Protein (MBP) that elicits the dominant response to this antigen in H-2u mice. We utilized synthetic peptides to determine the optimal length in stimulating an MBP-specific, T-cell hybridoma derived from a B10.PL mouse. We found that the minimal peptide required for stimulation of this hybridoma consisted of acetylated mouse MBP1-6 and the optimal length was acetylated mouse MBP1-11. Longer peptides were increasingly less effective in stimulating IL2 production. Acetylated 1-20 could stimulate a detectable response at concentrations similar to 1-11, however the maximal response generated by the longer peptide was substantially reduced. We also carried out studies using analog peptides and found that, in agreement with studies by others in the PL/J strain, an alanine substitution at position four enhanced stimulation by 1-11. Interestingly, the same substitution enhanced stimulation by 1-20 to an even greater degree, making this peptide a much more potent stimulator than 1-11Ala4. Further studies have characterized differences in the presentation of the 1-11 versus 1-20 peptide to this MBP-restricted hybridoma.

O 221 LOCATION OF B CELL ANTIGEN PROCESSING IN ENDOSOMES USING FUNCTIONAL, ENZYMATIC AND BIOCHEMICAL APPROCHACHES. G. Gradehandt, S. Milbradt, K. von Figura*) and E Rüde, Institut für Immunologie, Joh. Gutenberg Universität, W-6500 Mainz, and *) Zentrum Biochemie, Biochemie II, Georg-August-Universität, W-3400 Göttingen, FRG

In most but not all cases proteolytic degradation of exogenous protein antigens within the cell is involved in antigen presentation by MHC class II-positive APC to CD4+-T cells. Here a cell biological approach is used to resolve the question whether this proteolytic step of antigen processing occurs. By incubation of APC with antisera directed against Man-6-phosphate receptors (MPR), endosomes were depleted of proteases such as cathepsin B and D, known to be involved in proteolytic antigen processing, while lysosomes still obtain normal amounts of enzyms. This fact was confirmed by measurement of enzymatic activity in subcellular, percoll gradient-derived fractions of B-cell hybridomas which were anti-MPR-treated for 18 h. When B-cells were pulsed under these conditions with protein antigens as OVA or conalbumin, their presentation to CD4+ T cells is inhibited whilst presentation of insulin is even enhanced, since its processing does not involve proteolytic steps. This result, together with the finding that presentation of processing-independent peptides and stimulation of alloreactive as well as Mls^a-reactive T cells is not affected, did exclude overall inhibitory effects of anti-MPR-treatment on APC. In addition we could show that amount and status of MHC class II-molecules as well as invariant chain in different cellular compartments are very similar in anti-MPR-treated and control B cells.

O 222 DIFFERENTIAL EPITOPE SELECTION IN IMMUNE RESPONSE TO BOVINE & CHICK COLLAGENS: INTERACTION OF RT1 & NON-RT1 PRODUCTS, M.M. Griffiths, M.A. Cremer, VA Research Services Salt Lake City, UT; Memphis, TN Seven RT1-congenic rat strains were analyzed for arthritis (CIA) and serum IgG antibody binding to the cyanogen bromide peptides of bovine(BII), chick (CII), & rat (RII) type II collagen. Characteristic profiles of CB-peptide responses were identified and reflected both RT1 and non-RT1 genotypes plus the species source of immunizing collagens. BN non-RT1 genes moderated clinical arthritis but enhancedthe anti-CB11 IgG of $RT1^{av1,n}$ rats. WF($RT1^u$) rats (CIA+) showed high IgG to CB11 of CII but to CB11 & CB9,7 of BII. DA($RT1^{av1}$), with the most severe CIA, showed a broad response to CB11, CB9,7 & CB10 of CII but to predominantly CB12 and CB9,7 of BII. Two $RT1^n$ strains (DA.1N, WF.1N) were CIA[-] after BII injections; both had dominant immune reactivity to RII CB9,7 (70%, 50%) and CB12 (20%, 30%) with low (<5%) IgG to CB11; DA.1N & WF.1N were CIA[+] after CII injections and showed increased (>20%) IgG to RII CB11 but the immunodominant RII epitopes were CB8 (50%) in DA.1N and CB9,7 (38%) in WF.1N. Thus, differential induction of CIA in WF.1N and DA.1N by CII was associated with increases in the level of IgG reactive with rat CB11. The data suggest that rat CB11 may be one of several "arthritogenic" eptiopes on rat type II collagen. Selection amongthese is dictated by the interaction of RT1 and non-RT1 gene products which presumably represent class II and T cell receptor molecules.

O 223 RECEPTOR-MEDIATED ANTIGEN PRESENTATION & ANTIGEN-MEDIATED RECEPTOR PRESENTATION Jean-Charles Grivel, Gilles Jolly and Lee Leserman, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille CEDEX 9 France

The internalization of surface immunoglobulin of B cells is triggered by the antigen for which immunoglobulin is specific. Thus, stimulated B cells could express complexes of the amino acid sequences of their variable regions (idiopeptides) with class II, in addition to antigen-class II complexes. Nevertheless, while anti-antigen responses are rapidly induced, elicitation of idiotype-specific antibodies usually require hyper-immunization with antibodies coupled to protein carriers.

We have produced high affinity syngeneic monoclonal idiotype-specific antibodies against several well-characterized lysozyme-specific antibodies. These were derived by fusion of inguinal and popliteal B cells after one or two injections in mice of unmodified idiotype emulsified in adjuvant. These antibodies bind to transgenic B cells expressing the target idiotype but do not block antigen binding by these cells, nor do they elicit the production of responses which are anti-antigen or which express the initial idiotype following their administration in naive animals. Thus, monoclonal anti-idiotypic responses to protein-specific antibodies are easily generated in primary responses. This response, which implies the existence of idiopeptide plus MHC restricted T cells, poses the question as to possible immunoregulatory roles of these cells. We are currently evaluating this role in adoptive transfer experiments using transgenic B cells and cloned T cells.

O 224 ANCHOR RESIDUE DETERMINATION OF PEPTIDES BOUND TO MHC CLASS II MOLECULES WITH M13 PEPTIDE LIBRARIES,
Juergen Hammer, Bela Takacs and Francesco Sinigaglia, F. Hoffmann-La Roche Ltd., Basel, Switzerland
Oligonucleotides encoding peptides of known binding characteristics to HLA-DR1 and DR4 molecules have been inserted into the gene III of filamentous M13 phages. The gene III product is a minor coat protein of M13 and the region, containing the insert, is known to be exposed on the phage surface. Based on binding studies with several of these M13 constructs and on phage/peptide competition assays we were able to demonstrate that affinity purified MHC class II molecules can specifically bind to peptide sequences expressed on the phage surface. Our studies show that peptides can be replaced by corresponding M13 phages providing us with a new tool to study peptide-MHC class II interaction. As a first application we used this method to identify "anchor" residues of peptides binding to DR1 molecules. The oligonucleotides used in the experiments above were replaced by random oligonucleotides and a random M13 peptide library with 20 million independent clones was constructed. After several rounds of screening more than 80% of the phages were able to bind DR1. Competition experiments with both isolated phages and corresponding synthetic peptides showed that this binding is specific. This again confirmed the exchangeability of peptides and phages. Sequence analysis of 59 phages able to bind to DR1 revealed two major anchor positions. The first is an aromatic residue (Tyr, Phe or Trp) at the N-terminal part of the random peptides. The second is located 3 residues downstream of the first and consists of Met or Leu. This new approach should enable one to easily determine the binding motifs of other class II alleles and isotypes. Furthermore, it could have interesting applications in autoimmune disease intervention.

O 225 In vitro processing of insulin for recognition by murine T cells results in the generation of A-chains with free CysSH
Johannes Hampl, Gernot Gradehandt, Hubert Kalbacher*), and Erwin Rüde, Institut für Immunologie der Joh. Gutenberg Universität, W-6500 Mainz, and the *) Naturwissenschaftlich-Medizinisches Forschungszentrum der Universität Tübingen, Ob dem Himmelreich 7, W-7400 Tübingen, FRG

Insulin-specific T cells recognize a peptide comprising amino acid residues 1-14 of the insulin A chain in association with the appropriate MHC class II molecules. This A1-14 peptide contains three Cys-residues which were either protected by S-sulfonate groups or engaged in disulfide bridges. The A1-14 peptide as well as native insulin needed processing for efficient presentation. However, fixed APC are able to present these antigens without further processing if their Cys-residues are converted into the Cys-SH form by reduction with thiols.
Substitution of Cys-residues by Ser within the A1-14 peptide revealed that only one out of three Cys-residues, CysA7, is critical for Ia binding and/or T cell recognition. In intact insulin this residue links the A-chain containing the T cell epitope to the B-chain. In agreement with previous results we propose that insulin processing is not dependent on proteolysis or on the generation of a conformational determinant but on the separation of A and B chains resulting in A chains whose Cys-residues are converted into CysSH. In general, our findings indicate that reduction of Cys-residues into the SH-form can be an essential step of antigen processing if the T cell relevant epitope contains Cys-residues.

O 226 Functions of endosomes and lysosomes in antigen processing. C.V. Harding, D.S. Collins, and E.R. Unanue. Washington University, St. Louis, MO 63110
We have explored the roles of different subcellular compartments in antigen processing. Antigens were targetted to endosomes or lysosomes of macrophages by encapsulating them within liposomes of different membrane compositions. Acid-sensitive liposomes released their contents in endosomes, whereas acid-resistant liposomes sequestered their contents from potential endosomal processing events, releasing their contents only after delivery to high density lysosomes. Multiple protein or peptide antigens encapsulated in acid-resistant liposomes were processed for class II MHC presentation in a chloroquine-sensitive manner and presented more efficiently than soluble antigen or antigen encapsulated in acid-sensitive liposomes. Thus, many exogenous antigens may be processed in lysosomes and then recycled for presentation to CD4+ T cells. A minor proportion of antigen encapsulated within acid-sensitive liposomes (but not acid-resistant liposomes) was also released into the cytosol and was processed for class I MHC presentation to CD8+ T cells. Reduction of disulfide bonds may be another important lysosomal function in antigen processing. Subcellular fractionation and analysis of disulfide-conjugated ligands showed that heavy density lysosomes (not light density endosomes) mediate reduction of disulfide bonds. Lysosomal disulfide reduction may allow increased access of lysosomal proteases to reduced, unfolded antigens as well as the production of linearized peptides from disulfide-bonded epitopes.

O 227 HOMOLOGOUS COLLAGEN INDUCED ARTHRITIS IN MICE AND RATS ARE ASSOCIATED WITH STRUCTURALLY DIFFERENT MHC DQ-LIKE MOLECULES,
Rikard Holmdahl, Carina Vingsbo, Christina Kvick, Mikael Karlsson, Tom J Goldschmidt and Kenth Gustafsson, Dept. of Medical and Physiological Chemistry, Box 575, Uppsala University, Sweden.
The susceptibility to collagen induced arthritis (CIA) and type II collagen (CII) autoimmunity in rodents, induced with homologous CII, is highly associated with the expression of certain MHC alleles. We have earlier shown that CIA in mice is dependent on the expression of an A^q molecule. The A^p molecule, on the other hand, is not associated with the disease. The difference between A^q and A^p is restricted to 4 positions (pos 85,86,88,89) in the ß-chain of the molecule. Therefore these positions are important for binding a specific CII-peptide. CIA in the rat is also highly associated with the expression of certain alleles. The DA strain is highly susceptible to CIA induced with homologous CII, while the Lewis strain is resistant. F1(DAxLew) is susceptible and backcrossing to Lewis reveals a close but not complete association of both arthritis and CII responsiveness to the $RT1^a$ haplotype. Analyses of congenic strains on DA and Lewis backgrounds suggest that expression of an MHC class II B^a molecule, encoded from the $RT1B^a$ locus, is associated with arthritis susceptibility and CII responsiveness. The second exons coding for the first domains of the α and ß chains of both the $RT1^a$ and $RT1^l$ haplotypes were sequenced and the deduced amino acid sequences compared with the corresponding molecule associated with susceptibility to CIA in the mouse (H-2 A^q). The sequences of the respective alleles revealed no obvious structural homology in the antigen binding site explaining the extensive similarities in the development of chronic autoimmune arthritis. Instead, this finding implies that different trimolecular constituents (i.e. class II, T-cell receptor, and CII peptides) may yield an antigen presentation event that is able to trigger a similar autoaggressiveness in the two rodent species.

O 228 The Amino Terminal Portion of the T Cell Receptor Alpha Chain Affect the Fine Specificity of MHC Recognition Soon-cheol Hong, Sangwook Tim Yoon, Susan Wolf, Adina Chelouche, Rong-Hwa Lin, David Shaywitz, Ned Braunstein, Laurie Glimcher, Charles A. Janeway Jr. HHMI and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510 and The Deparetment of Cancer Biology, Harvrd School of Public Health, Boston, MA 02115.

TCR α and β chains from two different T cell lines, D10.G4.1 (D10) and AK8 have been cloned and sequenced. Both T cell clones are AKR/J-derived CD4 helper clones that recognize peptides fragment of hen egg conalbumin presented by self I-A^k molecules. The D10 T cell clone is alloreactive to I-$A^{b,p,v,q}$ & d, however AK8 is not alloreactive. DNA sequencing results reveal that both T cell clones used identical β chains and related but different α chains. The α chain differences, located in the CDR1, CDR2 and CDR3 regions must be responsible for the differences of alloreactivity of D10. To understand the interaction of the D10 T cell receptor with its various MHC ligands, we have performed TCR transfection and mutation analysis. We find that the CDR3 of both apha and beta chains of the TCR are critical to all recognition events by the D10 TCR and for binding of anti-receptor antibodies. Furthermore, critical interaction sites for alloreactivity map to the major alpha helix of the I-Aβ chain, while no changes in the floor of the peptide binding groove affect alloreactivity of this cloned T cell line. In addition, we have also observed a marked shift, with both gains and losses, in alloreactivity when D10 TCR alpha chain CDR1 and CDR2 are interchanged with those of AK8. This gain in function maps to the I-Aa chain of class II MHC molecules. These results suggest that CDR1 and/or CDR2 of TCR alpha chain contact the Aα chain during non-self MHC recognition. The data support a model for the degenerate alloreactivity of the cloned D10 T cell line in which the major stimulus results from contact of the receptor with the MHC molecule, independent of specific peptide binding.

O 229 INFLUENCE OF C3 ON TETANUS TOXIN PROCESSING BY HUMAN EBV TRANSFORMED B CELLS, Muriel Jacquier, Françoise Gabert, Marie-Bernadette Villiers and Maurice G. Colomb, DBMS/Laboratoire d'Immunochimie, INSERM U238, CENG 85X, F-38041 Grenoble cedex, France

C3 is a ubiquitous conserved protein which appears to have played a singular role in ancestral host defence against foreign intruders, even before the emergence of specific immune defence.The severity of C3 deficiensis illustrates the importance of this protein. C3 belongs to a restricted family of thioester-containing proteins, together with α2-macroglobulin and complement protein C4. Opening of the highly unstable thioester upon modification of C3 (limited proteolysis, transconformation) leads to a double reactivity to form covalent bonds: 1. through the native carbonyl to form ester or amide bonds with OH or NH2 groups; 2. through the newly available SH to form disulfide links with other SH groups.

On the other hand, modified or cleaved C3 is also able to react non covalently with a family of proteins among which are membrane C3 receptors. It has been shown that C3b chemically cross-linked with tetanus toxin (TT-C3b) favors a targeting of the toxin unto C3b receptors of antigen presenting cells, as revealed by a reduction of the amount of antigen inducing the proliferation of T lymphocytes.

To analyse the influence of antigen-bound C3 on antigen processing and presentation, we set up a system in which EBV-transformed B cells specific for TT were first loaded with purified TT and then incubated with ^{125}I-labelled C3. ^{125}I-C3-TT complexes were immunoprecipitated from cell lysates by antibodies against C3 and TT. Analysis by SDS-PAGE after reduction revealed two radioactive bands (Mr 115 and 75 kDa) indicating that non proteolyzed C3 was bound to the toxin by a disulfide bond. A residual amount of non reducible complexes were disrupted upon incubation in 1M hydroxylamine at pH 9, for 2 hours. The stability of TT-C3 complexes and their evolution inside presenting cells are now under investigation.

In parallel, other observations using TT-C3b preformed complexes to saturate the specific presenting B cells indicate that C3b binding to TT increases and prolongs the stimulation of specific T cells measured by thymidine incorporation.

O 230 EVIDENCE AGAINST A SIMPLE TRAPPING MECHANISM RESPONSIBLE FOR ENHANCED PEPTIDE/CLASS II BINDING AT LOW pH. Jensen P.E., Dept. Pathology, Emory Univ., Atlanta GA 30322

We have previously reported that the association of peptide Ags with class II MHC is greatly facilitated at pH values approximating those found in acidic endosomal compartments in APC. We now report that the rate of association and the apparent number of binding sites is increased at low pH, with no change in the peptide dissociation rate. The results of binding experiments using a variety of peptide/MHC combinations suggest that the protonation state of critical groups in Ia serves to regulate the transition from unstable to stable complexes, perhaps by affecting conformational flexibility. Once stabilized by interactions involving bound peptide, complexes are insensitive to changes in proton concentration between pH 7 and pH 4. Lower pH is required to induce peptide dissociation. Thus there is discordance between the pH values required for enhanced binding as opposed to peptide dissociation. Our results exclude a simple trapping mechanism for the pH-depenence of peptide binding where pH neutralization is required to trap peptide in the binding groove. We provide convincing evidence that stable complexes are formed at pH 5 in binding experiments where the pH was never shifted back to neutrality. Acidification also allows binding of certain native protein Ags indicating that proteolysis of Ag may *follow*, rather than precede, MHC binding. Despite the substantial enhancement of binding observed at low pH, our results suggest that this effect alone does not fully account for the rapid kinetics of peptide binding that occurs when class II is in its natural environment.

O 231 A NOVEL METHOD FOR THE ANALYSIS OF BINDING BETWEEN PEPTIDES AND PURIFIED MHC CLASS II MOLECULES. I. Joosten*, M.H.M. Wauben*, E.J. Hensen* and S. Buus**. * Dept. Infectious Diseases and Immunology, Fac. Veterinary Medicine , University of Utrecht, The Netherlands. ** Dept. Experimental Immunology, University of Copenhagen, Denmark.

Biochemical assays for peptide binding to MHC class II molecules are cumbersome and of low through-put. Establishing such a binding assay often involves screening of many - frequently radiolabelled - peptides. We here describe a novel assay that allows rapid screening of large sets of non-radiolabelled peptides. The MHC molecules used were: the rat B^l affinity purified from the cell line Z1A using the monoclonal antibody Ox6, and the mouse E^d affinity purified from the cell line A20 using the monoclonal antibody 14-4-4. The peptides used were : the B^l restricted encephalogenic epitope 72-85 of guinea pig myelin basic protein and the Ed restricted T cell epitope 67-83 of the nucleocapsid protein of infectious bronchitis virus. These peptides were labelled with biotin with no apparent deleterious effect in functional T cell assays. Affinity purified MHC molecules were incubated together with biotinylated peptides and a protease inhibitor mix at pH5. After 2 days of incubation samples were analyzed on SDS-PAGE and blotted onto nitrocellulose. Peptide - MHC complexes were detected by incubation of the blots with HRP-streptavidin and subsequent chemiluminescence. Allele-specific binding could be demonstrated. The sensitivity of the biotin assay was high as peptide concentrations of 5nM together with 3uM of MHC still provided a clear signal. Competition studies showed that next to inhibition by unlabelled MBP 72-85, B^l restricted analog peptides derived from MBP 72-85 and from the mycobacterial 65 kD protein sequence 180-188, but not the E^d restricted IBV 67-83 peptide could inhibit binding of MBP 72-85 to B^l. These data were confirmed by functional competition assays. To our knowledge this is the first demonstration of peptide-MHC binding in the rat. Conversely, in the E^d system unlabelled IBV 67-83, but not B^l restricted MBP 72-85 could inhibit binding of the IBV 67-83 peptide. Thus, the sensitivity and specificity of this assay seems comparable to the previously reported assay (Buus et al., Cell 47, 1071 (1986)). Currently, studies on the effect of biotinylation and the specificity of the assay are being extended.

O 232 PROCESSING OF ANTIGEN TARGETED TO THE PHAGOLYSOSOME. Paul M.Kaye, *Christina Cuomo, *Cara Coburn and *Steven M. Beverley. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K. and *Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Many important pathogenic micro-organisms have adapted to reside within host macrophages. Little is known, however, about their ability to be handled by the host cell for antigen presentation. We have recently established that for the presentation of the protozoan parasite Leishmania donovani, a processing period of at least 3-4h is required in activated macrophages. Immunogold labelling of class II and the use of Brefeldin A suggest that the processing route most likely involves processed antigen leaving the parasite containing phagolysosome and interacting with class II elsewhere (Lang and Kaye, Eur.J.Immunol in press). In order to further define this route of antigen traffic and its efficiency, we have utilised a recently described vector (Coburn et.al. Mol.Biochem.Parasit. 46169-180 (1991)) to transfect Leishmania with the gene encoding chicken ovalbumin. The stable transfectants produce OVA at levels approximating 0.01% total protein. This is not degraded by the parasite over a 6h time period as determined by pulse-chase with ^{35}S-methionine nor is it secreted in detectable quantity. Functional studies using polyclonal CD4$^+$ T cell populations demonstrate that macrophages infected with these parasites are able to stimulate OVA specific proliferative and cytokine responses, demonstrating that access to the class II pathway of antigen processing occurs. Further data will be presented on the relative efficiency of delivery via this route compared to via fluid phase or receptor mediated uptake of soluble protein.

O 233 HUMAN CLASS II MHC RESTRICTED T CELL RECOGNITION OF AN ENDOGENOUSLY SYNTHESIZED VIRAL GLYCOPROTEIN: EVIDENCE FOR A POST-ER MHC CHARGING EVENT, D. J. Kittlesen*‡, L. R. Brown*, V. L. Braciale*‡, L. M. Roman°, J. Sambrook°, M.J. Gething° and T. J. Braciale*‡, *Washington University School of Medicine, St. Louis, MO 63110 and ‡Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22908 and °University of Texas Health Sciences Center, Dallas, Texas 75235

Infection of a human B lymphoblastoid cell line with a recombinant vaccinia virus, directing the expression of the influenza hemagglutinin (HA) protein, leads to the rapid sensitization of these cells for recognition and lysis by a class II major histocompatability complex (MHC) restricted CD4+ cytolytic T lymphocyte (CTL) clone. This is in contrast to what is seen with murine cells, where the newly synthesized glycoprotein charges class I MHC for recognition by CD8+ CTL, but not class II MHC for recognition by CD4+ CTL.

To further characterize and dissect the intracellular pathway for this mechanism of class II antigen presentation in the human, we have studied a number of HA mutants, some of which are transport defective. Our data show that surface expression of the native antigen is not required, arguing against the classical "exogenous pathway" being necessary. The native protein must therefore gain access to a processing compartment (probably the endosome) from within the cell. One mutant (HA-JS67) can charge class II, yet does not transit the Golgi, implying that the shunt to the processing compartment is early in the secretory pathway. Finally, the mutant HA-DR1 which also does not transit the Golgi and has a relatively short half-life (consistent with ER degradation) fails to sensitize the cell, leading us to conclude that the endoplasmic reticulum is not the relevant processing compartment for class II in this system.

O 234 CLONAL ELIMINATION OF H-2E REACTIVE T CELLS AND ANTIGEN PRESENTATION BY H-2A MOLECULES DETERMINE TOLERANCE TO SELF E^k_α PEPTIDES, Christopher J. Krco, Thomas G. Beito, and Chella S. David, Dept. Immunology, Mayo Clinic, Rochester, MN 55905

There is considerable interest in characterizing mechanisms of T cell repertoire selection. Roles for H-2E molecules, Mls determinants, retroviral products and superantigens have been reported. With an emerging understanding of the structure of class I and class II molecules, a concerted effort has been made to characterize biochemical parameters of peptide binding to histocompatibility molecules. However, relatively little information is available concerning the utility of using synthetic histocompatibility peptides as probes for deciphering pathways for T cell selection. In view of the importance of H-2E molecules in clonal selection we have investigated whether synthetic peptides spanning H-2E$^k_\alpha$ chain residues (90-110), (110-130) and (130-150) could be used as "self" peptides in assays of T cell recognition. Mice representative of H-2E$^-$ (B6, B10.M, B10.Q, B10.S) and H-2E$^+$ (B10.D2, B10.K, B10.RIII) were immunized with individual peptides and lymph node cells challenged in vitro. B6 mice respond to in vitro challenge to peptides (90-110) (cpm 20,000), (110-130) (cpm 40,000) and (130-150) (cpm 60,000). In contrast all H-2E$^+$ haplotypes were unresponsive to all three peptides (cpm's <10,000). Furthermore, B10 mice could be rendered hyporesponsive to E^k_α peptide challenge following expression of an E^k_α transgene or mating to an H-2E$^+$ strain. To formally address the role of the H-2A molecule in E^k_α peptide responsiveness, the responses of B10.RKB ($K^kA^kE^bD^b$) and H-2A$_\beta$ mutant bm12 T cells were determined. It was established that both B10.RKB and bm12 mice were hyporesponsive to challenge with peptide (90-110). Moreover, B10.RDD ($K^dA^dE^bD^d$) mice responded less than 10% (cpm's of 3,000 vs 50,000) of the level of B6. These results raise the interesting possibility that tolerance to self E^k_α molecules may be a result of negative selection of T cells capable of stimulation of E^k_α determinants presented in the context of H-2A molecules. Further H-2A molecules differ in their capacity to present various E_α peptides.

O 235 CHARACTERIZATION OF T CELLS REACTIVE WITH *Borrelia burgdorferi* OR *Yersinia* ANTIGENS ISOLATED FROM PATIENTS WITH LYME DISEASE OR REACTIVE ARTHRITIS. Lahesmaa, R.[1], Shanafelt, M.-C.[2], Yssel, H.[3], Steinman, L.[1], and Peltz, G[2]. [1]Department of Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305, [2]Department of Inflammation Biology, Syntex Research, Palo Alto, CA 94303, and [3]DNAX Research Institute, Palo Alto, CA 94305.

T cell clones isolated from arthritic patients with Lyme and reactive arthritis recognizing *B. burgdorferi* or *Yersinia enterocolitica* antigens were characterized for their antigenic specificity, profile of lymphokines secreted and HLA restriction elements. Both *Yersinia* and *B. burgdorferi* selectively activated T helper cells with a Th1-like profile of lymphokine secretion in these patients. Lymphokine production and proliferation in response to antigen by the T cell clones was regulated by IL10. Sixteen out of 43 *Borrelia*-reactive T cell clones were characterized in detail for their antigenic fine specificity, HLA restriction and TCR gene usage. Two of the clones recognized *Borrelia* hsp70 and one of them *Borrelia* hsp60. These responses were specific for bacterial hsp's; no reactivity was detected to human hsp-homologues. Comparison of the profile of cytokines produced by a T cell clone, whose response to antigen was restricted by HLA-DQ to HLA-DR restricted T cell clones revealed that the DQ-restricted clone exhibited a Th0-like lymphokine profile with higher production of IL4 and IL5. Analysis of TCR V-region gene usage revealed that four of the clones recognizing an unidentified spirochetal antigen used the same Vbeta but different Valpha TCR genes. Two of these clones recognized *Borrelia* in association of APCs expressing diverse HLA restriction elements. Based on these results we conclude that the selective activation of Th1-like T cells by arthritogenic pathogens plays an important role in the pathogenesis of joint inflammation.

O 236 MULTIPLE PROCESSING PATHWAYS FOR THE PRESENTATION OF CYTOSOLIC ANTIGEN TO CLASS II-RESTRICTED T CELLS, Eric O. Long, Mauro S. Malnati, Merce Marti and Robert DeMars, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852, and Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

Assembly and transport of class I MHC molecules is controlled in part by the requirement for a peptide supply factor (PSF). As a result, class I molecules are generally limited to the presentation of endogenous antigen. In contrast, class II MHC molecules have the ability to efficiently present exogenous antigen endocytosed into acidic compartments, as well as endogenous antigen synthesized within the cytosol. The H3 hemagglutinin of influenza virus A, and an engineered cytosolic form of H3, expressed by a recombinant vaccinia virus, were efficiently presented to HLA-DR1-restricted T cells. To test whether class II-restricted presentation of cytosolic antigen followed the class I processing pathway, a mutant human B-cell line (.134) defective in PSF was used for presentation to H3-specific T cells. The cytosolic form of H3 was efficiently presented to DR1-restricted T cells by the PSF-defective B-cell line. In contrast to the cytosolic H3 protein, a short cytosolic peptide, expressed from a mini-gene encoding the DR1-restricted H3 epitope, was not presented by the PSF-defective mutant cells, even though it was presented by the normal B-cell line. Helper T-cell epitopes can thus be generated from cytosolic proteins by different mechanisms, one of which is totally distinct from the class I pathway of antigen presentation.

O 237 ANTIGEN SPECIFIC B CELLS PROCESS AND PRESENT SELECTED SELF PEPTIDES THAT PRIME AUTOREACTIVE T CELL RESPONSES. Mark Mamula, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510.

The initiation of autoimmune responses by self antigens or by foreign pathogens (molecular mimics) is not well understood. In the present study, cytochrome *c* (cyt *c*) was used as a model autoantigen to investigate how self proteins are processed and presented in the priming of autoimmune T cell responses. Mice are tolerant at both the B and T cell level to immunization with native mouse cyt *c*. In previous studies, however, we have found that B cells elicited with cross-reactive foreign (human) cyt *c* protein or short polypeptides can bind, process and present self (murine) cyt *c* that, in turn, prime autoimmune $CD4^+$ T cells. The T cell response is specific for an epitope within amino acids 54-68 of murine cyt *c* in association with $I\text{-}E^k$. No autoimmune T cell responses were observed to the carboxy terminal self polypeptide 81-104 , a site that dominates T cell responses to foreign cyt *c*. To investigate this further, mice were immunized with the carboxy terminal peptide of either human or mouse cyt *c*. T cells elicited to the human peptide 81-104 could be stimulated by APCs with human peptide 81-104 or by whole human cyt *c* and not by mouse cyt c or its peptides. Interestingly, T cells could be elicited to the murine 81-104 peptide but these cells were unresponsive to native mouse cyt *c*. These results suggest that either foreign or self 81-104 fragments can associate with class II^k, however, APCs cannot generate the 81-104 peptide from native whole mouse (self) cyt *c*. My observations demonstrate fundamental differences in how foreign and self cyt *c* proteins are processed which may subsequently influence the repertoire of autoreactive T cells.

O 238 THE HLA DR4 PEPTIDE BINDING SITE IN ANTIGEN SPECIFIC T CELL RECOGNITION. J.M.Mc Nicholl, F. Oftung, R.W Karr and T.M. Shinnick. Emory University, Atlanta, the Norwegian Radium Hospital, Oslo, Norway, the University of Iowa, Iowa City and the Centers for Disease Control, Atlanta.

The association of HLA DR4, particularly subtype Dw4 (DRβ1*0401) and amino acids 71-74 of this molecule, with rheumatoid arthritis and other autoimmune diseases prompted an investigation of the importance of these and other putative peptide or T cell receptor interacting amino acids in antigen recognition. In proliferation assays we examined the ability of 2 HLA DR4Dw4 restricted T cell clones, derived form the same donor, to recognize a 13 amino acid peptide of the 18Kd heat shock protein of M.leprae presented by wild type and mutant HLA DR4 molecules transfected into L cells. Mutations were to corresponding amino acids in: other DR4 subtypes (DRβ positions 57-85); DR7 (DRβ positions 9-30); DPα, DQα, IEα or IAα (DRα positions 7-72). In DRβ, α-helical substitutions at positions 55, 70, 71, 74 and 86 significantly diminished or abrogated recognition, even with a conserved K→R substitution at position 71, suggesting that these residues are critical in antigen and/or TCR interactions. Floor substitutions at 3 or 4 of positions 9, 11, 13, 28, 30, 37 prevented recognition by clones 6/10F and 2/4F respectively. With DRα substitutions, similar heterogeneity was seen. Five (clone 6/10F) or 4 (clone 2/4F) of 6 floor substitutions and 2 (6/10F) or 1 (2/4F) of 6 α-helical substitutions significantly diminished recognition suggesting that the clones have different molecular requirements for the formation of a stimulatory trimolecular complex of peptide, MHC and T cell receptor. In both α and β chain substitutions amino acid charge was important as 8 of 11 substitutions involving a charge change at putative peptide-interacting MHC residues almost completely ablated recognition. Amino acid side chains were also important in these and in more conservative substitutions. These data support the current model of Class II MHC molecules and begin to define paradigms of MHC Class II-peptide interactions relevant to understanding autoimmune disease.

O 239 A Gene Required for Class II - restricted Antigen Presentation Maps to the Major Histocompatibility Complex, Elizabeth Mellins[1], Laura Smith[2], Tatsui Monji[3], and Donald Pious[2,4], Departments of Pediatrics[2], Microbiology[3], and Immunology[4], University of Washington, Seattle, WA 98195.

We have previously described a set of mutants of a B-LCL that are defective in the presentation of intact proteins to class II-restricted T cells, but effectively present immunogenic peptides. The mutations in these mutants are recessive in somatic cell hybrids and are not in class II structural genes. We now report on a new mutant, 5.2.4, in which a similar defect in class II-restricted antigen presentation occurred in association with a one megabase homozygous deletion in the class II region of the MHC. Complementation analyses using 5.2.4 and 3 of the previous mutants and using a second, unrelated MHC deletion mutant, T2, strongly suggest that the defects in the mutants are in a single, MHC linked gene. This gene appears distant from PSFI, an MHC linked gene required for class I surface expression.

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O 240 IDENTIFICATION OF A T-CELL EPITOPE ON HETEROLOGOUS TYPE II COLLAGEN IN H-2Q MICE. SELF TOLERANCE ON THE LEVEL OF DETERMINANT SELECTION. Erik Michaëlsson, Mikael Andersson, Åke Engström* and Rikard Holmdahl, Department of Medical and Physiological Chemistry and Department of Immunology*, Uppsala University, Uppsala, Sweden

Type II collagen-induced arthritis is a tissue-specific, autoimmune disease which is induced by immunization with type II collagen(CII). Immunization with heterologous CII(human, bovine, rat, chicken) results in a strong proliferative T-cell response in H-2q mice against epitopes present on heterologous but not on autologous CII. These epitopes are located on CB 11, a cyanogenbromide-cleaved fragment of CII. A comparision of mouse CB11 with human, bovine and chicken CB11 reveals a strong homology between the species and few potential heterologous T-cell epitopes. Using synthetic peptides, one T-cell epitope, corresponding to amino acid number 256-270 on CII, was determined. This peptide differs only at position 266 between man and mouse(Glu in man , Asp in mouse). While the human peptide gave rise to a strong T-cell response, both in rat CII-primed primary cultures and by rat CII-reactive T-cell hybridomas, the autologous peptide totally lacked antigenicity. The self tolerance could either be explained by the lack of reactive T-cells(due to negative selection or anergy) or incapability of the self peptide to bind I-Aq(determinant selection). To investigate this, inhibition studies were made. The response of a T-cell hybridoma specific for human CII[256-270] was inhibited by the addition of a peptide corresponding to myelin basic protein, amino acid 89-101, which has been shown to induce experimental allergic encephalomyelitis in H-2q mice. Mouse CII[256-270] on the other hand, did not inhibit the response of the hybridoma suggesting that the tolerance to the autologous CII-peptide is due to determinant selection.

O 241 SEQUENCE ANALYSIS OF PEPTIDES PRESENTED BY CLASS II MHC MOLECULES. Hanspeter Michel, Andrea L. Cox, Theresa Davis, Tracey Dickinson, Jeffrey Shabanowitz, Donald F. Hunt, Chemistry Dept., Univ of Virginia, Charlottesville, VA 22901, Kazuyasu Sakaguchi and Ettore Appella, Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20892, Alessandro Sette and Howard M. Grey, Cytel Corporation, San Diego, CA 92121

Class II MHC molecules present a complex mixture of peptides derived from both self and foreign proteins. This report describes the use of microcapillary HPLC in conjunction with electrospray ionization/ tandem mass spectrometry to achieve high resolution separation and sequence analysis of peptides presented by $I\text{-}A^d$ and $I\text{-}E^d$ molecules. With the mass spectrometric technique, the approximate number and quantity of individual peptides in the mixture can be estimated and maximum length of each peptide can be defined. Sequences are obtained by the technique of collision activated dissociation. In this process ions derived from a particular peptide in the mixture are selected by the mass spectrometer and induced to fragment more or less randomly at the various amide bonds in the sample. Subtraction of masses for two fragments differing by a single amino acid provides a value that specifies the mass and thus both the identity and the location of the extra residue in the larger fragment.

Peptides presented by class II MHC molecules are typically 14-19 residues in length and exhibit ragged C-termini. Those analyzed to date are all derived from proteins found in the extracellular medium. Examples include the sequences: WANLMEKIQASVATNPI and EEQTQQIRLQAEIFQAR, which are derived from mouse apolipoprotein E; VPQLNQMVRTAAEVAGQL, found in the transferrin receptor; ASFEAQGALANIAVDKA, which is part of the $I\text{-}E^d$ alpha chain; KPVSQMRMATPLLMRPM, which derived from in the H-2 invariant chain; and DAYHSRAIQVVRARKQ, found cystatin C, an inhibitor of cysteine proteases. All of the above peptides exhibit binding motifs that are consistent with those deduced in earlier work by three of the present authors (Sette, Grey, and Appella). Results of studies to deterimine the binding affinities of the above peptides for the class II MHC molecules will be reported.

O 242 UNUSUAL MAJOR HISTOCOMPATIBILITY ANTIGENS ENCODED WITHIN THE MHC OF BIRDS. Marcia M. Miller, and Ronald Goto, Department of Molecular Biochemistry, Beckman Research Institute of the City of Hope, Duarte, CA 91010

B-G antigens are polymorphic dimeric transmembrane proteins encoded within the major histocompatibility complex (MHC) of the chicken. Although considered for years to be solely blood group antigens, it is now evident that B-G antigens are present on cells in a number of different tissues. Recent cloning of erythrocytic B-G transcripts has provided evidence that the antigenic polymorphism of the B-G molecules is associated with a domain that resembles variable-region domains of immunoglobulin. This finding has lead to the consideration that B-G molecules may serve in immune recognition. Two approaches have been used to determine the nature of the polymorphism in the B-G extracellular domain. Traditional cloning and sequencing has provided insights into the organization and size of the *B-G* gene family. Further evidence for the diversity of the *B-G* genes expressed on cells in different tissues and in different MHC genotypes has been obtained from the analysis of transcripts in different tissues by SSCP (single-stranded conformational polymorphism) assays and by sequence analysis of PCR-generated clones. Evidence has been found for a complex array of multiple B-G molecules expressed in different MHC haplotypes in non-erythroid tissues. Moreover multiple *B-G* genes are expressed in bursa, thymus and small intestine.

O 243 BINDING OF IgG FROM DR3 POSITIVE RECONVALESCENT DONORS TO SYNTHETIC PEPTIDES OF THE MEASLES FUSION PROTEIN

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Infection with measles virus or immunization with the attenuated virus induce a life long protective immune reaction directed both against hemagglutinin and fusion protein. In an effort to map the immunodominant recognition structures of the measles virus, we have synthesized overlapping pentadecapeptides with sequence homology to the F protein. Sera from HLA-typed donors with elevated anti-measles titers were used in a solid phase immunosorbant assay to determine antibody reactivity with the above FMOC peptide conjugates. Unselected sera recognized clusters of specific peptides spread over the total sequence. Affinity purified IgG from DR3 positive donors were tested against the same peptides. The reactivity was relatively weak but cumulating the standard deviations of 22 DR3 positive heterozygous donors defined 6 distinctive regions (31-82; 111-140; 151-215; 271-315; 419-438; 486-525) within the above regions. The sequences were to a large extend either transmembranal or adjacent to the membrane and located both on F1 and F2. They did not coincide with computer assisted B cell epitope prediction based on secondary structure, hydrophylicity, hydropathy, acrophily, flexibility. However, the binding sites closely coincided with putative T cell epitopes according to a modified Rothbart and Taylor prediction. The predicted binding sites of peptides to DR3 (Kalbacher et al. unpublished results) were directly adjacent or overlaping with the binding regions in 23 out of 29 peptides, or seperated by 2-5 amino acids in 4 of 29 peptides or unrelated in 2/29 peptides. Our data strongly suggest that the sequences recognized by the antibodies to the measles F protein are situated in close proximity of MHC class II binding sites.

O 244 Molecular Approach to Mapping CD4+ T Cell Epitopes, T.Y. Nakagawa, H. Von Grafenstein, C.A. Janeway, and R.A.Flavell, Section of Immunobiology, Yale Univ. School of Medicine, New Haven, CT 06510

Attempts to identify CD4+ T cell epitopes have been largely based on biochemical methods. Recent advances purifying peptides off of isolated MHC molecules has been highly successful but limited by the amount of starting material required. Molecular cloning techniques have allowed the use of recombinant antigen fragments to delineate T cell epitopes, particularly of large antigens. Using PCR technology and inducible bacterial expression vectors, the epitopes of two well-characterized Th2 clones, D10.G4.1 and Ak8, have been mapped to a 17 amino acid region. Both clones recognize a protein moiety derived from chicken conalbumin in association with class II I-A^k. Overlapping fragments of the 2 kb conalbumin cDNA were subcloned into a modified pGEX-2T expression vector expressing a c-myc derived TAG sequence which is recognized by the monoclonal antibody 9E10. Fusion protein expression was monitored by Western analysis. Small scale cultures were induced, harvested, boiled, and T cell activation was measured by ^{3}H-thymidine incorporation and IL-4 response.

We have adapted this method for the cloning of potential autoantigens involved in IDDM in the NOD mouse. A β cell cDNA library will be screened with diabetogenic CD4+ T cell clones. The sensitivity of the assay will be increased by the use of an antigen-focusing APC expressing the unique I-A^{NOD} and a surface Ig specific for the TAG. Using the method developed by Greg Winter to clone antibody V regions, the binding domain of the 9E10 hybridoma has been cloned and expressed in an IgM membrane bound construct. This "highly unprofessional" APC may increase the sensitivity of the assay several orders of magnitude.

O 245 Vα4 DOMINANCE IN A NON-CROSSREACTIVE, MIXED ISOTYPE $A\beta^dE\alpha^d$-RESTRICTED T CELL RESPONSE, Kannan Natarajan and Maurice Zauderer, Immunology Division, University of Rochester Cancer Center, Rochester, NY 14642.

Using the linker-facilitated polymerase chain reaction to analyse T cell repertoires we have determined that genes of the T cell receptor Vα4 family are the dominant receptor genes expressed in Balb/c (H-2^d) T cell lines specific for the synthetic peptide FFEELLKFFEELLK. Experiments using appropriately transfected L cells as antigen-presenting cells indicate that these T cells are restricted to the unusual mixed Ia isotype, $A\beta^dE\alpha^d$, expressed in the H-2d haplotype. T cells specific for the related peptide YYEELLKYYEELLK also show dominant use of the Vα4 T cell receptor family even though the T cell responses induced by FFEELLKFFEELLK and by YYEELLKYYEELLK are non-crossreactive. We propose that Vα4 expression is selected by an MHC feature conserved in a large number of different $A\beta^dE\alpha^d$:peptide complexes. Different peptides with a common binding motif will be similarly oriented in the Ia binding cleft so that they either are presented in a geometry which selects a common surface MHC interaction element or stabilizes a particular MHC conformational determinant recognized by Vα4.

O 246 ACIDIC AMINO ACID RESIDUES OF DR4β DETERMINE T CELL RECOGNITION OF AN HIV-1 gp120 PEPTIDE, Richard R. Olson, Janet McNicholl, Carol Alber, Ellen Klohe, Kevin Callahan, Robert Siliciano, and Robert W. Karr, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242; and Division of Molecular and Clinical Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We examined the structure-function relationships of HLA-DR4 molecules using a series of transfectants expressing wild-type or mutant DRβ chains as antigen-presenting cells for an HIV-1 gp120 specific, DR4-restricted T cell clone (Een217). Antigen-specific proliferation of Een217 was seen to occur with transfectants expressing the Dw10 and Dw13 subtypes of DR4, but not with Dw14, Dw15, or Dw4. We examined the effects of single amino acid substitutions at 57, 67, 70, 71, 74, and 86 in the β chains of each of these DR4 subtypes, and positions 9, 11, 13, 28, and 37 of Dw10 and Dw13. We found a predominant role of third hypervariable region residues in antigen presentation. The pattern of restriction according to DR4 subtype for Een217 is demonstrated to be due to the presence of aspartic acid and glutamic acid at positions 70 and 71 in Dw10, and glutamic acid at position 74 in Dw13. Gain of ability to present peptide was seen with mutation to acidic residues at positions 70, 71, or 74 in Dw14 and positions 71 or 74 in Dw4. The demonstration that a class II molecule functional in T cell stimulation could be generated by acidic residue substitutions at alternative positions in non-stimulatory DR molecules suggests that a functional subsite of the DR4 molecule includes amino acids 70, 71, and 74.

O 247 BINDING OF A MAJOR T-CELL EPITOPE OF MYCOBACTERIA TO A SPECIFIC POCKET WITHIN HLA-DRw17 (DR3) MOLECULES.

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CD4+ T cells recognize antigenic peptides bound to the polymorphic peptide binding site of MHC class II molecules. The *M.leprae* hsp65 peptide aa 3-13 is an immunodominant epitope in the *M.leprae* directed immune response of DR3+ individuals and is not recognized in the context of any other DR molecule. We therefore have tested whether this peptide is selected as a DR3 restricted T cell epitope on the basis of exclusive binding specificity for DR3 molecules.

Using biotinylated peptides, we find that p3-13 binds to DRw17 (DR3) but not any other DR molecule. A control peptide bound to all DR molecules. The DRw17 β1 chain differs uniquely at 5 positions from all other DR β1 chains. Three of these amino acids are potential peptide contacting residues and map closely together as a positively charged pocket in the hypothetical 3D structure of the DR molecule.

The DR3 variant DRw18 molecule differs from the common DRw17 β1 chain at only 2 major positions, close to or within this pocket. Interestingly, p3-13 does not bind to DRw18. The DRw18 substitutions drastically change the structure and charge of the pocket and thus presumably abrogate its ability to bind the peptide.

Further studies aiming at the precise definition of peptide and DRw17 residues critical for binding are underway.

O 248 DIFFERENCES IN PERCENT OCCUPANCY OF HLA DR4Dw4 OBSERVED BY SDS-PAGE AND SOLID PHASE BINDING ASSAY, Lisa R. Paborsky, Robin D. Gantzos, Maya R. Tanaka, Angela F. Liu, and Jonathan B. Rothbard, ImmuLogic Pharmaceutical Corporation, Palo Alto, CA 94304. Two features distinguish MHC class II proteins from other cell surface receptors: only a small percentage of class II molecules are able to bind exogenously added peptide ligands and these molecules are able to bind a structurally diverse range of peptide ligands. Once formed, the peptide ligand-class II complexes are remarkably stable and do not dissociate even in the presence of SDS. To define the requirements for peptide binding, DR4Dw4 molecules were immunoaffinity purified from both detergent solubilized EBV-transformed B cells (Priess), which are capable of antigen processing and from transfected CHO cells, which lack human invariant chain and cannot process antigen (CHO DR4Dw4-TM). In addition, a soluble form of this molecule was isolated from the supernatant of transfected CHO cells expressing DR4Dw4 containing a phoshatidylinsitol (PI) tail and treated with phosphatidylinositol specific phospholipase C (CHO DR4Dw4-PI). These three preparations of purified DR4Dw4 vary in the proportion of individual α and β monomers to α/β heterodimer and represent a spectrum of stability. All three forms of DR4Dw4 were capable of binding an equally small amount of added peptide, as assessed by a solid phase binding assay utilizing a monoclonal antibody to capture the peptide-class II complexes. In contrast, preincubation with appropriate peptide ligands prior to SDS-PAGE dramatically increased the proportion of α/β heterodimer and indicated that a much higher percentage of these molecules were able to bind exogenous peptide ligands. In addition, the kinetics of binding differed depending on the method used to measure the formation of peptide-class II complexes. The stable complex observed by SDS-PAGE formed within minutes, whereas a much longer incubation time was required to demonstrate peptide binding using the solid phase assay. The peptide-class II complex observed by SDS-PAGE may represent a fast-forming intermediate which was less stable and dissociated either due to the stringent washing conditions or the presence of the monoclonal antibody used in the solid phase binding assay.

O 249 **STRUCTURE AND KINETICS OF FORMATION OF PEPTIDE / IE^k COMPLEXES**, Philip A. Reay and Mark M. Davis. Stanford University. CA 94305.

We have quantitatively characterized the pH dependency of specific binding by IE^k, produced as a PI-linked heterodimer, in an aqueous environment to two biotinylated peptides. These peptides were chosen to represent either a "high responder" (moth cytochrome c, MCC, residues 82-103) or a "low responder" (sperm whale myoglobin, SWM,128-153) epitope. Both peptides show significantly enhanced binding to IE^k at pH values approximating those of late endosomal compartments (4.8-5.2). This is primarily due to an enhanced rate of association, being 50-fold faster at pH 5 than at pH7. The dissociation of MCC(82-103) is extremely slow and pH-independent (in the range 5 to 7). By contrast, the "low responder" peptide epitope has a significantly faster, pH-dependent, off-rate. Using this system we have shown that a large fraction of IE^k exhibits peptide exchange in vitro.

A competition binding assay has also been used in conjunction with a complete "replacement set" of peptides representing MCC(93-103) to elucidate the exact biochemical nature required at each position for this peptide sequence to act as a T cell epitope. This implicates conformational flexibility directed by residues within the minimal epitope as a major feature contributing to antigenicity. Several monoclonal antibodies have been raised that are specific for the combination of IE^k with MCC(88-103). These have been used to determine the orientation of this peptide within the IE^k molecule, and are being used to probe the location and nature of formation of this complex in vivo.

O 250 PAIRING STUDIES OF MOUSE MHC CLASS II HETERODIMERS, Tina Rich and Paul Travers, Department of Crystallography, Birkbeck College, London, England, WC1E 7HX.

We have hypothesised that the favourable packing of the class II α and β chain transmembrane domains are required for the successful pairing of the molecule and subsequent cell surface expression.To test this we have constructed hybrid molecules bearing various combinations of α and β transmembrane regions; wild type α/β, α/β$_{\alpha tm}$(β with α transmembrane), and αβ$_{tm}$/β$_{\alpha}$ tm. Transfectants expressing 'mismatched' transmembrane domains show a pairing deficit, with the majority of the class II α and β chains remaining within the cell. However, those that do pair successfully are transported to the cell surface. The 'mismatched' heterodimer has lost the epitopes for the IA$^k\alpha$ chain specific antibodies 1E9 and 2A2, although the epitopes of the antibodies 3F12 and 3G2 are retained . In the 'swapped' heterodimer in which the transmembrane domain pairing is restored, the pairing defect is not seen and the 2A2 and 1E9 epitopes are restored. In functional assays, the ability of the 'mismatched' heterodimer to present peptide antigens is impaired relative to the wild type or to the 'swapped' heterodimers. Thus we have identified two separate roles for the class II transmembrane and cytoplasmic domains. Firstly, correct pairing of the transmembrane domains is required for the efficient assembly of the class II heterodimer. Secondly, we feel that the best explanation for the effect on the conformation of the membrane distal domains and on function is that the β chain transmembrane and/or cytoplasmic domains encode an intracellular routing signal that directs the nascent class II molecule through a peptide loading compartment. In the absence of such a signal the molecule would be transported to the cell surface in a 'peptide-free' state. Validation of this model is in progress.

O 251 UNEXPECTED CHARACTERISTICS OF SPONTANEOUS MUTAGENESIS AND ITS POTENTIAL FOR EXPLAINING SOME CHARACTERISTICS OF HLA DIVERSITY, Lynn S. Ripley, Department of Microbiology and Molecular Genetics,UMDNJ-New Jersey Medical School, Newark, NJ 07103

Spontaneous mutations occasionally change more than one nucleotide during a single mutational event. For example, multiple base substitutions produced in concert with frameshifts represent a significant fraction of spontaneous frameshift mutations. These substitutions often arise by mechanisms that depend on templated, but misaligned DNA synthesis. That is, the DNA sequence changes are complementary to an ectopic template. Three mechanisms have been identified and none of these require that the final DNA sequence change include a frameshift mutation. Thus, multiple base substitutions are expected to occur at low rates by templated mechanisms. Despite the low rates, these multiple substitution mutations might contribute frequently to the DNA changes fixed in populations during the evolution of genes like the MHC genes that are under positive Darwinian selection. The DNA sequences of a large numbers of alleles of class II loci of the Major Histocompatibility Complex differ from other alleles by many base substitutions. Examination of the clustered DNA base substitutions has revealed that many of the substitutions are exactly complementary to nearby conserved DNA sequences and thus, have precisely the characteristic expected of multiple substitutions arising by templated mutational mechanisms. The distribution of the base substitutions suggests their independent occurrence in substantially diverged species and perhaps in separate lineages within a single species.

O 252 CHARACTERIZATION OF I-A^q BINDING AND T-CELL RECEPTOR INTERACTION SITES IN A CHICK TYPE II COLLAGEN PEPTIDE. Edward F. Rosloniec, Linda K. Myers, Patricia Kent, and John M. Stuart. Departments of Medicine and Pediatrics, University of Tennessee, and the VA Medical Center, Memphis, TN, 38104.

The immunization of genetically susceptible mice with chick type II collagen (cCII) results in an autoimmune polyarthritis (CIA) that resembles human rheumatoid arthritis. The susceptibility to CIA is restricted to the expression of the I-A^q molecule, although strains expressing other I-A haplotypes also generate an immune response to cCII. Previously, we have shown that the peptide fragment cCII(245-270) is preferentially recognized by mouse strains expressing I-A^q. We have examined this peptide in detail to determine which residues formed the minimal antigenic determinant. Using a panel of I-A^q-restricted, cCII specific T-cell hybridomas, we have determined that the minimum antigenic determinant for two different T-cell hybridomas lies within the residues 260 through 270 of cCII. In addition, we have identified residues which are involved in T-cell receptor (TcR) interaction and residues that are important for binding to I-A^q. Using synthetic peptides containing amino acid substitutions based on the sequence of type I collagen, we have determined that residues 261 and 263 of this peptide are critical for binding to the I-A^q molecule. For recognition of this peptide by the TcR, amino acids 266, 267 and 269 constitute important residues for the TcR expressed by the T cell hybridoma qCII98, and residues 266 and 269 are important for the recognition by the TcR expressed by 2qCII92. These two TcR also differ in their ability to recognize the murine homolog of CII(260-270). Analysis of TcR expression by these T-cell hybridomas indicates that they both utilize a member of the Vb8 gene family. We are in the process of determining the Va TcR gene segments expressed by these T-cell hybridomas, and assessing the importance of cross reactivity with murine CII in conferring susceptibility to CIA.

O 253 PRESENTATION OF ANTIGEN BY MIXED ISOTYPE CLASS II MOLECULES IN NORMAL H-2^d MICE, Giovina Ruberti#, Karen S. Sellins#, C. Mark Hill*, Ronald G. Germain^,C. Garrison Fathman# and Alexandra Livingstone##, #Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA 94305; *ImmuLogic Pharmaceutical Corporation, Palo Alto, CA 94304; ^Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, N.I.H., Bethesda, MD 20892; ##Basel Instute for Immunology, Basel, CH-4005

The α and β chains of MHC class II molecules show very strong preferences for matched isotypic pairing. High level expression of mixed isotype molecules can be achieved following transfection of certain combinations of α and β gene pairs into L cells. There is also biochemical, serological and functional evidence for low level expression on some B cell tumours and human EBV-transformed cells. EαA^dβ molecules can be detected on the spleen cells of mouse strains with defective Eβ expression, and also in mice expressing high levels of an Eα transgene, where they can be recognized both as alloantigens and as antigen presenting molecules. Despite these latter results it was thought that mixed isotype molecules were expressed at levels too low to play any physiological role in individuals with balanced class II chains expression. Here we show that the mixed isotype molecule Eα^{d}Aβ^d constitutes a major presenting element in H-2^d mice for a determinant on sperm whale myoglobin. The quantitative aspects of this mixed isotype molecule suggest that the very low number of MHC molecules expressed is not limiting in T cell activation.

O 254 BIOCHEMICAL AND FUNCTIONAL ANALYSIS OF ENDOGENOUS PEPTIDES ASSOCIATED WITH MHC CLASS II. Alexander Rudensky, Paula Preston-Hurlburt, Satyajit Rath*, Donal B. Murphy# and Charles A. Janeway,Jr. Section of Immunobiology and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510, *National Institute of Immunology, New Delhi, 110067 India and #Laboratory for Immunology, Wadsworth Center, NY State Deptartment of Health, Albany, NY12201.

Endogenous peptides associated with MHC class II molecules of two different haplotypes have been isolated and sequenced. The analysis revealed fixed N-termini of MHC class II-associated peptides and differences in one and/or two amino acids at the C-termini, perhaps due to trimming by carboxypeptidases. Based on this finding and on the variable length of peptides isolated so far (13-17 residues) model of a peptide binding to the class II MHC molecule has been proposed. A unique monoclonal antibody recognizing one of the peptides in the complex with I-A^b has been used for further analysis of peptide:MHC class II complexes. The binding of a naturally processed peptide to cell surface MHC class II molecules has been shown to be dose, time, temperature and pH-dependent. The abundant class II MHC:peptide complexes are largely depleted of invariant chain. The binding of peptide to MHC class II occurs after transport through the Golgi as demonstrated by endoglycosidase H sensitivity analysis. In studies on T cell recognition of naturally processed endogenous peptides, tolerance has been shown in mice expressing an abundant MHC:peptide complex. Mice not expressing this complex develop a strong T cell response to the corresponding peptide. In these mice low level of expression of a cross-reactive epitope on APCs has been detected by a monoclonal antibody to MHC:peptide complex. The implications of these findings for positive and negative selection of the T cell receptor repertoire will be discussed.

O 255 FAST PEPTIDE BINDING TO CLASS II MHC DIMERS AT LOW pH AND STRUCTURAL STABILIZATION OF THE COMPLEXES UPON NEUTRALIZATION Scheherazazade Sadegh-Nasseri and Ronald N. Germain, Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892

The kinetic behavior of MHC class II in binding to peptides is complex. A fast-forming, fast-dissociating intermediate complex converts to a stable form following a slow forward rate with a half time of hours. This presumably is limited by slow dissociation of the previously bound peptides in MHC binding groove and/or the rigidity of the MHC molecule. In vivo, however, stable peptide binding to newly synthesized MHC molecules is significantly more rapid. This difference could be due to either a) newly synthesized α/β heterodimer being free of bound peptide and/or b) peptide binding occurring within acidic vesicles where class II molecules may be more flexible. Thus, simulation of the in vivo conditions may enhance the forward rate for peptide binding in vitro. We therefore studied peptide binding to, as well as structural changes induced in Eα^k Eβ^k upon exposure to low pH. In a non-reduced SDS PAG gel system, the majority of unboiled purified MHC-II migrate as compact heterodimers with a molecular weight of 57 kD. However, low pH exposure induces partial unfolding of the a/b heterodimer visualized as either a dimer with slower migration (66 kD) than the compact form or dissociation of the heterodimer into a and b chains during the SDS-PAGE. The extent of unfolding correlates with loss of bound peptide. We have demonstrated that under acidic conditions specific peptides bind MHC efficiently and re-construct the compact form upon neutralization. This binding and stabilization occurs within less than 5-10 minutes and the complexes formed are effective in T cell stimulation. We propose that at acidic pH, previously bound peptides dissociate leading to an open molecule with more flexibility. Newly offered peptide binds to this molecule with a low affinity at acid pH and induces a compact conformation which stabilizes upon neutralization. This mechanism can cause 'trapping' (slow off rate binding) of peptides that may not have a high intrinsic affinity for binding to MHC class II at acidic pH.

O 256 Discrepancy between the ability of peptides to bind to MHC class II molecules and their capacity to modify the immune response.
Corinne M. Savill, Vanessa C. Vickers, C. Jane Cunliffe, Richard E. Shute, Eric J. Culbert, Robert D. Gordon ICI Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG, UK.

The use of non-stimulatory MHC class II binding peptides to block recognition and resultant $CD4^+$ T cell activation represents a potential therapeutic strategy in a number of autoimmune diseases for which the relevant MHC class II association is known. To explore the potential of this approach we have investigated the correlation between the ability of peptides to bind to MHC class II molecules and their capacity to modulate antigen-specific, human T cell responses.

We have demonstrated binding of a peptide based on the sequence of a previously described T cell epitope, (residues 307-320 of influenza virus haemagglutinin) to HLA-DR4Dw4, HLA-DR4Dw14 and HLA-DR1 in an intact cell binding assay and have also confirmed binding to HLA-DR4Dw4 using solubilized purified protein. A similar peptide HA307-319 has been previously shown to bind to soluble HLA-DR1 molecules (Jardetzky et al, EMBO J. 9, 1797, 1990) and to a range of cell associated HLA-DR types (Busch et al Int.Immunol. 2, 443, 1990). A control peptide, with a sequence unrelated to any known T cell determinant, showed negligible binding to all HLA-DR types tested. In contrast to these findings, neither peptide had a significant inhibitory effect on an HLA-DR1 and HLA-DR4Dw14 restricted T cell response to mycobacterial antigens. A possible explanation for this lack of effect is that the stimulatory mycobacterial epitopes bind to HLA-DR with a much higher affinity than the HA307-319 peptide. Alternatively, factors other than simple binding to the cleft, such as access of exogenously added peptides to the intracellular site at which peptides derived from antigenic processing associate with newly synthesised MHC class II, could be important in determining inhibitory activity.

O 257 A SINGLE AMINO ACID CHANGE IN AN MBP PEPTIDE CONFERS THE CAPACITY TO PREVENT RATHER THAN INDUCE EAE.
Dawn E. Smilek*, David C. Wraith†, Lawrence Steinman‡§, and Hugh O. McDevitt.*¶ Departments of *Microbiology and Immunology, ¶Medicine, ‡Neurology, §Pediatrics and Genetics, Stanford University School of Medicine, Stanford, CA 94305; and †Cambridge University Department of Pathology, Cambridge CB2 2QQ, U.K. Experimental autoimmune encephalomyelitis (EAE) is an experimental demyelinating disease of rodents. In (PL/J x SJL)F1 mice, it is induced by immunization with the myelin basic protein peptide Ac1-11. Ac1-11[4A], a myelin basic protein peptide analog with a single amino acid substitution: 1) binds to the class II MHC molecule $A\alpha^u A\beta^u$ and stimulates encephalitogenic T cells *in vitro* better than Ac1-11; 2) is nonimmunogenic and non-encephalitogenic *in vivo* in (PL/J x SJL)F1 mice; 3) prevents EAE when administered before or at the time of immunization with Ac1-11; 4) prevents and reverses EAE when administered later, near the time of disease onset. EAE induced with Ac1-11 is not prevented by MBP 89-101 [not encephalitogenic in (PLJ x SJL)F1 mice, and non-binding to $A\alpha^u A\beta^u$]. Initial studies of the mechanism of disease prevention suggest that Ac1-11[4A] does not prevent EAE by competitive inhibition, since another peptide (Ac1-11[3A,4A]) which binds to $A\alpha^u A\beta^u$ as well as Ac1-11[4A] fails to inhibit EAE. Moreover, protection from disease by Ac1-11[4A] is not readily transferred. In summary, substitution of a single amino acid in a myelin basic protein peptide confers the capacity to prevent rather than induce EAE, even after peptide-specific encephalitogenic T cells have been activated.

O 258 EXPRESSION OF HLA-DR1 IN INSECT CELLS
Lawrence J. Stern and Don C. Wiley, Department of Biochemistry and Molecular Biology, Harvard University. We have expressed the human class II MHC protein HLA-DR1 as a soluble, secreted, glycoprotein, in insect cells infected with baculoviruses carrying truncated α and β subunit genes. DR1 from insect cells binds nearly a stoichiometric amount of peptide, at a rate ten-fold faster than DR1 from human lymphoblastoid cells. Moreover, peptide binding to DR1 from insect cells was relatively pH independent, while peptide binding to DR1 from human cells increased at lower pH. The low binding capacity, slow association kinetics, and pH dependence of peptide binding to class II molecules isolated from human cells are all believed to be due to the presence of tightly bound peptide occupying the antigen binding site. The increased peptide binding capacity, increased binding rate, and decreased pH dependence of peptide binding for DR1 from insect cells indicate that the antigen binding site is largely empty. "Empty" DR1 as isolated from insect cells is sensitive to SDS-induced dissociation. After peptide loading, the $\alpha\beta$ heterodimer is resistant to SDS, like DR1 from human cells. The "empty" DR1 from insect cells tends to aggregate, but the aggregation is reversed by peptide loading. Although stabilized by peptide binding against denaturation and against aggregation, the empty class II heterodimers remain associated under physiological conditions. Thus heterodimer formation cannot itself be the means of "trapping" the peptide within the binding site. The slow peptide association kinetics even for "empty" DR1 suggest that a slow conformational change may occompany peptide binding.

O 259 EXAMINATION OF THE MURINE T CELL RESPONSE TO A NON-PROTEIN, SELF ANTIGEN; HEME.
Robyn Sutherland, Kim-Anh Nguyen, Tanya Scharton and Yvonne Paterson. Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA19104.

We have been studying the murine T cell response to a novel antigen; heme. This response differs from classical T cell responses by virtue of the structurally rigid and non-protein nature of the heme antigen, yet resembles T cell responses to protein antigens in so far as the response to heme is MHC restricted. Class II restriction was shown in *in vitro* bulk cultures by the capacity of anti-IA and anti-CD4 mAb to block the proliferative response to heme, and was corroborated using heme specific T cell hybrids and lines which required MHC matched APC for activation. A provoking aspect of this response is that despite the abundance of heme *in vivo*, and hence the expectation of tolerance, it is possible to demonstrate a high frequency of heme reactive T cells in the periphery of unprimed $H\text{-}2^s$ mice. *In vitro* limiting dilution analysis performed on lymph node T cells freshly explanted from various $H\text{-}2^s$ mouse strains (B10.S, SJL and ASW) has indicated that 0.2-0.4% of these T cells are heme responsive ie. a frequency which approaches the high frequency recorded for T cell responses to alloantigen. This analysis again supported the MHC restriction of the T cell response to heme, since by contrast to the high responder $H\text{-}2^s$ mice, $H\text{-}2^d$ mice (Balb/C and B10.D2) expressed a low responder phenotype (0.03-0.04% of unprimed lymph node T cells responsive to heme). In an effort to understand these phenomena we are currently examining whether the natural tendency of heme to co-ordinate with many proteins/peptides masks or contributes to its antigenicity for T cells.

O 260 ANALYSIS OF THE ROLE OF RECYCLED CLASS II MHC MOLECULES IN ANTIGEN PRESENTATION. Kevin Swier and Jim Miller, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637. Class II MHC molecules bind antigenic peptides in endocytic compartments of antigen presenting cells (APC) and transport them to the cell surface where they are presented to $CD4^+$ T lymphocytes. It is debated whether class II molecules have one opportunity to bind antigens, on their biosynthetic route after exit from the Golgi complex and prior to arrival at the cell surface, or multiple opportunities, on an internalization and recycling pathway. Consistent with the latter model, the half-lives of immunogenic MHC-peptide complexes measured by functional assays are shorter than the half-lives of MHC molecules measured biochemically. Furthermore, it has been shown that class II MHC molecules can bind a competitor peptide after being loaded with antigenic peptide. These results suggest that APC can efficiently internalize class II MHC-peptide complexes into antigen-containing compartments where exchange of peptides occurs and new MHC-peptide complexes are recycled back to the cell surface. Biochemical measurements of class II MHC internalization in B cells, however, have failed to reveal internalization patterns similar to other known recycling cell surface molecules. Moreover, the functional association of surface class II molecules with intracellular antigen has never been demonstrated. We have attempted to address these questions in two ways. First, we have developed a new method for the nonradioactive labeling of surface class II molecules, making it possible to distinguish intracellular MHC molecules derived from the cell surface from newly synthesized MHC molecules. Secondly, we have engineered internalization signals into the cytoplasmic domains of class II molecules. By transfecting these molecules into non-professional APCs we can test directly whether or not class II molecules can associate with antigen when given the opportunity to be internalized and recycled.

O 261 CONFORMATIONAL ANALYSIS OF MHC CLASS II MOLECULES, Christina Zamoyska and Paul Travers, ICRF Structural Molecular Biology Unit, Department of Crystallography, Birkbeck College, Malet St., London, England, WC1E 7HX

We have defined single amino acid mutations on the surface of both the α and β chains of the DR molecule that affect the conformation of the molecule. Some of these mutations are in residues that are not expected to interact with peptide and are therefore likely to have direct effects on the conformation of the molecule. We have found that the conformation of the DRβ2a (DRB5*0101) chain is labile compared to other DRβ alleles. In particular, in the isolated DRβ chain and in the peptide free heterodimer, the DRB5*0101 chain fails to bind any of the DRβ chain specific monoclonal antibodies that we have tested. Moreover, we have found that mutations in regions of the molecule expected to interact with the T cell receptor cause the expressed heterodimers to fail to adopt the 'compact' state, fail to bind DRβ antibodies and in some cases fail to bind exogenous peptide. The current understanding of the role of polymorphism is that allelic variation alters the antigen specificity of the class II molecule directly, by changing the residues in contact with the peptide antigen. Our results suggest that some polymorphism may affect antigen specificity indirectly, by altering the structure of the molecule to which the peptide antigens initially bind. Moreover, by identifying mutations that arrest the molecule in the 'floppy' state we have begun to define residues that play a critical role in the folding of the class II molecule.

O 262 BIOCHEMICAL ANALYSIS OF NATURALLY PROCESSED PEPTIDES BOUND TO HUMAN CLASS II MOLECULES, Robert G. Urban, Roman C. Chicz, Joan C. Gorga, William. S. Lane, Lawrence J. Stern, Dario Viginali, and Jack L. Strominger, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138 Class II Major Histocompatability Complex (MHC) molecules are heterodimeric glycoproteins expressed on the surface of antigen presenting cells. Antigen presentation begins with internalization and proteolytic processing of proteins into small peptides. From this pool class II molecules bind some peptides and then transport the bound peptides to the cell surface for presentation. Using synthetic peptides, it has been demonstrated that divergent MHC molecules bind identical peptides with different affinities; the fundamental property resulting in MHC restriction. However, to date little information is available on the characteristics of naturally processed peptides bound to different human class II molecules.

HLA-DR1, -DR2, -DR3, -DR4, -DR7 and -DR8 molecules have been purified in relatively large quanities from B lymphoblastoid cell lines by immunoaffinity chromotography (and crystallized, J. Gorga et al., Res. Immunol. 142, in press, 1991). The peptides bound to each have been obtained by acid extraction and separated by reverse-phase HPLC. The peptide elution profile for each allele is distinct. Preliminary microsequence analysis has been obtained. A full report of the peptides bound to the different alleles will be presented.

O 263 THE ROLE OF LIGAND BINDING IN STABILIZING HLA-CLASS II MOLECULES. Robert G. Ulrich, Jack L. Strominger, and Joan C. Gorga, Department of Immunology and Molecular Biology, Research Institute of Infectious Diseases, Frederick, MD 21702, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, and Children's Hospital of Pittsburgh, Pittsburgh, PA 15213. Protein antigens must pass through an acidic intracellular compartment before peptides become associated with the class II MHC molecule on the surface of antigen presenting cells. As it also has been observed with other class II MHC molecules, peptide binding to the HLA-DR molecule is enhanced under conditions of low pH as a result of a change in the apparent ligand on-rate. The unfolding of DR and DQ protein structure is pH-dependent, and exhibits a threshold for loss of secondary structure and subunit dissociation that occurs at a pH optimal for peptide ligand binding. Peptide binding stabilizes the class II molecule under a variety of denaturing conditions, although the molecules with apparently unoccupied binding sites are stable under standard conditions. It is possible that the class II/peptide molecular complex may have a role in protein maturation and cell surface expression in a manner similar to the class I MHC antigen presentation pathway. In addition, staphylococcal enterotoxins that bypass the usual processing requirement for binding to the class II MHC molecule can stabilize the complex. The enterotoxin binding can still occur even when mutations designed to disrupt secondary are introduced into conserved regions in both subunits of the HLA-DR heterodimer.

O 264 A HIGH EFFICIENCY ANTIGEN PRESENTING CELL LINE FOR USE IN T CELL DETECTION OF ISLET ANTIGENS, Dale Wegmann and Kathryn Haskins, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262. A variety of experimental approaches have been devised to increase the efficiency of presentation of antigens to class II restricted T cells by antibody-mediated targeting of antigen to the APC. This strategy generally yields several orders of magnitude increase in the sensitivity of detection of antigens by T cells and would therefore be advantageous for use in T cell identification of uncharacterized antigens available in limited quantities. Over the past several years we have developed and characterized a panel of islet-reactive T cells from NOD mice. One of our major goals is to identify the islet antigens toward which these T cells are directed. In order to overcome some of the problems associated with limited availability of antigen, we have constructed an APC for use in presentation of islet antigens to the panel of islet-reactive T cells. The key feature of this APC is the surface expression of TNP-specific IgM as a result of gene transfection. With this APC, the presentation of TNP-conjugated experimental antigens such as ovalbumin is up to 10^6 more efficient than that observed for non-conjugated antigens. We have also demonstrated that this APC line will present unfractionated, TNP-conjugated islet cell lysates to all members of the panel of islet-reactive T cells with a dramatic increase in efficiency compared to conventional APC. We intend to use this system to identify the antigens recognized by islet-reactive T cells.

O 265 THE IMMUNODOMINANT T CELL RESPONSE TO STAPHYLOCOCCAL NUCLEASE IS ALTERED BY A SINGLE AMINO ACID SUBSTITUTION IN THE PROTEIN, Kelly M. Welsh and Alison Finnegan, Dept. of Immunology/Microbiology and Dept. of Medicine, Sect. of Rheumatology, Rush University, Chicago, IL 60615

In examining T cell responses to complex antigens it has been observed that T cells are specific for a limited number of determinants in the overall antigen. The mechanisms responsible for the immunodominance of particular determinants over others has not been clearly established. For example, the majority of Nase-specific T cell clones recognize the Nase 81-100 peptide presented in association with the I-E^K class II molecule. We have demonstrated that a single amino acid substitution at position 89 in the Nase molecule (leu to phe)(L89F) alters the ability of a T cell clone to recognize the mutant protein. To determine the effects of a single amino acid substitution on the immunodominant T cell response *in vivo*, mice were immunized with either wild-type Nase or the mutant L89F. Epitope specificity of primed T cells was tested by examining T cell proliferation to 20-mer peptides spanning the length of the Nase protein. T cell lines derived from Nase immunized mice specifically proliferated to the 81-100 peptide and not to any other peptide. In contrast, T cell lines derived from L89F immunized mice did not proliferate to the 81-100 peptide but did respond to peptides 111-130 and 121-140. The substitution at position 89 either alters the processing of the Nase protein permitting other peptides to be presented or disrupts the binding of the dominant 81-100 peptide to the I-E^K molecule such that other peptides can now bind. To determine if the processed mutant peptide disrupts binding to the I-E^K molecule, *in vivo* competition between wild-type and mutant processed peptides for presentation was evaluated. Preliminary data demonstrates that T cell lines derived from mice co-immunized with Nase and mutant L89F proteins did not respond to either 111-130 or 121-140 peptides but did respond to 81-100. These data suggest that the immunodominance of particular T cell epitopes is determined by competition between peptides derived from the same protein for binding to the class II molecule.

O 266 DEFINITION OF MHC AND TCR CONTACT RESIDUES IN A HLA-DR2 RESTRICTED T CELL EPITOPE

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The trimolecular interaction of TCR, antigen and MHC was analyzed using a panel of HLA-DR2 restricted T cell clones recognizing the 49-61 region of a meningococcal class 1 outer membrane protein (OMP). The clones were selected by restimulation with the synthetic peptide OMP(49-61), which contains an immunodominant T helper determinant. Using a series of peptides that were sequentially truncated from the N- or C-terminus, four different epitope fine-specificity patterns were identified. Furthermore, each clone was found to exhibit a distinct recognition pattern for a panel of 20 single residue-substitution analogues of the minimal epitope OMP(50-58). By performing proliferation competition assays, putative TCR and MHC contact residues in the peptides were defined for each clone. Within the minimal epitope, T cell receptor and MHC contacts varied when comparing the differents clones. DR2 apparently allows several modes of interaction with a single peptide. Consequently, different residues of this peptide are exposed to the TCR. These findings are discussed in the context of the nucleotide sequences of the TCR V/J_α and $V/D/J_\beta$ junctional regions of the OMP(49-61)-specific T cell clones.

O 267 PEPTIDE SPACIAL ORIENTATION IN CLASS II MHC: INSULIN ISOFORM A-LOOP AMINO ACID CONTACT RESIDUES INFLUENCE MHC BINDING. Douglas B. Williams, Jim Ferguson, Jean Gariepy and Nobumichi Hozumi, Samuel Lunenfeld Research Institute Mount Sinai Hospital, Department of Immunology University of Toronto, Toronto, Ontario, Canada, M5G1X5. We have synthesized 24 different variants of the N-terminal beef insulin A-chain molecule to examine their interactions with MHC class II molecules and insulin-specific T-cell receptors. Using functional competition assays and computer modeling with peptides modified by either amino acid residue substitution or by truncation, permitted delineation of common and unique residue contact sites defining MHC binding motifs for several insulin isoforms. Activation of I-A^d restricted insulin-specific T-cell hybrids required presentation of a 14 amino acid residue insulin-A(1-14) peptide containing an intact intra-chain disulfide bond (A-loop). The importance of maintaining the A-loop structure is required for T-cell recognition and also for orientation of peptide within the antigen groove of class II MHC. Molecular modeling of electrostatic charge in native peptide sequences and analogs, dramatically emphasize shifts in spacial electron densities and distributions in localized regions of peptide, induced by single and multiple amino acid substitutions. The findings suggest altered electrostatic fields associated with insulin isoforms induced by regional amino acids, influence peptide orientation in MHC. As a result, the peptide agretope constitutes a major determining factor regulating selection of T-cell epitopes presented to reactive T-cells and in the case of insulin, constitutes a major factor governing insulin fine specifity. Supported by MRC.

Accessory Molecules, Ii, and Superantigens

O 300 B2-microglobulin upregulate class I MHC antigen and enhance peptide association with class I as detected by cytotoxic T lymphocytes.Abdel Motal ussama,Zhou,Louise and Mikael Deppartment of immunology,Karolinska Institute, S-10401 Stockholm,Sweden.
CTL recognize intra-cellular antigen as a complex consisting of short peptides,MHC classI heavy and light (B2-microglobulin) chains.External synthetic peptides can sensitize target cells t CTL killing and also upregulate the membrene expression of MHC-I.In the present work we have found that also B2-microglobulin can upregulate class I expression on RMA-S lymphoma, B16 melanoma and P815 mastocytoma cells in vitro. This upregulation is completely blocked by Brefeldin A, and partially by chloroquine,monensin,primaquine and colchicine, suggesting a dependence on both out-transport of class I chain from the Golgi system and recycling inearly endosomes. Using RMA-S cells, D^b upregulation was found to be additative when both peptide and B2-microglobulin was used for tretment of cells. This increase of D^b upregulation was also reflected as an increase killing of target cells, using CTL specific for the influenza A derived, NP peptide 364-379. The data suggest an immuno-regulatory role for B2-microglobulin, although more evidence is required for this conclusion.

O 301 DOMINANT T CELL RECEPTOR USAGE IN PEPTIDE-SPECIFIC L^d-RESTRICTED AND ALLO-L^d RESPONSES, MA Alexander-Miller, JC Solheim*, JM Martinko*, TH Hansen, and JM Connolly.

Dept. of Genetics, Washington University School of Medicine, St. Louis, MO and Dept. of Microbiology, Southern Illinois University, Carbondale, IL.

Recent evidence suggests that a correlation, in terms of limited heterogeneity, exists between particular TCR V_α and/or V_β gene segments and MHC/antigen recognition. We have generated a panel of CTL clones specific for a peptide antigen, tum$^-$, presented in the context of the H-2L^d molecule. Characterization of these clones revealed the existence of an inverse correlation between the CD8 dependency and determinant density requirements of an individual clone (Alexander et al.,J. Exp. Med. 173:849, 1991). This allowed us to investigate correlations which may exist between the TCR expressed by a clone and its functional phenotype. Ten of thirteen clones analyzed were found to express TCRs from the $V_\beta 8$ gene family as ascertained by positive staining with mAb F23.1. TCRs from a number of these clones were amplified via PCR techniques, cloned and sequenced. The choice of TCR V_β, V_α, and J_α genes expressed was limited; however, no correlation with CD8 dependency or determinant density requirements was apparent. In addition to the characterization of a peptide-specific CTL response, we are examining the TCR usage in an allogeneic model system using the L^d molecule in an attempt to demonstrate whether CTLs responding to the L^d molecule when recognized as an allo-antigen would also employ predominantly $V_\beta 8$ TCRs. In initial studies involving FACS analysis of bulk allogeneic populations, a significant percentage of responding CTLs were found to be positive with the F23.1 mAb. Therefore it appears that $V_\beta 8$ usage is significant in both L^d restricted as well as allogeneic L^d responses.

O 302 THE EFFECT OF N-TERMINAL DELETIONS OF INVARIANT CHAIN ON CLASS II BIOSYNTHESIS AND TRANSPORT AND ON CLASS II-RESTRICTED ANTIGEN PRESENTATION, Mark S. Anderson and Jim Miller, *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637*

During their biosynthesis, class II major histocompatibility complex (MHC) molecules are intimately associated with invariant chain (Ii). This association has been implicated in controlling the intracellular sorting of class II and its association with antigenic peptides, however, the exact role of Ii in this process remains unclear. A recent set of studies have identified a potential endosomal retention signal in the cytosolic tail of Ii. To clarify the role this signal may have in class II transport and class II-restricted antigen presentation, N-terminal deletions were made in the Ii gene and transfectant cells were constructed that expressed class II with and without mutant and wild type forms of Ii. This mutant form of Ii is able to associate with class II early in biosynthesis, however, unlike wild type Ii, the mutant form of Ii remains associated with the majority of class II at the cell surface. This altered phenotype of class II-Ii association at the cell surface suggests that the mutant Ii could dramatically affect the ability of class II to present antigens to T cells. Therefore, we tested the ability of this altered Ii molecule to affect antigen presentation by taking advantage of a recent observation in our laboratory in which it was shown that Ii can facilitate antigen presentation with some antigen specific T cells. Interestingly, the N-terminal deletion mutant of Ii is still able to facilitate antigen presentation at nearly the same level as wild type Ii. Studies to further clarify the role that the mutant Ii has in class II transport and antigenic peptide association will be discussed.

O 303 THE EFFECTS OF INVARIANT CHAIN IN MODULATING CLASS II CONFORMATION DURING BIOSYNTHESIS. Lynne S. Arneson and Jim Miller. Molecular Genetics and Cell Biology, University of Chicago. Chicago, Illinois. 60637.

Invariant chain has been shown to be important in class II MHC biosynthesis and transport. One indication of the effects of invariant chain on class II is that its presence is required for the formation of several class II specific monoclonal antibody epitopes. We have now identified a panel of antibodies that react to class II in a conformation dependent manner. This panel includes antibodies whose epitopes form early in class II biosynthesis and persist as class II matures, and others whose epitopes only form late in class II maturation. Late epitope formation has recently been shown to be associated with peptide loading and the formation of compact class II dimers. Interestingly, invariant chain does not correlate with early or late forming epitopes since both early and late forming epitopes can be invariant chain dependent and independent. In addition, an antibody that reacts more strongly in the absence of invariant chain has also been identified. This panel of antibodies is currently being used to study the compact dimer, to determine whether the compact form of class II is associated with invariant chain dependent epitopes and whether any of these epitopes can be associated with peptide loading of class II.

O 304 ROLE OF NON-POLYMORPHIC REGIONS OF CLASS I MHC MOLECULES IN THE ACTIVATION OF CD8+ CTL BY PEPTIDE AND PURIFIED CELL-FREE CLASS I MOLECULES. J. A. Berzofsky, T. Takeshita, S. Kozlowski, R. D. England, H. Takahashi, J. Schneck, and D. H. Margulies. Metabolism Branch, NCI, and Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

We developed a method of activating non-transformed CD8+ cytotoxic T lymphocytes (CTL) in the absence of presenting cells using synthetic peptide and purified recombinant soluble class I MHC molecules coated on plastic, in order to quantitate the requirements for each component in the activation process, in the absence of other accessory molecules on the APC. The CTL used were a BALB/c clone specific for peptide 315-329 of the HIV-1 IIIB envelope protein (P18, RIQRGPGRAFVTIGK) in association with D^d. When simultaneously titrating the concentrations of D^d and P18 required for CTL activation, we noted that the response was more sensitive to changing the concentration of D^d than to changing that of peptide. The D^d titration appeared to have a second order concentration dependence, whereas that for peptide was first order. This result suggested that D^d played more than one role in the reaction. One role of D^d was to present peptide to the conventional $\alpha\beta$ T-cell receptor (TCR), a role that could not be replaced by other class I molecules. We asked whether the D^d was also serving a function involving the interaction of non-polymorphic regions with other accessory molecules on the CTL. We observed that titrating in purified recombinant K^b (with the $\alpha 3$ domain of D^d), which cannot present P18, could enhance the activation of the CTL clone by D^d and P18. Moreover, when we titrated D^d in the presence of an amount of K^b adjusted to keep the sum of $D^d + K^b$ constant, we found that the slope of the dependence of the response on D^d was now half of what it was in the absence of K^b. Thus, the additional interaction between D^d and the CTL that increases the order of dependence of CTL activation on D^d concentration involves only a non-polymorphic region of the class I molecule, and can be substituted by a class I molecule incapable of presenting the peptide. We are testing the hypothesis that this effect is mediated by the interaction between the $\alpha 3$ domain of class I and the CD8 molecule on the CTL. If so, this result would indicate that CD8 interaction with class I molecules not presenting peptide to the $\alpha\beta$ TCR can also enhance CTL activation, when the $\alpha\beta$ TCR is also concurrently engaged.

O 305 DENDRITIC CELLS ARE POTENT ANTIGEN PRESENTING CELLS FOR MICROBIAL SUPERANTIGENS. Nina Bhardwaj*§, Steven M. Friedman§, Barry C. Cole# and Anahid J. Nisanian*. The Rockefeller University* and the Hospital for Special Surgery§, New York, N.Y. 10021 and the University of Utah College of Medicine#, Salt Lake City, UT 84123.

Microbial superantigens (SAG) cause a spectrum of disease in both man and animals. SAG are potent mitogens and bind with high affinity to MHC class II molecules. Little is known regarding the SAG presenting function of different types of MHC class II+ cells, particularly dendritic cells (DC). DC are a subset of human blood mononuclear cells that are potent stimulators of several T cell functions. We have compared the capacity of blood DC with B cells and monocytes to present staphylococcal toxins (SEA, SEB, SEE and TSST-1) and M.arthritidis mitogen (MAM) to human T cells. Compared to these APCs, DC have 10-100 fold more DR, DP or DQ molecules on their surfaces. Using standard pM doses of SAG we found that DC are 10-50 fold more efficient than other APCs in stimulating T cells. For MAM, in particular, monocytes and B cells were strikingly less efficient than DC. Sorted DC (>95% pure) were effective even when present at 1 cell per 3000 T cells. At these doses DC did not stimulate allogeneic cells in the MLR. The ability of different APCs to stimulate T cells in the presence of a wide range of enterotoxin concentrations was compared. At pM to fM concentrations of SEE, DC induced T cell proliferation even when present in limiting numbers (1:1000). Monocytes and B cells can stimulate T cells at these doses but relatively high stimulator to responder ratios (1:10-30) are required. Furthermore, DC but not B cells, pulsed for 1 hour with pM levels of SAG maximally activated T cells. Our results suggest that very small amounts of SAG will be immunogenic in vivo if presented on DC.

O 306 I-E MEDIATED TOLERANCE IN T CELL RECEPTOR Vß5-EXPRESSING TRANSGENIC MICE. F.R. Carbone, Department of Pathology and Immunology, Monash Medical School, Commercial Rd. Prahran, Australia 3181, and M.W. Moore, Genentech Inc., South San Francisco CA USA 94080.

It has recently been shown that virtually all T cell receptor (TCR) Vß5-expressing cells are deleted in mice that coexpress the class II molecule I-E and a product encoded within the endogenous MTV9 proviral insertion site found in a number of common mouse strains such as the C57Bl/6 line. We have derived transgenic mice where the majority of peripheral T cells express a Vß5.2 TCR ß chain. As these transgenic mice became older they showed a significant decrease within the CD4+ splenic T cell population without any comparable deletion in the thymic CD4+CD8- T cell subset even in the absence of I-E expression. Mating of a wild-type I-E expressing mice with the TCR transgenics resulted in a more rapid and pronounced deletion of CD4+ T cells in the periphery and in the mature CD4+CD8- T cells within the thymus. These results suggest that the CD4+ T cells within these mice are being deleted by a mechanism that is firstly dependent on the increasing expression of peripheral superantigen and secondly enhanced by the coexpression of the MHC class II I-E molecule.

O 307 PROCESSING OF A PROTEIN ANTIGEN FOR PRESENTATION BY CLASS II MHC REQUIRES CLEAVAGE BY THE NON-LYSOSOMAL PROTEINASE CATHEPSIN E. B.M.Chain, J. Kay, K.Bennett, T. Levine, R.J. Peanasky, and I.M. Samloff. Dept Biology, Medawar Building, University College London, Gower St., London WC1 6BT. In order to initiate a T cell dependent immune response, most proteins must undergo sufficient proteolysis within antigen presenting cells to generate fragments that bind to and are presented by Class II MHC antigens. Previous investigations have implicated both cysteine and aspartic proteinases in this antigen processing pathway but the identity and location of the specific enzyme(s) responsible are unknown. Evidence is now presented that the major aspartic proteinase activity in murine lymphocytes, and in the murine antigen presenting B cell lymphoma, A20, is a non-lysosomal proteinase of restricted tissue distribution, cathepsin E. Functional studies using a selective inhibitor isolated from the nematode, Ascaris lumbricoides, suggest that cathepsin E plays an important role in the processing of the antigen ovalbumin, for subsequent presentation to ovalbumin-specific T cells. At least in murine B cells, therefore, processing of this antigen is carried out by a proteinase that is distinct from the lysosomal "housekeeping" hydrolases. The restricted tissue distribution of ovalbumin makes this an attractive potential target for the design of novel anti-inflammatory drugs aimed at impairing immune system function.

O 308 HIGHLY CONSERVED AMINO ACID (aa) SEQUENCE WITHIN MHC CLASS II β-CHAIN CONTROLS THE INTRACELLULAR TRAFFICKING, MOLECULE CONFORMATION AND IMMUNE RECOGNITION.

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Non-polymorphic regions of MHC Class II molecules are anticipated to control conserved functions of the protein i.e. general structure, interaction with accessory molecules and intracellular trafficking, all of which can affect the immune recognition of the MHC/peptide complex by the T cell. Among these sequences is a stretch of 5aa (80-84) in Aβ chain. In order to delineate the significance of this region we made conservative aa changes by site directed mutagenesis. The findings are as follows: a change at aa 80 led to changes in Class II conformation as judged by relative reactivity with a panel of invariant chain (Ii) dependent and independent monoclonal antibodies (mAb). Mutation of aa 81 did not change mAb reactivity but abrogated presentation of most antigens tested, superantigens and alloreactivity. Peptides presentation, although diminished in the mutant, was readily detectable. Thus this aa seems to be important in many types of MHC Class II restricted T cell recognition events. The change at aa 83 appeared to be neutral in terms of antigen recognition and molecule conformation. Two independent mutations of aa82 both led to protein transport arrest in a Golgi/post-Golgi compartment. A model is discussed which links these findings with the intrinsic property of class II molecules: delay in transport late in the biosynthetic pathway, allowing them to release the Ii, and bind antigenic peptide.

O 309 Structural analysis of a mouse mammary tumor virus superantigen

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The 3'-long terminal repeat of the murine mammary tumor virus contains an open reading frame encoding a Vβ specific superantigen (vSAG). A set of truncated vSAG genes were used in both transfection and in vitro translation experiments to show that the functional vSAG is a type II integral membrane protein with a large glycosylated extra-cellular C-terminal domain and a small, non-essential, intracellular N-terminal cytoplasmic domain.

αβ T-cell antigen receptors (TCRs) recognize peptide antigens in the context of self major histocompatibility complex molecules (MHC). The specificity of TCRs for MHC plus peptide antigen is generally determined by the combination of the variable elements of the α and β chains of the TCRs. Unlike conventional peptide antigens, superantigens, when combined with class II MHC molecules, interact with TCRs mainly through the Vβ portions of the receptors, with little contribution from the other variable components. In the mouse, several endogenous superantigens, previously known as minor lymphocyte stimulating antigens (Mls), have recently been shown to map concordantly with mouse mammary tumor virus (MTV) integrants in the genomes of certain strains of mice. It is likely that these MTV produced superantigens (vSAG) are encoded by open reading frames (ORFs) in the 3' long terminal repeats (LTRs) of the MTVs, since we and others have shown that this gene encodes the vSAG associated with the exogenous, milk-borne MTV (exoMTV) of C3H/HeJ mice. We report here that the exoMTV vSAG is a type II-membrane bound glycoprotein, with a core protein of 37kD, the predicted molecular weight of the ORF gene protein product.

O 310 MOUSE ENDOGENOUS PROVIRUS MMTV-9 PRODUCT IS A DOMINANT, BUT NOT EXCLUSIVE, COTOLEROGEN IN CLONAL DELETION OF TCR $V_\beta5^+$ AND $V_\beta11^+$ T CELLS - A STUDY IN HLA-DQb TRANSGENIC MICE. Chella S. David and Paul Zhou. Dept. Immunol., Mayo Clinic, Rochester, MN.

Previously, we have demonstrated that in the H-2E negative, HLA-DQw6b single transgenic mice, human DQ_β chain pairs with mouse A^f_α chain and is expressed in a tissue-specific manner. These hybrid molecules cause the clonal deletion of Vb-5.1, Vb5.2 and Vb11-bearing T cells in thymus. We postulated that A^f_α/DQ_β hybrid molecules may possess the same superantigen presenting epitope as E_α/E_β molecules. We investigated whether the product of MMTV-9 genome, which has been shown to be a cotolerogen for the clonal deletion of H-2E-reactive Vb5 and Vb11-bearing T cells, is also a cotolerogen for the clonal deletion of the A_α/DQ_β reactive $V_\beta5$ and $V_\beta11$-bearing T cells. B10.M$^-$ DQb+ (H-2E$^-$, MMTV-9$^+$) were crossed to DBA/1 (H-2E$^-$ and MMTV-9$^-$), and F1 hybrids were backcrossed to DBA/1. The correlation between $V_\beta5$ and $V_\beta11$ expression and the presence of MMTV-9 genome were tested. The results clearly demonstrated that the DQb transgene is required for the clonal deletion of $V_\beta5$ and $V_\beta11$-bearing T cells. Furthermore, a strict inverse correlation between the presence of MMTV-9 genome and the $V_\beta5$+/CD8+ and $V_\beta11$+/CD4+ expression was observed in the DQ_β+ backcrosses. The percentage of $V_\beta5$+/CD8+ T cells among 16 DQ_β+, MMTV-9 negative backcrosses was also quite different, which ranged from 5.3% to 11.5%. Based upon the percentage of $V_\beta5$+/CD8+ T cells, seven mice were classified into an intermediate group (6.30-+0.30) and the other nine mice were classified into a higher group (9.30+0.33) ($P<0.001$). This suggested that, besides MMTV-9 gene products, an additional gene product derived from B10.M background might serve as a second layer of cotolerogen. The nature of this second cotolerogen is currently being investigated.

O 311 CD4 CAN DIRECT THE MATURATION OF CD8-EXPRESSING T- CELLS IN THE ABSENCE OF CLASS I MHC MOLECULES, Craig B. Davis and Dan R. Littman, Department of Microbiology and Immunology, UCSF.

Mature CD4+ (class II MHC-reactive) and CD8+ (class I MHC-reactive) T cells are generated from a population of immature CD4+8+ thymocytes. In order to determine if there is a population of class II MHC-reactive thymocytes that fail to mature because they shut off CD4 instead of CD8 before positive selection, two strains of transgenic mice were mated. One strain expresses CD4 under the control of the *lck* proximal promotor, allowing for expression of CD4 at high levels in the thymus and on all T cells in the spleen and lymph nodes. The second strain fails to express class I MHC molecules due to the disabling of the β2-microglobulin (β2-m) gene by homologous recombination. Mice that are homozygous for the disabled β2-microglobulin gene have mature CD4+8- but not mature CD4-8+ T cells. In contrast, mice that express the *lck*CD4 transgene in association with the β2-m deficiency have mature CD4+8- and CD4+8+ T cells. The CD4+8+ T cells are mature by the criteria of expression of HSA and TCRαβ and proliferation in MLR assays. Differentiation of these cells in the thymus appears to be normal. S1 and FACS analysis indicates that the endogenous CD4 and CD8 genes are regulated appropriately. The cytoplasmic domain of CD4 is required to generate this effect. The implications for the role of CD4 in the acquisition of T-cell MHC specificity and function will be discussed.

O 312 MOLECULAR ANALYSIS OF THE INTERACTIONS OF THE MOUSE ENDOGENOUS SUPERANTIGENS AND THE HUMAN MHC CLASS II MOLECULES. François Denis, Jacques Thibodeau, Helen McGrath, Nathalie Labrecque and Rafick-P. Sékaly. Laboratoire d'Immunologie, Institut de Recherches Cliniques de Montréal, Montréal, Quebec, Canada, H2W 1R7.

The LTR of the mouse mammary tumor virus (MMTV) encodes for a highly glycosylated type 2 protein which acts as a superantigen. Unlike conventional antigens, the superantigens are not processed by the antigen presenting cells. The superantigens bind to the major histocompatibility complex (MHC) class II molecules and interact with a specific Vb element of the T cell receptor (TcR), causing the stimulation, followed by the clonal deletion of the T cells bearing these TcRs.

The murine fibroblast cell line DAP-3, which does not express murine MHC class II molecules, was transfected with various wild-type human MHC class II molecules, and endogenous (MTV-9) and exogenous (MTV-GR) MMTV superantigens, and were able to stimulate mouse T cells. We have produced mutants of HLA-DR which differ in their binding of *S. aureus* superantigens. These mutants will allow us to find out which residues of the MHC are critical in the binding of endogenous superantigens. In order to study the importance of the glycosylation state of the MMTV protein, a soluble form of this protein was produced in various expression systems. The soluble protein was expressed in *E. coli* (no glycosylation), *Streptomyces* (partial glycosylation) and *Drosophila* (complete glycosylation). These various systems will allow to define the molecular interactions between the human MHC class II molecules and the mouse endogenous superantigens.

O 313 GENERATION AND ANALYSIS OF MUTANT CELLS DEFICIENT IN SURFACE EXPRESSION OF MHC CLASS I AND CLASS II MOLECULES, Alexander D. Diehl*‡, Vivian L. Bracialé‡, and Thomas J. Bracialé‡, *Program in Molecular Biology, Washington University School of Medicine, St. Louis, MO 63110, and ‡The Beirne Carter Center for Immunology Research, University of Virginia Charlottesville, VA 22908.

We have generated mutants of the mouse B-lymphoma A20-1.11 exhibiting deficits in their surface expression of both Class I and Class II MHC molecules. Our selection procedure was as follows: A20 cells were stably transfected with the A/Japan/57 influenza HA gene under the control of the CMV promoter to produce constitutive targets for our laboratory's HA-specific Class I and Class II restricted CTL's. Mutant cells were selected by coculturing with a Class II I-A^d restricted CTL for two rounds of 7 days each to allow the CTL's to kill off the cells which successfully presented antigen. Surviving cells were cloned by limiting dilution and multiple independent lines were grown up and analyzed. All mutants lines examined to date have proved to be phenotypically similar. All lines show reduced recognition by both Class II I-A^d and I-E^d restricted CTL's and by Class I K^d restricted CTL's upon superinfection by influenza virus as compared to the parental cell line. Similarly, all lines show reduced surface expression of Class II and Class I, in some cases down to background levels. Other cell surface molecules such as mIgG and Pgp-1 also show reductions in their expression, but such reduction is not general as at least one molecule, CD45, is not reduced in its surface expression. Studies of transcription and translation have not thus far indicated a reduction in overall gene expression of these molecules, and immunoprecipitation of total cellular MHC suggests a block in the secretory pathway that affects these molecules. Thus a deficit in a componant responsible for the assembly or transport of these molecules may account for the phenotype described.

O 314 THE INTERACTION OF CD4 AND CLASS II MHC PROTEINS, Carolyn Doyle, Michael S. Kinch and Jennifer Brogdon, Division of Immunology, Duke University Medical Center, Durham, NC 27710.

A cell binding assay has been used to study the interaction of CD4 and class II MHC proteins. Class II+ human B lymphocytes adhere to monolayers of Chinese hamster ovary (CHO) transfected with the human CD4 cDNA (CHO-CD4 cells). Stable cell adhesion only occurs at 37°C and requires the continuous presence of intracellular ATP. Preliminary characterization suggests that the cell adhesion occurs as a result of the formation of focal contacts which involve cytoskeletal elements. Moreover, expression of the p56*lck* protein tyrosine kinase in the CHO-CD4 cells enhances the formation of focal contacts resulting in increased cell adhesion. Thus, it appears the p56*lck* interacts with cytoskeletal proteins to stabilize cell adhesion.

Finally, we have engineered mutations in both the β1 and β2 domains of the HLA-DR1 protein. The altered gene products have been expressed in a class II- murine B cell lymphoma which has previously been transfected with an HLA-DRα cDNA. Transfected cells, expressing mutated class II proteins, have been analysed for CD4 binding and T cell recognition.

O 315 **USE OF DISEASE-MODIFYING AGENTS TO ELUCIDATE PATHOGENIC MECHANISMS IN SUPERANTIGEN-INDUCED ARTHRITIC Vβ8.2 T-CELL RECEPTOR MRL-*LPR/LPR* MICE,** Carl K. Edwards, III[1], Tong Zhou[2], Jun Zhang[1], Richard E. Long[1], David R. Borcherding[1], and John D. Mountz[2], Depart. of Immunology[1], Marion Merrell Dow Res Institute, Kansas City, MO 64134 and Depart. of Medicine[2], UAB, Birmingham, AL 35294

Certain bacterial products have the ability to combine with class II MHC and stimulate T-cell specificities through the T-cell receptor (TCR) Vβ chain. We recently demonstrated that the superantigen *Staphylococcus enterotoxin B* (**SEB**) can lead to a chronic arthritis in Vβ8.2 TCR transgenic I-E^+ *lpr/lpr* mice (Mountz et al. Arthritis Rheum 34:S61, 1991). To validate this model, we injected **SEB** (10μG) into four different genetic control strains (TCR (+) +/+ [N=12], TCR (-) +/+ ([N=5], TCR (-) *lpr/lpr* [N=9], TCR (+) *lpr/lpr* [N=17]) to observe the relative influence of TCR and/or the *lpr* gene effects on the histopathological **joint severity score (SEV)**. The data suggests that the *lpr* gene effects are as important as the presence of the Vβ8.2 TCR since there were no significant ($p>0.05$) differences in **SEV** with these two strains of mice. (TCR (+) *lpr/lpr*:**SEV** = 1.00 vs TCR (-) *lpr/lpr*:**SEV** = 1.13). Other aspects of histological disease including synovial cell hypertrophy/hyperplasia (47% vs. 55%, respect.), fibrosis/fibroplasia (53% vs. 50%, respect.), and cartilage proliferation (24% vs. 25%, respect.) also showed no major differences. TCR (+) +/+ and TCR (-) +/+ mice both had significantly ($P>0.05$) lower **SEV scores** (17% and 30%, respect.). To study the effects of disease-modifying agents on **SEB** arthritis using TCR (+) *lpr/lpr* mice, we utilized Cyclosporin A [CsA] (10mg/kg/d; 25d [N=12]) and MDL201112 (25mg/kg/d; 25d [N=12]), a purine nucleoside which has previously been shown to have anti-inflammatory activities *in vivo*. Both of these agents were effective in eliminating the **SEB**-induced weight-loss during the first 5d of disease by inhibiting TNF-α release, reducing MHC class II I-E^+ expression by nearly 30% in splenic and peritoneal macrophage at d+10 and d+25, T-cell proliferation after TCR crosslinking with KJ16 by nearly 60-70%, and inhibited **SEB**-induced production of serum IgG_{2a} and RF by nearly 30-45%. Histopathology showed that MDL201112 reduced **SEV** by nearly 60% in comparison to disease controls. These data indicate that CsA and MDL201112 appear to regulate **SEB** interactions with TCR, and may also have direct 2° inhibitory effect(s) on TCR signalling. In conclusion, this transgenic disease model appears to be an excellent *in vivo* modeling tool to assess disease-modifying immunosuppressive agents.

O 316 HLA CLASS I AND II ANTIGENS ARE ASSOCIATED TOGETHER WITH 90 kDa AND 210 kDa PROTEINS ON THE CELL SURFACE.

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Human MHC (HLA) class I proteins are expressed as a heavy chain (about 45 kDa) non-covalently associated with β_2-microglobulin (12 kDa). MHC class II proteins consist of an α-chain (about 35 kDa) non-covalently attached to a ß-chain (about 29 kDa). In this report we show that MHC class I (MHC-I) and class II (MHC-II) proteins can also be associated together with a 90 kDa protein and a 210-220 kDa protein in the cell membrane.

Surface radiolabeled cells were treated with dithiobis succinimidyl propionate (DSP) in order to preserve multimer protein complexes during cell lysis. The lysates were immunoprecipitated and analysed by SDS-PAGE and autoradiography. Immunoprecipitation (ip) of human MHC-I proteins coprecipitated another protein of about 90 kDa in molecular weight - p90. p90 was coprecipitated from all the MHC-I expressing cells tested, including fresh peripheral blood mononuclear cells (PBMC). MHC-I ip from MHC-II expressing cells revealed additional protein bands, corresponding to MHC-II proteins. A protein of about 210-220 kDa was also found in these precipitates. Ip of MHC-II from class II expressing cells co-precipitated proteins corresponding to MHC-I proteins, as well as the 90 kDa- and 210 kDa proteins. MHC-II precipitates from Daudi cells (MHC-I neg.) contained class II proteins and the 90 kDa- and 210 kDa proteins but did not show any bands corresponding to MHC-I.

Our results suggest that MHC-I and II proteins can be associated together with 90 kDa- and 210-220 kDa proteins in the cell membrane, probably by close but weak, non-covalent interactions. We conclude that these proteins probably can form a multimer complex together.

O 317 MUTATIONAL ANALYSIS OF THE CELLULAR INTERACTION BETWEEN HUMAN CD4 AND DIFFERENT HUMAN CLASS II MHC ISOTYPES AND ALLELES.

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The CD4 molecule is a member of the immunoglobulin gene superfamily. The CD4 molecule is a non-polymorphic membrane glycoprotein of Mr 55,000 which is expressed on a subpopulation of mature T cell. It is composed of four extracellular domains, D1 to D4, a transmembrane region and a cytoplasmic tail. It has been proposed that CD4 function as a co-receptor and/or as an adhesion molecule that binds a monomorphic region of class II MHC molecules.

To better define functional domains of CD4 involved in binding of class II MHC molecules, we have used a cellular system which is dependent on the CD4-class II interaction. CD4 wild-type and CD4 mutants were expressed in the murine $CD4^-$ $CD8^-$ T cell hybridoma, the 3DT52.5.8. After a co-culture between CD4 hybridomas and class II target cells, supernatant were tested for IL-2 production. This study allowed us to identify which regions of the CD4 molecule is involved in the CD4-class II interaction. Residues in the CDR1- and CDR3-like loops of the first domain and residues of the FG loop of the second domain seem to be part of the class II MHC binding site. The CD4 crystal structure shows that class II MHC binding sites are amino acid residues located on the same face of the CD4 molecule and pointing out from CDR1- and CDR3- loops of D1 and FG loop of D2. We are currently characterizing the other residues on this face of the molecule which could potentially interact with class II molecules. These mutations into the CD4 molecule affected the interaction of CD4 with all the isotypes and alleles which we have tested.

O 318 THE α3 DOMAIN OF CLASS I MOLECULES AFFECTS BOTH NEGATIVE AND POSITIVE SELECTION OF ANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTES, Forman, J.*, *, Robert E. Hammer*, Sharon Jones-Youngblood,*, Ulrich Koszinowski°, Lee Hood', Iwona Stroynowski', and Carla J. Aldrich*, *Department of Microbiology; *Department of Biochemistry, UT-Southwestern Medical Center, and Howard Hughes Medical Institute, Dallas, TX 75235-9048; ° Abteilung Virologie der Universitat, D-7900 Ulm, Germany; and ' Department of Biology, California Institute of Technology, Pasadena, CA 91125.

The α1 and α2 domains of major histocompatibility complex class I molecules function in the binding and presentation of foreign peptides to the T-cell antigen receptor and control both negative and positive selection of the T-cell repertoire. Although the α3 domain of class I is not involved in peptide binding, it does interact with the T-cell accessory molecule, CD8. CD8 is important in selection of T cells as anti-CD8 antibody injected into perinatal mice interferes with this process. We previously used a hybrid class I molecule with the α1/α2 domains from L^d and the α3 domain from $Q7^b$ and showed that this molecule binds an L^d restricted peptide but does not interact with CD8-dependent cytotoxic T lymphocytes. Expression of this molecule in transgenic mice fails to negatively select a subpopulation of anti-L^d cytotoxic T lymphocytes. In addition, positive selection of virus-specific L^d-restricted cytotoxic T lymphocytes does not occur. We conclude that besides the α1/α2 domains of class I, the α3 domain plays an important role in both positive and negative selection of antigen-specific cells.

O 319 BINDING OF SOLUBLE T CELL RECEPTOR β-CHAIN TO SUPERANTIGENS, Nicholas R.J. Gascoigne, Michael J. Irwin and Charles Doyle, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

The superantigens are a group of molecules that are recognized in association with MHC class II by the Vβ region of the TCR. Superantigens are defined as such because they cause activation or thymic deletion of essentially all cells bearing specific Vβ-elements. The strong association of particular Vβ-regions with T cell responses to superantigens suggests that their interaction with the TCR is fundamentally different from that of most antigens. We have used a soluble form of the TCR β-chain to investigate the interaction of the β-chain with superantigens produced by staphylocci or endogenous murine retroviruses. We have preliminary data indicating that the binding of soluble β-chain to superantigen is saturable and are in the process of determining affinity and rate constants for the interaction.

O 320 THE LEVEL OF ENDOGENOUS SUPERANTIGEN EXPRESSION DETERMINES THE EXTENT AND KINETICS OF T CELL DELETION. Tatyana V. Golovkina* and Alexander Chervonsky+, and Susan R. Ross*. *Dept. of Biochemistry (m/c 536), University of Illinois College of Medicine, Chicago, IL 60612 and +Dept. of Pathology, University of Chicago, Chicago, IL 60637.

It has recently been shown that some retroviral gene products may participate in shaping the T cell repertoire, acting as superantigens, and that endogenous mouse mammary tumor virus (MTV) proviruses co-segregate genetically with various minor lymphocyte stimulating (Mls) loci. In particular, T cells bearing Vβ14 and Vβ15 receptors are deleted by the product(s) of the the open reading frame (ORF) encoded in the long terminal repeat (LTR) of C3H milk-born MTV. With the ultimate goal of understanding the role of the ORF protein in the MTV life cycle, we created transgenic mice that contained an internally deleted version of the C3H exogenous MTV genome, such that only the ORF protein from this virus would be produced in those same tissues in which endogenous MTV is normally expressed. Eight different strains of transgenic mice in the C3H/HeN inbred strain were generated, containing from approximately 1 to 20 copies of the transgene. All of the mice deleted Vβ14+ T cells, but the percentage of deletion was dependent on the level of expression of the transgene. Mice containing a high transgene copy number and a high level of transgene expression showed 100% deletion of this class of T cells as early as day 1 after birth, while transgenic strains expressing low levels of ORF-specific RNA showed only 50% deletion. In addition, the kinetics of deletion of the low-expressing strains was much slower than of the high-expressing strains. These results show that clonal deletion of T cells interacting with the proteins produced by the Mls loci is dependent on the level of expression of these superantigens. One hypothesis to explain these results is that only Vβ14+ T cells bearing high affinity receptors are deleted in strains expressing low levels of the ORF protein, while in transgenics containing high levels of this antigen, both high and low affinity T cells are deleted.

O 321 ROLE OF EXPRESSION OF T CELL COSTIMULATORS ON PARENCHYMAL TISSUE IN THE DEVELOPMENT OF AUTOIMMUNITY?, Sylvie Guerder, Peter S. Linsley*, Richard A. Flavell, Section of Immunobiology, Howard Hughes Medical Institute, Yale University, School of Medicine, New Haven, CT 06510, *Oncogen Division, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

The insulin promoter has been used succesfully to direct expression of several transgenes on the islets of Langerhans of the pancreas. In light of the "passenger leukocytes hypothesis", since the ß cells do not express costimulatory signal(s) (2nd signal) required for lymphocytes activation, such a transgene should not be presented in an immunogenic form and therefore the mice should be tolerant *in vivo*. And indeed, in all cases, the mice were tolerant *in vivo* to the transgene. If this interpretation is correct, one would predict that ß cells expressing a costimulatory signal, should present the transgene in an immunogenic form leading to an autoimmune response towards the islets cells. To test this hypothesis we have generated mice transgenic for the B7/BB1 antigen, a recently cloned costimulatory molecule, driven by the rat insulin promoter. Such a construct should allow tissus specific expression of the B7 costimulatory molecule by the ß cells. The effect of the expression of this transgene, with or without co-expression of MHC class II on the ß cells, on tolerance to pancreatic antigens will be discussed. If *in vivo* tolerance is only due to lack of signal 2 which can be provided by B7, the ß cells now expressing the B7 antigen should present their class II molecule in an immunogenic form and lymphocytic infiltration as well as *in vitro* responsiveness should occur.

O 322 IDENTIFICATION OF THE STAPHYLOCOCCAL ENTEROTOXIN A (SEA) SUPERANTIGEN BINDING SITE IN THE ß1 DOMAIN OF HLA-DR. **Andrew Herman[1], Nathalie Labrecque[2], Jacques Thibodeau[2], Rafick-P. Sekaly[2], Philippa Marrack[3], and John Kappler[3]. [1]Virginia Mason Research Center, Seattle, WA 98101 [2]IRCM, Quebec, Canada [3]Howard Hughes Medical Institute, Division of Basic Immunology, Dept. of Medicine, NJCIRM, Denver, CO 80206.**

SEA is a superantigen that must bind to MHC class II molecules in order to be recognized by T cells. In humans, HLA-DR class II allelic and isotypic forms differ in their ability to bind SEA. The HLA-DR1 molecule binds very well, while HLA-DRw53 binds SEA extremely poorly. We have constructed mutants of DR1 and DRw53, in which amino acid residues that differ between these two molecules were substituted in a reciprocal manner, in order to identify the residues that are crucial for SEA binding. Binding analyses with these transfectants have shown that a single residue (aa 81) in the ß1 domain dictates SEA binding. A histidine at this position allows SEA binding, while a tyrosine does not. This residue is predicted to lie on an α-helix on the surface of the MHC molecule, with its side chain pointing up and away from the peptide binding pocket. This finding suppports the idea that superantigens and conventional antigens bind to different sites on the MHC class II molecule. We are currently examining whether other residues in this region of class II play a role in presentation of bacterial and viral superantigens.

O 323 MOLECULAR CHARACTERIZATION OF MLS-1, Brigitte T. Huber, Ulrich Beutner, Christine Rudy and Meena Subramanyam, Department of Pathology, Tufts University School of Medicine, Boston, MA 02111

The murine Mls-1 antigen is the prototype of endogenous superantigens, activities which lead to deletion of T cells expressing certain TCR Vb genes from the mature repertoire. However, Mls-1 also stimulates T cells expressing these particular Vb genes (Vb6, 7, 8.1 and 9) in vitro, making it one of the strongest known T cell activators. We have recently reported that the *Mls-1* gene is closely linked to the endogenous mammary tumor virus (MMTV) *Mtv-7*, and that Mls-1 is encoded by the open reading frame in the U3 region of the LTR of *Mtv-7*. The sequence of the *Mtv-7* open reading frame differs from all other known MMTV open reading frame sequences in the 3' end, suggesting that the TCR Vb specificity is conferred by the C-terminus of the molecule. The predicted structure of the open reading frame encoded protein is consistent with a type II transmembrane molecule where the C-terminus ins extracellular. - Furthermore, we have defined that the Mls-1-like activity in the MA/MyJ mouse is encoded by a new *Mtv* provirus, *Mtv-43*, that is not seen in any other inbred strains. We have sequenced the open reading frame of *Mtv-43* and found that its predicted amino acid sequence is closely related to that of the *Mtv-7* open reading frame. This analysis defines the epitope on the Mls-1 molecule that is responsible for the TCR Vb specificity. - Analysis of the tissue distribution of MMTV specific transcripts revealed that CD8+ T cells may express Mls-1, apart from B cells.

O 324 INTERACTION BETWEEN MHC CLASS II AND CD4: MUTATIONAL ANALYSIS OF THE BINDING SITE FOR CD4 ON MHC CLASS II

Rolf Koenig and Ronald N. Germain, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

In the thymus, precursor T cells differentiate into mature cells distinguished by the expression of either CD4 or CD8. Each of these membrane glycoproteins is associated with a bias in T cell interaction with major histocompatibility complex (MHC) antigens: CD8+ T cells for class I MHC and CD4+ cells for class II MHC. Evidence for direct interaction between CD4 and MHC class II has come from cell binding assays. Although mapping of the regions of CD4 controlling interaction with class II has been reported, there is little data on the regions of class II that are the targets of CD4 binding.

We have used site-directed mutagenesis to alter individual amino acid residues of I-A^d and expressed the mutant molecules in mouse L-cells. To analyze the effect of the mutations on the interaction of I-A^d with CD4, T-cell hybridomas with I-A^d-restricted T-cell receptors of various specificities and expressing different co-receptor molecules (CD4, CD8, or none) were stimulated by antigen presented by L-cells that were transfected with mutant I-A^d. All produce lymphokines upon stimulation, the stimulation of the CD4+ hybridomas, however, occurs at lower antigen concentrations. A mutation affecting only the CD4-binding region of I-A^d should not affect stimulation of the CD4- T-cell hybridomas, but should reduce the stimulation observed with the CD4+ populations. Using this assay, we have identified several mutations in the α2 and the β2-domains of I-Ad that have the predicted phenotype for a site affecting CD4 binding. The results of this mutational analysis have been incorporated into a computer-generated model of I-A^d using the molecular modelling programs QUANTA and CHARMM.

O 325 THE MOUSE MAMMARY TUMOR VIRUS LONG TERMINAL REPEAT ENCODES A TYPE II TRANSMEMBRANE GLYCOPROTEIN. Alan J. Korman, Pierre Bourgarel, Tommaso Meo & Gabrielle E. Rieckhof INSERM U.276, Institut Pasteur, 28 Rue du Docteur Roux, 75724, Cedex 15, Paris, France

Superantigens are products of bacterial or viral origin which stimulate large numbers of T cells. This stimulation is regarded as the result of the interaction of particular Vβ chains of the T cell receptor with a complex of class II major histocompatibility complex (MHC) molecules and superantigen on the stimulating cell. Bacterial superantigens act by binding directly to class II MHC molecules. It has recently been shown that minor lymphocyte stimulatory (Mls) antigens, originally discovered as strong lymphocyte stimulatory determinants *in vitro* and subsequently shown to delete T cells expressing specific Vβ chains during development, are associated with endogenous mouse mammary tumour viruses (MTV). In particular, an unidentified product encoded by an open reading frame (orf) present in the 3' long terminal repeat (LTR) of exogenous MTV, has been shown by transfection experiments to stimulate T cells. A related orf sequence from GR mice, when present as a transgene, results in the deletion of T cells expressing Vβ14. We have shown using *in vitro* translation in the presence and absence of dog pancreatic microsomes that (1) the orf of MTV encodes a type II transmembrane glycoprotein (N-terminus intracellular, C-terminus extracytoplasmic), and (2) a cotranslationally secreted orf protein is not produced. In addition, we have also isolated and sequenced several endogenous MTV orfs (MTV-1, MTV-6, and MTV-13) which are involved in the deletion of Vβ3-bearing T cells; each of these sequences is nearly identical to each other. These observations lead to a model of action of viral superantigens.

O 326 A PEPTIDE CORRESPONDING TO THE CD8 BINDING REGION OF HLA CLASS I BLOCKS THE GENERATION OF CYTOLYTIC T LYMPHOCYTES (CTL),

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CD8 is a cell surface glycoprotein expressed by T lymphocytes which recognize HLA class I molecules. The CD8 molecule is invariant, and we and others have used naturally occuring and/or genetically engineered MHC molecules to show that CD8 molecules bind to a highly conserved portion of the HLA class I $alpha_3$ domain. We prepared a synthetic peptide corresponding to this putative CD8 binding site, residues 222-235 of HLA class I, and tested it for effects on T lymphocyte function in vitro. This peptide, which contains the majority of amino acids previously shown to interact with the alpha chain of CD8, potently inhibited the differentiation of precursor CTL to mature effector cells in a limiting dilution analysis. There were no measureable effects, however, on cytotoxicity or proliferation by fully differentiated alloreactive T lymphocytes. These studies show that CD8 interacts directly with this region of HLA class I and that an efficient CD8-HLA class I interaction is necessary for the generation of class I restricted CTL. Furthermore, these studies suggest that peptides corresponding to this and other binding regions of cell surface molecules involved in receptor-ligand interactions can be used to modulate the immune response.

O 327 FUNCTIONAL ROLE OF CD8 IN T CELL ACTIVATION,

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CD8 functions as a co-receptor in class I restricted responses by increasing the avidity between T cells and their targets and by associating with the tyrosine kinase $p56^{lck}$, which may participate in signal transduction when brought into proximity with the T cell receptor. It has also been suggested that ligation of CD8 by antibodies can generate a "negative signal" which anergises the cell. We have been investigating this by comparing the responses of CD8 negative hybridomas generated from K^b-specific T cell clones which vary in their susceptibility to blocking with anti-CD8 antibodies. We have found that CD8 "dependence" as defined by the susceptibility of a particular clone to blocking with anti-CD8 antibodies does not always correlate with a requirement for CD8 expression in the hybridomas in order to generate a response to antigen i.e. CD8 "dependent" clones can give rise to CD8 "independent" hybridomas. Furthermore CD8-independent hybridomas, when expressing transfected CD8α molecules, may remain sensitive to blocking by anti-CD8 antibodies. These data suggest that CD8 dependence is not necessarily a function of T cell receptor affinity for antigen/MHC but may reflect a requirement for an interaction between the T cell receptor and the CD8 molecule on the surface of the responding cell.

O 328 MOLECULAR ANALYSIS OF THE INTERACTION BETWEEN *S. AUREUS* SUPERANTIGENS AND THE HUMAN MHC CLASS II MOLECULES.
Nathalie Labrecque, Jacques Thibodeau, Helen McGrath and Rafick-P. Sékaly, Laboratory of Immunology, Clinical Research Institute of Montreal, 110 West Pine Avenue, Montreal, Quebec, Canada, H2W 1R7.

Staphylococcal enterotoxins (SEs) bind to major histocompatibility complex (MHC) class II molecules. This complex stimulates T cells in a Vβ specific manner. We have previously shown that alleles and isotypes of MHC class II differ in their ability to bind and present SEs to T cells. Moreover, experimental evidence show the presence of non overlapping binding sites for different SEs. Indeed, SEA is able to compete for the binding of SEB and TSST-1 on MHC class II molecules but SEB and TSST-1 are not able to cross-compete. In order to discriminate the binding site on MHC class II molecules of SEA, SEB and TSST-1, site-directed mutagenesis of MHC class II molecules was performed. Several reciprocal single or multiple substitutions in residues of the α helix and outside of the Ag binding groove were carried out in both the α and β chains of class II.
A murine class II negative fibroblastic cell line DAP-3 was transfected with wild-type and mutants class II molecule. These transfectants were analyzed for their ability to bind SEA, SEB and TSST-1 using a fluorescent binding assay. We have already shown that the residue 81 of the β chain of DR1 is critical for the binding of SEA. The different mutants are presently tested for their ability to present SEs to T cell hybridomas expressing different T cell receptors. This will enable us to discriminate the residues in the class II molecule involved in the interaction with the TcR from those involved in binding of SEs.

O 329 MHC CLASS II MOLECULES AND INVARIANT CHAIN: USE OF Ii MUTANTS TO STUDY CLASS II/Ii TRAFFICKING,
Corine Layet[1], Oddmund Bakke[2], Paola Romagnoli[1], and Ronald N. Germain[1], [1]Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892 and [2]Univ. of Oslo, Oslo, Norway.

To examine the consequences of class II MHC molecule and invariant chain (Ii) interaction on intracellular folding and transport, we have co-transfected into COS cells constructs encoding various combinations of class II MHC α and β chains and wild-type or mutant murine or human Ii. Overall cell surface expression was assessed in relationship to total chain synthesis, acquisition of endoH resistance, and degradation during chase. The intracellular compartmentalization of the class II and Ii proteins was evaluated by immunofluorescence microscopy.

We previously reported (PNAS 88:2346,1991) that murine Ii p31 and p41 can increase surface expression of haplotype-mismatched murine αβ pairs. Using a series of truncated human p31 constructs with varying cytoplasmic tails, we found that the capacity of human Ii to improve surface expression of such pairs increased as the length of the cytoplasmic tail was decreased, with a 23 residue deletion giving the best result. This was correlated with the ability of the Ii itself to acquire endoH resistance and be expressed on the cell surface. An Ii chain missing the N-terminal 30 residues, much of which is secreted, failed to augment class II expression. Although Ii lacking 20 or 23 residues showed detectable cell surface expression alone, class II markedly enhanced this Ii surface expression. Intracellular staining showed that class II alone was found predominantly in the ER and Golgi, as well as on the surface. Wild-type Ii alone was localized in peri-nuclear vesicles, whereas the Δ23 deletion had a Golgi and surface distribution or was found in peripheral small vesicles. Class II followed the distribution of co-expressed Ii in both cases. Thus, (a) signal(s) in the cytoplasmic tail of Ii appears to dictate the site of steady state accumulation of class II-Ii complexes, whereas Ii and class II have mutual effects on transport to or residence on the cell surface. Experiments are in progress to dissect the role of intracellular sorting signals and the effects of aggregation on biochemical maturation and cellular localization of class II and Ii, and to relate these factors to peptide binding.

O 330 CLASS II MHC IS DISTRIBUTED WIDELY IN THE ENDOCYTIC PATHWAY
Timothy P Levine and Benjamin M Chain, Medawar Building, Dept. of Biology, University College London, WC1E 6BT, UK

Exogenous antigen is presented after intracellular processing to produce short peptides which bind to class II MHC. Peptides isolated from class II include epitopes from invariant chain and class II itself, as well as major serum proteins. Thus processing involves antigen, endoprotease and newly synthesized class II interacting within the same endocytic compartment. It has been suggested that class II is concentrated in an organelle related to the lysosome, but other evidence points to a more uniform distribution of class II in the endocytic pathway. We have used immuno-isolation of intact class II positive intracellular vesicles to determine their distribution across the exocytic and endocytic pathways.

A20 cells, a murine B lymphoma cell line, were labelled with either ^{125}I-transferrin (I-Tf) to fully saturate the early endosome, or ^{125}I-$\alpha_2$macroglobulin (I-α_2m) to label the late endosome and lysosome. Surface bound I-Tf was removed by acid stripping. The cells were homogenized, and post nuclear supernatant was incubated wiht affinity purified rabbit antiserum to the cytoplasmic tail of murine class II α chain (anti-α_{cyt}). Unbound anti-α_{cyt} was removed by gel filtration. Serial dilutions of antibody labelled vesicles were then rotated with magnetic beads coated with sheep anti-rabbit Ig (Dynal). Upto 65% of I-Tf was immunoisolated, compared to upto 35% of I-α_2m. Therefore, class II MHC had a wide distribution throughout the endocytic pathway, particularly in the early endosome as defined by the presence of transferrin. This finding was confirmed by density gradient centrifugation, which showed that most class II MHC positive vesicles have the same low density as plasma membrane and early endosome; a small sub-population has the higher density of late endosomes.

O 331 DIFFERENTIAL PROCESSING OF VIRAL AND ALLOGENEIC PEPTIDES,
Roger Lippé, Gerry Kolaitis and Wilfred A. Jefferies, Biotechnology Laboratory, Depts of Microbiology, Medical Genetics and Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Recognition by cytolytic T cells (CTL) of foreign antigens is dependent on the production of antigenic peptides and their presentation at the cell surface in association with major histocompatibility complex (MHC) molecules. Although these requirements apply to allogeneic and viral antigens, it is unclear whether both immunogens are processed by a unique pathway or by specialized biochemical routes. We have investigated this issue by analyzing the allogeneic and viral specific CTL responses using a mutant cell line. This cell line is deficient in the production of HSV virions due to a block in the transport of the assembled particles to the surface of the infected cells. Our results indicate that the allogeneic CTL response against this mutant is comparable to wild type despite a slightly slower processing of MHC molecules in this cell line compared to its parental cell line. However, when infected with HSV, this cell line was significantly less sensitive to killing by anti-HSV CTL than the parental infected cell line. Preliminary results indicate that this is specific for HSV virus. These data suggest the existence of a pathway for the production of HSV peptides at least partially distinct from the pathway used to generate allogeneic peptides and other viral peptides.

O 332 THE HEAT-STABLE ANTIGEN IS A COSTIMULATORY MOLECULE FOR CD4 T CELL GROWTH

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The induction of clonal expansion of T cells requires two biologically distinct signals. Signal 1 is generated by interaction of the T cell receptor (TCR) with a MHC-peptide complex, while signal 2 is generated by interaction of the costimulatory molecules on antigen-presenting cells with the receptors for such costimulators on the T cells. Recent studies have demonstrated that the consequences of engaging T cell receptor with antigens are determined by delivery of the signal 2. In order to identify the B cell surface components which may mediate the costimulation of T cells, we have generated a hamster monoclonal antibody which can block the costimulatory activity of splenic accessory cells. Treatment with this antibody together with a T cell receptor ligand abolishes the proliferation of CD4 T cells. This treatment also induces non-responsiveness of the T cells to subsequent stimulation. Molecular cloning and DNA sequencing indicate that this antibody recognizes an antigen which is identical to the heat-stable antigen (J11d). The costimulatory activity of this protein has been confirmed by gene transfer experiments. Thus, the heat-stable antigen is a costimulator for T cell growth.

O 333 THE DIFFERENTIAL EFFECTS OF CHLOROQUINE ON CLASS II TRANSPORT AND EXPRESSION IN INVARIANT CHAIN POSITIVE AND NEGATIVE CELLS, George E. Loss, Jr. and Andrea J. Sant. Dept. of Pathology, University of Chicago, 5841 S. Maryland, Chicago, IL, 60637. Major Histocompatibility Complex (MHC) class II molecules are type I cell surface glycoproteins which associate intracellularly with a third polypeptide termed invariant chain (Ii). Ii noncovalently binds class II in the Endoplasmic Reticulum (E.R.) and the class II/Ii complex is transported through the E.R. and Golgi undergoing glycosylation. After sialylation in the Golgi, the class II/Ii complex enters a post-Golgi acidic compartment where Ii is proteolytically cleaved from class II, a process inhibitable by chloroquine (CQ). Class II is then transported to the cell surface and expressed free of Ii. In this report we examine the effects of CQ (a lysosomotropic amine) on the biogenesis and intracellular trafficking of class II molecules in cells transfected with Ii and MHC class II or transfected with class II alone. Dissociation of Ii/MHC complexes was inhibited by CQ. Prolonged treatment of Ii positive cells with CQ resulted in decreased cell surface class II expression and accumulation of intracellular class II molecules. These effects were rapidly reversible with removal of CQ from culture medium. In contrast, CQ had no effect on intracellular or cell surface class II expression in Ii negative cells. Likewise, CQ did not affect MHC class I expression in Ii positive or negative transfectants. Similar effects were observed treating cells with primaquine, another lysosomotropic amine. These data indicate that CQ treatment results in cell surface depletion and intracellular retention of class II molecules in an Ii-dependent manner and suggest that Ii serves as an intracellular retention protein for MHC class II.

O 334 NON-TCR Vß ELEMENTS DETERMINING RESPONSIVENESS TO STAPHYLOCOCCAL ENTEROTOXIN B IN TCR Vß6 T CELLS ARE NOT IN THE ß CHAIN CDR3 REGION. Stuart Macphail, Department of Surgery, North Shore University Hospital and Cornell Medical College, Manhasset, NY 11030.
We have generated a panel of 14 T cell receptor (TCR) Vß6 expressing T hybrids by fusing Mls1^a activated T cell blasts with the TCR a/ß non-expressing variant of the T lymphoma, BW5147. Although all the T hybrids expressed the same Vß element, they displayed qualitative heterogeneity in their responsiveness to the bacterial superantigen, Staphylococcal enterotoxin B (SEB). Three (approx. 20%) out of the 14 T hybrids were strongly responsive to SEB while the rest showed no responsiveness, over a wide concentration range (0.2 - 10.0 ug/ml). In the Vß6 T hybrids, SEB responsiveness did not correlate with the strength of the response of the T hybrids to Mls1^a and although there was variability in the level of expression of TCR and the CD4 accessory molecule, neither of these parameters correlated with responsiveness to SEB. Thus it seemed that some recognitive element of the TCR in addition to Vß determines responsiveness to SEB. We have sequenced the CDR3 regions of the ß chain cDNA from the 14 TCR Vß6 T hybrids. The 3 SEB responsive T hybrids expressed Jß1.3, Jß1.4, and Jß2.3. Three of the SEB non-responsive T hybrids expressed Jß1.1, 3 Jß1.4, 2 Jß1.6, 1 Jß2.2, 1 Jß2.4 and 1 Jß2.5. Thus, since Jß1.4 was expressed by 1 SEB responsive and 3 non-responsive Vß6 T hybrids, it seems that Jß does not alone bestow SEB responsiveness on TCR Vß6 T cells. Furthermore, neither the length nor amino acid content of the D/Nß region showed correlation with SEB responsiveness. Thus we believe that a TCR α chain element, possibly in combination with certain Jß or D/Vß elements, determines this responsiveness. We are currently defining the α chain elements in our panel of TCR Vß6 T hybrids.

O 335 MOLECULAR ANALYSIS OF T CELL FUNCTIONAL UNRESPONSIVENESS,
Kiyoshi Migita and Atsuo Ochi, Division of Neurobiology and Molecular Immunology, Samuel Lunenfeld Research Institute, and The Dept. Immunology and Medical Genetics University of Toronto, Mount Sinai Hospital 600 University Ave. Toronto, Ontario Canada M5G 1X5
Two major mechanisms, deletion and anergy, have been defined in thymic and peripheral T cell tolerance. However anergy plays a major role in the periphery and that contrasts to deletion dominant in the thymus. Previous work from this laboratory, demonstrated both clonal anergy and deletion of peripheral T cells bearing reactive Vβ8 T cell receptor (TCR) with *in vitro* administration of one of the bacterial superantigens, *Staphylococcus* enterotoxin B (SEB), which engages Vβ7 and 8-bearing T cells. T cell tolerance was evident when spleen cells of SEB-primed mice were examined for the *in vitro* proliferative response and IL-2 production to SEB after a week. In order to study the anergy of SEB-primed T cells in more detail, purified anergic Vβ8, CD4 T cells have been examined for the response to TCR and IL-2R mediated stimulation. These purified T cells were unresponsive to TCR and IL-2R stimulation but responsive to co-stimulation with PMA and Ca^{++} ionophore. We have also found that Vβ8 T cell anergy is reversible in the thymectomized mice. These results indicate that the *in vivo* tolerized T cells have multiple defects in both TCR and IL-2R mediated activation pathways. It is also shown that these defects are reversible *in vivo*. The molecular mechanism of T cell anergy will be discussed.

O 336 CD4+ CLASS I-RESTRICTED MURINE T CELLS SPECIFIC FOR HIV gp160 315-329, Richard L. Moore and Barbara S. Fox, Department of Microbiology and Immunology and the Division of Rheumatology and Clinical Immunology, University of Maryland School of Medicine, Baltimore, MD 21201

CD4+ T cells generally recognize peptide antigens in association with class II MHC molecules. CD8+ T cells generally recognize peptide antigens in association with class I MHC molecules. We generated a panel of 13 T cell hybridomas by fusion of BW5147 with spleens from mice primed with HIV gp160 315-329 in complete Freund's adjuvant. Twelve of these hybridomas were class I-restricted and one was restricted to I-A^d. Surprisingly, two of the class I-restricted hybridomas had a mixed CD4+/CD4- phenotype. This mixed pattern of expression of CD4 was stable in multiple clones of these hybridomas. Therefore, the class I-restricted hybridomas could have resulted from fusion of BW5147 with CD4+ class I-restricted T cells. In support of this, class I-restricted CD4+ normal T cell clones specific for 315-329 were generated from the same splenocyte population used to generate the hybridomas. These two normal T cell clones, L3.1 and L3.7, expressed high levels of CD4 and are CD8-. Their activation was inhibited by the anti-class I antibody 34-1-2 and not by the anti-class II antibody MK-D6. This is the first report of CD4+ T cells that recognize a peptide antigen in association with class I. We postulate that these T cells were educated in the thymus to recognize antigen in association with class II MHC molecules; the association of 315-329 with class I may resemble the association of some unknown antigen with class II MHC.

O 337 AUTOREACTIVE T CELLS RESULT FROM INCREASED CO-STIMULATORY SIGNALING IN TCR TRANSGENIC *LPR/LPR* MICE, John D. Mountz, Tong Zhou, Hörst Blüthmann and Carl K. Edwards, III, Department of Medicine, UAB, Birmingham, AL 35294, Hoffmann-LaRoche, Switzerland and Marion Merrell Dow, Kansas City, MO 64134

We have analyzed defects in clonal deletion and tolerance induction of autoreactive T cells *in vivo* using two different TCR transgenic *lpr/lpr* mice. D^b/HY reactive TCR$\alpha\beta$ transgenes were analyzed in D^b male C57BL/6-*lpr/lpr* mice. There was an increased specific cytotoxicity against the D^b+ HY bearing targets and increased specific proliferative response to irradiated D^b male stimulator cells by purified T cells from Tg C57BL/6-*lpr/lpr* males compared to Tg C57BL/6 +/+ males. Expression of TCR and CD8 was not up-regulated in *lpr/lpr* male mice suggesting that the anergy defect resulted from an augmentation of an accessory signalling pathway. A class II/SEB reactive Vβ8.2 TCR transgene was analyzed in MRL-*lpr/lpr* mice. Vβ8 TCR transgenic MRL-*lpr/lpr* and control TCR transgenic MRL-+/+ mice were injected with SEB (10 ug IP) from birth to two weeks of age. Injection with SEB(100 ug/IP) at six weeks of age resulted in a 20% weight loss and a 50% mortality in *lpr/lpr* mice but no weight loss or mortality in +/+ mice. Neonatal administration of SEB induced clonal deletion and anergy of V$\beta8^+$ T cells in both *lpr/lpr* and +/+ mice. There was a rapid increase of V$\beta8^+$ T cells and responsiveness to SEB within 1 week after stopping SEB in *lpr/lpr* whereas +/+ mice remained anergic to SEB up to 10 weeks. Signaling in *lpr/lpr* T cells was induced by SEB associated with the B7+ AG8 cell line, but not SEB associated with B7- cell lines, suggesting a defect in the CD28/CTLA-4 co-receptor signaling pathway. Increased signaling through the co-receptor pathway of *lpr/lpr* mice was associated with unique tyrosine phosphorylated protein products at 50 kd and 100 kd which were distinct from phosphotyrosine products after CD3 co-crosslinking. Anergy loss in *lpr/lpr* mice was not inhibited by Cs-A. These data provide the first evidence for a specific signaling defect leading to autoreactive T cells in lupus-prone mice.

O 338 INVARIANT CHAIN ALTERS THE MALIGNANT PHENOTYPE OF MHC CLASS II+ TUMOR CELLS, Suzanne Ostrand-Rosenberg and Virginia K. Clements, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228

T lymphocytes usually recognize endogenously encoded antigen in the context of MHC class I molecules, while exogenous antigen is presented by MHC class II molecules. *In vitro* studies in model systems suggest that presentation of endogenous antigen by class II molecules is inhibited by the association of class II with it's invariant chain. In the present study we test this hypothesis in an *in vivo* system in which endogenously encoded tumor peptides are presented by tumor cell MHC class II molecules. In this system, transfection of syngeneic MHC class II genes ($A\alpha^k$ and $A\beta^k$) into a highly malignant, invariant chain negative, mouse tumor (SaI sarcoma) produces an immunogenic tumor (SaI/A^k) which is rejected by the autologous host, and which effectively immunizes the host against a challenge of wild type class II- tumor. We have suggested that the SaI/A^k transfectants induce protective immunity because they function as antigen presenting cells (APC) for endogenously synthesized tumor peptides, and thereby stimulate tumor-specific T helper cells and by-passing the need for professional APC. In order to test the role of invariant chain as an inhibitor of presentation of endogenous peptides, SaI/A^k tumor cells were super-transfected with invariant chain gene (SaI/A^k/Ii cells). If invariant chain interferes with the binding of endogenously synthesized peptide to MHC class II molecules, then SaI/A^k/Ii cells should be unable to present tumor peptides and should not stimulate tumor-specific T_h lymphocytes. In 6/7 SaI/A^k/Ii lines, invariant chain expression restores the malignant phenotype to the tumor, suggesting that it blocks the class II presentation of tumor peptide. Invariant chain therefore regulates antigen presentation in these cells, and can be a critical parameter for *in vivo* tumor immunity. (Supported by NIH CA52527 and Elsa Pardee Foundation grants.)

O 339 DETERMINATION OF INTRACELLULAR LOCATION BY THE CYTOPLASMIC TAIL OF THE MHC CLASS II ASSOCIATED INVARIANT CHAIN

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Oligomeric complexes of MHC class II molecules and invariant chain (Ii) assemble in the endoplasmic reticulum and are then transported via the Golgi complex to endocytic compartments. During this intracellular transport, the invariant chain is proteolytically processed in distinct steps and dissociates from class II molecules. MHC class II molecules are then transported to the cell surface whereas the invariant chain is degraded. In order to investigate signals that are responsible for endosomal sorting of the oligomeric complex of class II molecules and Ii, we analyzed putative sorting signals in Ii. When Ii was expressed in Ii and class II negative CV-1 cells, it was found to be localized in endosomes, as analyzed by the colocalization of endocytosed marker molecules at the immuno electron microscopy level. At the light microscopy level we show that, using several markers for intracellular compartments, Ii accumulates in structures that costain with antibodies against the (cation-independent) mannose-6-phosphate receptor, but not with antibodies against resident endoplasmic reticulum proteins (SSR, PDI) or lysosomal proteins (LAMP). To search for a putative sorting signal in Ii, deletions were made in the Ii cytoplasmic tail. The intracellular transport and location of these Ii mutants was analyzed. Endosomal location required either amino acids 1-15 or amino acids 12-30.

In order to investigate whether the Ii cytoplasmic tail is sufficient to redistribute a plasma membrane protein to endosomes, we fused the Ii cytoplasmic tail to the transmembrane and lumenal domain of the plasma membrane protein influenza neuraminidase.This hybrid molecule was found in endosomal structures as judged by immunofluorescence analysis at the light microscopy level. In contrast to the wild type invariant chain, this fusion protein was not retained and degraded in the endoplasmic reticulum but is rapidly transported to the Golgi complex. This suggests that the transmembrane and/or lumenal domain of the invariant chain play a role in retention and degradation of Ii in the endoplasmic reticulum.

O 340 **DISTINCT CONFORMATIONAL ISOMERS OF RAT CLASS II MOLECULES ARISE IN α,β HETERODIMER TRANSFECTANTS IN THE ABSENCE OF THE INVARIANT γ chain,** Konrad Reske, Karin Demleitner, Andreas Fisch, Wolfgang Henkes, Susanne Querner and Jessica Syha-Jedelhauser, Institute for Immunology, Johannes Gutenberg University of Mainz, W-6500 Mainz, FRG

Earlier work from our laboratory suggested that rat class II molecules acquire distinct conformational states along their biosynthetic maturation path to the cell surface (Eur. J. Immunol. 1985. 15:1229). This was evidenced by discrete reactivity patterns of mAbs OX6/OX3 towards RT1.B^{l} specific (I-A equivalent) class II constituents. Additional mAb pairs OX6/20.8 and OX17/14-4-4S permitted to extend these observations to RT1.B^{a} allelic products and to the RT1.D^{a} isotype. To more thoroughly test the concept of class II conformational rearrangement and to explore the possibility that γ might contribute to this process full length cDNA clones of the corresponding class II subunits (RT1.Bα^{l},β^{l}; RT1.Dα^{l},β^{l} and RT1.Bα^{a},β^{a}) including the rat invariant γ chain were established and were transfected into class II nonexpressing recipient cells in discrete combinations. In addition a panel of mAbs against the C-terminal portion (aa142-216) of rat invariant γ chain was derived by subcloning procedures combined with somatic cell hybridization. The findings indicate that (i) class II conformational isomers detected by the mAb pairs occur in the absence of the γ chain and (ii) stable conformational forms originate in cells that are not professional class II expressing antigen-presenting cells.

O 341 THE ANTIGEN PROCESSING MUTANT T2 SUGGESTS A ROLE FOR MHC-LINKED GENES IN CLASS II ANTIGEN PRESENTATION, Janice M. Riberdy and Peter Cresswell, Section of Immunobiology, Howard Hughes Medical Institute and Yale University School of Medicine, New Haven, CT 06510

.174xCEM.T2 (T2) is a human cell hybrid that has a large homozygous deletion within the MHC, including all of the functional class II genes. We have generated stable HLA-DR3 and H-2 I-A^{k} transfectants of T2 that express parental levels of class II molecules at the cell surface. T2.A^{k} transfectants fail to stimulate a hen egg lysozyme (HEL) specific, I-A^{k} restricted T cell when incubated with intact HEL. However, stimulation occurs if the appropriate HEL peptide is provided. The T2 cell line therefore has a defect in class II-restricted antigen processing. Biosynthetic studies demonstrate that the kinetics of I-A^{k} transport in T2.A^{k} are similar to the parental rates of transport, although the percentage of I-A^{k} molecules transported appears somewhat lower. I-A^{k} glycoproteins in T2. A^{k} associate normally with the invariant chain, which appears to be proteolytically cleaved after transport through the Golgi apparatus in a similar fashion to that in the parental cell line, .174xCEM.T1 (T1). The DR αβ heterodimers in T2 differ from the parental phenotype in two ways. First, HLA-DR3 expressed in T2 does not have the epitope recognized by the DR3-speicific monoclonal mAb 16.23, while DR3 expressed in the parent does have the epitope. Secondly, the αβ subunits in the parent remain associated when exposed to SDS at room temperature, while those in T2 dissociate.

O 342 THE CYTOPLASMIC TAIL OF Ii TARGETS CLASS II αβIi COMPLEXES TO ENDOSOMES, P.A. Roche, C.L. Teletski, D.R. Karp, B. Dobberstein, O. Bakke, and E.O. Long, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852, and European Molecular Biology Laboratories, Heidelberg, Germany, and University of Oslo, Norway

The influence of the cytoplasmic tail of the Invariant chain (Ii) on the intracellular transport of HLA-DR was investigated in transfected human fibroblasts. Cells transfected with HLA-DR α- and β-chains were re-transfected with either full-length Ii or a construct in which 15 amino acids of the cytoplasmic tail of Ii had been deleted. Pulse-chase studies demonstrated that the absence of the cytoplasmic tail of Ii did not significantly affect the rate of transport of HLA-DR through the Golgi apparatus, but did increase the rate of arrival of these molecules at the cell surface. In addition, cells producing the truncated form of Ii failed to liberate Ii from the class II molecules intracellularly, resulting in the generation of αβIi complexes stably expressed on the cell surface. These studies demonstrate that the cytoplasmic tail of Ii targets class II molecules to endosomes and that this targeting is necessary for the liberation of Ii from the class II αβIi complex.

O 343 EXPORT PATHWAY OF MHC CLASS II MOLECULES, Paola Romagnoli[1], Corine Layet[1], Jonathan W. Yewdell[2], Jack R. Bennink[2] and Ronald N. Germain[1], [1]Laboratory of Immunology and [2]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892

To investigate the export pathway of MHC class II molecules and the role that invariant chain plays in it, we used a transient transfection system. Cos cells were transfected with IA^{b} or a mismatched pair a^{d}b^{b} in the presence or in the absence of hIi31 (human invariant chain p31-form) or hIiD23 (a truncated form of invariant chain lacking 23 a.a. of the cytoplasmic tail). 40 h after transfection the cells were fixed, permeabilized and mAbs M5/114 and LN2 were used to detect MHC class II molecules and invariant chain respectively . In the absence of invariant chain, MHC class II molecules are found in the Golgi compartment, as previously shown. The same pattern is observed in the presence of hIiD23, while in cotransfection with hIi31 MHC class II molecules are found in a vesicular compartment. Of interest, in transfectants with the mismatched pair a^{d}b^{b} and hIi31, MHC class II molecules are present in the Golgi compartment and to a lesser extent in vesicles, where they colocalize with hIi31. Thus, hIi31 determines the localization inside the cells of correctly folded MHC class II molecules.
The vesicles where hIi31 and MHC class II molecules are found were further characterized: they are connected to the endocytic pathway, they are not reached by a short pulse of transferrin (5'), they do not contain M6PR and they are not terminal lysosomes. Further studies are in progress in order to better define this compartment.

O 344 T CELL ANTIGEN RECEPTOR MEDIATED PASSIVE ACQUISITION OF MAJOR HISTOCOMPATIBILITY ANTIGENS ON MOUSE T CELLS. Mitsuyoshi K. Saizawa and Satoru Suzuki. Max Planck Institute for Immunobiology, Freiburg im Breisgau, D-7800, Germany.
When cloned T-cells or heterogeneous T-cells and antigen presenting cells(APC) are mixed, products of major histocompatibility complex(MHC) on APC are transferred to the T-cell surface. CD4 positive T-cells acquire MHC class II molecules while CD8 positive T-cells acquire predominantly MHC class I molecules. These MHC molecules are immuno-precipitatable from the T cell surface and have same apparent molecular weight as those on APC. Release of MHC molecules from APC is not spontaneous but dependent on their contact with T cells. Although down-modulation of MHC determinants on APC after contact with T cells is not restricted to the relevant MHC products, the magnitude of acquisition of MHC determinants on T cell surface is closely related to TCR's specificity. Moreover, the acquisition of MHC products by T cells is inhibited by the addition of mixture of anti-TCR mAbs suggesting collectively that the transaction is mediated by the TCR.
The magnitude of acquisition is about 100 fold greater when T cells and APC are incubated together than when T cells are incubated with supernatant from mixed cultures, suggesting that most of the transfer is achieved by direct receptor-ligand interactions at the cell surface. The contributions of specific antigen and of accessory/co-receptor molecules (CD4 and CD8) to the specific acquisition, however, are marginal, thus documenting the TCR's strong affinity towards the self-restriction element.

O 345 PRESENTATION OF ENDOGENOUSLY SYNTHESIZED ANTIGENS BY THE MOUSE MHC CLASS II MOLECULES.. Andrea J. Sant and George E. Loss, Jr. Department of Pathology, Committee on Immunology, University of Chicago, Chicago, IL 60637

MHC Class II molecules have the unique capacity to bind and present peptides derived from exogenous, soluble antigens for recogntition by T lymphocytes. Their role in presenting internally synthesized antigens is less clear. In order to examine the requirements for, and the regulation of endogenous antigen presentation by MHC Class II molecules, we have established a model system in which peptides derived from a normal endogenous cellular protein (MHC Class I) are presented in the context of murine MHC Class II molecules. Our studies have indicated Class II can associate with peptides derived from internally synthesized antigens by intracellular mechanisms that are distinct from currently described presentation pathways. Presentation of our model antigen does not appear to occur by a conventional Class II endocytic mechanism in that: 1. exogenous antigens do not compete for presentation of the internally synthesized antigen 2. modified forms of the target antigen that are secreted are presented by the MHC Class II molecule, by a mechanism that does not involve re-uptake by the APC, 3. cell lines that are able to process exongenous antigens via Class II do not present our model antigen and 4. invariant chain does not facilitate presentation. A typical Class I endoplasmic reticulum loading pathway does not appear to be involved in presentation of the target antigen by Class II in that cytosolic forms of the antigen are inactive, and invariant chain expression does not block presentation. Together, our results suggest that Class II has access to some, but not all types of internally synthesized antigens, that entry into the vesicular compartments may be requisite for Class II restricted presentation, and that Class II may bind to antigenic peptides late in biosynthesis in a non-endocytic exocytic compartment.

O 346 STRESS PROTEINS INHIBIT HLA-DR EGRESS FROM THE ENDOPLASMIC RETICULUM IN INVARIANT CHAIN NEGATIVE CELLS, W. Timothy Schaiff, Keith A. Hruska, Jr., David W. McCourt, Michael Green*, and Benjamin D. Schwartz, Department of Medicine, Division of Rheumatology, Washington University School of Medicine, St. Louis, MO 63110 and *Department of Microbiology, St. Louis University School of Medicine, St. Louis, MO 63104

The human class II major histocompatibility DR molecules (HLA-DR) are heterodimeric molecules which, in cells normally expressing class II molecules, associate with a third molecule, the invariant chain (Ii), soon after synthesis. Although the exact function of Ii is not completely understood, Ii has been described to affect several aspects of class II biology including biosynthesis and intracellular trafficking of class II molecules, peptide binding by class II molecules, and antigen processing and presentation. During the course of studies on the effect of Ii on the biosynthesis of HLA-DR molecules, we observed that at least three high molecular weight proteins co-immunoprecipitated with class II molecules in transfected cell lines expressing HLA-DR in the absence of Ii. These molecules did not co-immunoprecipitate with class II molecules from cells co-expressing Ii or with class I molecules. N-terminal sequence and western blot analyses revealed the identity of two of these proteins as the endoplasmic reticulum resident stress proteins GRP94 and ERp72. Immunofluorescence experiments revealed that in the cells lacking Ii, the majority of the HLA-DR molecules were retained in the endoplasmic reticulum or a pre-Golgi compartment. We hypothesize that in the absence of Ii, HLA-DR molecules are bound by endoplasmic reticulum resident stress proteins and that these stress proteins retain the HLA-DR molecules in the endoplasmic reticulum or a pre-Golgi compartment.

O 347 CREATION OF A MOUSE STRAIN LACKING MOUSE MAMMARY TUMOR VIRUS SUPERANTIGENS, Mark T. Scherer, John W. Kappler and Philippa Marrack, Howard Hughes Medical Institute, 1400 Jackson St., Denver, CO., 80206. All laboratory strains of mice have numerous endogenous superantigens which are responsible for the clonal deletion of T cells bearing particular V_β elements of the T cell receptor. These superantigens are encoded by a gene found in the 3' long terminal repeat of Mouse Mammary Tumor Viruses (Mtv) integrated at various locations in the chromosomes of mice. CBA/CaJ carries three Mtv proviruses, Mtv-8, Mtv-9, and Mtv-14, while C58/J carries proviruses Mtv-3, Mtv-7, Mtv-17, and Mtv-30. Both strains are H-2^k. (CBA/CaJ x C58/J)F_2 mice from crosses of these two strains can have from 0 to 7 of the parental Mtv's. After several generations, a mouse strain free of the Mtv superantigens can be created. We are in the process of creating this strain. This will allow for the analysis of T cell repertoire usage unaffected by superantigen deletion or tolerance. In addition, strains containing only one of the MMTV superantigens can be created, allowing for a finer analysis of the effects of individual superantigens.

O 348 **Lymphocyte function in mice lacking functional CD4 and CD8 genes.** Marco W. Schilham, Julia Potter, Li Zhang, Wai-Ping Fung-Leung, Amin Rahemtulla, Tak Mak.
Department of Medical Biophysics, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ont. M4X 1K9, Canada.

Using the embryonic stem cell technology, mouse strains have been developed lacking either a functional CD4 gene or a functional CD8 gene. By interbreeding the two mouse strains a novel strain lacking both genes has been obtained. The mice seem to be healthy when kept in microisolator cages. Thymus, spleen and lymph nodes are of normal size. FACS analysis of the thymuses of these mice revealed that the majority of mature T cells in the thymus, as defined by high CD3 expression, are missing, but the immature $CD3^{low}$ cells are present in normal numbers. In the peripheral lymph nodes, numbers of $CD3^+,\alpha\beta^+$ T cells are reduced from 80-90% to 5-50%, varying from mouse to mouse. Numbers of $\gamma\delta$ T cells are not drastically increased. The function of the $CD4^-8^-\alpha\beta^+$ T cells is being studied under a variety of in vitro and in vivo conditions. Initial experiments indicate that spleen cells from these mice are capable of giving rise to a small but specific alloresponse in a mixed lymphocyte culture. In these cultures antigen specific cytotoxic T lymphocytes were generated. When stimulated with anti-CD3 or anti-$\alpha\beta$ antibodies, cytotoxic T cells were generated. The cytotoxic activity of these cells was measured on Fc receptor bearing P815 cells in the presence of anti-CD3 antibodies. The mice without CD4 and CD8 were also examined in skin transplantation experiments.

O 349 ALLELES AND ISOTYPES OF CLASS II MHC MOLECULES DIFFER IN THEIR CAPACITY TO INTERACT WITH CD4. Rafick-P. Sékaly, Gilbert Croteau and Sylvain Fleury. Laboratory of Immunology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada. H2W 1R7.

The CD4 molecule, which is expressed on the surface of all T cells which recognize their specific antigen in the context of class II MHC molecules, is thought to interact with a monomorphic determinant on class II MHC molecules. We have recently identified critical residues on CD4 involved in the interaction with class II MHC molecules. These residues are very conserved in evolution leading to the suggestion that human CD4 could interact with murine class II molecules and vice-versa. We have used a murine T cell hybridoma specific for a murine class I molecule and which is dependent on the CD4-class II interaction for its function in order to assess the contribution of allelic and isotypic diversity of class II MHC molecules. Products of the DRβ1, DRβ3, DRβ4 and DRβ5 were included in our study in addition to several alleles of DP, DQ, I-E and I-A. A heterodimeric molecule composed of the DRα chain and the DOβ chain was also analyzed. Our results indicate that human CD4 interacts with the majority of human and murine class II MHC molecules which we have tested. Chimeric molecules composed of a human or murine DRα chain paired with murine of human β chain confirmed the contribution of both chains to the interaction with CD4. Furthermore products of the DRb1 gene were consistently more efficient in interacting with CD4 than the other DRβ chains. Results will be presented as to the contribution of these CD4-class II interactions on the development of the T cell repertoire.

O 350 DIFFERENCES IN RAT T CELL V BETA REPERTOIRES - EVIDENCE FOR SELF SUPERANTIGENS IN RATS ?, Karen S. Sellins[1], Anne-Catherine Lagarde [1],Daniel P. Gold[2], and Donald Bellgrau[1], [1] Barbara Davis Center for Childhood Diabetes, Denver, CO 80262 and [2] La Jolla Institute for Experimental Medicine, La Jolla, CA 92037

The mouse T cell repertoire is influenced by positive and negative selection events that occur primarily in the thymus. It has been shown that T cells bearing particular V beta gene families are either expanded or deleted after interaction with endogenous or "self" superantigens. In rats and humans there is little evidence at present for deletion/expansion of V betas by endogenous superantigens. Using a polymerase chain reaction assay, we have examined the T cell V beta repertoire in several strains of rats. T cells from two MHC-incompatible strains, DA ($RT1^a$) and Lewis ($RT1^l$) have quite different V beta family profiles. Most notably, V beta 13 in DA T cells is expressed at extremely low levels as are V betas 7 and 11 in Lewis T cells. Several possible explanations including genomic deletion, nonfunctional V beta gene products, and superantigen-induced deletion may account for the low V beta expression. In Lewis T cells, V betas 7 and 11 can be stimulated to high levels by stimulation with appropriate staphylococcal enterotoxins. This result suggests that the low expression of these V betas is not due to genomic deletion or to a non-functional gene product. In DA T cells, we have been unable to stimulate V beta13 with an enterotoxin that stimulates this V beta in Lewis T cells. We have demonstrated by Southern analysis that V beta 13 is present in the genome of DA animals. Thus, in both strains of rats it is possible that superantigen-induced deletion may account for the low V beta expression.

O 351 THE SURFACE ANTIGENIC PROFILE OF DENDRITIC CELLS FROM MOUSE THYMUS AND SPLEEN: CD8 EXPRESSION SUGGESTS A VETO FUNCTION, Ken Shortman, Michelle Zorbas and David Vremec, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia

Dendritic cells (DC) were isolated in high purity and high yield by collagenase digestion, selection of light density cells, depletion of non-dendritic cells using a cocktail of lineage-specific mAb and anti-Ig coated magnetic beads, then finally sorting for class II MHC bright cells. The pattern of surface markers differed between thymic and splenic DC. Thymic DC expressed several characteristic T-cell markers, but were distinct from T-lineage cells in lacking CD3-TCR expression and having TCR genes in germline state. A large proportion of both thymic and splenic DC expressed high levels of CD8; this was shown to be authentic CD8 and message for CD8α was expressed by both types of DC. However, thymic DC were $Ly\,2^+3^+$, and so expressed the CD8$\alpha\beta$ heterodimer, whilst splenic DC were $Ly\,2^+3^-$, and presumably expressed only the CD8$\alpha\alpha$ homodimer. It is suggested that the presence of high levels of CD8 by certain DC, as well as high levels of MHC class I and class II, could endow them with the ability to delete or "veto" interacting T cells, in line with the work of Sambhara and Miller.

O 352 BINDING OF CD4 TO SOLUBLE HLA-DR MOLECULES PRODUCED IN INSECT CELLS: IDENTIFICATION OF A CLASS II BINDING SITE ON THE β-2 DOMAIN. Giancarlo Cammarota, Armella Scheirle, Bela Takacs, Dan Doran, Reinhard Knorr, Willy Bannwarth, John Guardiola and Francesco Sinigaglia. Pharma Research Technology, F. Hoffmann-La Roche Ltd., Basel, Switzerland and International Institute of Genetics and Biophysics, Naples, Italy.

Based on functional and cell adhesion studies it has been proposed that CD4 binds a nonpolymorphic region of class II molecules thereby increasing the avidity of the T cell receptor for its ligand.

We have used soluble HLA-DR molecules produced in Sf9 insect cells to measure a specific binding between soluble DR antigens to immobilized recombinant CD4. The specificity of this interaction was demonstrated by the ability of a panel of mAbs against CD4 to block CD4/DR binding. The binding was saturable and scatchard analysis gave a K_D value of $3.2x10^{-6}M$. Subsequent experiments showed that in the same system the the DR-β chain alone, purified from Sf9 cells, was able to specifically interact with CD4. This finding prompted us to analyse the binding ability to CD4 of a series of overlapping peptides derived from the β chain. One out of 14 peptide tested (DRβ sequence 134-148) was able to bind to CD4 and the binding could be specifically inhibited by soluble DR molecules. Our results indicate that CD4 binds to the β_2 domain of class II MHC molecules.

O 353 ROLE OF THE T CELL RECEPTOR ALPHA CHAIN IN THE RECOGNITION OF MLS-1 SUPERANTIGENS. Hedy Smith, Phuong Nguyen, David Woodland, and Marcia Blackman, Department of Immunology, St.Jude Children's Research Hospital, Memphis, TN 38105.

The majority, but not all Vβ8.1 bearing T cells recognize Mls-1 and are deleted in the thymus of mouse strains that express this superantigen. We have generated a large panel of T cell hybridomas from Vβ8.1 T cell receptor transgenic mice, and found that less than 40% of the hybridomas reacted to Mls-1 in vitro. To evaluate the contribution of the α-chain to Mls-1 reactivity, we determined Vα gene usage in a panel of hybridomas that expressed an identical Vβ8.1 receptor chain, by dot blot analysis, using 11 Vα specific DNA probes. A comparison of Vα usage in hybridomas from Mls-1^+ and Mls-1^- transgenic mice revealed two striking effects: 1. An increased percentage of Vα2^+ hybrids in Mls-1^+ mice (35% compared with 14% in Mls-1^- mice) and 2. an absence of Vα11^+ hybrids in Mls-1^+ transgenics (0% compared with 16% in Mls-1^-). Further analysis showed that only 14% of Vα2^+, but as many as 95% of Vα11^+ hybridomas were Mls-1 reactive. Thus, there is a clear influence of the α-chain on Mls reactivity and clonal deletion of T cells expressing this transgenic Vβ8.1 chain. In an attempt to identify the α-chain element involved in Mls-1 recognition, we sequenced the alpha chains from a panel of 20 Mls reactive and non-reactive hybrids. Our data showed no simple correlation between Vα2 family members and Jα gene usage, and reactivity to Mls-1 antigens.

O 354 MOLECULAR ANALYSIS OF THE INTERACTION BETWEEN CLASS II MOLECULES AND CD4. Jacques Thibodeau, Nathalie Labrecque, Sylvain Fleury, Helen McGrath, France Ampleman, Claude Cantin and Rafick-P. Sékaly, Laboratory of Immunology, Clinical Research Institute of Montreal, 110 West Pine Avenue, Montreal, Quebec, Canada, H2W 1R7.

$CD4^+$ T cells recognize antigens of exogenous origin which are presented at the surface of cells (APCs) in the context of MHC class II molecules. The CD4-MHC class II-Ag interaction is critical both for the effector phase of T cell recognition and for positive and negative selection.

A functional and an adhesion assay has been developed in to identify the residues on CD4 which are implicated in the contact with class II molecules. A T cell hybridoma specific for a murine class I molecule and which is dependent on the CD4-class II interaction for adhesion and IL-2 production has been used. The CD4 binding site suggests that at least two domain of class II are involved in binding to CD4. We have used the same adhesion and functional assay to identify the residues on the class II molecule which contact CD4. A 3D model of the two extracelluar domain of the α and β chains of class II MHC molecules has been developed based on the homology between class I and class II and the crystal structure of class I. We have thus mutated to alanines the non-polymorphic and solvent-exposed residues of the β1 and α1 domains of DR1. Some mutants were also created in the membrane proximal β2 domain in a region which has a high degree of homology with these segments of class I that interacts with CD8. Results will be presented on the capacity of the different class II mutants expressed at the surface of murine fibroblasts to interact with CD4 and stimulate T cells in a CD4-dependant manner.

O 355 DOES CD4 HELP TO MAINTAIN THE FIDELITY OF T CELL RECEPTOR SPECIFICITY? Dario A.A. Vignali, Bernard Chang and Jack L. Strominger, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

During antigen presentation, a close association between CD4 and the T cell receptor (TCR) occurs as a result of 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 four different murine T cell hybridomas specific for the hen egg lysozyme (HEL) peptide 46-61 (DV, J. Moreno, D. Schiller and G. Hämmerling, International Immunology, Submitted). While all the CD4+ and CD4- variants tested responded comparably to immobilized anti-TCR mAbs, they differed dramatically in their responses to naturally processed HEL, synthetic peptide 46-61 or staphylococcal enterotoxins (SE). While one hybridoma was unaffected by the loss of CD4, another completely lost responsiveness to HEL and 46-61 while retaining reactivity to SE. In contrast, two other hybridomas still responded to HEL but lost reactivity to 46-61 and SE. Responsiveness to all stimuli was restored upon transfection of wild-type CD4. These data could not be readily explained on the basis of affinity or signal transduction requirements alone, and thus suggest that the intimate association of CD4 with the TCR may result in a subtle modulation of its fine specificity. Here, we attempt to define these observations and identify the CD4/TCR interaction sites by transfecting a series of CD4 mutants into the loss variants and analyzing their functional capacity. The following mutants have been made; CD4 which (a) lacks a cytoplasmic tail, (b) lacks the two MHC binding domains, (c) lacks three critical MHC contact residues, and (d) has had the two membrane proximal domains replaced with either (i) two Ig-like domains of ICAM-1, (ii) the two homologous domains from human CD4, and (iii) the membrane proximal stem-like portion of CD8. The conclusions of this study will be presented and discussed.

O 356 BIOCHEMICAL CHARACTERIZATION OF MOUSE MAMMARY TUMOR VIRUS SUPERANTIGENS, Gary M. Winslow, Yongwon Choi, John W. Kappler and Philippa Marrack, Howard Hughes Medical Institute, 1400 Jackson St., Denver, CO., 80206. Endogenous superantigens in the mouse are responsible for the clonal elimination of T cells that bear particular variable elements as part of their T cell receptor. It has been demonstrated that the endogenous murine superantigens are products of a gene located in the 3' long terminal repeat of Mouse Mammary Tumor (MTV) proviruses. Monoclonal and polyclonal antibodies have been generated against MTV superantigens. The monoclonal antibodies precipitate a 58 kDa glycoprotein from the surface of B cell hybridomas and DBA/2 spleen cells. Polyclonal antibodies specific for the C-terminus of Mtv-7 inhibit T cell activation in vitro, indicating that the superantigen adopts an inverted membrane orientation characteristic of Type II membrane proteins. The surface expression of the MTV superantigen suggests that it functions, like other superantigens, by direct association with the Class II ligand and the TCR.

O 357 MHC RESTRICTED RECOGNITION OF RETROVIRAL SUPER-ANTIGENS BY $V\beta17^+$ T CELLS, David L. Woodland, Sherri Surman, Frances Lund, Ronald B. Corley, Phuong Nguyen and Marcia A. Blackman, Department of Immunology, St Jude Childrens Research Hospital, Memphis, TN 38105 and Division of Immunology, Duke Medical Center, Durham, NC 27710.

We have previously shown that $V\beta17^+$ T cell hybridomas interact with an endogenous super-antigen encoded by the murine retrovirus Mtv-9. Here we analyse the role of MHC class II molecules in presenting this super-antigen, vSAG-9, to $V\beta17^+$ hybridomas. A panel of ten hybridomas was analysed for their ability to respond to A20/2J ($H\text{-}2^d$) and CH12.LBK ($H\text{-}2^k$) cells which had been transfected with the vSAG-9 gene. Whereas some of the hybridomas recognised vSAG-9 exclusively in the context of $H\text{-}2^k$, other hybridomas recognised vSAG-9 exclusively in the context of $H\text{-}2^d$ or in the context of both $H\text{-}2^d$ and $H\text{-}2^k$. Similar results were obtained using B10.D2 ($vSAG\text{-}9^+$, $H\text{-}2^d$) and B10.BR ($vSAG\text{-}9^+$, $H\text{-}2^k$) spleen cells as presenting cells. Antibody inhibition studies revealed that $I\text{-}A^k$, $I\text{-}E^k$ and $I\text{-}E^d$ were all capable of presenting vSAG-9 to T cells but that individual hybridomas discriminated between different vSAG-9/class II complexes. There was no correlation between the Jβ element of the T cell antigen receptor (Tcr) and the MHC class II/vSAG-9 complex recognised by each hybridoma. Taken together, these results suggest that (i) there is a direct interaction between the class II molecule and the Tcr during the recognition of retroviral super-antigen by $V\beta17^+$ T cells and that (ii) other, as yet unidentified components of the Tcr are involved in this interaction.

O 358 THE B7/BB1 ANTIGEN PROVIDES ONE OF SEVERAL COSTIMULATORY SIGNALS FOR THE ACTIVATION OF $CD4^+$ T LYMPHOCYTES BY HUMAN BLOOD DENDRITIC CELLS *IN VITRO*, JW Young*‡, L Koulova‡, SA Soergel*, EA Clark§, RM Steinman*, B Dupont‡, *The Rockefeller University and ‡Memorial Sloan-Kettering Cancer Center, New York, NY 10021; and §University of Washington, Seattle, WA 98195.

T cells respond to peptide antigen in association with MHC products on antigen-presenting cells [APCs]. A number of accessory or costimulatory molecules have been identified that also contribute to T cell activation. Several of the known accessory molecules are expressed by freshly isolated dendritic cells, a distinctive leukocyte that is the most potent APC for the initiation of primary T cell responses. These include ICAM-1 [CD54], LFA-3 [CD58], and class I and II MHC products. Dendritic cells also constitutively express the accessory ligand for CD28, B7/BB1, which has not been previously identified on circulating leukocytes freshly isolated from peripheral blood. Dendritic cell expression of both B7/BB1 and ICAM-1 [CD54] increases following binding to allogeneic T cells. Individual MAbs against several of the respective accessory T cell receptors, *e.g.*, anti-CD2, anti-CD4, anti-CD11a, and anti-CD28, inhibit T cell proliferation in the dendritic cell-stimulated allogeneic MLR by 40-70%. Combinations of these MAbs are synergistic in achieving near total inhibition. Other T cell-reactive MAbs, *e.g.*, anti-CD5 and anti-CD45, are not inhibitory. Lymphokine secretion and blast transformation are similarly reduced when active accessory ligand-receptor interactions are blocked in the dendritic cell-stimulated allogeneic MLR. Different APCs do not differ qualitatively so much in their capacity to express these accessory ligands, but in their quantitative expression and factors regulating same. We conclude that dendritic cells are unusual among circulating leukocytes in their comparably higher expression of accessory ligands, among which B7/BB1 can now be included; and that these are pertinent to the efficiency with which dendritic cells in small numbers elicit strong primary T cell proliferative and effector responses.

O 359 INTERACTION OF PHOSPHOINOSITOL-LINKED CLASS I MHC MOLECULES WITH T CELL RECEPTORS AND CD8.

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PI-linked class I MHC molecules are poor stimulators of primary T cell responses. To ask whether this is due to a failure of interaction between PI-linked class I MHC and CD8, we generated CD8 negative hybridomas specific for $H\text{-}2K^b$, and asked whether they could respond to PI-linked K^b molecules. Hybrid $H\text{-}2K^b/Q7$ molecules were created which are identical to K^b throughout the α1, α2 and α3 domains but retain the Q7 connecting peptide and PI-linkage signals. Despite acting as efficient targets for the parent CTL clone, transfectants expressing PI-linked K^b are very poor stimulators of the corresponding hybridomas. These same hybridomas can respond well to K^b transfectants with conventional transmembrane linkages. It is possible therefore, that PI-linked class I molecules do not cross-link T cell receptors sufficiently to cause activation despite acting as efficient target molecules for CTL recognition.

O 360 **DEFINING A ROLE FOR EACH DOMAIN OF THE CD4 MOLECULE.**

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The CD4 molecule is a cell surface glycoprotein of the Ig super family which defines the T cell lymphocytes subset that recognizes their antigen (Ag) when presented by a MHC class II molecule. When this specific recognition occurs, the CD4 molecule has been shown to interact with the class II molecule and the TcR and then increasing the stimulation of this T cell. As the CD4 molecule is associated by its cytoplasmic tail to the tyrosine kinase $p56^{lck}$, it is not clear if the interaction of CD4 with its ligands, stabilizes the complex TcR/Ag/MHC and/or transduces a signal to the cell via the $p56^{lck}$. It has been also shown that an inhibitory signal can be send to the cell in certain conditions when using an anti CD4 antibody. Using two different Ag specific murine hybridomas, we show that the cytoplasmic truncated form of the human CD4 when expressed in those cells, is as efficient as the wild-type CD4 to potentiate the Ag stimulation of this T cell. Since this truncated molecule does not associate with $p56^{lck}$ in those systems CD4 fulfills its role only by interacting with its ligands. Those hybridomas will allow us to define the amino acids involved in the association between CD4 and the MHC molecule. As we have previously shown that most them are located in the first two domains of the molecule, we will show the consequences of deletions of the third and/or fourth domains. Results will show the ability of these deleted molecules to potentiate an Ag stimulation and to transduce a negative signal to the cell when anti CD4 antibodies are used. These evidences will enable us to better understand how this molecule plays its role in these multiple interactions events.

O 361 THE ROLE OF ACCESSORY MOLECULES IN T CELL ACTIVATION. Linda A. Zuckerman and Jim Miller, Committee on Immunology, University of Chicago, Chicago, IL 60637. *Engagement of the TCR with MHC alone leads to an* unresponsive state of T cells called anergy. While many APC surface molecules have been implicated in providing the second signal necessary to drive T cells past anergy, the exact role of these molecules is unclear. We have recently shown that Class II transfectants of an islet tumor cell line failed to express costimulatory function and induced anergy in T cell clones. In order to better understand the function of candidate costimulatory molecules, we are utilizing this novel islet tumor cell transfection system. Specifically, we are examining the function of ICAM-1, B7, fibronectin, as well as the chondroitin sulfate form of invariant chain which has previously been shown to have a costimulatory capacity. We have begun to analyze the effect of these molecules on T cell hybridomas as well as T cell clones for both allogeneic and antigen specific responses. We hope to be able to correlate the presence of specific accessory molecules on APCs with their ability to stimulate T cells to both proliferate and secrete IL-2 at different stages of cell priming.

T Cells Gamma Delta Cells, NK Cells and Non-Classical MHC

O 400 FLANKING SEQUENCES AND THE CTL RESPONSE TO AN HIV GP160 EPITOPE Cornelia C. Bergmann, Stephen A. Stohlman, Denise M. McKinney and Minnie McMillan, Dept. of Neurology University of Southern California School of Medicine, Los Angeles, CA 90033.

Recognition of virus infected cells by cytotoxic T lymphocytes (CTLs) requires endogenous processing of viral antigens to generate peptides that are selectively presented at the cell surface by MHC class I molecules. Efficient antigen presentation is highly dependent on residues flanking the antigenic epitope and on structural features intrinsic to the peptide. To identify structural requirements necessary for efficient processing and selection of antigenic epitopes we are studying the CTL response to an epitope in the HIV-gp160 glycoprotein comprising amino acids 315-329. This epitope, designated p18, elicits a CTL response in both humans and mice.

Although it has been demonstrated that the synthetic p18 peptide only binds to its restriction element Dd and not Ld, the addition of an N terminal glutamic acid residue (E), which serves as a class I binding motif, allows its association with the Ld molecule. To test the effect of the E residue on T cell recognition, gp160 specific CTLs were generated by immunizing Balb/c mice with recombinant vaccinia virus expressing the entire gp160 protein. These CTLs specifically killed Dd tranfected L cells stably expressing gp160 and cells coated with peptide p18. Ep18 was also able to sensitize target cells , however only in the context of Dd not Ld; these results imply that the E residue does not disrupt peptide conformation necessary for Dd restricted CTL recognition. Brefeldin A treatment did not inhibit the presentation of either synthetic peptide demonstrating that these peptides presumably associate with the Dd molecule at the cell surface. Surprisingly, endogenous expression of the Ep18 epitope in the context a heterologous glycoprotein also resulted in lysis of Dd, but not Ld expressing targets by gp160 specific CTLs, implying that the cellular requirements for proteolytic cleavage are not very stringent. Lipopeptides and minigenes encoding the p18 sequence with and without the E residue are currently being investigated for their ability to generate (E)p18 specific CTLs in vivo.

O 401 Analysis of B-EBV deletion mutants at the MHC region for the susceptibility to lysis by alloreactive NK clones.

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We analyzed a family (characterized for MHC haplotypes) for the segregation of 5 different characters "susceptibility to lysis" by 5 different groups of alloreactive NK clones. Having demonstrated that, all these characters are inherited in an autosomic recessive manner and the corresponding genes are carried by chromosome 6, we selected an heterozygous donor (and thus resistant to lysis) for specificities 1, 2 and 5. B-EBV cell lines were derived from this donor and deletion mutants were obtained by γ-irradiation and selection with mAbs specific for MHC alleles. Several mutants hemizygous for MHC were selected. Some of these mutants completely lost the MHC region while others only deleted a single MHC locus. These mutants were tested for their susceptibility to lysis by anti- 1, -2 and 5 specific NK clones. Only those mutants that lost the MHC region were susceptible to lysis. These studies provided evidence that the genes governing susceptibility or resistance to NK-mediated lysis are localized inside the MHC region.

O 402 THE PERIPHERAL REPERTOIRE OF Vα2 CELLS IS SKEWED IN MLS-1 EXPRESSING Vβ8.1 TRANSGENIC MICE, Marcia A. Blackman, Phuong Nguyen, Hedy Smith, David L. Woodland, Department of Immunology, St Jude Children's Research Hospital, Memphis, TN 38105.

We have generated Vβ8.1 (β chain only) transgenic mice in order to study mechanisms of tolerance to Mls-1 and to examine the role of the alpha chain in Mls-1 recognition. Previous analyses have shown that tolerance to Mls-1 in these mice is mediated by two mechanisms- clonal deletion in the thymus and clonal anergy in the periphery. Analysis of Vα2 expression in the periphery of these mice, using an antibody kindly provided by Bernard Malissen, revealed a 2-fold increase in $V\alpha2^+$ T cells (42% versus 19%) in $Mls\text{-}1^+$, compared with Mls^-, transgenic mice. Analysis of the Mls-1 reactivity of a panel of $V\alpha2^+$ T cell hybridomas generated from $Mls\text{-}1^-$ transgenic mice (hence, a population not influenced by mechanisms of tolerance) revealed that only 27% of the Vα2 hybrids recognized Mls-1 in vitro, and that the general reactivity was weak. The high frequency of $V\alpha2^+$ T cells in the periphery of $Mls\text{-}1^+$ transgenic mice is consistent with both thymic and peripheral tolerance mechanisms. For example, the increased levels of $V\alpha2^+$ T cells in the periphery of $Mls\text{-}1^+$ transgenic mice could be the result of selective lack of clonal deletion of Vα2 in the thymus. Thus, fewer $V\alpha2^+$ T cells are clonally eliminated in $Mls\text{-}1^+$ mice compared with T cells expressing Vαs that are more inherently Mls-1-reactive. Alternatively, $V\alpha2^+$ cells with weak Mls-1 reactivity may escape thymic deletion, and subsequently expand in the periphery, perhaps as a precedent to anergy. An alternative explanation for the Vα2 skewing could be unrelated to tolerance, and due, for example, to preferential positive selection in the $Mls\text{-}1^+$ transgenic mice. These alternatives will be examined experimentally and the implications of the findings will be discussed.

O 403 TSA-1: A NOVEL THYMOCYTE MARKER EXPRESSED ON IMMATURE THYMOCYTES, Richard L. Boyd, Dale I. Godfrey, Marilina Masciantonio, Carolyn Tucek, Mark Malin and Patrice Hugo. Dept. Path. & Immunol., Monash University, Prahran, Melbourne, Vic. 3181, Australia. We recently identified a novel plasma membrane antigen expressed both on a subset of thymic medullary epithelial cell and cortical thymocytes. Here the relevance of TSA-1 as a marker of immature T cells was investigated in details by multi-color flow cytometry in comparison with an extensive panel of standard reagents. TSA-1 was distinct from ThB, HSA, Ly6C, Ly6A/E, H-2K, Thy-1, PNA receptor, CD5, IL-2R, CD44 and LFA-1. TSA-1 was present on 85% of adult thymocytes; this includes all immature subsets ($CD4^-CD8^-CD3^-$, $CD4^+CD8^-CD3^-$, $CD4^-CD8^+CD3^-$, $CD4^+CD8^+CD3^-$ and $CD4^+CD8^+CD3^{low}$), and >95% of thymocytes from SCID or day 14-16 embryos. It was negative on cells with high expression of TcR/CD3 complex: mature thymocytes, early thymic migrants and peripheral T cells. Interestingly, $CD4^+CD8^+CD3^{high}$ cells a post positive- and negative-selection subset which convert into mature thymocyte were $TSA\text{-}1^-$. In fact, there was a direct inverse relationship between the expression of TcR/CD3 complex and TSA-1. It was characterized as a 17kd PI-linked protein, which showed no capacity in inducing a calcium influx upon cross-linking. TSA-1 is thus a unique marker which exquisitely separates mature from immature thymocytes and should prove invaluable for defining selection events occurring during thymopoiesis.

O 404 PRIMING OF HSV-SPECIFIC MHC CLASS I RESTRICTED CTL ACTIVITY IN MICE WITH RECOMBINANT gB. D. Cataldo, G. Barchfeld, J.P. Valensi, A. Dennis, J. Kazzaz, G. Ott, C. Walker, A. Erickson, M. Selby, G. Van Nest. Chiron Corporation Emeryville, CA 94608

Successful presentation of antigen to MHC Class I restricted cytotoxic T cells was originally thought to require endogeneous synthesis of antigen. More recently it has been demonstrated that artificial delivery of antigen to the cytoplasm can also result in Class I presentation. Mice infected with Herpes Simplex Virus (HSV) have been shown to mount a Class I restricted CTL response against virus infected target cells. One of the antigens recognized by CTLs from $H\text{-}2^b$ mice is glycoprotein B. Using this HSV mouse model, we have investigated requirements for primary and secondary stimulation of CTL activity with gB subunit antigen. We found that gB truncated in the transmembrane region expressed in CHO cells delivered by standard methods did not elicit a CTL response. In contrast, truncated gB was able to generate a strong CTL response *in vivo* when associated with pH insensitive fusogenic liposomes. This antigen-liposome combination was also able to restimulate CTLs from HSV infected mice *in vitro*. In addition, gB containing the transmembrane domain associated with pH sensitive fusogenic liposomes was able to generate a primary CTL response *in vivo*. Reduced and alkylated gB with CFA/IFA generated a strong CTL response *in vivo*. A 15 amino acid synthetic peptide representing the gB CTL epitope delivered alone, with CFA/IFA or liposomes gave variable results *in vivo*, but very efficiently restimulated a secondary response *in vitro*. We are further defining the requirements for effective Class I presentation of subunit antigens both in terms of antigen form and delivery vehicle.

O 405 WHAT PRECEDES ANTIGEN PRESENTATION? Michael J. Caulfield and Deborah Stanko, Section of Immunology, Research Institute of the Cleveland Clinic Fndn., Cleveland, OH 44195.

Although it is clear that antigens can be processed by several different cell types, including macrophages, dendritic cells and antigen-specific B cells, in many cases, the antigen does not reach the presenting cell in its free form. In fact, particulate antigens are usually opsonized by antibody, and soluble antigens are more likely to first encounter specific antibody instead of antigen-specific B cells or macrophages. This is particularly true in secondary responses in which high levels of antibodies are present in body fluids but may also be true in the case of primary immune responses in which antigen may be complexed with "natural antibodies". In previous studies, we compared the response to free antigen with that to preformed immune complexes and found that the response to antigen presented in an immune complex was genetically restricted; i.e., in certain mouse strains, the response to the immune complex (formed in antigen excess) was markedly lower than that to free antigen whereas in other strains, the response to both forms of the antigen were equivalent. This genetic restriction did not correlate with the MHC or *Igh* haplotype of the mouse strains, rather. the response to immune complexes was correlated with the expression of a recurrent idiotype (T15) in response to immunization with free antigen (PnC). In current studies we have employed Ig transgenic mice whose B cells express the MOPC-167 heavy and light chain genes encoding phosphorylcholine (PC)-specific antibodies to examine the response to PC-containing immune complexes. We find that TG^+ and TG^- mice yield an approximately equivalent antibody response to free antigen (PnC) or to PnC/MOPC-167 immune complexes. Interestingly, however, when mice were immunized with immune complexes prepared with PnC and the TEPC-15 antibody, the resulting response was 10 - 50 fold higher than that to complexes formed with the transgene antibody (MOPC-167). It should be pointed out that the mice were not primed with TEPC-15 before challenge with the complex and that MOPC-167 and TEPC-15 (which express different idiotypes) are both IgA myeloma proteins derived from BALB/c mice. The results suggest that the antibody response to antigen presented in the form of an immune complex can differ from the response to free antigen and suggests that the formation of immune complexes *in situ* may precede and markedly influence antigen processing and the subsequent immune response. (Supported by NIH grant number RO1 AI27573).

O 406 **Evidence of a Natural Killer (NK) cell repertoire for (allo)antigen recognition: definition of five distinct NK-determined allospecificites in humans.**
E. Ciccone*, D. Pende*, O. Viale**, C. Di Donato*, G. Tripodi*, A.M. Orengo°, A. Moretta°, L. Moretta*+. *Istituto Scientifico Tumori, Genova; **Istituto di Anatomia Umana, Univ. di Genova; °Ist. di Istol. ed Embriol. Gen., Univ. di Genova; +Univ. di Torino, Sede di Novara.
Previous studies indicated that CD3-CD16+ NK cells are capable of specific alloantigen recognition. Thus, alloreactive NK clones lysed normal allogeneic target cells (PHA blasts) bearing the stimulating alloantigen but did not lyse autologous cells or the majority of unrelated allogeneic cells. In this study we investigated whether NK cells isolated from single individuals could exhibit different allospecificities. To this end, we derived large numbers of CD3-CD16+ clones (in the presence of PHA) from fresh CD3- peripheral blood lymphocytes. Cloning efficiencies ranged between 5 and 10%. The resulting CD3-CD16+ clones were tested for their reactivity against a panel of allogeneic PHA blasts (derived from 6 donors). In donor A, 4 distinct groups of clones could be identified according to their pattern of reactivity (over 300 clones have been analyzed). Clones that could be assigned to one or another group of specificity represented <40% of all clones derived from donor A. The remaining clones did not display cytolytic activity against any of the allogeneic target cells used in the panel. None of the clones lysed autologous (A) cells, however, PHA blasts from donor A were lysed by the representative clones G10 and H12 specific for donor A. Clones displaying a cytolytic pattern of reactivity identical to that defined for donor A were present in other individuals studied, however not all groups of allospecific clones were necessarily represented in different individuals. All clones belonging to the various groups of specificities were homogeneous in the expression of EB6/GL183 triggering surface molecules and could thus be assigned to one or another of the previously defined subsets of NK cells. Genetic analysis of the new NK-defined alloantigens was performed in representative families. The corresponding characters were found to segregate independently and to be inherited in an autosomic recessive mode. Moreover, since the genes governing susceptibility or resistance to lysis segregated with certain HLA haplotypes, they appeared to be carried by chromosome.

O 407 PROLIFERATIVE RESPONSE TO BORRELIA BURGDORFERI BY $\alpha\beta^+$ AND $\gamma\delta^+$ T LYMPHOCYTES FROM NORMAL INDIVIDUALS. Sheldon M. Cooper, Karen D. Roessner, Jennifer Q. Russell, and Ralph C. Budd. The University of Vermont, Burlington, VT 05405.
With the exception of superantigens, significant T cell proliferative responses to particular antigens are unusual from normal individuals not previously immunized with that antigen. In the course of examining the proliferative response of fresh peripheral blood lymphocytes (PBL) from Lyme disease patients to the causative spirochete, B. burgdoferi, we observed that virtually all normal individuals tested also manifested a brisk proliferative response equivalent to that of patients. To examine whether the response from normals was the result of cross-reactivity from previous naturally acquired immunity to another agent, we sorted fresh $CD4^+$ PBL based upon their expression of the memory T cell marker CD29. Whereas the proliferative response to the recall antigen, tetanus, was largely confined to the memory $CD29^+$ subset, the response to a sonicate of B. burgdorferi was equally distributed between the $CD29^-$ and $CD29^+$ subsets, similar to the PHA response. This suggested that the T cell response to B. burgdorferi was not merely due to cross-reactivity. T cell clones specific for B. burgdorferi have been established from PBL of normal volunteers. Most of these clones are $CD4^+$ and HLA-DR-restricted. However some clones appear capable of responding in the presence of a variety of unrelated HLA-DR backgrounds. This has similarities to previously described superantigens.
In addition to the above $\alpha\beta^+$ T cell response, we have observed a marked $\gamma\delta$ T cell response from one individual. Whereas this person's fresh PBL are 20% $\gamma\delta^+$, stimulation with B. burgdorferi resulted in a responding population that was up to 75% $\gamma\delta^+$. In contrast, the response to PHA or tetanus yielded only $\alpha\beta^+$ T cells. Studies are in progress to characterize the clonal responses to various Borrelia antigens and the $V\gamma$ and $V\delta$ usage.

O 408 ROLE OF CLASS I MHC AND SELF PEPTIDES IN NK RECOGNITION, Isabel Correa, Nan-Shih Liao and David Raulet, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley CA 94720
Natural killer (NK) cells can destroy tumor cell lines as well as virus infected cells. The antigen receptors on NK cells have not been identified. On the other hand, MHC class I molecules have been implicated as part of the antigen they recognize, or at least are associated with it. Do NK cells recognize class I molecules or self-peptides bound to class I molecules? Is the lack of self-peptide recognition the signal for cell lysis? To address these questions, we analyzed the NK sensitivity of RMA/S cells, which are deficient in the assembly of self peptides with class I MHC. Preformed peptides, when supplied to RMA/S, can assemble with class I molecules on the cell surface. RMA/S is sensitive to NK cells, and this is not reversed by synthetic foreign peptides that bind its class I molecules. Peptides isolated from normal cells, however, reverse the NK sensitivity of RMA/S. HPLC fractionation indicates that a few peptide species are responsible for the effect. No effect of these peptides on CTL lysis was observed. These results suggest that some NK cells may recognize self peptides associated with class I molecules, and that this recognition inhibits the killing by NK cells. Class I molecules that are empty or associated with specific foreign peptides appear to have no effect in reversing the NK sensitivity of target cells.

O 409 THE ROLE OF PKC IN THE TRANSDUCTION OF THE IFN-γ SIGNAL FOR HLA-DR EXPRESSION IN THE PROMONOCYTIC LINE THP-1. Bruce H. Devens, Rich Gumina, Jose Freire-Moar, and David R. Webb. Institute of Immunological and Biological Sciences, Syntex Research, Palo Alto, CA 94303.

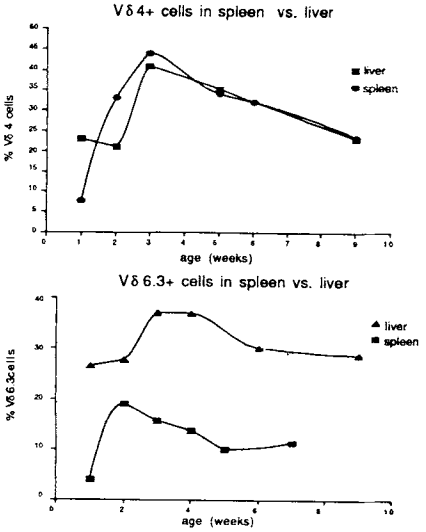
Interferon γ (IFN-γ) is the most potent known lymphokine for activation of macrophages and induction of HLA-DR expression. IFN-γ has also been shown to induce HLA-DR expression on THP-1 cells, a monocytic line that expresses many of the properties of monocytes. We have used the cell line THP-1 to study the mechanisms regulating the induction of HLA-DR expression. Expression was analysed by both FACS analysis and by measurement of mRNA levels. The expression of cell surface HLA-DR and HLA-DR message induced by IFN-γ was blocked by the protein kinase C inhibitors sphingosine, staurosporine, and H7 while non-specific kinase inhibitors had no effect. Inhibitors of PKC were also shown to decrease induction of HLA-DR on normal human peripheral blood monocytes. Inhibition of HLA-DR expression on by PKC inhibitors was seen when they were added up to 20 hours after the initial induction with interferon, suggesting a role for PKC throughout the course of events leading to HLA-DR expression. Stripping of PKC from the cells with PMA led to decreased induction of HLA-DR. It was not possible to induce HLA-DR expression by direct activation of PKC via PMA alone or by PMA plus calcium ionophore A23187. These results suggest that PKC has an undefined role in the IFN-γ-induced signal transduction pathway leading to expression of HLA-DR in cells of the mononuclear phagocytic lineage, and that PKC activity is required throughout the course of metabolic events leading to the actual expression of HLA-DR.

O 410 PRIOR T CELL RECEPTOR β CHAIN REARRANGEMENT DOES NOT IMPROVE A CELL'S ABILITY TO SUCCESSFULLY DIFFERENTIATE IN THE THYMUS, Stacey R. Dillon and Pamela J. Fink, Department of Immunology, University of Washington, Seattle, WA 98195

Developing thymocytes undergo gene rearrangement of their T cell receptor (TCR) α and β chain genes, followed by a stringent selection process survived only by those cells that bear functional receptors that can distinguish self from nonself. Using transgenic mice constructed on a C57BL/6 (H-2^b, Thy-1.2+) background which carry a rearranged TCR β chain gene (Vβ5.2Dβ2Jβ2.6, Cβ2), we have investigated the effects of giving the immune system a choice between transgenic and nontransgenic (B6.PL/J, H-2^b, Thy-1.1+) cells by reconstituting lethally irradiated host mice (B6.PL/J or C57BL/6) with several mixtures of bone marrow stem cells of each type. At certain timepoints post-irradiation, we stained thymocytes and peripheral lymphocyte populations from these chimeric mice with fluorescently labeled antibodies specific for various cell surface markers (including Thy-1) in order to assess the cell surface phenotype and maturational stage of transgenic versus nontransgenic cells. Surprisingly, our data strongly indicate that the transgenic cells do not enjoy any appreciable competitive advantage over the nontransgenic cells--either in immature or mature thymocyte subsets, or in peripheral lymphoid organs--despite essentially being provided with half of a functional TCR. Given these results, we speculate that either TCR α chain gene rearrangement is a substantial hurdle for developing thymocytes, or that this transgenic β chain is able to pair with an unusually limited number of α chains.

O 411 γ/δ T LYMPHOCYTES IN THE MURINE LIVER, Christina L. Ellis, Willi Born, Rebecca O'Brien, Department of Medicine, National Jewish Hospital, Denver CO 80206

Previous studies have demonstrated a high proportion of γ/δ T cells in the liver. However, very little is known about these cells. We have investigated two subsets of γ/δ T cells in B10 mice at various ages. We found that, while Vδ4+ cells were approximately equal in percentages in both spleen and liver, Vδ6.3+ cells were enhanced 2-2.5 fold in the liver. The Vδ6.3+ γ/δ cells show a very high correlation with HSP-60 reactivity. We are currently making liver γ/δ T cell hybridomas and plan to characterize the receptors that are present and test the reactivity of these cells.



O 412 LIPOSOME-MEDIATED DELIVERY OF RECOMBINANT PROTEINS TO THE CLASS I MHC ANTIGEN PRESENTATION PATHYWAY. Erickson, A.E., M. Selby, D. Cataldo, G. Barchfeld, J.P. Valensi, G. Van Nest, and C.M. Walker, Chiron Corp., 4560 Horton Street, Emeryville, CA. 94608

Glycoprotein B (gB) of Herpes Simplex Virus (HSV) type 2 is a target antigen for class I MHC restricted, HSV-specific CTL from virus infected C57Bl/6 (H-2^b) mice. We have investigated the ability of liposomes to sensitize target cells for class I MHC restricted CTL recognition of recombinant gB protein. Target cells were not sensitive to lysis by HSV-specific CTL after incubation with gB-containing fusogenic, pH sensitive liposomes that destabilize upon exposure to acidic environments. In contrast, efficient killing of target cells was observed when gB protein was incorporated into pH insensitive fusogenic liposomes. A dose response analysis revealed that as little as 1 ug of gB was sufficient for target cell sensitization when delivered in these liposomes. In contrast, up to 100 ug of gB in the absence of liposomes did not sensitize target cells for lysis. Treatment of target cells with Brefeldin A, which blocks class I MHC presentation of antigens, prevented recognition of the gB-liposome sensitized target cells by CTL. This method of antigen delivery will provide an efficient means for sensitizing target cells for lysis by class I MHC restricted CTL.

O 413 SEPARATION OF TH1 CELL CYTOTOXIC AND PROLIFERATIVE FUNCTIONS USING ALTERED IMMUNOGENIC PEPTIDE. B. D. Evavold and P. M. Allen Dept. of Pathology, Washington University School of Medicine, St. Louis MO 63110. The T cell response to the single immunogenic peptide from the beta chain of murine hemoglobin [Hb(64-76)] was examined and dissected using a panel of altered peptides that contain single, conservative amino acid substitutions. The absence of a T cell response to a given altered peptide identified amino acids critical for activation of that T cell; however, in any single T cell clone, different functional phenotypes may require distinct critical amino acid residues. The proliferative, lymphokine, and cytotoxic responses of three cloned T helper 1 (Th1) cells were measured to identify any changes in the pattern of critical amino acids. For proliferative responses, the Th1 clones typically identified multiple amino acids (>4) important for T cell activation, although none of the Th1 clones exhibited the same pattern of reactivity to the substituted Hb peptides. Critical amino acids for a proliferative response in the Th1 clone 3.L2 were found at positions 69, 71, 72, 73, and 76. When lymphokine production (IFN-γ or IL-3) was measured, the same pattern of critical residues for each particular Th1 clone was also observed. Conversely, fewer amino acids were identified as critical for cytotoxic responses from the Th1 clones. For clone 3.L2, only the conservative amino acid substitution at position 72 was crucial for a T cell cytotoxic response. Other amino acids needed for proliferation/ lymphokine responses, such as the glutamic acid at position 73, did not affect the cytotoxic response. In the same T cell, T cell activation can be separated using altered immunogenic peptides such that cytotoxic function disassociates from the proliferative response.

O 414 MHC-RESTRICTED RECOGNITION OF LEUKEMIC CELLS BY CYTOTOXIC T LYMPHOCYTE (CTL) CLONES ISOLATED FROM HLA-GENOTYPICALLY IDENTICAL SIBLING DONORS OF BONE MARROW TRANSPLANT PATIENTS.

J.H.Frederik Falkenburg, Laura M. Faber, Henriëtte M. Goselink, Simone A.P. van Luxemburg-Heijs and Roel Willemze, Laboratory of Experimental Hematology, Department of Hematology, University Medical Center Leiden, 2333 AA Leiden, the Netherlands.

Allogeneic bone marrow transplantation (BMT) has been associated with a graft versus leukemia (GVL) reactivity. We demonstrated that minor Histocompatibility (mH) antigen-specific CTL clones that can be generated from patients after HLA-identical BMT, are capable of MHC restricted recognition of (clonogenic) myeloid leukemic cells. Blocking studies with CD8 or anti-class I antibodies illustrated MHC class I restricted recognition. Furthermore, anti-leukemic CTL clones could be generated in-vitro from the HLA-genotypically identical bone marrow donors of patients with AML or CML using irradiated leukemic blasts of the patient as stimulator cells. Various types of clones could be established. The first type of CTL clone was CD8+ and showed MHC class I restricted mH specific lysis of all recipient tissues tested. A second type of clone, CD8+, recognized the recipient leukemic cells only, and not normal lymphocyte or EBV transformed cell lines from the recipient. Two of 15 other AML leukemic samples sharing different HLA class I antigens with the CTL clone were recognized by the same clones. Their recognition appeared to be class I restricted, since the lysis of all three leukemic samples could be inhibited with anti-class I or CD8 antibodies. Furthermore, cytotoxic CD4+ CTL clones were isolated that showed HLA-class II restricted reactivity with the recipient leukemic cells as well as EBV transformed cell lines. Thus, both MHC class I and MHC class II restricted CTL clones that show reactivity with neoplastic hematologic cells can be generated from healthy HLA-genotypically identical donors of patients with leukemia The specificity of the CTL clones will be further analyzed. If these clones recognize leukemia specific peptides presented by the MHC molecules, they may be used as immunotherapeutic agents in the treatment of leukemia.

O 415 THE ROLE OF NON-CLASSICAL CLASS I MHC IN THE RESPONSE OF $\gamma\delta$TCR$^+$ T CELLS TO L CELLS.

Madeline M. Fort, Farhad Imani, Mark Soloski, and Drew M. Pardoll, Immunology Training Program, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Previous studies have suggested that nonclassical class I MHC may be the antigen presenting molecules for $\gamma\delta$ TCR$^+$ T cells. Unprimed splenic $\gamma\delta$TCR$^+$ T cells (40-50% $\gamma\delta$TCR$^+$, >5% $\alpha\beta$ TCR$^+$ or B220$^+$) from naive C3H/HEN MTV$^-$ (H-2^k) or C57bl/6 (H-2^b) mice were found to proliferate rapidly in the presence of irradiated L cells (H-2^b). This response is not blockable with monoclonal antibodies to K^k and D^k. B cell depleted splenocytes from the same animals (85% $\alpha\beta$TCR$^+$, 1% $\gamma\delta$TCR$^+$) do not proliferate in the presence of L cells. $\gamma\delta$TCR$^+$T cell expansion in response to L cells is confirmed by pre- and post-stimulation FACSCAN anaylsis. We wondered if Tla region products might in fact be the ligands recognized by these $\gamma\delta$TCR$^+$ T cells. L cells stain positively for some, but not all, anti-Tla antibodies. This suggests that L cells express a nonclassical MHC molecule similar to Tla. Currently we are doing blocking studies to see if this is the molecules responsible for the stimulation of $\gamma\delta$TCR$^+$ T splenocytes. Also we have created L cell reactive $\gamma\delta$TCR$^+$ T cell hybridomas to study which Vγ and Vδ chains these cells express and to study what antigens expressed by L cells the $\gamma\delta$TCR$^+$ T cells recognize

O 416 COMPLEMENTARY SPECIFICITIES OF HSP-60 REACTIVE $\gamma\delta$ and $\alpha\beta$ T CELLS IN C57BL/10 MICE.

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We have found previously that a subset of unprimed murine $\gamma\delta$ T lymphocytes ($\gamma\delta$ cells) is capable of recognizing mycobacterial 60 kD heat shock protein (HSP-60). HSP-60 homologs are expressed in all prokaryotic and eukaryotic cells and expression may be altered by various stressful stimuli such as infection, inflammation and neoplasms. The mycobacterial and other HSP-60 homologs are also antigens for $\alpha\beta$ T cells and B cells. In this study, we have directly compared $\alpha\beta$ and $\gamma\delta$ cell responses to mycobacterial HSP-65. We found that $\alpha\beta$ cells can be primed with the native molecule (recombinant HSP-65 derived from *M. bovis*) but not with a peptide corresponding to amino acids 180-196 of this protein. In contrast, all HSP-60 reactive $\gamma\delta$ cells recognize this putative epitope and our data suggest that $\gamma\delta$ cells can be primed with the peptide. Whereas HSP-60 reactive $\alpha\beta$ hybridomas require $\alpha\beta$ antigen presenting cells, $\gamma\delta$ hybridomas do not. Comparing splenic $\alpha\beta$ and $\gamma\delta$ cells derived from C57BL/10 mice, and using various HSP-60-derived antigens (the native recombinant molecule, truncated recombinant molecules and synthetic peptides), we found that $\alpha\beta$ cells recognize several regions of the molecule but not the putative $\gamma\delta$ cell epitope, 180-196. This comparison *in vivo* and *in vitro* represents an example of complementary ligand specificities of $\gamma\delta$ and $\alpha\beta$ cells. Complementarily could be a more general feature of $\gamma\delta$ and $\alpha\beta$ cell specificities. Regardless of whether this is the case or not, the complementary reactivity in C57BL/10 mice provides an opportunity for studying a $\gamma\delta$ cell response *in vivo*, in the absence of $\alpha\beta$ cell reactivity to the same antigen.

O 417 Antigen Presentation in an Interleukin-4 Induced Anti-tumor Immune Response

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When a murine renal carcinoma (Renca, H-2^d) was engineered to secrete high levels of IL-4, and injected subcutaneously into syngeneic BALB/c mice, it induced a rapid infiltration by macrophages(Mo) and eosinophils(Eo). These infiltrating cells were able to kill the majority of the IL-4 producing Renca cells. Shortly afterwards a CD3$^+$ T cell infiltrate was present at the site and tumor specific CD8$^+$ T lymphocytes could be detected systemically. It is generally accepted that these cytotoxic T lymphocytes are restricted by the Class I MHC molecules that present endogenous tumor peptides.

We are investigating whether early infiltrating Mo are capable of processing exogenous tumor antigen for presentation to the CD8$^+$ T cells. By utilizing bone marrow (BM) chimeric BALB/c X C57BL6 F1 mice (H-2^{bxd}), it has been possible to distinguish the effects of antigen presentation by the tumor cells and the infiltrating Mo. Preliminary in vivo data suggest that when the BM MHC type (H-2^b; Mo,Eo,T cells) is mismatched to the tumor MHC(H-2^d), there is a substantial decrease in the level of tumor specific CD8$^+$ T-cells generated. We are now seeking to directly demonstrate by CTL assay, CD8$^+$ T-cells directed in vivo against a defined, transfected tumor antigen (influenza virus nucleoprotein,NP) restricted by the class I MHC of the infiltrating macrophages.

O 418 EVIDENCE FOR A ROLE OF MHC CLASS I OR STRUCTURALLY RELATED MOLECULES IN ANTIGEN RECOGNITION BY HSP-60 REACTIVE γδ T CELLS. Kalatardi Harshan, Robin Cranfill, Angela Dallas, William Townend, Terry Potter, Beverly Koller*, Rebecca O'Brien and Willi Born, Department of Medicine, National Jewish Center for Immunology and Respiratory Diseases, Denver, CO 80206 and *Pathology Department, University of North Carolina, Chapel Hill, NC 27599

The immunological role of γδ T cell receptor (TCR) bearing lymphocytes (γδ cells) has remained unclear. Although γδ TCRs resemble structurally αβ TCRs, specificities and ligand recognition by γδ cells are not understood. We have found previously that a subset of murine γδ cells respond to mycobacterial 60 kD heat shock proteins (HSP-60). Using synthetic peptide antigens, a putative epitope stimulatory for the entire HSP-60 reactive subset has been mapped to a region around amino acids 180-196 of *M. leprae* HSP-65, suggesting that γδ cells recognize small protein fragments similar to αβ cells. Here we report a correlation between MHC class I surface expression and reactivity with HSP-60 derived peptide antigens, suggesting a requirement for MHC class I or related molecules in antigen recognition by an entire subset of γδ cells. HSP-60 reactive γδ T cell hybridomas were selectively depleted of MHC class I by cell sorting. Clones expressing very little or no MHC class I molecules no longer responded to antigen but were capable of responding in the presence of appropriate antigen presenting cells. Moreover, exogenous human ß2 microglobulin was found to enhance the peptide antigen response. The data suggest that MHC class I or related molecules function as presenting elements for a subset of γδ T cells recognizing a molecularly defined peptide antigen.

O 419 SPECIFICITY OF T CELLS BEARING INVARIANT γδ RECEPTORS IN MURINE SKIN. Wendy L. Havran* and James P. Allison+, *Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037 and +Cancer Research Laboratory, University of California, Berkeley, CA 94720

We have demonstrated that the vast majority of dendritic epidermal T lymphocytes (dEC) found in murine skin express an invariant antigen receptor composed of Vγ3 and Vδ1 chains. The strict tissue localization and absence of clonal diversity in the TCR of these cells suggested that the immunological role of the dEC may be distinct from that of peripheral αβ and γδ T cells expressing diverse TCR. The role of γδ T cells with invariant TCR may be to recognize damage-induced self antigens. We have demonstrated that stressed keratinocytes were able to stimulate IL-2 release and proliferation of freshly isolated dEC and dEC clones in vitro. The response is mediated by the TCR and does not appear to be classically MHC restricted. There is evidence that the antigen recognized by the dEC is presented as a peptide. Tryptic digests of cultured keratinocytes, but not other cell types, stimulate the dEC to secrete IL-2 when presented by non-stimulatory live or fixed fibroblasts. Keratinocyte digests remain stimulatory when presented by cells which lack both class I and class II MHC gene products, providing further evidence that classical MHC molecules are not involved in this presentation. Fractionation of digests are being performed to isolate the active keratinocyte peptides. Monoclonal antibodies directed against keratinocyte cell surface molecules involved in dEC stimulation have been isolated and used to further characterize the interaction between the dEC and keratinocytes. In vivo the dEC may perform surveillance for conserved epitopes of stress-related proteins to allow recognition of damaged cells regardless of the agent inducing the damage and provide these T cells with invariant TCR with a critical role in protection from infection and malignancy.

O 420 LYMPHOKINE EXPRESSION OF GAMMA DELTA VS. ALPHA BETA T CELLS IN RESPONSE TO MYCOBACTERIA - TUBERCULOSIS

Hayashi, Robert J., Pardoll, Drew M., Departments of Medicine and Oncology, The Johns Hopkins School of Medicine

Gamma delta T cells play a prominent role in the primary response to mycobacterial infections. To further characterize this response, we have developed a system to measure lymphokine expression using quantitative PCR. Mice immunized with mycobacterial antigens are analyzed at various time points in the evolving response. Using monoclonal reagents coupled to magnetic beads, gamma delta and alpha beta lymph node cells are isolated from contaminating cells with a magnetic column to greater than 90% purity. RNA isolated from these purified T cell populations are then analyzed for expression of IL-2, IL-4, and gamma interferon using PCR coupled to a reverse transcriptase reaction. Quantitation of this lymphokine expression is achieved by comparing the amplified message with the amplification of a synthetic RNA standard template which contains the sequences of the primers specific for the lymphokines of interest. Using this technique, the pattern of lymphokine expression of both gamma delta and alpha beta cells have been characterized, demonstratingthe evolution of the expression of lymphokines during the primary response to mycobacteria tuberculosis.

O 421 DIFFERENTIAL REGULATION OF T AND NK CELL REPERTOIRE DEVELOPMENT IN MHC CLASS I TRANSGENIC AND β_2m KNOCK OUT MICE, Petter Höglund, Charles Bieberich, Takayuki Yoshioka, Claes Öhlén, Gilbert Jay, Beverly Koller, Klas Kärre. Dept. of Tumor Biology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden.

We have addressed the question of repertoire development in the NK and T cell compartments. We have previously shown that the D8 strain can reject hematopoietic grafts of the B6 haplotype; a rejection which is rapid and mediated by NK1.1+ cells. This result indicates that the D^d transgene brings about a redefinition of self with regard to NK cell function as well as T cell function. A similar redefinition of self was observed in the β_2m -/- mice. Normal cell from these mice was sensitive to NK cells from normal (β_2m + /-), but not from β_2m -/- mice. This "tolerance" in the NK compartment of β_2m -/- mice towards their own class I deficient normal cells was not secondary to their deficiency of CD8+ T cells. When β_2m -/- bone marrow was allowed to mature in a lethaly irradiated β_2m + /- mouse, reappearance of the CD8+ T cell pool was seen while the inability to recognize β_2m -/- hematopoietic cells persisted. A similar linkage of the NK repertoire to the bone marrow compartment was seen in chimeras between B6 and D8. The ability to reject H-2^b lymphoma was dependent on D^d transgene expression in the bone marrow. Differential effects on T and NK cells were seen in the transgenic MT-D2 and MT-D6L mice, where D^d is expressed only in the liver and in the intestine. These mice were T cell tolerant towards D^d (accepted skin grafts and did not respond in MLR) just like the D8 strain, but did not show the D8 type of NK reactivity against H-2^b lymphoma. In conclusion, we have shown that MHC class I in the host influences NK cell function by altering the target repertoire. This influence is distinct from that on the T cell repertoire development; the influence on NK recognition is only seen when the transgene is present in bone marrow derived cells, hypothetically on the NK cells themselves, whereas effects on T cell development occur also when only peripheral expression of the transgene is present.

O 422 PEPTIDE RECOGNITION BY CLONED γδ T CELLS, Joseph Holoshitz, Luis M. Vila, Lynn Wagner and Nadine Romzek, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109-0531

The function and antigenic specificity of γδ T cells, and the identity of their antigen presenting molecules are largely unknown. The majority of human peripheral γδ T cells bear a Vγ9/Vδ2 T cell receptor (TCR), and display non-clonal reactivity to mycobacteria, without restriction by the major histocompatibility complex (MHC). It is unknown whether these cells have clonal antigenic specificity as well. Here we describe rheumatoid arthritis-derived Vγ9/Vδ2 T cell clones, displaying dual antigenic recognition; A non-clonal, MHC-unrestricted recognition of mycobacteria, and a clonal recognition of a short tetanus toxin peptide presented by HLA-DRw53, a non-polymorphic class II MHC molecule closely associated with susceptibility to rheumatoid arthritis. Recognition of the mycobacterial antigen and the peptide were both mediated by the TCR and involved LFA1-ICAM1 interaction. This is the first evidence that Vγ9/Vδ2 T cells can recognize nominal antigenic peptides presented by class II MHC molecules. These results suggest that much like αβ T cells, Vγ9/Vδ2 cells may contribute to the immune response against foreign antigens in an antigen-specific and MHC-restricted manner. The reactivity of these γδ T cells to mycobacteria may represent a superantigen-like phenomenon.

O 423 T CELL RECEPTORS OF T CELLS THAT HAVE DIFFERENT FINE SPECIFICITIES FOR THE SAME EPITOPE, Benjamin L. Hsu, Scott G. Williams, Brian D. Evavold, and Paul M. Allen, Dept. of Pathology, Washington University School of Medicine, St. Louis, MO 63110

T cell clones and hybridomas raised against murine hemoglobin ß chain (Hbb^d) all respond to a synthetic peptide of Hbb^d (64-76). To more finely map critical residues within this epitope, we used peptide analogs of Hbb^d (64-76) containing single, conserved amino acid substitutions in its sequence. Of ten I-E^k-restricted, Hbb^d-specific T cells tested against a set of substituted analogs, each responded uniquely to the peptide analogs. Each T cell appears to have a different fine specificity for the same epitope. For example, the substitution of Ser for Thr69 was >1000-fold less stimulatory for four of the T cells, while other T cells responded as well to the substituted form as to wild-type. Only the Gln for Asn72 analog was nonstimulatory for all ten T cells assayed.

To understand the basis for these differences in fine specificity, we want to determine the T cell receptor (TCR) usage of the Hbb^d-specific T cells. We used variable region-specific and consensus primers in the polymerase chain reaction to amplify and obtain the sequences of the TCR α and ß chains from these T cells. Their variable gene (Vß) usage was found to be restricted: five (out of ten) use Vß1, three use Vß15, one uses Vß4, and another, Vß8.3. However, Jß usage is not restricted; no Jß segment is used by more than 20% of the T cells. Also, comparison of the V-D-J junctional sequences of the TCRß chains revealed a diversity of length and amino acid sequence. This suggests that the differences in fine specificity among this panel of T cells are largely due to differences in determinants in their TCR, particularly the junctional sequences. These results highlight the underlying diversity of the T cell response to this single antigenic epitope.

O 424 EXPRESSION AND FUNCTIONAL ANALYSIS OF A RECOMBINANT HUMAN GLYCOSYL PHOSPHATIDYL-INOSITOL (GPI) ANCHORED CLASS I HUMAN LEUKOCYTE ANTIGEN (HLA). Jui-Han Huang, Neil S. Greenspan, and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106.

The murine class I major histocompatibility complex (MHC) molecule Qa-2, which can act as an alloantigen, is naturally linked to the plasma membrane via a GPI-anchor. No human GPI-anchored class I MHC molecules are known. To test whether a GPI-anchored human class I antigen could be expressed and function as an alloantigen, we produced a recombinant GPI-modified class I HLA-A2.1 polypeptide derivative. By PCR fusion, a chimeric sequence was assembled consisting of the coding sequence for the extracellular domains of HLA-A2.1 and was linked in-frame with the GPI-modification signal sequence from the 3' end of decay-accelerating factor (DAF). This was subcloned into an extrachromo-somally replicating, EBV-based, human expression vector. K562, a human erythroleukemic cell line, and HeLa, a human cervical carcinoma cell line, were each stably transfected. Monoclonal antibodies specific for HLA-A2.1, but not for HLA-B7, bound to the cell surface of pHLA-A2.1:DAF transfectants. Pan-class I and anti-beta-2 microglobulin monoclonal antibodies also bound to pHLA-A2.1:DAF on K562 but not control transfectants. GPI modification of the pHLA-A2.1:DAF encoded product (HLA-A2.1:GPI) was confirmed by phosphatidylinositol-specific phospholipase C (PI-PLC) enzymatic cleavage analysis. Human GPI-anchor variants differ in their susceptibility to cleavage by PI-PLC from *B. thurigiensis* . PI-PLC cleaved both cell surface HLA-A2.1:GPI and endogenous surface DAF on Hela transfectants. In contrast, the membrane anchors for both DAF and HLA-A2.1:GPI on the K562 subline used were both PI-PLC resistant. As a negative control, transmembrane peptide-anchored HLA-A2.1 was PI-PLC resistant. To assess the alloantigenicity of HLA-A2.1:GPI, irradiated HLA-A2.1:GPI positive transfectants were added as stimulators in one-way mixed cell co-cultures. Significant alloantigenic stimulation for the GPI variant was observed with HLA-A2.1 negative peripheral blood lymphocyte responder cells. This is the first demonstration of the feasibility of expressing an artificial recombinant human class I antigen that is GPI-anchored. Studies are currently underway to determine the nominal antigen peptide binding capacity of HLA-A2.1:GPI.

O 425 HEAT SHOCK PROTEIN-DERIVED PEPTIDES INTERACT WITH CLASS Ib MOLECULE Qa-1b. Farhad Imani[1] , Thomas M. Shinnick[2] and Mark J. Soloski[1]. 1. Dept. of Molecular and Clinical Rheumatology, The Johns Hopkins University School of Medicine, Baltimore, MD. 2. CDC, Atlanta, GA.

In addition to the highly polymorphic class I and class II molecules, the murine MHC also encodes several less polymorphic structures referred to as class I-like or class Ib encoded within the Qa/Tla region. We report that transfection of mouse fibroblasts with gene $T23^b$ leads to the surface expression of a molecule which is structurally identical to lymphocyte Qa-1^b. Although in transfected cells the predominant Qa-1 species was present in an immature intracellular form, the expression of mature cell surface Qa-1 species was dramatically and selectively increased following heat shock. Importantly, the addition of a tryptic digest of *M. bovis* Hsp65 could stabilize the surface expression of Qa-1. Furthermore, In bindng studies, we have shown that several peptides corresponding to the deduced amino acid sequence of *M. tuberculosis* Hsp65 can bind to empty Qa-1 molecules. Recent data have suggested that Qa-1 may present a synthetic antigen (Poly Glu:Tyr) to T cells expressing γδ receptors. In addition, several reports have demonstrated that γδ T cells respond to mycobacterial heat shock proteins. Collectively, these observations suggest that Qa-1 molecule may be involved in the presentation of heat shock protein-derived peptides to the immune system.

O 426 IS HSP65 181-196 A SPECIFIC LIGAND FOR GAMMA-DELTA T CELLS? Gary E. Kikuchi, John E. Coligan, and Ethan M. Shevach, Biological Resources Branch and Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda MD 20982

It was proposed by W. Born and co-workers that heat shock proteins are specific ligands for autoreactive γδ T cells, as lymphokine production by γδTCR hybridomas bearing the Vγ1.1Vδ6 T cell receptor (TCR) could be augmented by addition of PPD, purified HSP65, or a synthetic peptide corresponding to residues 181-196 of HSP65 from Mycobacterium tuberculosis.

To test this, we transfected cDNAs encoding the Vγ1.1Vδ6 TCR from the epidermally-derived T cell hybridoma T195/BW into the cell line TG40, a variant of the cytochrome-c reactive hybridoma 2B4 that was previously mutagenized to delete expression of the αβ TCR. This transfectant, TG524, is constitutively activated and secretes IL-2 spontaneously as detected by CTLL assay although the amount of IL-2 production is always less than maximal compared to stimulation with plate-bound anti-CD3 or PMA and ionophore. A synthetic peptide corresponding to HSP65 181-196 was synthesized, and was found to augment spontaneous IL-2 production by this transfectant.

To test if this effect was due to recognition of HSP65 181-196 as a specific ligand of the Vγ1.1Vδ6 TCR, we used as a model system the autoreactive αβ T cell hybridoma B423, which recognizes an HIV gp160 peptide (peptide 18) in the context of D^d expressed on itself or on APC. Surprisingly, we found that IL-2 production by B423 in response to peptide 18 could also be augmented by HSP65 181-196. Maximal effect required peptide at concentrations above 100 μg/ml, approximately the same dose as used to augment IL-2 production by the γδ T cell TG524. Preliminary experiments suggest that the effect on B423 is due to an effect on the antigen presentation pathway, as overnight pretreatment of BALB/c spleen cells with HSP65 181-196 prior to treatment with peptide 18 also caused an augmentation in IL-2 production by B423 as compared to control spleen cells.

Based on these results, we suggest that HSP65 181-196 may not be a specific ligand for the γδ TCR. The mechanism whereby this peptide increases IL-2 secretion by both αβ and γδ T cells is under further investigation.

O 427 TRANSGENIC MICE CARRYING A T-CELL RECEPTOR SPECIFIC FOR THE INFLUENZA VIRUS NUCLEAR PROTEIN, Dimitris Kioussis, Clio Mamalaki, James Elliott, Yujiro Tanaka, Nicholas Yannoutsos, Alain Townsend, Philip Chandler and Elizabeth Simpson, NIMR, London; John Radcliffe Hospital, Oxford; C.R.C., Harrow, U.K.

Transgenic mice have proven a valuable tool in studying the establishment of a functional T-cell repertoire. Here we describe the analysis of transgenic mice carrying the genes for the α and β chains of a T-cell receptor from a cytotoxic T-cell clone (F5) specific for a defined peptide of the nuclear protein (NP) of the influenza virus in the context of $H\text{-}2^b$. The cDNA for the α and the β chains were inserted in the 5' untranslated region of a human CD2 mini gene cassette carrying the locus control sequences (LCR) of this gene. The constructs were coinjected in fertilized eggs of C57Bl/10 (H-2b) mice. Resulting transgenic mice expressed the transgenic receptor on >80% of the thymocytes and peripheral T-cells. As described before, we observed positive selection of thymocytes expressing the transgenic Tcr in the thymus of b/b, but not of k/k mice. As a result of the positive selection the majority of the mature thymocytes and peripheral T-cells had a CD8+CD4- phenotype in b/b mice. Surprisingly, k/k mice in which no positive selection was observed in the thymus, accumulated mostly CD8+CD4- T-cells expressing the transgenic Tcr in the periphery.

Functional analysis of T-cells from b/b or b/k transgenic mice showed that these lymphocytes were able to kill target $H2^b$ (EL4) cells in an antigen (NP) dependent fashion. T-cells from k/k mice were also able to kill target cells in an antigen dependent manner, suggesting that transgenic T-cells may not need to grow in a positively selecting thymus to be able to carry out their function. Third party reactivity studies showed that T-cells from the transgenic mice were able to respond to allo-antigen, albeit to a lesser extent.

We have also generated transgenic mice expressing the epitope recognised by the F5 receptor under promoters of different tissue specificity. We have crossed these mice to the F5 transgenic mice and we studied the effect on thymus development and tolerance induction.

In parallel experiments we injected the peptide recognised by this receptor into F5 transgenic mice and we analysed the effects on developing thymocytes and peripheral T-cells.

O 428 SPECIFIC RECOGNITION OF AUTOLOGOUS AND ALLOGENEIC B CELLS BY SYNOVIAL TISSUE DERIVED Vδ1⁺ T CELL CLONES, Daniela L.M.Orsini, Pieter C.M. Res, Jacob M. van Laar, and Frits Koning. *Depts of Immunohaematology/Blood Bank and Rheumatology, University Hospital, Leiden, The Netherlands*

Increased numbers of γ/δ TCR⁺ cells in the synovial tissue and fluid of arthritis patients have previously been reported. We have isolated a number of α/β and γ/δ TCR⁺ cell clones from the synovial tissue of a chronic arthritis patient. The majority of the γ/δ clones used the Vδ1 gene, but these γ/δ TCR⁺ cell clones appear not to originate from a monoclonal Vδ1 expansion in the joint. Although both the α/β and the γ/δ TCR⁺ clones were isolated after primary stimulation of the synovial tissue T cells with *Mycobacterium tuberculosis* (M.tub), none of the γ/δ TCR⁺ clones proliferated to M.tub, whereas the α/β TCR⁺ clones did. Two Vδ1⁺ γ/δ T cell clones, however, proliferated in response to stimulation with autologous and allogeneic (HLA-matched and -mismatched) EBV-B lymphoblastoid cell lines (EBV-BLCL). In contrast, the Vδ1⁺ γ/δ and α/β TCR⁺ T cell clones isolated from the same synovial tissue T cell line did not display proliferation towards the EBV-BLCL. In addition, Vδ1⁺ T cell clones from the peripheral blood of a healthy individual did not proliferate to the EBV-BLCL either. The proliferative response of the synovial tissue derived Vδ1⁺ cell clones could not be blocked by mAbs specific for HLA class I and class II molecules, nor by a mAb to CD48, a target structure for certain Vδ1⁺ T cell clones that has recently been identified. In contrast, significant blocking of the proliferative response was observed using an anti-CD3 mAb. Cytotoxicity of these Vδ1⁺ clones to the EBV-BLCL or other potential target cells such as Daudi, Molt-4 or K562 could not be detected. The capacity of these synovium derived Vδ1⁺ T cell clones to proliferate in response to autologous B cells may imply the recognition of an endogenous epitope, in which case the Vδ1⁺ T cells could play an important role in the pathogenesis of inflammatory arthritis.

O 429 FACTORS CONTROLLING UPREGULATION OF CD4 AND α-β TCR EXPRESSION ON THYMOMA CELLS, Hiroshi Kosaka and Jonathan Sprent, The Scripps Research Institute, La Jolla, CA 92037.

Positive selection of α-β TCR^+ T cells in the thymus is preceded by a transition of $CD4^-8^+TCR^-$ precursor cells to the major population of $CD4^+8^+TCR^{lo}$ thymocytes. The factors controlling this transition are still unclear. To seek information on this question we are studying a B6.PL (Thy 1.1)-derived thymoma line isolated from a long-term bone marrow chimera. When cultured in medium alone, clones derived from this line are all $CD4^-8^+$ and most are TCR^-. When transferred intrathymically into B6 mice or various F_1 hybrid mice, the thymoma cells colonize the thymus and seed the spleen and lymph nodes. Of the cells reaching these sites, 50-70% are $CD4^+8^+TCR^+$ ($V\beta11^+$). When maintained in vitro, the thymoma cells show upregulation of TCR/CD3 expression (from 3% to 40%) when cultured in the presence of TGF-β; this treatment does not induce CD4 expression. Attempts to upregulate CD4 expression in vitro have been largely unsuccessful, although limited CD4 expression occurs when the cells are cultured with a thymic stromal line plus IFN-γ. With regard to susceptibility to tolerance induction, incubating the thymoma cells with anti-CD3 antibody fixed to plastic leads to widescale death within 1 day. This effect is not seen with soluble anti-CD3 antibody plus spleen cells as APC.

O 430 A COMPONENT OF FETAL CALF SERUM PROCESSES A CLASS I - RESTRICTED PEPTIDE INTO ITS ACTIVE FORM. S. Kozlowski, M.P. Corr, T. Takeshita, L. Boyd, J.A. Berzofsky and D.H. Margulies. Laboratory of Immunology, NIAID and Metabolism Branch, NCI, NIH, Bethesda, MD 20892.

We have previously shown a dependence of functional peptide binding to purified soluble class I MHC molecules on the concentration of free β2-microglobulin. We also noted an additional enhancement of functional peptide binding due to fetal calf serum (FCS) that could not be replaced by β2-microglobulin alone. This FCS function could be replaced by ovalbumin or a number of other related proteins. To evaluate the target of this ovalbumin effect we treated the HIV IIIB gp-160 peptide 18 (residues 315-329) with ovalbumin prior to adding it to purified plate-bound H-2D^d. The ovalbumin was removed by spinning the treated peptide through a 3 kilodalton MW cutoff filter and then the peptide was added to H-2D^d. This ovalbumin treated peptide was able to functionally bind H-2D^d as assayed by growth inhibition of a specific T-cell hybridoma. Peptide treated with bovine serum albumin in a similar fashion failed to functionally bind H-2D^d. Both ovalbumin and FCS were shown to modify the reverse phase HPLC profile of the peptide in a similar manner, suggesting processing of the peptide. The greatest T-cell stimulatory activity was found in a minor peak of lower MW than the original peptide. The observation that ovalbumin preparations of greater purity have a lesser effect suggests that this processing activity is due to a contaminant of ovalbumin. The similar activity in serum suggests that this serum component may play a role in the extracellular processing of class I antigenic peptides.

O 431 FUNCTION OF NONCLASSICAL MHC CLASS I MOLECULES Mitchell Kronenberg[1], M. Teitell[1], P. Eghtesady[1], H. Cheroutre[1], S. Balk[2], M. Jackson[3], M. Mescher[4], P. Peterson[3], and L. Pond[3], [1]Department Microbiol. & Immunol., UCLA, Los Angeles, CA 90024-1747, [2]Beth Israel Hospital, Boston, MA, [3]Scripps Clinic & Research Foundation, La Jolla, CA, [4]Medical Biology Institute, La Jolla, CA.

We have studied two mouse, nonclassical class I molecules, TL and CD1. Both of these molecules are recognized by specific monoclonal antibodies and they are both expressed by intestinal epithelial cells. TL and CD1 are divergent with respect to their ability to interact with CD8 and their requirement for β2-microglobulin to maintain stable, surface expression. Mouse CD1 is truly 'nonclassical' in that it does not interact with CD8 and does not require β2-m for surface expression. Transfected *Drosophila* embryo cells can express CD1 in a stable form on their cell surface at 37°C in serum free medium. Classical class I molecules are empty and therefore are not expressed under these conditions in the *Drosophila* cells. This suggests that CD1 does not require bound peptide antigen for surface expression, or that it loads peptide antigen under conditions where the classical class I molecules do not.

We have found that mouse intestinal intraepithelial lymphocytes (IELs) that express a γδ TCR recognize autologous epithelial cells. A TCR containing the Vγ5 gene segment is important for this response. Indirect evidence suggests that a stress-response protein is recognized by these cells, as epithelial cells from germ free mice are effective stimulators, and heat shock of the epithelial cells increases the ability of IELs to respond to epithelium. To be effective at stimulating a response, the intestinal epithelial cells must have a functional β2-microglobulin gene. Inhibition studies using two anti-TL monoclonal antibodies suggest that TL may be presenting the putative stress protein to TCR γδ + IELs. Blocking with either anti-CD1 or anti-D monoclonal antibodies was not effective. The differences between TL alleles in the mouse occur outside of the peptide antigen binding site. The conservation of the TL antigen-binding cleft may reflect its function in the presentation of a conserved stress-response protein(s) to IELs.

O 432 IDENTIFICATION OF A NOVEL LYMPHOCYTE SUBSET: THE NK1$^+$CD4$^-$CD8$^-$αβTCR$^+$ T CELL H. Lantteky and D. M. Pardoll. Division of Rheumatology

The majority of peripheral T cells express the T cell receptor (TCR)/CD3 complex and either a CD4 or CD8 "accessory molecule" on their surface. During T cell activation, these structures participate in antigen/MHC recognition and MHC restriction on antigen presenting cells respectively. In the murine system, we have discovered a novel T cell subset that expresses an αβ TCR but is devoid of CD4 or CD8. This T cell subset bears NK1, a surface antigen previously thought to be expressed exclusively by TCR$^-$ natural killer cells. A corresponding population was found to exist in the thymus with the phenotype NK1$^+$CD4$^-$CD8$^-$TCRαβ$^+$. Both the thymocyte and the peripheral NK1$^+$TCRαβ$^+$ cell have a skewed TCR repetoire in which Vβ8 is overrepresented when compared to CD4$^+$ or CD8$^+$ T cells. Thymus transplant experiments into congenically marked athymic (nude) mice revealed that the NK1$^+$TCRαβ$^+$ subset is exclusively thymus-derived and represents a distinct subset from the thymus-independent NK1$^+$TCR$^-$ "natural killer cell" population. The NK1$^+$TCRαβ$^+$ cells preferentially localize to the bone marrow. These results demonstrate that this T cell subset is exported to the periphery after developing in the thymus. Mice challenged with an MHC class I low tumor genetically engineered to secrete IL2 have an accumulation of NK1$^+$TCRαβ$^+$ cells at the site of challenge. In vitro analysis of lymphocyte subsets reveal these cells to have CTL activity against the parental tumor. These results suggest an immune function distinct from classical T cells.

O 433 *PLASMODIUM BERGHEI* SPOROZOITE ANTIGENS DO NOT REQUIRE PROCESSING FOR T CELL ACTIVATION . **Heidi T. Link and Urszula Krzych. Department of Biology, The Catholic University of America and Dept. Immunology, Walter Reed Army Inst. Research. Washington, D.C. 20307-5100.**

P. berghei sporozoites (SPZ)-primed murine splenic and lymph node cell responses can be elicited in vitro to the priming antigen and to the circumsporozoite (CS) protein, the SPZ major surface antigen, but not to synthetic peptides of the CS protein. Parallel studies using either partially denatured preparations of SPZ or soluble recombinant CS protein revealed that the CS protein peptide-specific T cell repertoire is inducible. These observation suggested that a particular order of processing/presentation of the different forms of SPZ influenced the expression of CS protein-specific T cell repertoire. Hence we began our investigation by analyzing the requirements for antigen presenting cells (APC) as well as the mechanism of processing and presentation of SPZ antigens to specific T cells. APC depletion and reconstitution studies demonstrated that SPZ-primed T cells maintain their proliferative reactivity in the absence of macrophages, but not B cells, and that the T cell proliferative reactivity is restored when SPZ antigens are presented by activated B cells. SPZ-primed and naive LPS B cell blasts were equally efficient in presenting SPZ antigens to T cells. Although at present the mechanism of T cell activation by SPZ antigens remains unknown, it was found to require antigen priming and to be dependent upon MHC class II molecules in that monoclonal antibodies directed against the correct MHC haplotype were effective in blocking anti-SPZ T cell responses. Furthermore, SPZ-specific T cell activation occurred independent of SPZ internalization and processing, since gluteraldehyde fixation or monensin treatment of B cells did not affect the T cell proliferative responses. Based on these observations we suggest that unlike a nominal protein antigen, the CS protein in its native conformation within the SPZ contains an available, presumably conformational T cell epitope, and unlike the superantigens, the CS protein interacts with antigen specific T cells. Therefore, we hypothesize that SPZ-associated CS protein might represent an alternative way of antigen specific T cell interaction. Work is currently in progress toward elucidating some of the mechanisms involved in SPZ-specific T cell activation.

O 434 EXPRESSION OF FUNCTIONAL GAMMA DELTA T CELL RECEPTORS RECOGNIZING TETANUS TOXIN, Elwyn Loh[1], Martin Wang[1], Zhien Wang[2], Danuta Kozbor[2], [1]Hematology-Oncology Section, Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and [2]Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA 19107.

A series of human γδ T cell clones has been isolated that recognizes tetanus toxin, possibly in the context of MHC Class II, (Kozbor, et al., J. Exp. Med. 169:1847, 1989). Some of these clones can also specifically recognize other antigens including the Daudi leukemia cell line. They include two clones that express a Vγ9-Jγ2.3-Cγ2, Vδ1-Jδ1-Cδ; and two clones with Vγ9-Jγ1.2-Cγ1, Vδ2-Jδ1-Cδ. The cDNAs coding for the TCR of these clones have been isolated and sequenced. These receptors have been expressed in a recipient host T-cell, Jurkat variant, J.RT3-T3.5, that does not express an endogenous β chain. The adoptive receptors are expressed on the cell surface as identified by V region specific monoclonal antibodies; however, preliminary results suggest that Vδ2 may be incompatible with some combinations of Vγ-Jγ-Cγ. The TCR-CD3 complexes of the transfected cells are functional in that the cells can be stimulated to secrete IL2 by immobilized anti-CD3 antibody. This system is being used to analyze the requirements of the γδ T cell receptor to recognize tetanus toxin and superantigens.

O 435 SPECIFIC KILLING OF HHV6-INFECTED CELLS BY $CD3^-CD56^+$ NATURAL KILLER LYMPHOCYTES, Mauro S. Malnati, Paolo Lusso, and Eric O. Long, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, and Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Rockville, MD 20852

Several pieces of evidence indicate that Natural Killer (NK) cells exert a role in the control of viral infection before the establishment of a specific cytolytic response mediated by $CD3^+$ MHC-restricted lymphocytes. To test whether NK cells may be able to kill HHV6-infected cells, pure $CD3^-CD56^+$ cell populations were derived from PBL of healthy donors. After activation in rIL2 these cells efficiently lysed PHA blasts infected with HHV6. As expected for NK cells, the recognition of HHV6-infected cells was not restricted by HLA. To define this phenomenon further, 86 $CD3^-CD56^+$ clones were isolated and tested against autologous HHV6-infected PHA blasts. Surprisingly, only about half of the clones (41/86) were able to kill HHV6-infected cells, while all of them lysed the NK-sensitive cell line K562. A group of clones (17) was further characterized for their ability to recognize autologous or allogeneic infected cells, while another group (15) was analyzed to asses the lysis of cells infected with other viruses (EBV and HSV). The results indicated for the first time that cells infected with different viruses are recognized by different sets of clones. This implies specificity in the recognition by $CD3^-CD56^+$ lymphocytes and that these cells play a role in the immune response against HHV6 and more generally against viral infection.

O 436 SELECTIVE REMOVAL OF PEPTIDE-MHC COMPLEXES FROM THE SURFACE OF PRESENTING CELLS INTERACTING WITH SPECIFIC T LYMPHOCYTES. Fabrizio Manca, Daniela Fenoglio, Annalisa Kunkl, Maria T. Valle, Dept. of Immunology, University of Genoa, San Martino Hospital, 16132 Genoa, Italy.

Human T cell lines specific for different peptides of the HIV env glycoprotein gp120 have been used as probes to identify the availability of MHC-peptide complexes (MPC) on the surface of antigen presenting cells. Both PBMC and adherent monocytes (AM) were used as antigen presenting cells (APC) with similar results. Proliferative response of the peptide specific T cell lines was used as read-out of the system.
Persistence of presenting function and persistence of antigen on antigen pulsed APC over a period of time was determined. Pulsing of APC with high concentration of peptides resulted in persistence of presenting function, suggesting that intracellular compartments may operate as storage sites for antigen peptides. The availability of MPC was studied on APC that had undergone a previous contact with specific T cells. MPC recognized by T cells specific for peptide 24 (aa 225-240) were no longer available on the surface of APC following interaction with irradiated (binding-not proliferating) T cells with the same fine specificity. MPC recognized by T cells specific for peptide 30 (aa 285-300) were available and could stimulate T cells with this specificity. The reciprocal experiments yielded similar results. These data demonstrate that upon clustering of APC with specific T cells, a selective depletion of MPC engaged with interacting T cells occurs at the surface of APC. The depletion does not affect other MHC molecules complexed with unrelated peptides, that are left for interaction with a second cohort of T cells with a different fine specificity.

O 437 TOLERANCE INDUCTION IN MHC TRANSGENIC MICE. Andrew L. Mellor, Stephen J. Simpson, Helen Yeoman and Peter Tomlinson. Division of Molecular Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA. UK. Recombinant MHC class I genes have been used to make CBA ($H\text{-}2^k$ haplotype) transgenic mice which display tissue-specific expression of $H\text{-}2K^b$ molecules. Promoterless $H\text{-}2K^b$ genes were recombined with transcriptional control elements taken from the human CD2 or β-globin genes to create KCE (two lineages, KCE2 and KCE3) and Kβ transgenic mice respectively. T-cell and erythroid cell lineage specific-expression of $H\text{-}2K^b$ molecules was demonstrated by FACS analysis of peripheral blood lymphocytes (PBL's) and red blood cells from KCE and Kβ mice respectively. All transgenic mice are tolerant of $H\text{-}2K^b$ molecules as determined by failure of the mice to reject skin grafts from donor mice expressing $H\text{-}2K^b$ molecules. Cytotoxic T-cells recognizing $H\text{-}2K^b$ molecules cannot be detected amongst spleen T-cells from Kβ or KCE2 mice. In contrast, $H\text{-}2K^b$ target cells are killed by responder T-cells from KCE3 mice despite expression of $H\text{-}2K^b$ in the thymus of these mice. However, KCE3 responder cells failed to kill target cells from KCE3 mice even though these cells express $H\text{-}2K^b$. Splenic T-cells from Kβ mice proliferate in response to $H\text{-}2K^b$ molecules indicating that at least some $H\text{-}2K^b$ specific T-cells are present in the peripheral repertoire of mature T-cells in these mice. However, only very weak proliferative responses were detected in KCE2 mice. $H\text{-}2K^b$ restricted T-cell responses against C57BL/10 minor histocompatibility antigens were detected in Kβ and KCE2 mice suggesting that positive selection of the T-cell repertoire can take place in these mice. These results indicate that the mechanisms of T-cell tolerance induction when self antigens are expressed in distinctive tissue-specific patterns are diverse and suggest that spatial and temporal differences in self antigen presentation to thymocytes or T-cells leads to profoundly different modes of tolerance induction.

O 438 EXPERIMENTAL CUTANEOUS LEISHMANIASIS: LANGERHANS CELLS ARE POTENT STIMULATORS OF AN ANTIGEN-SPECIFIC T-CELL IMMUNE RESPONSE, Heidrun Moll, Antje Will, Christine Blank, Harald Fuchs, Nikolaus Romani,* and Martin Röllinghoff, Institute of Clinical Microbiology, University of Erlangen, FRG, and *Department of Dermatology, University of Innsbruck, Austria.

Cutaneous leishmaniasis is initiated by the bite of an infected sandfly and inoculation of **Leishmania major** parasites into the mammalian skin. Macrophages are known to play a central role in the course of infection because they are the prime host cells and function as antigen-presenting cells (APC) modulating the specific immune activity. However, in addition to macrophages in the dermis, the skin contains epidermal Langerhans cells (LC) which can present antigen to T cells. Therefore, we examined murine LC for their ability to serve as host cells and to induce a T-cell response to **L.major**. Our results demonstrated that LC can be infected by **L.major** both in vitro and in vivo. Furthermore, in vitro studies showed that epidermal LC can process and present **L.major** antigen to T cells from primed mice and to MHC class II-restricted **L.major**-specific T cell clones. The T cell-stimulatory effect was antigen-specific and could be induced by crude parasite antigen, by live organisms and by a highly purified host-protective lipophosphoglycan. In addition, we have evidence that LC are able to retain parasite antigen in immunogenic form while migrating from the skin to the regional lymph node. The data suggest that LC take up parasites in the skin and are important APC in cutaneous leishmaniasis. They may perform a critical function by transporting antigen into lymphoid tissues for initiation of the immune response and by stimulating T cells that infiltrate the cutaneous lesion.

O 439 A MAJOR SUBSET OF HUMAN γδ T CELLS REACTIVE TO MYCOBACTERIUM TUBERCULOSIS IS NOT RESTRICTED BY CLASSICAL MHC MOLECULES. Craig T. Morita, Hamid Band, Govindaswamy Panchamoorthy, and Michael B. Brenner Laboratory of Immunochemistry, Dana Farber Cancer Institute, and Department of Rheumatology and Immunology, Harvard Medical School, Boston, MA 02115.

Human T cells bearing the T cell receptor γδ represent a distinct T cell subset. A major subset of these γδ T cells recognize antigen(s) present in mycobacteria. We have characterized this response to gain insights into γδ T cell recognition. Exposure of peripheral blood lymphocytes to mycobacteria expanded γδ T cells expressing Vγ2Vδ2 TCR gene segments. This expansion is independent of prior exposure to antigen as Vγ2Vδ2 bearing γδ cells from umbilical cord blood also expanded upon mycobacterial exposure. Analysis of a panel of γδ T cell clones expressing various combinations of TCR Vγ and Vδ gene segments showed that only those bearing Vγ2Vδ2 responded by proliferating or by killing mycobacteria-pulsed target cells. Antigen presenting cells were required for optimal stimulation suggesting that presentation via a cell surface molecule is required. Recognition by Vγ2Vδ2 T cell clones was not restricted by the polymorphic regions of MHC molecules as antigen presenting cells from MHC disparate donors could present mycobacteria. Non-polymorphic regions of known MHC-encoded antigen presenting molecules were also not required for recognition as mutant tumor cell lines lacking classical Class I HLA-A,-B,-C or Class II HLA-DR,-DQ,-DP expression were able to present mycobacteria to γδ T cells. We conclude that the major human γδ T cell response to mycobacteria is restricted to cells bearing Vγ2Vδ2 TCR, does not require prior antigenic exposure, and does not require classical MHC Class I and MHC Class II molecules. We hypothesize that γδ T cells recognize mycobacterial antigens associated with a novel non-polymorphic antigen presenting molecule(s).

O 440 THE PHENOTYPE AND FUNCTION OF VIRUS-SPECIFIC T-CELLS IS DETERMINED BY THE VIRAL PROTEIN

Peter J.M. Openshaw, Fiona M. Record and Wafa H. Alwan, Department of Medicine, St. Mary's Hospital, Norfolk Place, London W2 1PG, United Kingdom.

Mice sensitized to individual RS (respiratory syncytial) virus proteins show distinct patterns of pulmonary immunity and immunopathology when challenged with live RS virus. In order to explore the immune mechanisms responsible for these patterns of post-vaccination disease, BALB/c (H-2^d) mice were primed by scarification with recombinant vaccinia viruses (rVV) expressing the major glycoprotein (G), fusion protein (F), phosphoprotein (P), nucleoprotein (N), or matrix (22K) proteins of RS virus. Antigen stimulated spleen cell cultures gave rise to $CD3^+$, $\alpha\beta TCR^+$ cell lines. Those from rVV-F primed mice were a mixture of $CD8^+$ and $CD4^+$ T cells; the $CD8^+$ cells were bright for CD45RB whilst the $CD4^+$ cells were a mixture of bright and dull. Most of the G, N, and P-specific T cells were $CD4^+$ and were dull for CD45RB. By contrast, the 22K-specific cells were mostly $CD8^+$ and bright for CD45RB. F and 22K-specific lines showed RS virus and protein specific cytotoxic T cell activity against H-2^d targets, but the lines from G, N, and P primed mice did not. The F specific line contained Th cells which released an exess of IL-2 and IL-3 but little IL-4/IL-5. By contrast, the G-specific line released IL-3, IL-4 and IL-5 but little IL-2. The 22K line contained Th cells releasing IL-3.

These results indicate that the classification of helper T cells into Th1/2 may be relevant to antiviral immunity *in vivo*. Since different RS virus proteins (given in the same route and form) prime for different types of T cell, the ways in which processing and presentation determines these differences are now being explored.

O 441 A LECTIN BINDING, PROTEASE RESISTANT MYCOBACTERIAL LIGAND SPECIFICALLY ACTIVATES Vγ9⁺ HUMAN γδ T CELLS

Klaus Pfeffer, Bernd Schoel*, Nick Plesnila, Grayson B. Lipford, Sandra Kromer, Kai Deusch*, and Hermann Wagner, the Institute of Medical Microbiology and Hygiene, Tech. University Munich, *II. Dep. of Internal Medicine, Tech. University Munich, D-8000 Munich, Germany, and *the Intitute of Med. Microbiology, University of Ulm, D-7900 Ulm, Germany

Bacterial (exogenous) superantigens have been defined as bifunctional proteinacious molecules. They bind to class II major histocompatibility complex (MHC) molecules of presenting cells and engage with particular T cell receptor (TCR) Vβ gene elements, thereby activating αβ T cells in a Vβ specific fashion. In previous studies we have elucidated that γδ T cells exhibit a propensity to vigourously respond towards mycobacterial antigens. Intrigued by this finding we now analysed whether mycobacteria express a superantigen for a subset of human γδ T cells, definable by the selective use of TCR V gene elements. Here we describe that a protease resistant, low molecular weight (1-3 kDa) component of mycobacteria selectively activates γδ T cells expressing TCR-Vγ9 gene segments. Contained in mycobacterial lysates it stimulates Vγ9-TCR positive γδ T cells at a frequency of (f) 1/6. Stimulation is critically dependent on the presence of class II MHC positive presenting cells, the important structure beeing HLA-DR molecules. The fine specificity of the Vγ9 seeking mycobacterial ligand differs from the γδ T cell stimulating structures expressed on Daudi cells. In addition, the mycobacterial, Vγ9 seeking ligand is bound selectively to lectins such as UEAI, SBA and DBA. We conclude that mycobacteria contain a component which acts as a superantigen for human γδ T cells and believe that it is this property that explains the vigourous participation of γδ T cells in mycobacterial infections.

O 442 INDUCTION OF TH1- AND TH2-LIKE CELLS IN VIVO - A ROLE FOR TCR-LIGAND DENSITY?, Christiane Pfeiffer, Joseph Murray and Kim Bottomly, Department of Immunobiology, Yale University, New Haven, CT 06510

Immunization of mice with the protein antigen human collagen IV induces humoral or cell-mediated immunity in a mutually exclusive fashion, the type of immunity displayed depending on the I-A genotype of the strain of mouse immunized. Both types of response are regulated by CD4+ T cells, which secrete IL-4 or IFNγ respectively. Employing H-2 recombinant inbred mice we could demonstrate that a single dodekapeptide at a given dose induces humoral immunity, as assessed by in vitro CD4+-B cell help accompanied by IL-4 release, in H-2b mice and cell-mediated immunity, as measured by in vitro proliferation with IL-2 and IFN-γ release, in H-2s mice. To further address the question, how the differential activation of Th1- and Th2-like cells in vivo is regulated, we studied the influence of T cell receptor ligand peptide:class II density during priming. Thus the inability of H-2b mice to mount a proliferative response could be overcome by priming with a hundred- to a thousandfold higher dose of peptide than induced optimal proliferative responses in H-2s. This response was accompanied by IFNγ-release and antibody production of antibodies of IgG2a-isotype.

O 443 THE OPEN READING FRAMES IN THE 3' LTRS OF SEVERAL MOUSE MAMMARY TUMOR VIRUS INTEGRANTS ENCODE Vβ3-SPECIFIC SUPERANTIGENS. Ann M. Pullen*, John Kappler and Philippa Marrack. Howard Hughes Medical Institute at Denver, National Jewish Center, Denver, CO 80206. *Department of Immunology, University of Washington, Seattle, WA 98112.

Murine endogenous superantigens, such as the minor lymphocyte stimulating (Mls) antigens, are encoded by mouse mammary tumor viruses (MMTV) that are integrated in the genome as proviruses. These endogenous superantigens induce thymocyte deletion during tolerance induction and thus are important in molding the T cell repertoire. Mice expressing Mls-1a, -2a, or -3a on the B10.BR background have been bred and have been used to demonstrate that these Mls superantigens are encoded by MTV integrants, Mtv-7, Mtv-1 and Mtv-6 respectively.

The open reading frame in the 3' long terminal repeats of infectious, exogenous MTVs encode superantigens. We have studied the four MMTVs (1, 3, 6 and 13) that encode Vβ3-specific superantigens and have cloned and sequenced their 3' LTR open reading frames (orf). Comparisons of the sequences of these Vβ3-specific superantigens and other published sequences for MMTV orfs or viral superantigens (vSAGs), indicate that it is the carboxy-terminal region of the vSAG that interacts with the T cell receptor Vβ element.

Interestingly, the data indicate that the Mtv-1 and Mtv-6 open reading frames encode superantigens with identical amino acid sequences. This was a surprising finding, since these vSAGs differ in their ability to delete Vβ3-bearing thymocytes. The basis for these different deletion phenotypes is being investigated.

O 444 DEFECTIVE ANTIGEN PRESENTATION LEADS TO NONRESPONDER STATUS: MECHANISM OF BY-PASSING NON-RESPONSIVENESS VIA ANTI-ID, Manoj Rajadhyaksha, Michael Pride and Yasmin Thanavala, Dept. of Molec. Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263

There are a well documented series of experiments which have established that the murine response to the group a determinant of Hepatitis B surface antigen (HBsAg) is H-2 restricted. There is a hierarchy of responsiveness to HBsAg with high responder being of the H-2^q and H-2^d haplotypes, intermediate being H-2^s > H-2^b > H-2^k and nonresponders being H-2^s H-2^f. We have examined B and T cell responses to HBsAg and an internal image anti-id that mimics this antigen in a responder (H-2^d) and non-responder (H-2^f) strain. In the responder strain (Balb/c) both HBsAg and anti-id elicited HBsAg specific antibody and *in vitro* T cell proliferative responses. In contrast, only the anti-id was able to generate these responses in the B10.M nonresponder strain. This result could be the consequence of possible defects in antigen processing/presentation specific to HBsAg or due to a lacuna in the T cell repertoire. To identify the cause of the non-responsiveness, a congenic strain B10.D2 (B10.M background, Balb/c H-2 haplotype) was immunized with HBsAg. HBsAg specific antibody and T cell proliferation responses were observed, implicating an H-2 linked defect in the B10.M strain. We next examined the possibility of a defect in antigen presentation in this strain. Dot blots of spleen cells and cell lines bearing 'd' and 'f' haplotype Class II molecules were incubated with radiolabelled peptides derived from HBsAg and the anti-id (2F10). Quantitative analysis and binding kinetics will be presented, which show that the peptide generated from our anti-id 2F10 associates substantially better with 'f' haplotype Class II molecules, compared to the peptide generated from HBsAg, suggesting 'antigen-presentation' as being a defect in the nonresponder B10.M strain preventing it from making an anti-HBsAg response.

O 445 AUGMENTED EXPRESSION OF MHC CLASS I AND RETROVIRAL ANTIGENS IN MURINE MELANOMA INDUCED BY HYDROSTATIC PRESSURE AND CHEMICAL CROSSLINKING, Venkatesh Ramakrishna*, Avi Eisenthal, Yehuda Skornick and Meir Shinitzky*, Weizmann Institute of Science*, Rehovot 76100, Israel and Tel Aviv Medical Center, Ichilov Hospital, 6 Weizmann Street, Tel Aviv 64239. Israel

Most spontaneously arising tumors show a marked decrease in cell surface expression of MHC class I antigens. The B16-BL6 melanoma is a poorly immunogenic tumor and is deficient in both MHC class I and class II antigens, as well as, any demonstrable tumor specific transplantation antigen(s). Treatment of these cells *in vitro* by hydrostatic pressure (2 MPa) for 15 minutes in the presence of 20 mM adenosine 2,3'–dialdehyde (AdA), a membrane impermeant crosslinker, caused elevated expression of class I (K^b and D^b) antigens as well as that of a retroviral antigen associated with this tumor. Alteration in antigenicity appears to be well correlated with functional *in vitro* assays. ^{51}Chromium labeled B16-BL6 target cells modified by pressure and crosslinking, as compared to unmodified target cells, were lysed in much greater numbers by LAK cells and alloCTLs (anti-H-2^b), while such modification rendered the melanoma cells refractory to lysis by NK cells and H-2 disparate (anti-H-2^k) effector cells. Preliminary studies on the immunogenicity of modified and unmodified B16-BL6 in vivo suggested that prevaccination of syngeneic B6 mice protected them against a tumorigenic dose of viable unmodified melanoma cells. Prolonged survival of immune mice was associated with regression of tumor size as well as reduction in the number of pulmonary metastatic nodules. The above experimental manipulation of melanoma may offer a novel strategy in the active immunotherapy of cancer.

O 446 ACTIVATION OF MURINE EPIDERMAL T CELL HYBRIDOMAS BY SMALL MOLECULAR WEIGHT ENDOGENOUS CELL PRODUCTS. **Christopher L. Reardon, Rebecca L. O'Brien, Willi K. Born. Departments of Dermatology, University of Colorado, and Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado**

$\gamma\delta$-TCR$^+$ cell hybridomas were produced by the fusion of mouse epidermal T cell (ETC) suspensions with the cell line, BW5147$\alpha^-\beta^-$. Hybridomas, produced from cells derived from four mouse strains, C57BL/10, C57BL/6, BALB/c, and C3H/HeJ, all expressed Vγ5$^+$-T cell receptors (TCR) based on staining with FITC-labeled monoclonal antibody 536. Vγ5$^+$-TCR expression is characteristic of ETC. In an attempt to stimulate these hybridomas, endogenous small molecular weight antigens were acid extracted from the melanoma cell line, B16FO, as well as from a keratinocyte cell line, PAM212, and from the T cell hybridomas themselves, using trifluoroacetic acid (TFA) (1% v/v). The extracted materials were passed through ultrafiltration columns (Centricon 3) at 10,000 X g permitting compounds less than 3000 daltons to be collected. ETC hybridomas incubated with these endogenous products were stimulated to produce IL2 as detected with an IL2-dependent cell bioassay system. These data indicate that mouse epidermal T cell hybridomas can be stimulated by small molecular weight endogenous materials in TFA extracts from a variety of different cell types.

O 447 INDUCTION OF A CELLULAR IMMUNE RESPONSE TO A FOREIGN ANTIGEN BY A RECOMBINANT *LISTERIA MONOCYTOGENES* VACCINE. Rosana Schafer, Daniel A. Portnoy, Steve A. Brassell, and Yvonne Paterson. Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104.

Listeria monocytogenes is a gram positive, facultative, intracellular bacteria which has been used for decades to study cell mediated immunity. Studies on the cell biology of *L. monocytogenes* have shown that after initial infection of the host cell, the bacteria escapes from the phagosomal vacuole into the cytoplasm of the cell. *L. monocytogenes* then grows and spreads from cell to cell primarily in the host cytoplasm. The unique ability of *L. monocytogenes* to escape phagosomal restriction and to live in the cytoplasm suggested that this bacteria may be particularly effective as a vector for targeting the class I restricted pathway of antigen processing for cytotoxic T cell recognition. We have therefore studied the ability of recombinant *L. monocytogenes* to present a foreign antigen to a host and elicit responses to that antigen. A recombinant strain of *L. monocytogenes*, DP-L967, which stably and constitutively expresses *E. coli* ß galactosidase, was used as a live vaccine vector. BALB/c mice were immunized orally or parenterally with DP-L967 and their cellular and humoral immune responses to ß galactosidase were measured. Spleen cells taken one week after oral inoculation or 5 weeks after oral or parenteral inoculation (with a boost at 4 weeks) had strong ß galactosidase specific CTL responses. The CTL were class I restricted and Thy 1.2$^+$, CD8$^+$, TCR $\alpha\beta^+$. All mice immunized with DP-L967 had positive DTH responses to HKLM, but only 15% had a positive DTH reaction to ß galactosidase. Individual serum samples from mice immunized i.p. or i.v. were tested for antibody to ß galactosidase. Approximately 11% had low positive titers for ß galactosidase antibodies. These results demonstrate that both oral and parenteral immunization with recombinant *L. monocytogenes* results in a strong cellular immune response to the foreign protein, which is primarily a specific CD8$^+$ CTL response.

O 448 PRODUCTION OF $\gamma\delta$-T CELLS BY THE ADULT MURINE THYMUS, Katherine Kelly and Roland Scollay, The Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia.

Although $\gamma\delta$ cells only make up a small proportion of all T cells in human and mouse, it seems likely that they have particular importance in certain inflammatory conditions. Very little is known of the developmental steps these cells go through in the thymus or when they separate from the main $\alpha\beta$ lineage. In an attempt to learn more about these cells, we have examined the export of $\gamma\delta$ cells from the adult thymus, using the intrathymic injection of fluorescein isothiocyanate (FITC) to label thymocytes. Sixteen hours after intrathymic labeling, fluorescein positive cells in spleen and lymph nodes were identified and their phenotype assessed by 4 colour immunofluorescence. Amongst emigrant cells, which make up about 0.1% of lymph node cells at this time-point, about 1% were CD4$^-$CD8$^-$ and of these, about 70% expressed TCR-$\gamma\delta$. Thus the $\gamma\delta^+$ emigrants in lymph nodes occurred with a frequency of about 1 per 10^5 cells. This leads to an estimate that the adult thymus is exporting about 10^4 $\gamma\delta$ cells per day to the lymph nodes and spleen.

In the thymus $\gamma\delta$ cells make up about 0.1-0.5% of all cells, and are predominantly CD4$^-$8$^-$, but they can be divided into at least 3 subsets based on relative expression of additional markers such as Thy 1 and HSA. However, only one of these subsets is represented amongst emigrant $\gamma\delta$ cells, raising the possibility that some thymic $\gamma\delta$ cells are not exported and may be an intrathymic dead end, rather like the population of $\alpha\beta^+$ CD4$^-$8$^-$ thymocytes.

O 449 ABERRANT TRANS-REGULATION OF THE DIABETOGENIC H-2^{g7} HAPLOTYPE IN NOD MICE, D.V.Serreze, H. R. Gaskins and E. H. Leiter, The Jackson Laboratory, Bar Harbor, ME 04609

Multiple genetic components for susceptibility of NOD mice to autoimmune insulin-dependent diabetes reside within the unique *H-2g7* haplotype. The generation of ß cell autoreactive T cells in NOD mice results from dysfunctions of hematopoietically-derived APC. To determine whether APC dysfunction in NOD results from aberrant regulation of *H-2g7*, total MHC class I and class II expression (cell surface and intracellular) was evaluated by FACS in peritoneal macrophages (PM) from NOD and control strains following 6 days of culture in the presence or absence of IFNγ. As expected, IFNγ increased total MHC class I expression in PM from C57BL/KsJ (Bks) control mice. In contrast, MHC class I expression was down-regulated in IFNγ-treated PM from NOD mice. In addition, APC from NOD were found to be poor stimulators in a MLC. NOR is a diabetes-resistant control strain which shares the *H-2g7* haplotype of NOD, but contains BKs-derived genomic elements on chromosomes 2, 4, 11, and 12. Thus, the NOR strain is useful in identifying non-MHC linked modifiers of diabetes susceptibility. Total MHC class I expression increased in IFNγ-treated PM from NOR mice. This demonstrates differential trans-regulation of class I loci within the diabetogenic *H-2g7* haplotype in NOD versus diabetes-resistant NOR mice. The *H-2g7* trans-regulatory loci of NOR function in a dominant fashion since total MHC class I is up-regulated in IFNγ-treated PM from diabetes resistant (NOD x NOR)F1 hybrids. Since segregation analysis of (NOD x NOR)F2 probands indicates that Bks-derived loci on chromosome 2 impart diabetes resistance to NOR, we are investigating whether this chromosomal segment trans-regulates *H-2g7*. This may provide insight as to how a diabetogenic MHC haplotype interacts with non-MHC linked modifiers to effect disease.

O 450 INDUCTION OF PERITONEAL GAMMA/DELTA T CELLS BY MICROBIAL TOXINS. Marianne J. Skeen and H. Kirk Ziegler, Dept. of Microbiology & Immunology, Emory Univ., Atlanta, GA 30322.
We have found that γ/δ T cells represent ~12% of the CD3+ population in the murine peritoneal cavity. Injection i.p. of gram-positive or gram-negative bacteria results in an approximate doubling of this percentage and in a 5-10 fold increase in the number of peritoneal γ/δ T cells. Similar increases follow i.p. injections of bacterial toxins such as LPS and listeriolysin-o. Induction was not observed with common peritoneal inflammatory stimulants (e.g., peptone) or routinely used antigens such as ovalbumin or sheep erythrocytes. Induction of γ/δ T cells is not seen in splenic or lymph node populations. Thus, γ/δ T cells appear to be induced by microbial products and this induction is localized to the peritoneal cavity, the site of the initial infection. Peritoneal γ/δ T cells are CD4-, CD8-, and 29% Thy-1+. Consistent with an activation phenotype, all γ/δ T cells express PGP1 and 68% are CD45R lo. Immunization with *L. monocytogenes* increases Thy-1 expression to 66%+, but other markers are unchanged. Culture of peritoneal cells from immunized mice with either bacterial antigens, mitogens or superantigens results in a preferential expansion of γ/δ T cells resulting in up to 80% γ/δ T cells in the blast population. If γ/δ T cells are magnetically separated from α/β T cells, however, their proliferation is reduced. Cell mixing experiments suggest an interdependence between peritoneal α/β and γ/δ T cells for expansion in vitro. Thus, γ/δ T cells appear to require α/β T cells as either accessory cells or a cytokine source. The results suggest that the murine peritoneal cavity may provide an excellent model for cell interactions and for elucidating the role of γ/δ T cells in infectious processes. (supported by grants NIAID RO1 AI 20215 and RO1 AI25132)

O 451 TUMOR NECROSIS FACTOR AMELIORATES THE SEVERITY OF MYOSIN-INDUCED MYOCARDITIS, Stacy C. Smith, and Paul M. Allen, Division of Cardiology, Department of Internal Medicine and Department of Pathology, Washinton University School of Medicine, St. Louis, MO 63110

Tumor necrosis factor (TNF) and interferon-γ (IFN-γ) are pleuripotent cytokines and have multiple functions during the inflammatory response. Using a murine model of autoimmune myocarditis we studied the role of TNF and IFN-γ in myocardial inflammation. Neutralizing monoclonal antibodies against TNF-α/β and IFN-γ were administered to myosin-immunized A/J mice to assess the effect on the severity of myocardial inflammation. Anti-TNF treatment significantly reduced the severity of myocarditis compared to rat IgG or saline controls ($p < 0.0007$) when given prior to myosin immunization. Myosin-specific lymph node T cell proliferation studies showed no difference in the proliferative response between the anti-TNF treated mice and controls. Administration of anti-TNF to mice after myosin immunization had no effect on the severity of inflammation. This suggests that TNF is an important mediator early in the pathogenesis of myocardial inflammation in this model of myocarditis. Neutralization of IFN-γ significantly increased the severity of myocarditis compared with rat IgG and saline controls ($p < 0.0065$), suggesting that IFN-γ may function as an important regulatory cytokine early in the pathogenesis of myocardial inflammation. Understanding the functions of cytokines during the inflammatory response to myocardial injury may provide important information on possible methods to limit myocardial damage.

O 452 TREATMENT OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG) WITH SOLUBLE MHC CLASS II:ACETYLCHOLINE RECEPTOR (AChR) PEPTIDE COMPLEX, Edward G. Spack, David Passmore, Bishwajit Nag, and Somesh D. Sharma. Anergen, Inc., Redwood City, CA 94063
Membrane bound MHC class II:peptide complexes are the natural ligand of the antigen specific receptor on $CD4^+$ T cells. We tested whether administration of soluble MHC class II:AChR α peptide 100-116 complexes could alter the function of AChR-reactive T cells and thereby modulate the course of EAMG, an antibody mediated but T cell dependent autoimmune disease. EAMG was induced in Lewis rats by immunization with Torpedo californica (Tc) AChR, followed by i.v. injections of saline, MHC II alone, AChR 100-116 alone, MHC II:AChR 100-116, or MHC II:HSP 180-188 (an irrelevant peptide from a 65 kd heat shock protein) on days 1, 4, and 7. T cells from rats treated with soluble MHC II:AChR α 100-116 had a 95% reduction in proliferation to Tc AChR 100-116 and a 82% reduction in proliferation to whole Tc AChR relative to the response of T cells from rats treated with saline. In contrast, the proliferative response to PT was equivalent in rats treated with soluble MHC II:AChR α 100-116 or saline. Treatment with AChR α 100-116, MHC II, or MHC II:HSP 180-188 did not significantly affect the T cell response to any antigen.

The efficacy of the soluble MHC II:peptide complex was also studied in rats with established clinical symptoms of EAMG. Five weekly injections of 25 μg soluble MHC II:AChR α 100-116 given to rats with clinical stage 1 EAMG resulted in a survival frequency of 67% at 140 days post induction, compared to a maximum survival rate of 20% in the control treatment groups (16.7% saline, 0% Tc AChR 100-116 alone, 20 % MHC II alone, 20 % MHC II:HSP 180-188). Therefore, *in vivo* treatment with soluble MHC class II molecules complexed with peptides can induce antigen-specific unresponsiveness, offering an unique approach to autoimmune therapy.

O 453 PERIPHERAL TCR γδ V-GENE REPERTOIRE MAPS TO THE TCR LOCI AND IS INFLUENCED BY POSITIVE SELECTION. Anne I. Sperling, Randy Q. Cron, and Jeffrey A. Bluestone. The Ben May Institute, University of Chicago, Chicago, IL 60637.

The genetic influences involved in TCRγδ repertoire development are unclear. Unlike the TCRγδ populations that localize in epithelial tissues, the circulating peripheral TCRγδ V-region repertoire is quite diverse. Previous studies have shown that three TCRγ chains and at least six TCR-Vδ genes are expressed by splenic TCRγδ cells. However, the relative frequency of individual γδ subsets among genetically diverse mice has not been determined. Therefore, the repertoire of TCRγδ cells was examined using anti-TCR V-region specific mAbs against Vγ2 and Vδ4 on expanded $TCR\gamma\delta^+$ cells from total splenocytes. We found that there was variation in TCRγδ usage between different strains. Mice housed in germfree conditions were found to express similar levels of $V\delta4^+$ and $V\gamma2^+$ γδ T cells as their normally housed counterparts. These data suggests that genetic, not environment, factors are the major influence on γδ T cell repertoire development in mouse. F1 analysis between parental strains which differed in relative frequency of either $V\gamma2^+$ or $V\delta4^+$ cells revealed that high expression was genetically dominant, suggesting that positive selection events play a major role in peripheral γδ repertoire. Variations in the levels of $V\gamma2^+$ cells and $V\delta4^+$ cells was not associated with Mls or MHC haplotype. Analysis of recombinant inbred strains revealed that high Vδ4 expression mapped to the TCRγ locus, while Vγ2 expression was influenced by the TCRδ locus. Backcross analysis supported these findings. Therefore, these data demonstrate that the levels of $V\gamma2^+$ cells and of $V\delta4^+$ cells are due to the positive selection of cells utilizing particular Vγ2-Vδ and Vγ-Vδ4 pairs, and that positive selection plays a major role in TCRγδ repertoire development in the spleen.

O 454 MOLECULAR CHARACTERIZATION OF THE TCR OF SPONTANEOUSLY LYMPHOKINE SECRETING γ/δ T CELLS, *Knut Sturmhöfel, Angel Ezquerra, David Wilde, Ethan Shevach, John E. Coligan, National Institutes of Health, NIAID, Biological Resources Branch, Bethesda, MD 20892.*

The nature of the antigen(s) recognized by γ/δ T cells and the function of these cells remains to be elucidated. Amongst γ/δ T cell lines derived from the skin and hybrids derived from the thymus or skin of C3H mice, a particular subset of γ/δ T cells was found that spontaneously secrete lymphokines. We have obtained evidence that these γ/δ TCR bearing cells recognize an self antigen. Strikingly all the spontaneously lymphokine secreting cells (autosecretors) bear the same type of TCR namely the Vγ1.1Cγ4 / Vδ6Cδ1 chains. We further investigated the receptor of these autosecretors to determine structural features critical for the recognition of the self antigen in order to eventually identify the self-antigen and the antigen presenting molecule. Comparison of the amino acid sequences of the γ and δ chains of the autosecretors with those of non-spontaneously lymphokine secreting cells revealed that the joining region of the γ chain from all autosecretors was highly conserved compared to those from non-secretors. On the other hand, the Vδ6 chains of autosecretors and non-secretors were not as readily distinguishable. Gene transfection experiments were explored to further investigate the role of the individual chains for the recognition of the selfantigen. TG40, an α/β TCR loss mutant, was transfected with the Cγ4 and Vδ6 genes isolated from an autosecreting cell which resulted in the spontaneous secretion of IL-2 by the transfectant. The fact that a Vδ5 chain could be substituted for the Vδ6 chain indicated that the Vδ6 chain was not essential for self recognition. Additionally we found that a Cγ4 gene from a non-secreting hybridoma, GD81, which is almost identical to the prototypic γ chain sequence present in autosecreting cells could convey the property of spontaneous lymphokine secretion when transfected into TG40 cells.

O 455 $CD4^+$ T CELLS WHICH CARRY PASSIVELY ACQUIRED CLASS II MHC MOLECULES ON THEIR SURFACE CAN SPECIFICALLY STIMULATE OTHER $CD4^+$ T CELLS. Satoru Suzuki and Mitsuyoshi K. Saizawa. Max Planck Institute for Immunobiology, Freiburg im Breisgau, D-7800, Germany.

As reported in our accompanying paper(T cell antigen receptor mediated passive acquisition of MHC antigens on mouse T cells), T cells can acquire extrinsic MHC molecules on their surface when they are mixed with relevant MHC bearing cells. To analyse the roles of passively acquired MHC molecules in detail, $CD4^+$ T cells carrying extrinsic class II MHC molecules were tested for their sensitivity to the stimulation by 1) syngeneic or allogeneic class II bearing APC with or without antigen or 2) anti-TCR mAbs. Whereas the stimulation by syngeneic cells plus antigen or by allogeneic cells was only slightly augmented after acquisition of MHC molecules, the stimulation by anti-Vβ but not anti-idiotypic anti-TCR mAbs was significantly augmented. In addition, syngeneic class II carrying T cells can be triggered by the antigen solution without APC, provided that it contains naturally digested peptide fragments. It was also tested whether acquired class II molecules can be recognized by other $CD4^+$ T cells. Syngeneic or allogeneic class II molecules passively transfered onto the T cell surface are in turn recognized by other $CD4^+$ T cells and can stimulate these T cells to proliferate, thus documenting the fully functional properties of those transfered MHC molecules. Our present observations may indicate a novel short-cut pathway in the antigen presentation possibly operating, for example, at the local inflamatory legions.

O 456 γδ T CELL DEVELOPMENT IN FETAL THYMUS: EVIDENCE FOR NEGATIVE AND POSITIVE SELECTION. Yoichi Tatsumi, Dominick Deluca*, Randy Q. Cron and Jeffrey A. Bluestone, Committee on Immunology, The Ben May Institute, University of Chicago, Chicago, IL 60637 and *The Department of Microbiology and Immunology, University of Arizona, Tucson, Az 85721

The development of TCRγδ cells in fetal thymus was analyzed. First, fetal thymus organ culture system (FTOC), a well known model used for the study of TCRαß cell development, was employed to study TCRγδ cell development. It was found that different waves of TCRγδ cells develop from precursors within the fetal thymus at the time of in vitro culture. In order to study the effect of TCR/antigen interaction in the development of TCRγδ cells, anti-TCR mAbs were added to the FTOC. Anti-CD3 mAb added to day 5 and 12 FTOC inhibited the development of TCRγδ cells, especially $V\gamma4^+$ cells. Anti-TCRγδ mAb also decreased the same TCRγδ subset. The primary mechanism of inhibition of TCRγδ cells by anti-TCR mAb was activation induced programmed cell death, as previously shown for developing TCRαß cells. The clonal deletion of TCRγδ cells induced by addition of anti-CD3 mAb was reversed by Cyclosporin A (CsA), which is known to rescue TCRαß cells from activation induced programmed cell death. Furthermore, preliminary experiments suggest that CsA can inhibit positive selection of TCRγδ cells. TCRγδ cell development was inhibited when CsA was added to deoxyguanosine treated fetal thymus cultured with fetal liver cells or when CsA was injected to early gestation stage pregnant mice prior to the development of TCRγδ cells. These results provide evidence that FTOC mimic normal TCRγδ cell development, and both negative and positive selection based on T cell receptor occupancy may play an important role in determining the TCRγδ repertoire.

O 457 BIOCHEMICAL SPECIFICITY OF AN MHC CLASS I MOLECULE USING PEPTIDE ANALOGUES, Jatin M. Vyas, Said S. Shawar, John R. Rodgers, Richard G. Cook, and Robert R. Rich, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

The maternally transmitted antigen (Mta) is a cell surface product of three gene products: Hmt, a class I MHC heavy chain, β_2-microglobulin, and Mtf, the N-terminus of the mitochondrially-encoded ND1 subunit of NADH dehydrogenase. This class I molecule has been shown to be an N-formyl peptide receptor. Although the N-formyl moiety is necessary for binding to Hmt, it is not sufficient. We proposed that the R group of the amino acid in position 1 plays a key role in binding to Hmt. To test this hypothesis, analogues differing in size and stereospecificity of the R group were synthesized. Substitutions with other hydrophobic amino acids such as N-formyl phenylalanine and N-formyl valine had no significant effect on the ability of the peptides to sensitize target cells (a,β) to $Mta^{a,\alpha}$ specific CTLs. In contrast, the non-substituted, formylated and acetylated forms of the glycine analogue of Mtf^{α} peptide bound equally to Hmt in a peptide competition assay. Moreover, the alanine analogue bound in an N-formyl dependent manner. In order to determine the limitations of the putative N-formyl pocket, Mtf^{α} analogues were constructed incorporating D-isomer amino acids. When formylated D-alanine or D-methionine replaced the native methionine, these peptide derivatives did not show significant binding to Hmt^{a}. Therefore, the presence of a space-filling R group (greater than hydrogen) is necessary for an antigenic peptide to bind Hmt in an N-formyl dependent manner. Additionally, the ability of Hmt^{a} to discriminate between the optical forms of methionine and alanine demonstrates that this N-formyl pocket is stereospecific in its ability to bind peptide. Thus, we have defined three requirements for peptide binding to Hmt: an N-formyl moiety at the amino terminus of the peptide, a space-filling R group at position one to main this N-formyl specificity, and the correct stereoismoer of the first amino acid.

O 458 REGULATION OF TL GENE EXPRESSION BY IFNγ. I-Ming Wang, Vidya Mehta, and Richard G. Cook, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030. TL antigens are encoded by genes in the MHC of the mouse chromosome 17 telomeric to the H-2K, D, L and Q regions. Although their general protein and genomic structures are similar to the other classical class I antigens, they show more restricted tissue distribution and polymorphism. The present study seeks to identify cis- and trans-acting elements which are involved in the regulation of TL gene expression. Our data show that TL is upregulated by IFNγ more significantly than the H-2 class I antigens; however, it responds poorly to IFNα/β, TNFα, and dexamethasone. When added together, IFNα/β even reduce the enhancing effect of IFNγ. Comparison of the promoter regions between TL and H-2 genes shows no apparent homology except for the presence of an interferon-responsive-element (IRE). Transfection assays confirm that TLcat constructs with the IRE do respond to IFNγ and deletion of the IRE abrogates the response. In addition, a negative regulatory element (NRE) is located within 0.5-1.4 Kb upstream of a TL gene $T18^d$; deletion of this NRE increases CAT activity 3-10 fold. Northern blot analysis indicates that IRF-1 and ICSBP, transcription factors that bind to IRE, are upregulated by IFNγ in the T tumor cell lines. Co-transfection of ICSBP with TLcat constructs decreases CAT activity 2-3 fold. Preliminary band shift data suggest that nuclear extracts derived from TL^+ and TL^- cells give different retardation patterns when a $T18^d$ promoter region probe is used.

O 459 ANALYSIS OF Qa-1.2 EXPRESSION IN Qdm^k VERSUS Qdm^+ MOUSE STRAINS. Paula R. Wolf and Richard G. Cook. Department of Microbiology and Immunology, Baylor College of Medicine, Houston TX 77030.

Biochemical analysis of the molecules reactive with Qa-1 alloantisera and antisera directed against a peptide deduced from the cytoplasmic domain of the $T23^b$ gene (gene 37) sequence demonstrated that $T23^b$, a low polymorphic ubiquitously expressed class I TL region gene encodes Qa-1.2 of $Qa\text{-}1^b$ strains but not Qa-1.1 of $Qa\text{-}1^a$ strains. Immunoprecipitation of Qa-1.2 with the anti-37 peptide sera results in the isolation of the expected 48K protein as well as a slightly higher mol wt (50K) species not detected with anti-Qa-1.2 sera. This species was shown to result from a modification of N-linked oligosaccharides on Qa-1.2. The inability to detect this 50K form with the alloanti-sera most likely results from a modification that alters folding of the molecule or sterically blocks recognition of allodetermi-nants by the anti-Qa-1.2 sera. Analysis of Qa-1.2 under reduc-ing and nonreducing conditions demonstrates that the 50K Qa-1.2 molecule is distinct from the 48K Qa-1.2 in its ability to form interchain disulfide bonds. The position at which the protein falls off the diagonal suggests that the 50K form may dimerize on the cell surface. To further investigate the two different mol wt forms, we have examined the role of Qdm (Qa-1 determinant modifier gene) in the regulation of Qa-1.2 expression. By SDS-PAGE analysis, $Qa\text{-}1^b/Qdm^k$ strains expressed only the 48K mol wt form whereas $Qa\text{-}1^b/Qdm^+$ mice expressed both the 50K and 48K mol wt forms. Thus, the presence of the 50K form of Qa-1.2 corre-lates with the Qdm^+ phenotype, suggesting that Qdm may regulate the expression of the higher mol wt species. Transfection experiments to compare the expression pattern of the two mol wt forms of Qa-1.2 in Qdm^k versus Qdm^+ strains and studies using $(Qa\text{-}1^a/Qdm^+ \times Qa\text{-}1^b/Qdm^k)F_1$ mice to examine Qdm transdominance are currently underway.

Late Abstracts

MOLECULAR ANALYSIS OF TCR (αβ) OF HUMAN T-CELL CLONES SPECIFIC FOR A TETANUS TOXIN UNIVERALLY IMMUNOGENIC PEPTIDE. Oreste Acuto*, Brigitte Boitel*, Myriam Ermonval*, Paola Panina§, Roy, A. Mariuzza+ and Antonio Lanzavecchia§. *Laboratory of Molecular Immunology and +Laboratory of Structural Immunology, Pasteur Institute, Paris 75015, France and §Basel Institute for Immunology, Basel CH-4031, Switzerland.

To investigate the structural basis of the T-cell response to defined peptide/MHC class II complexes, we established a large panel of T-cell clones (n=58) from donors of different HLA-DR haplotype and reactive with a tetanus toxin-derived peptide (tt830-844) recognized in association with most DR molecules ("universal peptide"). We found preferential usage of a particular Vβ gene segment, Vβ2, in three individuals studied (64%, n=58), irrespective of whether the peptide was presented by DR5, DR6 or DR4 alleles, demonstrating that shared MHC class II antigens are not required for shared Vβ gene usage by TCRs specific for this peptide. On the contrary, Vα gene usage, determined for 20 of these clones from two donors was more heterogeneous as Vα segments belonging to five distinct families were able to pair with Vβ2 segments to form anti-tt830-844/DR specificities. However, the most frequently used Vα families displaied significant higher degree of similarity to each other than to most Vα family members. Surprisingly, the amino acid sequences of theVα and Vβ putative CDR3 of these TCRs showed no apparent conservation in either composition or length, even for TCR restricted to the same DR allele and utilizing the same Vα and Vβ gene segments. These data suggest that Vβ2 recognizes a structural feature common to the complex formed by this peptide and DR4, 5 and 6. In contrast, the putative CDR3 may not play a major role in recognition of these complexes and so are not structurally constrained. Alternatively, the observed junctional sequence diversity may reflect a variability in the conformation of the peptide side chains recognized by the TCR. Studies are underway to verify these hypothesis.

INTRACELLULAR SORTING OF THE MHC CLASS II MOLECULES IN VARIOUS CELL LINES.

Oddmund Bakke*, Anne Simonsen*, Frank Momburg#, Bernhard Dobberstein^, Günther Hämmerling#, . *Dept. of Biology, University of Oslo, ^EMBL, Heidelberg, #Deutches Krebsforschungszentrum, Heidelberg

The polymorphic MHC class II α and β chains assosiate with the invariant chain (Ii) in the endoplasmic reticulum (ER), and serve to present antigenic peptides to T-helper cells. We have recently shown that Ii contains a sorting signal for endosomes within the cytoplasmic tail and the MHC class II molecules and Ii have been co-localized in endocytic vesicles. In the present study we have transfected MHC class II molecules (human and murine) into various cell lines alone or supertransfected with the human Ii or a mutant Ii (Δ20Ii), lacking the endosomal sorting signal. By immunofluorescence we observed the intracellular distibution of the class II molecules alone or together with Ii. In the fibroblastic cell lines transfected with the MHC class II molecules alone these molecules are localized in vesicles (both early and late endosomes), whereas a full length invariant chain is needed for sorting to endosomes in HeLa cells. This indicate that the sorting machinery for these molecules is cell line dependent. Further, the mutated Ii (Δ20Ii) could not redirect the complex of Ii and αβ indicating that the cytoplasmic tail of Ii is responsible for the endosomal sorting of the MHC class II molecules in Hela cells.

TRANSFERRIN RECEPTOR MEDIATES UPTAKE AND PRESENTATION OF HEPATITIS B ENVELOPE ANTIGEN BY T LYMPHOCYTES. Alessandra Franco*#, Marino Paroli*#, Ugo Testa§, Cesare Peschle§, Francesco Balsano#, & Vincenzo Barnaba*#. *Lab. Immunologia, #Fondazione "Andrea Cesalpino", I Clinica Medica, Università "La Sapienza"; §Lab. Ematologia ed Oncologia, Istituto Superiore di Sanità, 00161 Roma, Italy.

Human activated T lymphocytes expressing class II molecules overcome their inability of antigen presentation only for those complex antigens that bind to their own surface receptors, and thus can be captured, internalized, and processed through class II major histocompatibility complex (MHC) processing pathway. In the light of this, here we use for the first time the antigen-presenting T cell system as a tool to identify a viral receptor, as that used by hepatitis B virus (HBV) to enter cells. In deed, we demonstrate that both CD4+ and CD8+ T clones can process and present HB envelope antigen (HBenvAg) to class II-restricted cytotoxic T lymphocytes (class II-CTLs) and that CD71 transferrin receptor (TfR) is involved in the efficient HBenvAg uptake by T cells. Since TfR is also expressed on hepatocytes, it might represent a portal of cellular entry for HBV infection. Furthermore, we suggest that the system of antigen presentation by T cells should serve as a model to study lympho cyte receptors used by viruses.

PROCESSING AND PRESENTATION OF SELF-MHC CLASS I PEPTIDES.

Gilles Benichou, Eugenia Fedoseyeva, Clifford A. Olson, Mario Geysen*, Minnie McMillan# and Eli E. Sercarz. Department of Microbiology and Molecular Genetics, University of California Los Angeles, CA 90024., *Coselco Mimotopes Pty Ltd, Clayton, Victoria 3168, Australia., #Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033.

It is now widely accepted that self-proteins are continuously processed and presented as peptides in association with self-MHC molecules for interaction with T cells. We have recently shown that T cell proliferative responses towards certain polymorphic regions of self-MHC class I and class II molecules can be detected. In the class I model, the self- peptides, Ld 61-85 and Dd 61-85 (at a corresponding portion of the α1 helix) were shown to bind with high affinity to self-class II restriction elements. Interestingly, only the peptide Ld 61-85 was capable of inducing in vivo lymph node T cell proliferation upon immunization of syngeneic B10.A or BALB/c mice. Dd 61-85 failed to stimulate a T cell response, presumably because it had been presented to induce T cell deletion during thymic selection (as confirmed by its immunogenicity in B10.BR and CBA mice). Alternatively, Ld 61-85, despite its high affinity for self Ia molecules, was not presented efficiently enough to ensure T cell tolerance possibly due to incomplete processing or the low level of expression of Ld molecules as compared to Dd. Our results in the class I model indicate that self-determinants can be divided into two main categories : 1) *the dominant self:* the self-determinants which are efficiently processed and presented and make impact on the T cell repertoire by inducing deletion/inactivation of the autoreactive T cells 2) *the cryptic self:* the self-determinants which were presented efficiently enough to ensure T cell tolerance during development (despite their high binding affinity for self-MHC), due presumably to incomplete processing. Here, we investigated the fine specificity of the T cell responses to the immunogenic class I peptide Ld 61-85 and analysed the molecular basis for dominance versus crypticity, studying class II-restricted responses to these class I determinants. Our results indicate that 1) different overlapping self-determinants can coexist within the same sequence and compete for stimulating autoreactive T cells 2) certain amino acid positions are crucial for crypticity versus dominance of a self-determinant 3) the processing and presentation of self-proteins are different in the lymph nodes and in the spleen.

ANTIGEN PROCESSING BY ENDOSOMAL PROTEASES DETERMINES WHICH SITES OF SPERM-WHALE MYOGLOBIN ARE EVENTUALLY RECOGNIZED BY T CELLS

Claire J.P. Boog°, Jacqueline Boon°, Alfons C.M. Van der Drift•, Josée P.A. Wagenaar°, Annemieke M. Boots° and Johannes M. Van Noort•, Department of Infectious Diseases and Immunology°, University of Utrecht, Yalelaan 1 3508 TD Utrecht and TNO Medical Biological Laboratory•, Rijswijk

This study reports an identification of the major processing products of an exogenous protein antigen, *viz.* sperm-whale myoglobin, as obtained after cell-free processing with partially purified macrophage endosomes. It is demonstrated that such a system yields fragments that are indistinguishable by high performance liquid chromatography analysis from those generated after uptake of myoglobin inside live macrophages.

The concerted action of the endosomal proteases cathepsin D and cathepsin B can account for nearly all cleavages observed. Cathepsin D appears to be mainly responsible for the initial cleavage of myoglobin, while cathepsin B catalyzes the C-terminal trimming of initially released fragments. The fragments released by cathepsin D contain most, if not all major epitopes for murine myoglobin- specific helper T cells.

Interestingly each known T cell epitope of myoglobin is located at the very N terminus of a different myoglobin fragment released upon processing. In order to explain this correspondence, noted also in several other protein antigens, a structural relationship is proposed between antigen processing by cathepsin D and antigen recognition by major histocompatibility complex (MHC) class II products. As is demonstrated here, this relationship may be used as a predictive tool for the identification of MHC-binding sequences as well as of T cell epitopes in their naturally occurring form.

REQUIREMENTS FOR ACTIVATION OF CLASS II-RESTRICTED T-CELL CLONES SPECIFIC FOR PEPTIDES OF INFLUENZA VIRUS, Lorena E. Brown, Aude M. Fahrer, H. Mario Geysen, David O. White and David C. Jackson, Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia.

The sequence 307-KYVKQNTLKL-316 was identified as the determinant recognized by a panel of T-cell clones raised against a 24-residue peptide from influenza virus hemagglutinin. Particular clones showed partial reactivity with the smaller peptides KYVKQNTLK or YVKQNTLK. Clone 4.51, which responded to the native peptide at concentrations 100-1000 fold less than the others, had as its minimal determinant the sequence VKQNTLKL. Peptide analogs, in which each residue within the minimal determinant of 4.51 was replaced in turn by every other amino acid, revealed either an absolute requirement for the native residue or very limited replaceability at 7 of the 8 positions. Only the N-terminal V could be replaced with almost any other amino acid without loss of reactivity; in fact, substitution at this position with residues containing bulky side-groups enhanced the response. The reactivity of the clone with analogs of the longer peptide KYVKQNTLKL revealed a quite different pattern of replaceability for certain residues; in particular, the requirement for K at position 310 was no longer apparent. Extension of a minimal T-cell determinant therefore allows relaxation of the requirement for particular residues within the determinant. When 4 other clones were examined for their reactivity with the analogs of peptide KYVKQNTLKL, each showed a distinctive pattern of residues critical for T-cell stimulation, indicating that the peptide is viewed by the different T-cell receptors in very different ways. Some residues were irreplaceable for the stimulation of certain clones but almost totally replaceable for others, suggesting their role may be primarily as TCR-contact or spacer residues respectively. No one residue within the determinant showed the same pattern of replaceability for all 5 clones, which would have been suggestive of a role in MHC interaction. However, limited replaceability was always observed at positions 312-315 but with different amino acids.

THE INTERACTION BETWEEN IMMUNOGENIC PEPTIDES AND MHC CLASS II IS OPTIMAL AT ACIDIC pH. Søren Buus, Bodil Laub Petersen, Anette Stryhn Hansen, and Søren Mouritsen. Institute for Experimental Immunology, Copenhagen.

We have examined the pH dependence of a number of peptide-MHC class II interactions known to be immunogenic both in a direct biochemical binding assay and in a functional antigen presentation assay. The effect of pH on peptide-MHC class II interaction was quantitatively quite significant, however, in no case did the specificity of the peptide-MHC class II interaction change. For all combinations optimal peptide binding was observed at acidic pH around 4 to 5 frequently with a well defined bell shaped pH dependence curve. In several cases very little, if any, binding could be detected at pH 7.0 and above, whereas the rate of association was more than 15 times greater at pH 5 than at pH 7. The equilibrium dissociation constants are lower reflecting the overall increased affinity of peptide-MHC class II interaction at pH 5. The findings of the direct binding assay were compared with those of a more physiological antigen presenting assay. In this assay prefixed antigen presenting cells were pulsed with antigen at various pH's prior to stimulating specific T cell hybridomas. Again a very pronounced pH dependence was observed with the optimal pH being around pH 5.

Taken together, both biochemical and functional experiments demonstrate that a quite acidic environment is optimal for peptide-MHC interactions. It must be kept in mind that our panel of peptide-MHC interactions are heavily skewed towards known immunogenic combinations and that our data thus might reflect the conditions under which the immunogenic peptide-MHC class II complex were formed during the initial immune induction. Our data indicates that the environment of interaction in vivo is acidic and implicates intracellular organelles, such as the most acidic endosomes or the lysosomes, as being the site where peptide meets MHC class II. This is highlighted by the fact that for many of the combinations examined, binding was observed only at acidic conditions.

A DETERMINANT CAPTURE MODEL IN THE NOD MOUSE RESPONSIVE TO HEL. Hong-kui Deng, Michael Clare-Salzler*, H. Mario Geysen# and Eli Sercarz. Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024-1489, *Department of Medicine (Endocrinology), University of California, Los Angeles, CA 90024 and #Chiron Mimotopes Pty. Ltd., Clayton, Victoria 3168, Australia.

The NOD mouse spontaneously contracts insulitis and diabetes during development. Interestingly, NOD animals transgenic for an additional unrelated MHC molecule, such as E^d_α or $A^k_2A^k_1$, are protected from disease. In trying to understand this protection, we have been studying the response of NOD mice to hen eggwhite lysozyme, HEL, and then observing the effect of adding in other MHC molecules (among other structures) through mating to produce F1 progeny. NOD mice display a proliferative response to two HEL determinants, one of which is dominant to the othe.. After mating the NOD with BALB/c, A^d, E^d, E^{NOD} are presumably the new molecules available in the F1 in addition to A^{NOD}. We could then study the effect of the very dominant E^d-restricted response to HEL (106-116) on the A^{NOD} response to its two HEL determinants in the F1. In fact, response to the dominant NOD determinant is retained but the F1 response to the subdominant NOD determinant disappears. These results can be interpreted to support a 'determinant capture' model of antigen presentation in which certain responses are lost in the presence of competitive restriction elements. This explanation would then fit the diabetes model where preferential responses to the MHC transgene product would protect the mouse from response to some subdominant self-determinants. (Supported by American Cancer Society IM-626 and NIH grant AI-28419.)

NON-COGNATE B CELL ACCESSORY FUNCTION DEVELOPS AFTER HLA AND ADHESION MOLECULES ARE EXPRESSED ON THEIR SURFACE, Dieter Dennig and Richard J. O'Reilly, Department of Bone Marrow Transplantation, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

For T cell proliferation, two signaling pathways are needed. One pathway involves the cognate antigen-mediated TCR/HLA interaction, the other uses the non-cognate cellular interaction of the responding T cell with an accessory cell. Accessory function is mediated by molecules for cell adhesion and co-stimulation. In this study, we investigated whether B cells could mediate non-cognate accessory function independent of the cognate TCR/HLA class II interaction. We also determined whether pre-B cells which express surface HLA class II and adhesion molecules can provide sufficient non-cognate signaling to allow T cell proliferation in response to the superantigens TSST-1 or SEB. We further investigated whether non-cognate accessory function could be enhanced in pre-B cells and whether new expression of surface molecules were associated with enhanced accessory function. Our results demonstrate that non-cognate B cell accessory function necessary for T cell proliferation in response to superantigens can be mediated independent of HLA class II expression. Furthermore, pre-B cells expressing HLA class II and adhesion molecules, are deficient in their non-cognate accessory function, but are efficient accessory cells after stimulation with PMA. Accompanied with this enhanced accessory function is a new surface expression of the co-stimulatory molecule BB-1. As published by L. Nadler et.al., the mRNA of the BB-1 homolog B7 was found in anti-Ig stimulated B cells only.

BLOCKING OF COLLAGEN TYPE II (CII) SPECIFIC HUMAN T CELLS BY AUTOANTIBODIES TO CII: INTERFERENCE WITH ANTIGEN PROCESSING? Frank Emmrich, Jan Ten Feh, Anette Beck-Sickinger', Siegbert Jung', Klaus von der Mark, Harald Burkhardt, IIB Zurich, CH, **Charité, FRG, Max-Planck Society, Clinical Research Units for Rheumatology, D-8520 Erlangen, FRG

Collagen type II (CII) is a possible target structure for T and B cell autoimmunity in the pathogenesis of rheumatoid arthritis. We have established a human class I cell clone restricted to HLA DR7 that recognizes an epitope within amino acid residue 272 - 295 of the triple helical region of CII. Six mouse monoclonal antibodies (mAb) to CII which seem to be disease related in the rodent CII-induced arthritis model an interesting observation was made. One of the mAb (C1) inhibited the proliferative T cell response to CII very efficiently while the other (D5) did not. The mAb C1 binds to an epitope within amino acid residue 277 - 288 of CII. A direct effect on T cells or the antigen presenting cells could be excluded. Together with the experimental findings is the idea that antibody C1 would protect a collagen sequence sensitive to regular endosomal degradation thereby giving rise to a peptide which is associated to HLA molecules and competes with the stimulatory peptide. The putative inhibitory peptide was synthesized and is in fact capable of inhibiting a CII-mediated T cell response as predicted by the concept. Furthermore, by testing a panel of human monoclonal antibodies established from patients with different autoimmune diseases an IgM antibody was identified that competes with the mAB C1 for CII binding. Studies are in progress to map precisely the epitope of the human antibody and to investigate whether this antibody is also able to inhibit CII specific T cell proliferation.

SUPERANTIGEN ACTIVATION OF T-CELLS IN THE ABSENCE OF CLASS II MHC. J.M.Green, L Turka and C.B.Thompson. Howard Hughes Medical Institute, Ann Arbor, MI USA.
The staphylococcal enterotoxins A and B (SEA and SEB) are superantigens that stimulate T-cells by binding to the V-beta chain on the T-cell receptor. Superantigens also bind to class II MHC and the interaction with MHC is felt to be necessary for T-cell activation. We examined the ability of SEA and SEB to activate human T-cells in the absence of MHC. Purified T-cells were treated with graded concentrations of either SEA or SEB in the presence or absence of a stimulatory antibody to CD28 (mAb 9.3) and/or a blocking antibody to class II MHC (L243). The absence of accessory cells was demonstrated by the lack of proliferation to PHA, an accessory cell dependent stimulus. Incorporation of ^{3}H-thymidine (^{3}H-TdR) was determined. Stimulation of the CD28 pathway augmented T-cell proliferation that was independent of class II expression. L243 did inhibit the proliferation of mixed peripheral blood mononuclear cells in a dose dependent manner. Acridine orange staining for cell cycle analysis and surface staining for IL-2 receptor expression demonstrated that enterotoxin alone induced entry into G1 without progression to S-phase. Addition of mAb 9.3 increased the percentage of cells entering G1 and induced progression to S-phase. Cyclosporin A suppressed enterotoxin-induced proliferation. These data demonstrate that T-cells can be activated by SEA or SEB in the absence of class II MHC, and that the CD28 pathway is capable of providing costimulatory activity. The significance of this in the in-vivo response to superantigen remains to be determined.

A VIRAL PEPTIDE CAN MIMICK AN ENDOGENOUS PEPTIDE FOR ALLORECOGNITION OF A CLASS I MHC PRODUCT, Annick Guimezanes and Anne-Marie Schmitt-Verhust, Centre d'Immunologie INSERM-CNRS de Marseille Luminy, Case 906, 13288 Marseille Cedex 9, France.
The question of what is really seen by alloreactive T cells was studied with cytotoxic clones (CTL) which recognize H-2K^{b} molecules on any cell of K^{b} haplotype, but are affected in their recognition of mutant haplotypes (H-2K^{b}m) when the mutations modify the peptide binding site. The different possibilities for allorecognition of H-2K^{b} by T cells involve: recognition of the K^{b} structure alone, of the K^{b} molecules associated with any peptide which stabilizes K^{b} expression at the cell surface, of a defined peptide in the context of K^{b} or of a particular peptide-induced conformation of K^{b}. These hypotheses were tested using the mutant tumor line RMA-S which expresses at the cell surface MHC class I molecules devoid of peptides that can be loaded and stabilized with exogenously added peptides binding to H-2^{b} molecules. K^{b}-specific recognition of the RMA-S cells by the cytolytic T lymphocyte clone was restored by peptide extracts from endogenous proteins which only minimally stabilized H-2K^{b} on the surface of RMA-S cells and with one out of three defined peptides which greatly enhance the expression of H-2K^{b}, indicating the requirement for a specific peptide on a limited number of H-2K^{b} molecules. The results presented indicate that endogenous peptides are specifically recognized by an alloreactive CTL clone and that a viral motif associated with K^{b} can mimick peptides from endogenous proteins.

AMINOPEPTIDASE N AND ANTIGEN PROCESSING AT THE SURFACE OF ANTIGEN PRESENTING CELLS.
Anette Stryhn Hansen, Søren Buus, Joan Rhodes Madsen, Søren Mouritsen, Ove Norén, Ole Werdelin. University of Copenhagen.

Protein antigens are processed (fragmented) by antigen presenting cell (APC) and the resulting peptide fragments bound to MHC class II molecules. Peptides protruding from the MHC binding groove might be further processed by ecto-peptidases. We have asked whether the membrane bound ecto-peptidase, Aminopeptidase N (AP_N), resides on the surface of APC and if so, whether it performs such antigen processing.

FACS analysis demonstrated a correlation between AP_N expression and MHC class II on various populations of APC's (monocytes, macrophages, B cells, peritoneal exudate cells and dendritic cells). In contrast, various T cell population did not express AP_N.

Affinity purified mouse AP_N completely degraded the antigenic peptide hen egg lysozyme peptide (HEL) 46-61. In contrast, the peptide once bound to its MHC class II restriction element, I-A^{k}, was fully protected against complete degradation. However, it was not protected against partial degradation since in some experiments intermediary degradation products, still bound to the MHC class II molecule, were detected. We interpret this to mean that the N-terminus of HEL 46-61 protrudes from the MHC binding groove and is vulnerable to AP_N attack.

The action of surface proteases was demonstrated in a functional assay using the T hybridomas, 2A11 and 3A9. Both are HEL46-61 specific and I-A^{k} restricted. 2A11 preferentially recognises short versions of the peptide, while 3A9 preferentially recognises longer versions. Prefixed APC were pulsed with HEL 46-61 in the presence or absence of a cocktail of protease inhibitors. The stimulation of 2A11 was markedly reduced when protease inhibitors were included during the pulse, whereas the stimulation of 3A9 was increased.

Thus, the major findings are: 1) AP_N is expressed by APC's, 2) AP_N can partially degrade MHC class II bound peptides, and 3) such "final" processing might affect the immune response. This, and the striking correlation between AP_N and MHC class II, suggest a general immunological role for AP_N.

STIMULATION OF THE CD28 PATHWAY PREVENTS INDUCTION OF ANTIGEN-ACTIVATED T CELL HYPORESPONSIVENESS. Estelle S. Harris[1], Tullia Lindsten[1], Laurence A. Turka[1], Carl H. June[2],and Craig B. Thompson[1]. [1]Howard Hughes Medical Institute, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI and [2]Naval Medical Research Center, Bethesda, MD
Discrimination between self and nonself antigens is a vital function of the immune system. Current models propose that the discrimination between self and foreign antigens involves a two signal system, one through the antigen-specific T cell receptor (TCR)/CD3 and a second through an activationally-induced co-stimulatory signal present on antigen-presenting cells. Antigen stimulation in the absence of this co-stimulatory signal has been suggested to render T helper cells hyporesponsive to restimulation by antigen. In contrast, stimulation in the presence of a co-stimulatory signal induces a proliferative response in T lymphocytes upon antigen restimulation. Based on our previous work, the CD28 receptor is a candidate molecule for the co-stimulatory signal involved in T cell activation. In order to examine this possibility, we have developed an assay system which evaluates the ability of T cells to respond to stimulation through the TCR/CD3 complex after an initial activation in the presence or absence of CD28 receptor costimulation. We exposed purified human CD28+ T cells to immobilized anti-CD3 antibodies in the presence or absence of anti-CD28 in a primary culture system for 48 hours. The cells were then washed to remove stimulatory monoclonal antibodies, rested overnight, and placed in a 72 hour secondary culture in the presence or absence of immobilized anti-CD3 antibodies. The results of the proliferation assay showed that T cells stimulated through the TCR/CD3 complex alone in the primary culture demonstrated a dramatic reduction in the proliferative response upon secondary challenge with immobilized anti-CD3. In contrast, T cells exposed to both anti-CD3 and anti-CD28 antibodies within the first six hours of culture showed significant enhancement in their proliferative response upon rechallenge with anti-CD3 antibody. Adding anti-CD28 antibody after six hours did not protect against the induction of hyporesponsiveness. In conclusion, the protective effect of anti-CD28 monoclonal antibodies when added during the first six hours of stimulation through the TCR/CD3 complex is consistent with the CD28 pathway providing a co-stimulatory signal for T cell activation. Further studies are underway to further elucidate mechanisms involved in preventing tolerance formation via the CD28 pathway.

VIRAL GENES BLOCK THE TRANSIT OF PEPTIDE FILLED MHC CLASS I COMPLEXES TO THE CELL SURFACE

Hartmut Hengel, Margarita Del Val, Thomas Ruppert, Hans Häcker and Ulrich H. Koszinowski, Department of Virology, University of Ulm, P.O.Box 4066, D-7900 Ulm, Federal Republic of Germany

The phosphoprotein pp89, an immunodominant viral antigen of murine cytomegalovirus (MCMV), is efficiently presented to MHC class I restricted T cells during the immediate-early phase of CMV infection, but is prevented by the subsequent expression of viral early genes without any alteration of protein synthesis and stability (Del Val et. al. Cell 58:305, 1989). This regulation is not restricted to pp89 since presentation of an unrelated antigen, ß-galactosidase, is subjected to the same principle when expressed from a recombinant cytomegalovirus under the same temporal control. Because endogenously processed antigenic peptide (Del Val et. al. Cell 66:1145, 1991) could be isolated in undiminished amounts from early-phase infected cells which were not recognized by CTL, a quantitative or qualitative failure in antigen processing, transport into the ER and binding to MHC was excluded. Also, the rate of MHC class I heavy chain biosynthesis and the subsequent association with $ß_2$microglobulin were found to be unaffected. Thus, the trimolecular complex of MHC heavy chain, light chain and peptide was formed. In the presence of early phase proteins, however, MHC complexes were prevented from maturation to endoglycosidase H resistant forms, indicative for a blockade in transit through the medial golgi compartment. This interference with the transport of MHC molecules resulted in a rapid decline of their cell surface expression during the course of CMV early phase. Therefore our results demonstrate a novel molecular mechanism by which CMV can escape cellular immune control.

THE INVARIANT CHAIN ENHANCES MHC CLASS II RESTRICTED PRESENTATION OF AN ENDOGENOUS ANTIGENS RETAINED IN THE ER.

Martine Humbert, *Patrick Bertolino, *F. Forquet, *Chantal Rabourdin-Combe, *Denis Gerlier, Jean Davoust and Jean Salamero. Centre d'immunologie INSERM-CNRS de Marseille Luminy, 13288 Marseille, *Laboratoire d'immunobiologie moleculaire CNRS-ENS Lyon, 69364 Lyon, France.

Transport and intracellular distribution of class I and class II molecules is presumed to reflect the functional dichotomy in presentation of antigen from endogenous and exogenous origin respectively. Invariant chain (Ii) is suspected to be involved in this seggregation by protecting the binding site of class II molecules and/or by targeting class II molecules in a compartment where they will meet exogenously derived peptides. Using stable L transfectants expressing class II molecules and stable double transfectants expressing both class II and Ii molecules , we here demonstrate that endogenously synthesized antigens, either secreted or retained in the endoplasmic reticulum can be presented to 3A9 T cell hybridoma specific for the 46-61 immunogenic determinant. Futhermore, we show that the presence of Ii dramatically enhances the capacity of class II positive L transfectants for the presentation of 46-61 determinant. In all cases, the endogenous presentation was chloroquine sensitive. It suggests that, whatever their degradation site, the association of peptides originating from endogenous antigens with class II molecules takes place in an acidic compartment. We hypothesize that dissociation of Ii from class II molecules in such an acidic compartment will provide empty class II molecules for the binding of peptides, originating either from an endogenous or exogenous source.

A NOVEL METHOD FOR THE ANALYSIS OF BINDING BETWEEN PEPTIDES AND PURIFIED MHC CLASS II MOLECULES.

I. Joosten*, M.H.M. Wauben*, E.J. Hensen* and S. Buus**. * Dept. Infectious Diseases and Immunology, Fac. Veterinary Medicine , University of Utrecht, The Netherlands. ** Dept. Experimental Immunology, University of Copenhagen, Denmark.

Biochemical assays for peptide binding to MHC class II molecules are cumbersome and of low through-put. Establishing such a binding assay often involves screening of many - frequently radiolabelled - peptides. We here describe a novel assay that allows rapid screening of large sets of non-radiolabelled peptides. The MHC molecules used were: the rat B^1 affinity purified from the cell line Z1A using the monoclonal antibody Ox6, and the mouse E^d affinity purified from the cell line A20 using the monoclonal antibody 14-4-4. The peptides used were : the B^1 restricted encephalogenic epitope 72-85 of guinea pig myelin basic protein and the Ed restricted T cell epitope 67-83 of the nucleocapsid protein of infectious bronchitis virus. These peptides were labelled with biotin with no apparent deleterious effect in functional T cell assays. Affinity purified MHC molecules were incubated together with biotinylated peptides and a protease inhibitor mix at pH5. After 2 days of incubation samples were analyzed on SDS-PAGE and blotted onto nitrocellulose. Peptide - MHC complexes were detected by incubation of the blots with HRP-streptavidin and subsequent chemiluminescence. Allele-specific binding could be demonstrated. The sensitivity of the biotin assay was high as peptide concentrations of 5nM together with 3uM of MHC still provided a clear signal. Competition studies showed that next to inhibition by unlabelled MBP 72-85, B^1 restricted analog peptides derived from MBP 72-85 and from the mycobacterial 65 kD protein sequence 180-188, but not the E^d restricted IBV 67-83 peptide could inhibit binding of MBP 72-85 to B^1. These data were confirmed by functional competition assays. To our knowledge this is the first demonstration of peptide-MHC binding in the rat. Conversely, in the E^d system unlabelled IBV 67-83, but not B^1 restricted MBP 72-85 could inhibit binding of the IBV 67-83 peptide. Thus, the sensitivity and specificity of this assay seems comparable to the previously reported assay (Buus et al., Cell 47, 1071 (1986)). Currently, studies on the effect of biotinylation and the specificity of the assay are being extended.

CHARACTERIZATION OF THE INTERMOLECULAR INTERACTIONS OF THE WILD TYPE AND MUTANT HLA-A2 MOLECULES,

Gil E. Katzenstein, Masanori Matsui, Owen C. Tatford and Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Major histocompatibility complex (MHC) class I glycoproteins are integral to the function of the immune system. These highly polymorphic cell surface molecules are found on nearly all cells and serve as cytotoxic T cell (CTL) targets. The functional molecule is a trimeric complex composed of a 44kD heavy chain, a non-covalently associated 12kD light chain, β2-microglobulin (β2m), and an endogenously derived nine amino acid peptide bound to a cleft on the heavy chain. These three components are necessary for the proper cell surface expression and CTL recognition of the MHC class I molecule. An extensive panel of single site mutations of HLA A201 has been generated by *in vitro* mutagenesis. The genes for the mutated class I molecule were transfected into C1R cells and those which were adequately expressed on the cell surface were assayed for their ability to present an influenza A derived peptide to HLA A201 restricted, influenza A specific CTL's. While the phenotype of the majority of mutants was unaltered, a number of single amino acid substitutions resulted in a radically diminished CTL recognition. The mutant phenotypes included the expected alterations in recognition mediated by those amino acids whose side chains pointed towards the peptide binding groove and those which point towards the T cell receptor. Surpisingly, residues which are predicted to interact with β2 microglobulin and residues which form conserved intramolecular interactions in the heavy chain structure also showed altered recognition by T cells. In order to understand the mechanisms of this phenotype, we have attempted to asssess the interaction of β2m with the heavy chain using both flow cytometry and immunoprecipitations to measure the affinity of these interactions.

Antigen specific (P30) mouse CD8+ T cells are cytotoxic against *Toxoplasma gondii* infected peritoneal macrophages[1] Lloyd H. Kasper*,[2], Imtiaz A. Khan*, Kenneth E. Ely*, Roland Buelow= and John C. Boothroyd= Departments of Medicine and Microbiology, Dartmouth Medical School Hanover NH 03755 and Department of Microbiology and Immunology Stanford University Medical School, Stanford CA 94305. The importance of CD^{8+} T cells in immunity against *T. gondii* is now well recognized. The mechanism by which these CD8+ T cells are able to confer this immunity is not yet understood. To examine the antigen specificity of this response, immune splenocytes from mice immunized with P30, a major surface parasite antigen were evaluated for their ability to lyse peritoneal macrophages infected with three different strains of *T. gondii*. Macrophages infected with either the RH or P wild type strain tachyzoites were lysed at varying E:T ratio by nylon wool non-adherent immune splenocytes, whereas macrophages infected with a P30 deficient mutant (B mutant) of the P strain were not. The gene encoding P30 for the wild type and B mutant were amplified by the polymerase chain reaction. This revealed a nonsense mutation in the B mutant such that its primary translation product is predicted to be about two-thirds the size of the wild type P30 molecule. Monoclonal antibody depletion studies indicate that the cytotoxic effect of the immune splenocytes is mediated by the CD8+ T cell population. Peritoneal macrophages infected with the three different strains (RH, P wild type, B mutant) from mice genetically restricted were not lysed by the immune CD8+ effector cell population. A cloned line (C3) of P30 antigen specific CD8+ T cells exhibited significant cytotoxicity against syngeneic peritoneal macrophages infected with either the RH or P strain tachyzoites. There was no macrophage lysis observed by these CD8+ effector cells of either syngeneic macrophages infected with the B mutant or nonsyngeneic macrophages infected with the three different tachyzoite strains.

EFFICIENT PROCESSING OF A CRYPTIC ANTIGENIC MOTIF FROM CHIMERIC PROTEINS, U.H. Koszinowski, A. Buchner, H.-J. Schlicht and T. Ruppert, Department of Virology, University of Ulm, P.O. Box 4066, D-7900 Ulm, Germany.

We have found that efficient processing of an antigenic sequence for presentation by MHC class I molecules depends on its neighboring residues in the protein (Del Val et al. Cell 66:1145, 1991). This conclusion was based on the quantifications of naturally processed peptides derived from pp89, a protein of murine cytomegalovirus, in virusinfected cells, on studying the recognition of such cells by epitope specific MHC I L^d restricted CTL, and on determining the immunogenic and protective activity of chimeric proteins in vivo. We now provide evidence that this phenomenon is of general relevance. We have analysed peptides derived from HIV proteins that are presented by different MHC I alleles, and find that the same principles, i.e. alteration of flanking sequences by mutagenesis, can be applied to provide presentation even of a peptide motif which is otherwise completely cryptic. In addition, we will present data showing that other modifications in a composite chimeric protein can also significantly affect the efficacy of processing.

CLONAL ANERGY OF AUTOREACTIVE HUMAN T-CELL CLONES CAN BE OVERCOME BY STIMULATION VIA THE ALTERNATIVE CD28 PATHWAY. Karel C. Kuijpers, Folkert J. van Kemenade, Rene A.W. van Lier, and Frank Miedema. Lab. Clinical Viro-Immunology and Lab. for Clinical and Experimental Immunology of the University of Amsterdam incorporated in the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam

Here we describe a set of 4 CD4+ T-cell clones obtained from a healthy donor reacting with a component of fetal calf serum (FCS). Proliferation was induced by monocytes in a HLA-DR1 restricted fashion. Interestingly, a marginal response to human serum (HS)-pulsed monocytes was observed, suggestive of anergic self (HS)-reactive T cells. They were non-cytotoxic and did not produce significant amounts of IL-2, even under optimal proliferative conditions. These clones failed to proliferate upon activation via CD3 or CD2. Non-responsiveness to CD3 or CD2 mAb could partially be overcome by addition of anti-CD28 mAb. However, early signal transduction such as increase in cytoplasmic calcium levels was induced by HS-pulsed monocytes as well as by CD3 or CD2 monoclonal antibodies (mAb).

Here we present evidence for clonal anergy in 4 autoreactive T-cell clones non-responsive to stimulation via the TCR/CD3 and CD2 pathway despite normal calcium responses. Anergy was overcome by alternative pathway stimulation through CD28. The molecular basis for the in vitro heteroclytic cross-reactivity to FCS is further investigated to provide clues for its in vivo relevance.

INTRATHYMIC PRESENTATION OF EXTRATHYMIC ANTIGENS IN SITU: CORTICAL EPITHELIAL CELLS VERSUS MEDULLARY DENDRITIC CELLS, Bruno Kyewski, Alexandra Livingstone and Brigitte. Stockinger, German Cancer Research Center, Heidelberg, FRG, and Basel Institute for Immunology, Basel, Switzerland.

Self-reactive T cells are tolerized within the thymus by deletion or anergy induction. This negative selection is mediated by interactions between TCRs on immature CD4+8+ thymocytes and self-MHC/self-peptide complexes on thymic antigen presenting cells. The precise intrathymic site and the stage of T cell differentiation at which tolerance induction occurs seem to vary with the self-antigen. In order to assess the relative contributions of different compartments to tolerance induction, we compared cortical epithelial cells and medullary dendritic cells with respect to in vivo presentation of circulating extrathymic antigens to T-T hybridomas. C5, an endogenous, and myoglobin, an exogenous antigen were tested. For both antigens dendritic cells proved ~ 5-10 more efficient on a per cell basis than epithelial cells despite a ~ 3 fold lower expression of total MHC class II determinants per cell. In the case of exogenously applied myoglobin this difference was independent of the route of antigen delivery (i.p. or i.v.). Differential access of antigens to both compartments or differential antigen uptake and processing in situ may account for this difference. In the case of limiting amounts of self-antigens dendritic cells may be the principal site of intrathymic self-antigen presentation and hence tolerance induction.

RESTORATION OF A TUMORIGENIC PHENOTYPE BY ß2-MICROGLOBULIN TRANSFECTION TO EL-4 MUTANT CELLS.

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It has frequently been suggested that loss of ß2m in tumor cells may lead to malignant progression due to escape from immunological recognition. Here, we directly tested the role of ß2m expression in tumorigenicity. A ß2m loss mutant, C4.4-25$^-$, was selected from the murine lymphoma EL-4. The C4.4-25$^-$ mutant line showed a marked reduction in tumorigenicity as compared to EL-4 in normal B6 mice. The reduced tumorigenicity was directly related to ß2m expression, since DNA mediated gene transfer of an intact murine ß2m gene markedly increased the tumorigeneic potential. The reduced tumorigenicity of C4.4-25$^-$ compared to ß2m transfected C4.4-25$^-$ cells was observed also in athymic nude mice but was abolished in B6 mice depleted of NK1.1 positive cells. These results show that restoration of ß2m can promote tumorigenicity and demonstrates for the first time that restoration of MHC class I expression by transfection can lead to escape from NK cells in vivo.

HUMAN CD4- CD8- αβ+ T CELLS EXPRESS A FUNCTIONAL TCR AND CAN BE ACTIVATED BY SUPERANTIGENS

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The CD4 and CD8 molecules play an important role in the stimulation of T cells and in the process of thymic education.Most mature T cells express the αβ TCR and either CD4 or CD8, however there is a small population of αβ$^+$TCR T cells which lack both CD4 and CD8.Little is known of the biology of the CD4$^-$CD8$^-$ (double negative) αβ$^+$ TCR T cells or the nature of the antigens to which they may respond. These cells not only represent a novel population of T cells, but also provide useful biological tools to study the roles which CD4 and CD8 play in T cell activation. In this study we have addressed two questions.Firstly, whether CD4$^-$CD8$^-$ αβ$^+$TCR T cells have functionally active T cell receptors, and secondly whether CD4 or CD8 are required for the activation of T cells by bacterial enterotoxins.Six double negative αβ$^+$TCR T cell clones, propagated from 2 healthy donors, were challenged with a panel of 9 bacterial enterotoxins. The Vα and Vβ usage of their TCR was determined by PCR.All of the CD4$^-$ CD8$^-$ clones proliferated in response to at least one of the enterotoxins in a Vβ-specific manner.The proliferative response of the CD4$^-$CD8$^-$ αβ$^+$TCR T cell clones was similar in magnitude to that exhibited by CD4$^+$ T cell clones of known Vβ expression. These data clearly show that the CD4 and CD8 molecules are not required for the activation of untransformed human T cells by bacterial enterotoxins. Furthermore, these results indicate that CD4$^-$ CD8$^-$ αβ$^+$TCR T cells, normally present in all individuals, are not functionally silent, as they can be stimulated via their T cell receptor. Their physiological role, like that of γδ T cells, remains to be elucidated.

RESTRICTION FRAGMENT LENGTH POLYMORPHIC ANALYSIS OF THE MOUSE MHC CLASS III REGION.

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The recent cloning of most of the human MHC class III region has led to the identification of a minimum of 25 new genes beside the classical MHC linked complement genes and the TNF and B144 genes previously linked to the HLA-B locus. Using human cDNA or gene probes for 17 of these new class III genes [G1, G2, G4, G6, G7a, HSP70, G8, G9, G9a, G10, G12, G13, G14, G15, G16, G17, G18] we have searched for the presence of homologous genes in the mouse H-2 region. By hybridizing the human probes to DNA from 11 different inbred or B10 congenic mouse strains we found that 16 of the 17 probes detected a single or a few discrete DNA fragments in Southern blots, indicating that the mouse genome indeed does contain homologous genes to most of the new human MHC class III genes. Only with the G8 probe did we fail to detect any cross hybridizing DNA fragments. We have furthermore searched the 11 different mouse strains, some of which are resistent to and others susceptible for autoimmune diseases, for restriction fragment length polymorphisms (RFLPs) using 12 different restriction enzymes. Six genes probes [G1, G7a, G9, G9a, G14, G17] did not detect any RFLPs. Three genes [G4, G13, G15] exhibited a single RFLP in one of the congenic H-2 strains used; whilst four probes [G2, G6, G16, G18] detected two allelic forms with one or more enzymes for several of the mouse strains analyzed. Two genes [HSP70 and G12] detected several different alleles in the different mouse strains analysed. One gene [G10] detected, in addition to non-polymorphic DNA fragments, one or two additional strongly hybridizing DNA fragments which may map outside the MHC region. Using intra H-2 recombinant strains most of the polymorphic genes could be mapped to the H-2 class III region.

INVARIANT CHAIN BUT NOT AN N-TERMINAL DELETION MUTANT ENHANCES PRESENTATION OF HEN EGG LYSOZYME (HEL) BY RAT-2 FIBROBLASTS

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The MHC class II-associated invariant chain (Ii) is thought to play a role in the intracellular transport of class II molecules. To study the influence of Ii on the presentation of protein antigens rat-2 fibroblasts, which completely lack Ii, were transfected with A^k together or without Ii. Transfectants expressing Ii were superior in the presentation of HEL protein to a variety of T cell hybridomas with specificity for different HEL peptides. This enhancement appeared to be correlated to the amount of Ii expressed. The presentation of synthetic HEL peptides was not influenced by Ii.

In contrast to the findings with HEL, the presentation of ribonuclease A (RNase) by the same set of transfectants was clearly independent of Ii. Both antigens, HEL and RNase, required the processing in a chloroquine-sensitive compartment. Only the presentation of HEL, however, could be blocked by Brefeldin A. This result suggests that RNase-derived peptides are predominantly presented by pre-existing class II molecules whereas the presentation of HEL depends on de novo synthesized class II molecules. Ii may influence the intracellular route of newly synthesized class II molecules so that they locate more efficiently to the compartment where HEL-derived peptides are available.

The cytoplasmic portion of Ii has been shown to contain a signal sequence which mediates sorting to the endosomal compartment. We transfected rat-2 cells with A^k and a mutated Ii lacking the N-terminal 20 residues (D20Ii). In contrast to full length Ii, expression of D20Ii did not enhance the capacity to present HEL suggesting that the sorting signal of Ii is relevant in these cells in spite of the fact that in rat-2 cells class II molecules could be detected in endocytic compartments in the absence of Ii.

ROLE FOR HSP70 IN TUMOR SPECIFIC RECOGNITION OF HUMAN EWING'S SARCOMA (ES) CELLS BY CYTOTOXIC EFFECTOR CELLS, Gabriele Multhoff and Rolf D. Issels, Inst. für Klinische Hämatologie der GSF and Klinikum Großhadern, LMU München, Marchioninistr. 25, 8000 München 70, FRG.

A cytotoxic effector cell population was generated from a primarily stimulated bulk culture (see abstr. *) with recombinant interleukin 2 (IL2). The specificity of this IL2 expanded cell-line with no need for restimulation was tested in a cell mediated lympholysis (CML) assay with the following targets: autologous Epstein-Barr-Virus (EBV) transformed B-lymphocytes (B-LCL) as a control, untreated Ewing's Sarcoma (ES) cells (37°C), ES cells heat treated with sublethal temperatures (41.8°C, 200') and K562 cells.

In contrast to the primarily stimulated CTL cultures, this CD8 positive effector cell population showed strong lysis for heat treated human ES cells and for K562 cells, whereas the lysis of autologous B-lymphocytes and untreated ES cells was very weak. Surface analysis of the target cells showed that the MHC class I expression of ES cells after sublethal heat treatment (41.8°C, 200') and a 3h incubation period at 37°C was drastically reduced. A sublethal heat shock of ES preferentially induces the synthesis of Heat Shock Protein 70 (HSP70) in the cytoplasm, whereas the protein synthesis in general is reduced. Most interestingly after heat treatment and a 3h incubation period at 37°C, a positive surface staining with a monoclonal anti HSP70 antibody was detected. Antibody blocking experiments with this antibody showed that lysis of heat treated ES cells can be inhibited, whereas blocking with an anti-MHC class I antibody has no influence. These results suggest a non-MHC restricted recognition of these effector cells. For further characterization of this unusual cell population cloning experiments and the definition of surface markers are in progress.

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*Influence of heat treatment on MHC expression in tumor cells. G. Multhoff, G. Eißner and R.D. Issels.

VARIABLE EFFECT OF POLYMORPHISM AT DR1 β CHAIN POSITIONS 85 AND 86 ON BINDING AND RECOGNITION OF DR1-RESTRICTED PEPTIDES, Debra K. Newton-Nash and David D. Eckels, Immunogenetics Research Section, Blood Research Institute, Milwaukee WI 53233.

Molecules encoded within the D region of the human leukocyte antigen (HLA) complex function to bind antigenic peptides and display them for recognition by appropriately restricted, peptide specific human helper T cells. HLA-D region polymorphism resulting in amino acid substitution can thus affect T cell responsiveness either directly, by affecting T cell receptor contact, or indirectly, by altering peptide binding affinity or conformation. Substitution of valine for glycine at position 86 of the DRβ chain has been shown to dramatically affect peptide binding to a number of alleles in which this dimorphism occurs. A natural polymorphism involving positions 85 and 86 of the DR1 β chain was utilized to determine the impact of substitution at these positions on binding and recognition of several DR1-restricted peptides. DR1-restricted T lymphocyte clones (TLC) specific for peptides derived from influenza hemagglutinin ($HA_{306-320}$) and matrix protein (MP_{19-31}) and from tetanus toxin ($TT_{830-843}$) were established. TLC specific for $HA_{306-320}$ and $TT_{830-843}$ demonstrated a dependency upon the presence of valine and glycine at positions 85 and 86 of the DR1 β chain for peptide recognition, whereas recognition of MP_{19-31} was not affected by substitution at these positions. Direct peptide binding studies confirmed that binding of $HA_{306-320}$ and $TT_{830-843}$, but not MP_{19-31}, was diminished on cells expressing DR1 molecules bearing substitutions of alanine and valine at DR1 β chain positions 85 and 86. All three peptides formed stimulatory peptide/DR1 complexes with equivalent rates and competed with one another for binding to the same site on DR1. These results demonstrate the existence of antigenic peptides capable of accommodating dimorphism at DR1 β chain position 86. The structural features of peptides which render them susceptible to amino acid substitution at DR1 β chain positions 85 and 86 and functional competition among peptides which appear to depend upon different DR β chain residues for binding are discussed.

HLA-DRw53 PRESENTS A DEFINED T CELL EPITOPE FROM A NOVEL RECOMBINANT M. LEPRAE PROTEIN ANTIGEN

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A DNA recombinant encoding a novel M. leprae protein antigen was isolated by screening a lamda gt11 M. leprae DNA library with M. leprae reactive human T cell clones as probes. Donors for T cell cloning were healthy persons immunized with killed M. leprae. Based on the deduced amino acid sequence of the C-terminal part of the antigen, the T cell epitope recognized was defined by synthetic peptides. Studies using allogeneic APC, HLA class I and II reactive MAbs as well as HLA-DRB4 *0101 transfected L cells showed that HLA-DRw53 represented the restriction element. Crossreactivity studies using reactive T cell clones revealed that the epitope is present in the pathogenic mycobacteria M. leprae and M. tuberculosis as well as vaccine strain M. bovis BCG. In agreement with this, both M. leprae and BCG induced polyclonal T cells from HLA-DRw53 positive individuals were able to recognize the relevant peptide.

Interestingly, unselected PBMC from the same donors also proliferated in response to the peptide epitope. In relation to synthetic vaccines, HLA-DRw53 restricted epitopes are of particular interest due to the high frequency of this haplotype in many populations.

THE INTERACTION BETWEEN PEPTIDE AND MHC CLASS I - A DIRECT FLUID-PHASE BINDING ASSAY

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MHC class I molecules preferentially present endogenous protein (eg. virus derived) to cytotoxic T lymphocytes. Several indirect functional assays, assembly assay and studies of mutant cell lines have indicated that MHC class I molecules specifically bind immunogenic peptides, however, biochemical fluid-phase assays for peptide-MHC class I interaction have until now been difficult if not impossible to establish. We have developed such an assay.

An influenza virus haemaglutinin peptide corresponding to residue 255-271 has been reported as being restricted to the MHC class I molecule, K^k (Sweetser et al. 1989, Nature 342, p180-182). This peptide was synthesised in a tyrosinated version, HPLC purified, and subsequently radioiodinated. The MHC class I molecule, K^k, was affinity purified from appropriate cell lysates. Complexes between the radiolabelled peptide and purified K^k were formed and assayed by gelfiltration. Only peptide which is complexed to K^k appears in the void volume, whereas free peptide appears in the total volume. The binding can thus be estimated. Alternatively, binding can be demonstrate by cross-linking of the peptide to the MHC class I heavy chain followed by SDS-PAGE and autoradiography.

The fact that we can detect binding in the above described assay indicates that peptide-MHC class I complexes once formed are quite stable in physiological buffers. The binding is specific since the peptide only bound to K^k and not to other purified MHC class I molecules. Other peptides did not bind to K^k. The binding was saturable with a measurable affinity. Furthermore, we have found that the addition of human β_2-microglobulin to the reaction mixture significantly increase the binding of peptide to MHC class I. The effect of β_2m is mainly seen on the association rate. We have found that the 18-mer, Ha255-271(Y) binds to K^k as effectively as the nonamer, Ha255-262(Y). This is in sharp contrast to the current dogma that MHC class I bind short octa- to nonameric peptides.

THE SUPERNATANT PRODUCED BY A CLONED THYMIC CORTICAL EPITHELIAL CELL STIMULATES AN INCREASE IN T CELL RECEPTOR (TCR) SURFACE DENSITY AMONG $CD4^+/CD8^+$ THYMOCYTES. Jennifer A. Punt, Susan Faas, Bruce Freedman and Yasuhiro Hashimoto, Department of Pathology, Division of Immunology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Two major phenotypic changes characterize the development of a mature thymocyte from its $CD4^+/CD8^+$ (double positive or DP) precursor: the loss of expression of either CD4 or CD8 and the increase in the level of surface T cell receptor (TCR). The interactions that govern these maturation events are unknown, although positive selection appears to initiate them. We have cloned and characterized a transformed cell, 1308.1, that has the morphologic features and the surface phenotype of a thymic cortical epithelial cell. The supernatants generated by this cell induce an increase in the surface density of TCR expressed by DP thymocytes developing in fetal thymic organ culture (FTOC). We are currently determining if the activity of this supernatant is related to the activity of anti-CD4 which, by disrupting an interaction between CD4 and class II expressed by thymic epithelial cells (*in vivo* or in FTOC), causes a similar increase in TCR expression among DP,TCR^{low} thymocytes.

EXPRESSION OF THE SAME Vβ GENE IN HUMAN T CELL CLONES DOES NOT CONFER IDENTICAL PATTERN OF STIMULATION BY BACTERIAL ENTEROTOXINS.

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Superantigens are the most potent T cell mitogens so far described and are believed to activate virtually all the T lymphocytes bearing the same Vβ fragment in their TCR. In order to determine whether the expression of the same Vβ gene confers the same pattern of responsiveness to bacterial superantigens, we have established a panel of twenty untransformed human T cell clones expressing the identical Vβ fragment (6.7a) in their TCR. The nature of the Vβ usage was established by immunostaining, using the Vβ 6.7a specific monoclonal antibody OT145, by PCR analysis and was further confirmed by direct sequencing. Although all the clones analysed possessed the same Vβ fragment, they had disparate patterns of proliferation to challenge with a panel of bacterial enterotoxins.These patterns were maintained on repeated testing. This result suggests that the mere expression of the identical Vβ region by T lymphocytes does not confer identical responsiveness to bacterial superantigens. Furthermore our data indicate that other, as yet undefined T lymphocyte components, apart from the Vβ segment, play a key role in the process of T cell activation induced by bacterial superantigens.

CHARACTERIZATION OF INVARIANT CHAIN FRAGMENTS GENERATED DURING ITS RELEASE FROM CLASS II MHC MOLECULES. **Victor E. Reyes, Valery Lam, Patricia S. Reisert, and Robert E. Humphreys. Department of Pharmacology. University of Massachusetts Medical Center, Worcester, MA 01655.**

The class II MHC-associated invariant chain (Ii) appears to block the association of self-peptides to the antigen binding site during the assembly of class II MHC molecules. The binding of antigenic peptides to class II MHC molecules occurs after Ii is cleaved and released by cathepsins B or D which can also generate antigenic peptides. In order to follow the events leading to the release of Ii from class II MHC molecules, we have characterized the intermediate fragments generated during Ii cleavage by cathepsin B and cathepsin D. Cathepsin B induces the complete dissociation of Ii from class II MHC molecules and is responsible for the generation of the N-terminal fragments p21 and p6. In contrast, cathepsin D does not induce the release of Ii from class II MHC but it does generate two different 25 kD species. One corresponds to the acidic exomembranal p25, while the other is a basic, variably glycosylated N-terminal fragment, which has not been described previously. We postulate that the action of these enzymes on Ii, in compartments where antigenic peptides are also available, might lead to a concerted exchange of Ii by antigenic peptides into the binding site.

IDENTIFICATION OF A CYTOPLASMIC PROTEIN ASSOCIATED WITH THE T-CELL SURFACE RECEPTOR CD28, Pamela J. Reynolds, Tullia Lindsten, and Craig B. Thompson, Howard Hughes Medical Institute and Departments of Internal Medicine and Microbiology/Immunology, University of Michigan Medical Center, Ann Arbor, MI 48109.

CD28 is a member of the Ig super family and is present on the surface of most T cells and plasma cells. Work from several labs support a co-stimulatory role for CD28 in T-cell activation. Engagement of the T-cell receptor (TCR) with antigen is necessary but not sufficient for T-cell activation. Several other T-cell surface molecules have also been implicated in T-cell activation. Co-stimulation of the TCR/CD3 complex and CD28 with monoclonal antibodies leads to the stabilization of lymphokine messenger RNAs and enhances lymphokine production. We have been investigating CD28's signal transduction pathway and its role in T-cell activation. Like many members of the Ig super family, CD28 has a modest cytoplasmic domain with no identifiable catalytic function. In metabolically-labeled T cells we have identified at least one protein that is co-immunoprecipitated with CD28 using a CD28 monoclonal antibody. Thus, like the T-cell surface molecules CD4 and CD8, CD28 may be physically associated with a cytoplasmic protein forming a signal transduction complex. We are in the process of purifying the CD28-associated protein for further analysis.

MURINE MHC CLASS II MOLECULES ARE DELIVERED TO THE ENDOSOMAL PATHWAY INDEPENDENTLY OF THE INVARIANT CHAIN. Jean Salamero, Martine Humbert, Pierre Cosson, *Denis Gerlier, *Frederique Forquet, Jean Davoust, Centre d'immunologie INSERM-CNRS de Marseille Luminy, 13288 Marseille, *Laboratoire de Biologie Moléculaire et Cellulaire ENS de Lyon, 69364 Lyon, France.

In murine B lymphocytes and macrophages, the invariant chain (Ii) binds to newly synthesized MHC class II molecules. These complexes remain intracellularly associated until they reach a compartment where Ii is degraded by acid proteases. Since newly synthesized class II molecules can only reach cell surface several hours after completion of their carbohydrate moities, they are thought to be addressed first to an endosomal compartment where they could gain access to processed exogenous antigens. To elucidate the putatively exclusive role of Ii in the intracellular targeting of class II molecules, we have compared stable L cell transfectants expressing class II molecules with stable double transfectants expressing both class II and Ii molecules. We have found that independently of Ii, class II molecules are transported to the cell surface at a very low rate and that most of the mature αβ heterodimers remain inside the cell. Using confocal microscopy coupled to double fluorescence image analysis, we then compared the intracellular distribution of class II molecules with markers of caracterised compartments. By this approach, we could not detect any differences in the intracellular location of class II molecules in Ii negative and Ii positive cells. However, as predicted, expression of Ii reduced by 10 to 50 fold the dose of HEL antigen required to stimulate 3 different IA^k restricted T helper cell hybridomas.

THE SCAVENGER SITE MAY BE IN OR NEAR T CELL-PRESENTED EPITOPES IN EXCISED FRAGMENTS OF ANTIGENIC PROTEINS. **Markus Salomon, Stephen Vazquez, Robert E. Humphreys, Robert A. Lew, Victor E. Reyes, Department of Pharmacology, University of Massachusetts Medical Center, Worcester, MA 01655**

The folding of helices in nascent proteins and scavenging of excised fragments is proposed to depend on the folding of recurrent hydrophobic residues against a hydrophobic surface. The template □○○○□○○□○○○□○○□○○○ was fitted to 247 known helices to maximize the mean hydrophobicity of □ positions. The template was extended beyond the helices to identify first virtual □ positions. Leu, Ile, Val, Phe and Met were increased in □ positions within true helices and were at random levels in first virtual □ ($p<0.001$). This and other correlations established the mechanism for initiation, propagation and termination of helices in native proteins. Predictions with a computer-based algorithm to find segments with high strip-of-helix hydrophobicity indices were made to find helices in proteins and structurally analogous, scavenger sites in excised fragments of antigenic proteins. The scavenger site may function to protect a segment from proteolysis, or to promote transfer by adsorption against a membrane or transfer molecule. Mutation of that site may affect potency of the T cell-presented epitope, but may also affect folding and function of therapeutic proteins. Because scavenger sites fell both in and near the T cell-presented epitopes, we conclude it is not congruent with the T cell epitope, but functions in the selection of excised fragments with potential T cell-presented epitopes.

PERIPHERAL TOLERANCE OF γδ T CELLS. David Spaner, K. Migita*, A. Ochi*, and Robert A. Phillips, Div. of Immunology and Cancer, Research Institute, Hospital for Sick Children, 555 University Ave., Toronto, Canada M5G 1X8, *Div. of Neurobiology and Mol. Immunol., Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Ave., Toronto, Canada M5G 1X5.

The role of γδ T cells in the immune response is still not completely understood. The study of these cells is limited by the lack of knowledge of the ligand of the γδ receptor in most cases, the low number of γδ cells in lymphoid organs, and the difficulty of extracting these cells from epithelial tissues. To overcome these problems we have made a *scid* mouse transgenic for a γδ T cell receptor (KN6) specific for Tla encoded gene products of all H2 haplotypes except for $H2^d$. This mouse contains high levels of transgenic T cells in the spleen and thymus in the complete absence of any other lymphocytes. Immunization of these mice with 10^7 C57BL/6 ($H2^b$, Tla^b) spleen cells resulted in proliferation and activation of host splenic γδ cells with clearing of the allogeneic donor lymphocytes. Subsequently the majority of activated cells died via apoptosis and the remaining cells were functionally anergic. They did not respond to restimulation by B6 spleen cells *in vitro* or *in vivo* or to stimulation by α-CD3 antibodies but they partially responded to exogenous IL2. They could inhibit a KN6-*scid* α B6 but not a third party mixed lymphocyte reaction. Similar results occurred using CBA ($H2^k$, Tla^k) cells to stimulate the γδ T cells where the affinity of binding of T cell receptor to antigen is less. These findings imply that functional tolerance may be the endpoint of an immune response, at least for γδ cells.

CHARACTERIZATION OF THE COAT PROTEINS INVOLVED IN VESICULAR SECRETORY TRANSPORT. Gudrun Stenbeck*, Doris Herrmann*, James E. Rothman+ and Felix T. Wieland*, *Institut für Biochemie I, Universität Heidelberg, D-6900 Heidelberg, Germany; +Program in Cellular Biochemistry and Biophysics, Rockefeller Research Laboratory, Sloan-Kettering Institute, New York, NY 10021

The eukaryotic cell is composed of a number of compartments bounded by membranes. Flow of material through these compartments is mediated by transport vesicles. These vesicles are formed by budding of one compartment and are precisely directed to their target membran, with witch they fuse. Targeting and fusion must be specific and controlled processes otherwise the highly differentiated compartmental structure of the cell were impossible.

The transport of proteins such as the MHC molecules between Golgi cisternae is mediated by non-clathrin-coated vesicles. These vesicles accumulate when Golgi membranes are incubated with ATP and cytosol in the presence of GTPγS, a nonhydrolysable analogoue of GTP (1). In order to investigate the mechanisms and function that underlie the coating of these vesicles our interest focuses on the structure of four high molecular weight proteins that form the main subunits of this coat. One of these coat proteins (COP's), β-COP (2,3), shares homology with the clathrin coated vesicle coat protein β-adaptin. The molecular characterization of another coat protein, γ-COP, will be presented.

References:
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2 Serafini, T. et al. (1991) *Nature* 349, 215-220
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HLA-CLASS II RESTRICTED PRESENTATION VIA A NON-ENDOSOMAL PATHWAY AND HLA-CLASS I RESTRICTED PRESENTATION OF EXOGENOUS MEASLES VIRUS FUSION PROTEIN,

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The routes used by antigen presenting cells (APC) to convert the transmembrane fusion glycoprotein (F) of measles virus (MV) to HLA-class I and class II presentable peptides have been examined using cloned human cytotoxic T lymphocytes (CTL) in functional assays. Presentation by Epstein Barr virus transformed B lymphoblastoid cell lines was achieved with live virus, ultraviolet inactivated MV (UV-MV), purified MV-F protein, delivered either as such or as immunostimulating complexes (ISCOM) and with synthetic peptides of MV-F. Apart from synthetic peptides, only live virus and ISCOM allow presentation by class I molecules, whereas all antigen preparations show class II restricted presentation. We observe presentation of MV-F by class II molecules in a fashion that is not perturbed by lysosomotropic agents. Our study allows visualization of novel presentation pathways of a type I transmembrane protein to class I and class II restricted CTL.

SIMULTANEOUS MULTIPLE PEPTIDE SYNTHESIS (SMPS) FOR THE ANALYSIS OF T CELL EPTOPES

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Previously we described a technique for the rapid analysis of T cell epitopes by a modification of the Pepscan method for the simultaneous preparation of multiple peptides (1). Although quite succesfully applied, this method is rather time consuming and the peptide amounts (ug's) are too low for quality control.
Therefore, for a better analysis of T cell epitopes we recently developed a fully automated simultaneous multiple peptide synthesis (SMPS) technique which we set-up using a standard autosampler programmed for delivery of the reagents and washing solvents into microvials containing small amounts of regular peptide synthesis resin. Standard Fmoc-chemistry is employed and peptides, synthesized as C-terminal amides, are obtained in amounts of 1-3 mg. With this method 30-60 peptides are prepared simultaneously within 2-3 days.
The mg-amounts in this way obtained not only enable quality control and purification of the peptides prior to epitope analyses, but also allow in vivo immune response studies.
The use of sets of peptides thus prepared for the rapid analysis of T cell epitopes (minimal critical sequence, fine specificity, blocking variant peptides) will be shown in a few applications: Analysis of the bounderies of an epitope recognized by a T cell line that responds to mycobacterial hsp65 derived peptides but not to the hsp65 protein itself, and the design of blocking peptides from single substituted peptides in our studies of peptide and protein induced experimental autoimmune diseases.

1) R. van der Zee et al. (1989), Eur. J. Immunol. 19, 43-47.

HLA CLASS-I AND -II MOLECULES PRESENT INFLUENZA VIRUS ANTIGENS WITH DIFFERENT KINETICS.

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The different routes of MHC class I and II molecules or the respective compartments where peptide association takes place might influence the efficiency of antigen presentation. We investigated the kinetics of antigen presentation by measuring $[Ca^{2+}]_i$ changes in influenza specific T-cell clones induced by infected EBV-transformed B-cells (B-LCL).
Class-II restricted peptides were presented 2 hours after infection, irrespective of restriction element or the viral protein involved, reaching plateau values after 4 hours. Class-I restricted peptides were presented with slower kinetics, which was not homogeneous: CD8+ T-cells (HLA-A2,B37) that recognize matrix protein-, and nucleoprotein derived peptides showed $[Ca^{2+}]_i$ changes after 4 hours, while a non-structural protein derived peptide (HLA-B7 context) was recognized only after 8 hours. These differences in class-I presentation did not depend on differences in intracellular transport: HLA-B7 molecules reach the cell membrane more rapidly than others.
In conclusion: a) class-II presentation was more rapid than that of class-I and b) class II presentation was homogeneous while heterogeneity of class-I presentation might reflect the generation of peptides during infection. This divergence of CD4+ and CD8+ T-cell recognition may be relevant in the anti-viral immune response.

INHIBITION OF EXPERIMENTAL AUTOIMMUNE DISEASES BY MHC BINDING PEPTIDES RELATED TO DISEASE ASSOCIATED EPITOPES

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Lewis rats ($RT1^l$) are susceptible to both experimental allergic encephalomyelitis (EAE) and adjuvant arthritis (AA). By substituting single amino acids within disease associated T cell epitopes, RT1 B^l binding competitor peptides were developed. Two competitor peptides, one AA associated- and one EAE associated peptide, inhibited equally well, in vitro proliferation of encephalitogenic and arthritogenic T cells. Furthermore, both peptides, when coimmunized with the encephalitogenic peptide inhibited development of EAE. However, AA was strongly inhibited by the AA competitor and not the EAE competitor. Further testing revealed the AA competitor peptide to be immunogenic and to induce "cryptic" T cells cross-reactive with the original AA associated peptide but not responsive to the mycobacterial hsp60 of which this sequence was derived or whole Mycobacterium tuberculosis. These data suggest that although peptides may compete successfully for MHC binding *in vitro* their disease suppressing activity can also be due to activation of non-pathogenic $CD4^+$ T cells interfering in the immune regulation of the disease. It is concluded that MHC competition and antigen specific immuno-modulation, integrated into one single competitor-modulator peptide, may create a powerful disease specific therapy.

VAL/GLY DIMORPHISM AT POSITION 86 OF THE DRß CHAIN IS CRITICAL TO ANTIGEN RECOGNITION BY T-CELLS,

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Recognition of peptide antigens in the context of HLA class II molecules on antigen presenting cells is fundamental to the establishment of helper T-cell mediated immune and autoimmune responses. We have established a T-cell line from a thymoma of a patient with myasthenia gravis which recognizes a recombinant fragment of the α subunit of the acetyl choline receptor, as judged by proliferative responses in [^{3}H] thymidine incorporation assays. In contrast, there was absolutely no response generated to the native or recombinant homolog from the electric fish, Torpedo. A series of overlapping peptides was used to map this epitope between amino acids 144-156 of the human sequence which differs by three amino acids from the electric fish in this region. Proliferative responses to recombinant acetylcholine receptor α subunit were blocked by monoclonal antibodies against HLA-DR but not -DQ nor -DP. The target antigen, either as recombinant fragment (37-181) or synthetic peptide (144-156), was recognized only when presented by the patient's own peripheral blood lymphocytes (Dw14.2) or by certain other DR4-positive donor cells (fresh peripheral blood lymphocytes and EBV transformed B-cell lines). Substitution of one basic amino acid for another, lysine for arginine, at position 71 in the putative antigen binding-site (Dw4) did not impair recognition of the synthetic peptide (144-156), whereas variants of DR4 with charged amino acid substitutions at positions 70 and 71 (Dw10) and position 74 (Dw13) abolished almost any stimulation of the cell line. In addition, substitution of valine (Dw14.1) for glycine (Dw14.2) at one end of the binding-site was sufficient to abolish recognition completely (stimulation index <1 compared to 150). This val/gly dimorphism exists on several HLA-DR haplotypes (DR1, DR3, DR4, DR5, DR6), implying a degree of functional selection. The profound effects on T-cell recognition that we have described probably account for this example of conserved major histocompatibility complex diversity.

ANTIGEN PRESENTATION BY CHIMERIC MOUSE — HUMAN MHC CLASS II MOLECULES.

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We are attempting to generate transgenic mice that express functional human MHC Class II molecules. To acheive this goal, we have used PCR mutagenesis to construct chimeric E^d/DR4Dw4 genes that encode MHC molecules in which the α1 and β1 domains of human DR4Dw4 replace the corresponding domains of mouse E^d. These chimeric MHC genes are under the control of endogenous mouse MHC Class II promoters and should thus be expressed in an appropriate tissue-specific manner in transgenic mice. The chimeric MHC Class II proteins contain the peptide binding domains from DR4Dw4 and are thus capable of binding DR4Dw4-restricted peptides. The rest of the chimeric molecules are derived from mouse E^d and are thus capable of interacting with mouse T-cell surface receptors. The properties of these chimeric MHC molecules in mouse B lymphoma transformants will be described.