EMERGING PRINCIPLES FOR VACCINE DEVELOPMENT: ANTIGEN PROCESSING AND PRESENTATION

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Keynote Address (Joint)

HZ 001 PROTEIN FOLDING AND OLIGOMERIZATION IN THE ENDOPLASMIC RETICULUM. Ari Helenius, Ineke Braakman, Utpal Tatu, Thorsten Marquardt, Craig Hammond, Jonne Helenius, Dept. of Cell Biology, Yale School of Medicine, New Haven, Ct 06510.

The conformational maturation of glycoproteins in the ER, determines how efficiently, how fast an in which form proteins are secreted, expressed on the plasma membrane or delivered to the membranes or lumen of vacuolar organelles. Maturation intermediates, misfolded proteins or misassembled oligomers are generally retained in the ER where they are degraded. In addition to providing a mechanism for restricting the potential damage caused by the deployment of defective proteins, this system is used by the cell to post-translationally regulate expression levels of specific proteins.

In our work, we are trying to define the characteristics that make the ER lumen an efficient folding and sorting environment for a multitude of important proteins. Using mainly viral glycoproteins(Influenza HA and VSV G protein) as model proteins, we study the folding, maturation and 'quality control' processes in living cells. Since these proteins, like most others made in the ER, depend on the formation of disulfides for proper folding, we can—using the pulse-chase approach —follow folding by monitoring the state of oxidation. The folding and oligomerization process can also be analyzed using monoclonal antibodies to conformation dependent epitopes, by morphological methods, by cell fractionation and by determining the degree of sugar processing.

The results show that folding of individual glycopolypeptides begins on the nascent chain and continues for several min post-translationally. Full length intermediates with different disulfides bonding patterns can be distinguished, as well as monomeric fully oxidized subunits prior to homo-oligomer formation. While the folding process takes place in the ER, the assembly of mature oligomers seems to occur mainly in the intermediate compartment between the ER and the Golgi complex.

The rate of folding and its efficiency depends on the conditions in the ER. These vary between cell types and physiological state. The important variables include the redox state(folding can be reversibly inhibited by addition of membrane permeable reducing or oxidizing agents such as DTT or diamide), the presence of lumenal ATP, the concentration of Ca^{++}, the level of ER-chaperons and folding enzymes such as BiP/GRP78 and protein disulfide isomerase, and temperature(folding is more efficient the lower the temperature). Frequently conditions arise in which a fraction of a given protein synthesized misfold while the rest fold properly, emphasizing the stochastic nature of the maturation process. Our results, moreover, demonstrate that the ER is a highly dynamic folding compartment where folding, unfolding, refolding, misfolding. oligomerization, aggregation and disassembly are continuously occurring catalyzed by a variety of folding factors and chaperons.

The quality control system(s), responsible for the retention and retrieval of defective proteins operates at different levels of the pathway: the rough ER, the intermediate compartment and the cis-Golgi. These proteins usually associate with BiP/GRP78. At a slow rate, some move from the ER to the Golgi complex from which they are recycled back to the ER. BiP/GRP78 is likely to play a central role in many aspects of quality control in addition to its role in translocation and folding.

Cell Biology for Immunologists

HZ 002 PROTEIN TRANSLOCATION INTO AND PEPTIDE EXPORT FROM THE ENDOPLASMIC RETICULUM, Randy Schekman, Jeff Brodsky, Dave Feldheim, and Karin Römisch, Department of Molecular and Cell Biology, Howard Hughes Medical Institute, Barker Hall, University of California, Berkeley, CA 94720.

At least four genes (SEC61, SEC62, SEC63, and SEC65) were identified in a selection for yeast mutants defective in polypeptide translocation into the ER. Three of the genes encode integral proteins localized to the ER membrane, and one (SEC65) encodes a subunit of yeast SRP. Sec61p is homologous to E. coli SecY which is required for secretion in bacteria. Yeast α-factor precursor trapped as a translocation intermediate can be chemically crosslinked to Sec61p and to the lumenal hsc70 isozyme, BiP, suggesting that at least these two molecules are intimately involved in the penetration event. Sec63p contains an internal domain, oriented toward the ER lumen, that shows ~40% sequence identity to the N-terminal domain of bacterial dnaJ, a heat shock protein that functions in concert with the dnaK protein. This domain may serve to recruit BiP to the translocation center. Antibodies directed against cytosolic domains of Sec62p or Sec63p precipitate a complex of five polypeptides from detergent solubilized membrane fractions. In addition to the three identified Sec membrane proteins, the complex includes two new proteins: a 31.5 kD glycoprotein (gp31.5) and a 23 kD nonglycosylated polypeptide (p23), molecular clones of which show no sequence homology to other proteins.

Protein translocation has been reconstituted in proteoliposomes prepared from yeast membranes solubilized with a mixture of octylglucoside and phospholipid and then dialyzed to remove the detergent. Reconstituted vesicles display ATP-dependent and cytosol-stimulated translocation and signal peptide-processing of α-factor precursor. Fractionation of the detergent-soluble material has allowed the isolation of a functional complex that includes Sec63p, BiP, p23, and gp31.5. The participation of BiP appears to be topologically specific: BiP but not cytosolic hsc70 restores translocation in reconstituted proteoliposomes formed from BiP mutant membranes.

Protein and peptide export from the ER have been examined using α-factor precursor and a synthetic tripeptide containing the acceptor site for N-linked glycosylation as substrates. The release of both glycosylated pro-α-factor and glycotripeptide from the ER is dependent on cytosol, temperature, and ATP. Antibodies against two proteins essential for the formation of transport vesicles inhibit glyco-pro-α-factor exit from the ER, but do not affect the release of the glycosylated tripeptide. Furthermore, in contrast to pro-α-factor, the exported glycopeptide is not associated with a membrane fraction and does not acquire Golgi-specific mannose residues. We conclude that the glycosylated tripeptide leaves the ER by a route different from the secretory pathway, possibly through an ATP-driven pump.

HZ 003 PEPTIDE LOADING AND INTRACELLULAR TRANSPORT OF MHC CLASS I MOLECULES, Michael Jackson, Pierre Langlade-Demoyen, Elisabeth Song, Young Yang, Anders Brumark and Per Peterson. The Scripps Research Institute, Dept. Immunology, 10666, N. Torrey Pines Road, La Jolla, CA 92037.

Functional MHC class I molecule consist of a trimeric complex of heavy chain, β2 microglobulin and a short peptide. The assembly of this complex in the ER and subsequent transport to the cell surface appears to be carefully controlled in mammalian cells, such that the number of peptide free or empty class I molecules arriving at the cell surface is minimized. In order to investigate the role of MHC class I dedicated accessory proteins in this process we have transfected Drosophila cells with cDNAs encoding murine and human MHC chains. In such cells we find that heavy chains and β2m assemble efficiently, however such complexes are thermolabile as they are devoid of peptide. The relative transport rates of the various class I haplotypes in mammalian and Drosphila cells was found to be quite different. Interestingly, in Drosphila cells the relative transport rates of both human and murine class I was found to be directly proportional to their thermostability. We have used these Drosphila cells to assess the role of potential accessory molecules in both the folding and assembly of the MHC class I and in the generation and loading of peptide.

Although Drosophila cells are unable to process and present cellular antigen, MHC class I molecules expressed at surface of these cells can be efficiently loaded with synthetic peptides at the cell surface. As binding of high affinity peptides to the empty class I molecules results in a significant thermostabilization of the complex we have used this system to screen for peptide epitopes in various viral and tumor proteins. In addition the ability to produce large quantities of either surface expressed or soluble empty MHC has allowed us to analyze in detail the biochemical properties and conformations of empty and homogeneous peptide containing MHC class I molecules and the parameters that affect peptide loading and stabilization.

Drosophila cells expressing a single MHC loaded with a specific peptide are readily recognized by T-cell clones showing that the molecules produced in the system are fully functional. Furthermore such cells were found to be potent inducers of CTL in primary cultures providing the basis of a potential new therapeutic approach.

Peptide-Binding Specificity of Class I Molecules

HZ 004 THE USE OF RECOMBINANT CLASS I MHC:PEPTIDE COMPLEXES TO DEFINE THE MOLECULAR BASIS OF PEPTIDE/MHC/TCR INTERACTIONS. James C. Sacchettini, Weiguo Zhang, Aideen C.M. Young and Stanley G. Nathenson. Departments of Biochemistry, Cell Biology, and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx NY 10461.

Most of the sequence diversity among MHC class I alleles is found in the amino acid residues that line the peptide binding groove. This diversity alters the chemical composition and spatial properties of the peptide binding groove and in turn dictates the characteristics of the peptide that can be accommodated.

In order to characterize those peptides that bind to specific class I molecules in vivo, we identifed the major H2-K^b restricted peptide from VSV as a unique octamer, VSV N52-59, Arg- Gly- Tyr- Val- Tyr- Gln- Gly- Leu. Alanine substitued peptide variants were used to define the role of each amino acid residue in the octapeptide in terms of its interaction with the H2-K^b molecule and with the TCR. As a result of binding studies we postulated that Tyr3, Tyr5 and Leu8 were MHC anchor residues, while studies using a panel of T cell clones recognizing VSV/K^b complexes on cells suggested that Arg1 Val4 , Gln6 and Gly7 were important in TCR recognition.

To evaluate these hypothesis at the structural level we exploited a high yield bacterial expression system and in vitro co-complex formation (protein folding) to prepare a homogeneous MHC class I molecule containing VSV N52-59 peptide. This complex was crystallized and its structure solved using molecular replacement techniques.

The structure of mouse H-2K^b revealed its similarity to three human class I HLA molecules, consistent with the high primary sequence homology and common function of these peptide-presenting molecules. Electron density was located in the peptide binding groove, to which a single peptide in a unique conformation was unambiguously fit. The peptide extended the length of the groove, parallel to the α-helices, and assumed an extended, mostly ß-strand conformation. The peptide was constrained within the groove by hydrogen bonding of its main-chain atoms and by contacts of its side-chains with the H-2K^b molecule. Its amino terminal nitrogen atom formed a hydrogen bond with the hydroxyl group of Tyr171 at one end of the groove, while the carboxyl terminal oxygen formed a hydrogen bond with the hydroxyl group of Tyr84 at the other end, amino acids which are conserved among human and mouse MHC molecules. This anchoring of each end of the peptide appears to be a general feature of peptide-MHC class I molecule binding and imposes restrictions on its length. The side-chains of residues Tyr3, Tyr5, and Leu8 of the peptide fit into the interior of the K^b molecule with no appreciable surface exposure, a finding in support of previous biological studies that showed the importance of these residues for binding. Thus the basis for binding of specific peptide sequences to the MHC class I molecule is the steric restriction imposed on the peptide side-chains by the architecture of the floor and sides of the groove. The side-chains of Arg1, Val4 and Gln6 as well as the main-chain of Gly7 of the peptide are exposed on the surface of the complex, thus confirming their availability for T cell receptor contact, as previously suggested by experiments which demonstrated that a specific subset of these residues were interactive with specific TCRs. The overall picture that arises from our studies is that the TCR/MHC interaction is unique since only 3 to 4 residues of the peptide have sufficient solvent accessibility for TCR interaction, with the majority of the peptide residues being buried. T cell recognition thus depends on only a few of the residues of a peptide presented in the context of the much larger pattern of amino acid side chains of the 2α helices of the antigen presenting domain of the MHC.

HZ 005 **STRUCTURAL STUDIES OF FREE AND BOUND MURINE MHC CLASS I**, Ian A. Wilson, Daved H. Fremont, Masazumi Matsumura, Enrico A. Stura and Per A. Peterson, Departments of Molecular Biology & Immunology, The Scripps Research Institute, 10666 North Torrey Pines Road La Jolla, CA 92037

The structures of murine MHC Class I H-2K^b in complex with two viral peptides have been determined to 2.3 and 2.5 Å (Fremont et al 1992, Matsumura et al, 1992). The production of soluble empty MHC Class I molecules in Drosophila cells (Jackson et al, 1992, Matsumura et al, 1992) has provided the opportunity to make these single peptide complexes with MHC molecules. H-2K^b has been crystallized with three different peptides, VSV-8, OVA-9 and SEV-9 (Stura et al, 1992) as well as the empty H-2K^b. The structure determination of the OVA-9 and empty K^b is in progress and will extend the structural knowledge of how MHC Class I molecules recognize peptide antigens. The comparison of the VSV-8 and OVA-9 structures has shown the importance of conserved MHC interactions with the peptide backbone. Different length peptides are then accommodated through insertion of a bulge in the center of the peptide. Small but important conformational differences are seen in H-2K^b depending on which peptide is bound. Thus, different peptide sequences may be recognized by the T-cell receptor through the synergistic effects and indirect readout of peptide sequence through conformational change in the MHC molecule itself. The question of whether empty MHC Class I molecules have a substantially different conformation to the peptide bound form will be addressed by the present studies.

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Protein Degradation in the Cytosol and the Endoplasmic Reticulum (Joint)

HZ 006 SELECTIVE DEGRADATION OF CYTOSOLIC PROTEINS BY LYSOSOMES, J. Fred Dice, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

Lysosomes are able to take up and degrade cytosolic proteins by several different pathways. Although lysosomal pathways of protein degradation have traditionally been viewed as degrading proteins to their constituent amino acids, it is now clear that certain peptides are spared degradation, released from the lysosomal compartment, and eventually released from cells. Some of these peptides are of an appropriate size to serve as antigens. We have studied a lysosomal pathway of proteolysis that is selective for cytosolic proteins containing peptide sequences biochemically related to Lys-Phe-Glu-Arg-Gln (KFERQ). This pathway is stimulated in confluent cell cultures that are deprived of serum growth factors and in liver and certain other tissues of fasted animals. Isolated lysosomes are able to selectively take up and degrade KFERQ-containing proteins, and this process is stimulated by ATP/$MgCl_2$ and the constitutively-expressed heat shock 70 kD protein (hsc73). Other members of the heat shock 70 protein family do not bind to KFERQ-containing proteins and do not stimulate uptake and degradation of proteins by isolated lysosomes. This lysosomal pathway of proteolysis is saturable and temperature-dependent. Intralysosomal degradation of the protein, but not lysosomal uptake of the protein, is inhibited by ammonium chloride and leupeptin. At 0^o C. proteins with KFERQ-like peptide sequences specifically bind to a protein on the lysosome surface, and such binding is stimulated in the presence of hsc73. This lysosomal binding protein is probably a receptor or a component of the protein translocation machinery within the lysosomal membrane. A heat shock 70 kD protein also resides within the lysosome, and this protein may play a role in the import or the intralysosomal digestion of substrate proteins.

Peptide-Binding Specificity of Class II Molecules

HZ 007 THE KINETICS OF PEPTIDE BINDING TO MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II MOLECULES. Stephan N. Witt and Harden M. McConnell, Department of Chemistry, Stanford University, Stanford, California 94305.

Major histocompatibility complex (MHC) class II proteins are non-covalently associated heterodimeric (ab) molecules that bind and display antigenic peptides to $CD4^+$ T cells. The reactions between peptides and detergent solubilized MHC molecules are remarkably slow ($t_{1/2}$ = 5-300 h). In order to deduce the molecular events that govern complex formation and dissociation, we have studied the kinetics of peptide binding to and dissociation from solubilized mouse MHC class II molecules under a variety of experimental conditions. The results and mechanistic implications are summarized as follows. (i) At pH 4.5 to 5.2, the cleavage of heterodimers into the individual a and b subunits is subject to *peptide-specific* inhibition. Thus peptide stabilizes the quarternary structure of MHC class II molecules. (ii) Unexpectedly, the half-times for the dissociation of a number of labeled peptides (P*) from preformed complexes (abP*) are nearly a *constant*, independent of sequence or length. The combined kinetic results are consistent with a reaction mechanism whereby low pH induces a slow, rate-limiting conformational change from a "closed", unreactive heterodimeric state to an "open", reactive heterodimeric state (Eq. 1), from which bound peptide dissociates (Eq. 2). In the presence of added labeled

(1) $\{abP\}_C \longrightarrow \{abP\}_O$ *slow*

(2) $\{abP\}_O \longrightarrow \{ab\}_O + P$

(3) $\{ab\}_O \longrightarrow a + b$

peptide, the reactive intermediate undergoes competing cleavage (Eq. 3) and rebinding, yielding individual a and b subunits and stable complexes $\{abP^*\}_C$. The data imply that the rate of reaction (1) does not depend strongly on peptide composition or length, whereas reaction (2) must. These conclusions may not apply to all peptide-class II combinations, if the rate of the conformational change is comparable to the peptide off rate. It is likely that mechanism (1) - (2) will apply to many peptide-class II MHC dissociation kinetics.

HZ 008 FINE SPECIFICITY OF CLASS II BINDING OF PEPTIDES, Alessandro Sette[1], Stephanie Ceman[2], Ralph T. Kubo[1], Kazuyasu Sakaguchi[3], Ettore Appella[3], Donald F. Hunt[4], Theresa A. Davis[4], Hanspeter Michel[4], Jeffrey Shabanowitz[4], Richard Rudersdorf[2], Howard M. Grey[1], and Robert DeMars[2], [1]Cytel, 3525 John Hopkins Court, San Diego, [2]Laboratory of Genetics, University of Wisconsin, Madison, WI, [3]National Cancer Institute, NIH, Bethesda, MD, [4]Department of Chemistry, University of Virginia, McCormick Road., Charlottesville, VA.

Intracellular formation of MHC class II/peptide complexes is impaired in human B cells of the mutant 721.174 that have been transfected with DR genes. Although normal quantities of cell surface DR molecules were expressed, essentially all DR heterodimers were unstable in the presence of sodium dodecylsulfate (SDS) and lacked reactivity with certain conformation-specific antibodies. Peptide binding studies indicated that a relatively large fraction (~30%) of these molecules were unoccupied, but since about 70% of the molecules contained bound peptide, deficient peptide binding *per se* could not explain their instability. Mass spectrometry analysis indicated that at least 80% of the self peptides bound to DR3 molecules produced in .174 were 21-24 amino acids long and were derived from a single 24-residue segment of the invariant chain. These data indicate the presence of a defect in the mechanisms normally employed by cells in processing antigens for presentation with class II molecules, and reveal an additional pathway for peptide-MHC class II association.

Mechanisms of Endocytosis and Membrane Recycling (Joint)

HZ 009 REGULATION OF ENDOCYTIC FUSION EVENTS IN VITRO, Jean Gruenberg. European Molecular Biology Laboratory. Postfach 10.2209, D-6900 Heidelberg. Germany.

We are studying the mechanisms of membrane-membrane interactions in the endocytic pathway with an in vitro fusion assay. Until now, we have reconstituted two separate endosomal fusion events, which reflect in vivo an early and a late stage of the pathway, respectively. The first one corresponds to the lateral (homotypic) fusion of early endosomes with each other (an observation suggesting that early endosomes form a highly dynamic network in vivo). We have shown that this process is specific, microtubule-independent, and regulated by the small GTP-binding protein rab5. In addition, this fusion event is inhibited by mitotic cytosol, a finding consistent with membrane trafic arrest during mitosis in mammalian cells. This inhibition is mediated by the cdc2 kinase specifically associated to cyclin B, but not cyclin A. The second fusion event occurs during transport from peripheral early endosomes to perinuclear late endosomes. We previously observed that microtubule-dependent endosomes with a multivesicular appearance are intermediates in this transport. We refer to them as endosomal carrier vesicles (ECVs). In vitro, we have reconstituted the fusion of ECVs with late endosomes both in polarized epithelial cells and in non polarized cells. This process is specific, facilitated by the presence of polymerized microtubules, and depends on MAPs and motor proteins.

Until now, studies in membrane transport have revealed the existence of complex mechanisms for the regulation of membrane-membrane interactions. However, relatively little is known about these interactions. We have, therefore, used an assay that measures the transfer of membrane-associated proteins from "donor" to "acceptor" endosomes upon fusion. [This assay fulfills all criteria of the fusion process.] We observed that transfer/fusion still occurred when donor endosomes were sonicated into small (0.1µm diameter) vesicles before the assay. Then, only 5 proteins were efficiently transferred, including two membrane proteins and three peripheral proteins associated to the membrane cytoplasmic face. Partial sequencing showed one of the latter protein to be annexin II, which has been previously implicated in membrane-membrane interactions. Our data, suggest that annexin II, together with the 4 other proteins we have identified, may be involved in the formation of close contacts between membranes and/or fusion.

HZ 010 MEMBRANE TRAFFIC AND TRANSCYTOSIS IN POLARIZED EPITHELIAL CELLS: SIGNALS, MECHANISMS, AND REGULATION.

K. Mostov, G. Apodaca, B. Aroeti, C. Okamoto, W. Song, and *M. Bomsel, Departments of Anatomy, and Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0452, USA, and *Unité de Pathogénic Microbienne Moléculaire, Institut Pasteur, Paris, France.

Polarized epithelial cells use two mechanisms to send proteins to the correct plasma membrane domain: direct delivery from the TGN to the final surface, and indirect delivery to one surface, followed by endocytosis and transcytosis to the opposite surface. We have used the polymeric immunoglobulin receptor (pIgR) as a model to study these processes. The membrane-proximal 17 residues of the cytoplasmic domain of the pIgR are an autonomous signal for delivery from the TGN to the basolateral surface. Transcytosis is regulated by two independent signals: phosphorylation of Ser^{664} on the cytoplasmic domain, and binding of ligand (IgA) to the extracellular domain. Activation of the heterotrimeric G protein, G_S, by cholera toxin stimulates transcytosis independently of these two signals. We have reconstituted budding of transcytotic vesicles from early endosomes in a perforated cell system. Both the α and $\beta\gamma$ subunits of G_S act to control sorting of pIgR into transcytotic vesicles. G_S is thus the first identified component of the machinery for polarized sorting of proteins in epithelial cells

In Vivo Peptide-Loading into Class II Molecules

HZ 011 ANALYSIS OF CLASS II MHC TRANSPORT IN NORMAL AND FUNCTIONAL MUTANT CELL LINES, Peter Cresswell, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Class II MHC α-and β-subunits associate with invariant chain trimers in the endoplasmic reticulum. Following assembly the $\alpha\beta$-invariant chain complex is transported through the Golgi apparatus and then diverted from the constitutive transport pathway into the endosomal system. Here the invariant chain is proteolytically degraded and $\alpha\beta$ dimers are released to bind peptides derived from endocytosed proteins. The precise mechanisms involved in peptide generation and binding, and the route by which $\alpha\beta$-peptide complexes are subsequently delivered to the plasma membrane, are unknown. A number of mutant cell lines, defective in an uncharacterized MHC-linked gene or genes, are impaired in their ability to generate functional $\alpha\beta$-peptide complexes. We have determined that many $\alpha\beta$ dimers from one such cell line, T2.DR3, are associated with a nested set of invariant chain-derived peptides, and that such $\alpha\beta$ dimers can be efficiently loaded with antigenic peptides *in vitro*. Comparative analyses of class II MHC transport, invariant chain processing, and subcellular distribution in wild-type and mutant cell lines will be presented.

HZ 012 **REGULATION OF MHC CLASS II INTRACELLULAR FOLDING AND TRANSPORT BY INVARIANT CHAIN AND PEPTIDE**, Ronald N. Germain[1], Oddmund Bakke[2], Elizabeth Bonney[1], Flora Castellino[1], Corine Layet[1], Austin G. Rinker, Jr.[1], Scheherazade Sadegh-Nasseri[1], and Paola Romagnoli[1], [1]Lymphocyte Biology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD USA 20892 and [2] Department of Biology, Univ. of Oslo, 0316 Oslo, Norway

MHC class I and class II molecules bind and transport to the cell surface peptides derived from self or foreign proteins. Each class is specialized for acquisition of peptides in distinct intracellular locations - class I for peptides present in the ER, class II for peptides in the endocytic pathway. Movement of class II dimers from their site of initial assembly in the ER to the cell surface involves a complex interplay between the class II molecules, peptide, and the invariant chain (Ii). Ii acts as a chaperone for class II in the ER, preventing aggregation and retention. In the endosomal pathway, Ii plays an active role in modifying movement through the early endocytic compartment, retarding the flow of fluid phase and membrane material *en route* to late endosomes. This function of Ii may be important in regulating the class II presentation system. Movement of class II to later compartments in the endocytic pathway is associated with cleavage of Ii and such cleavage is essential for peptide loading and expression of class II on the cell surface. Data will be discussed concerning the role of various segments of the cytoplasmic and lumenal portions of Ii in mediating interaction with class II, proper class II folding, control of ER egress, alteration of early endosome function, regulation of antigen loading, and release from endosomal retention.

Peptide plays an important role in the structure of the class II dimer, as revealed by stabilization of $\alpha\beta$ dimers *in vitro* against dissociation in SDS-containing buffers. A similar transition in class II structural stability can also be observed in living cells when newly synthesized, Ii-associated class II dimers are compared to those present in the cell several hours after synthesis. Ii-associated class II does not show evidence of associated peptide The proportion of class II molecules surviving for several hours increases following addition of antigen to the medium. An *in vitro* model of endosomal peptide loading indicates that peptide binding prevents class II that is free of intact Ii from undergoing aggregation with itself and/or other proteins. We suggest that in the endosomal pathway, when Ii is cleaved and removed from class II, the exposed molecules have two possible fates: 1) to bind peptide and move to the cell surface for T cell recognition, or 2) to aggregate if no peptide is bound, resulting in endosomal retention. This provides an intracellular editing mechanism that results in the selective surface expression of loaded class II dimers. This selection appears to be based on the change in protein structure resulting from peptide binding, which interferes with the aggregation properties of the class II molecules.

These studies indicate that class II molecules behave early after synthesis as improperly folded proteins requiring the action of Ii to protect them from the retention effects of ER chaperones. Peptide-free class II-Ii complexes leave the ER and move to the trans-Golgi network, where a signal in the cytoplasmic tail of Ii results in deviation from the export route to the membrane and localization first in early endosomes. In this compartment, Ii can affects the rate of transport, slowing down passage of the associated class II. Movement to later compartments is accompanied by cleavage of Ii, revealing the class II binding site. In a low pH environment, such class II either captures peptide and undergoes a conformational change, or aggregates because of its failure to achieve a properly folded state. The peptide-loaded dimers move by an uncharacterized pathway to the cell membrane for presentation of peptide to T lymphocytes. This view of class II transport, folding, and expression emphasizes the role of conformation change in the class II molecule in regulating its movement in both the early and late post-synthetic compartments, the reciprocal relationship between Ii and peptide in controlling the change in conformation required for class II expression at the cell surface, and the specialization of these molecules for peptide acquisition from the endocytic compartment. For both classes of MHC molecule, the selective acquisition of peptides from different sources is inherent in the structure of the molecule, and is facilitated by additional proteins that participate in peptide production, movement, or loading.

HZ 013 ROLE OF THE INVARIANT CHAIN IN MHC CLASS II ASSEMBLY, TRANSPORT FUNCTION, Luc Teyton, Research Institute of Scripps Clinic, La Jolla, CA 92037.

MHC class II molecules acquire peptides produced from exogenous proteins in the endocytic pathway. This function comes along with the targeting of class II molecules to a post-Golgi compartment by the p31 invariant chain (Iip31).Two additional forms of invariant chain, Iip33 and Iip41, had so far no assigned function. Recently, Iip33, which is present exclusively in humans, was shown to be resident of the endoplasmic reticulum (ER). The retention of Iip33 in the ER is mediated by the negatively charged stretch of amino acids present at the N-terminal part of the additional 15 residues of its cytoplasmic tail. Iip41 is found in all species. Using specific antisera we were able to demonstrate that Iip41 is also a ER resident protein. The presence of 6 cysteins in the additional 64 residues of Iip41 plays a critical role in the retention mechanism. $[A^d$ stable transfectants were supertransfected with the various invariant chains and their various combinations.The assembly, transport and targeting of class II molecules to the peptide loading compartment requires the presence of two forms of Ii, Iip31 and one of the ER retained forms. The right targeting comes with the ability of the transfectants to present whole exogenous antigens. In addition, we showed that in Raji cells were all three forms of Ii are present, the transport of Iip31 with alpha and beta out of the ER occurs in a trimeric complex. The ER forms of Ii allow the retention of Iip31 in the ER and promotes the formation of trimers able to be transported out.

Peptide Translocators and Pore-Forming Molecules (Joint)

HZ 014 SUBSTRATE SPECIFICITY OF HEMOLYSIN TRANSPORT, Fang Zhang, Jonathan Sheps, David Greig, Ya Yin, Cheryl Arrowsmith and Victor Ling. The Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, Toronto, Ontario, CANADA.

The secretion of the 107kDa hemolysin A (HlyA) protein from the cytoplasm of E coli directly into the surrounding medium is mediated by membrane proteins hemolysin B (HlyB) and hemolysin D (HlyD). Hemolysin B is a member of the so called ATP-binding cassette (ABC) transporter superfamily which includes the multidrug resistance P-glycoprotein, the cystic fibrosis CFTR protein, and the MHC-associated transporter of antigenic peptides. Recognition of HlyA by the HlyB/D transporter is dependent on a signal sequence mapped approximately to the last 50 amino acids of the C-terminus of HlyA. Many proteins which are not normally secreted by E. coli are secreted by the hemolysin transporter when engineered to contain the C-terminus of HlyA. We show that the recognition of the HlyA signal sequence by the hemolysin transport system may be mediated via specific sites on HlyB. This conclusion is based on experiments in which we show that transport defective mutants of HlyA containing deletions in the signal sequence can be compensated at least in part by reversion point mutations in HlyB. These point mutations all mapped close to the predicted transmembrane domains of HlyB on the cytoplasmic side. In another approach, we show that a 70 amino acid sequence of leukotoxin (LktA) can substitute functionally for the HlyA's signal sequence so that a chimeric molecule of HlyA containing the LktA sequence is transported as efficiently as wild-type hemolysin HlyA. The LktA peptide has no primary sequence similarity to the 50 amino acid signal sequence of HlyA. This finding implies that the transport signal of HlyA is not determined by a unique primary sequence. A secondary structure common to both the HlyA and LktA sequences can be predicted using a computer program involving six different methods. A common structure of helix-turn-helix and strand-loop-strand was predicted. This predicted structure is functionally consistent with many deletion mutants in the signal sequence of HlyA, although it is not consistent with a number of point mutants. Thus we conclude that the HlyA transport signal is complex and may involve an as yet unidentified higher order structure. Since the HlyA and LktA primary sequences are entirely different, it is likely that, despite an apparent similarity in secondary structures, the molecular surfaces presented by these peptides to the transporter will be quite different. This raises the possibility that the hemolysin transporter is able to recognise as substrates an unexpected diversity of structures. Three dimensional structure information at the atomic level is currently being sought for both the HlyA and the LktA signal sequence. An understanding for the basis of the apparent broad specificity of the hemolysin transporter may provide new insights into the mechanism of action of other ABC transporters. For example, it may be speculated that the MHC-linked translocation of a broad range of antigenic peptides from the cytoplasm into the lumen of the endoplasmic reticulum may function in a similar manner. The transporter may recognise antigenic peptides based on some as yet unidentified higher order structures.

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HZ 015 CFTR MISLOCALIZATION IN CYSTIC FIBROSIS, John R. Riordan, Xiu-Bao Chang, and Norbert Kartner, Research Institute, The Hospital for Sick Children, and the Department of Biochemistry and Clinical Biochemistry, University of Toronto, Toronto, Ontario, Canada.

From the perspective of the topic of this symposium, CFTR is a member of a superfamily of transporters involved in the translocation of a diverse range of substrates including polypeptides across membranes of either intracellular compartments or the cell surface. However, these is as yet no evidence that CFTR transports a polypeptide or any other substrate across the apical membrane of epithelial cells in which it resides. Rather, its primary function seems to be that of a low conductance Cl^- channel subject to a complex mechanism of regulation. In a speculative model, we have proposed earlier that part of this regulatory mechanism may involve an active movement of a unique portion of the protein itself (R domain) against the cytoplasmic aspect of the pore-forming domains. If this were true, CFTR would be at the same time an active transporter and a Cl^- channel. Of greater importance from the perspective of the disease, the mutation in the CFTR gene which occurs in the majority (70%) of patients (ΔF508) severely alters the biosynthesis and intracellular trafficking of the molecule. ΔF508 CFTR is apparently trapped in the ER and degraded; hence, it fails to reach its normal site of action in the apical membrane. We have confirmed that this occurs *in vivo* by directly observing the protein localization using monoclonal antibodies to CFTR. Immunocytochemical analysis of cryosections of skin biopsies from patients homozygous for the ΔF508 mutation showed in epithelial cells of the sweat duct, only weak intracellular staining and a complete absence of the normal strong apical staining. Heterologous expression studies in mammalian and insect cells reveal that this biosynthetic arrest of ΔF508 CFTR occurs in the former but not the latter. Furthermore, when not biosynthetically limited, the mutant protein is functionally competent. This focusses attention on the practical need to understand and maniuplate this biosynthetic block. Experiments in the laboratory of M. J. Welsh at the University of Iowa have already shown that reduced temperature can promote maturation and transport of ΔF508 CFTR to the cell surface. Additional means of accomplishing this are actively being sought. (Supported by the Cystic Fibrosis Foundations of Canada and the U.S. and NIH-NIDDK).

Novel MHC Genes and Their Functions

HZ 016 ORGANIZATION AND FUNCTION OF GENES IN THE HUMAN MHC CLASS II REGION. John Trowsdale, Human Immunogenetics Laboratory, Imperial Cancer Research Fund, 44 Lincolns Inn Fields, Holborn, London WC2A 3PX, U.K.

We have constructed yeast artificial chromosome (YAC) clones over the human MHC class II region. Cosmid libraries have been made from some of the YACS in order to obtain dense coverage. cDNA library screening, sometimes with the help of exon amplification and genomic sequencing, has been used to identify novel genes within the region. Some of the genes we have found may have no relationship with the immune system. The RING3 gene for example, centromeric of the *HLA-DNA* gene, is expressed in most tissues and is related to Drosophila *female sterile homeotic*. Other genes encode a class II-related molecule, *DM*, which may have emerged in evolution at around the same time as class I and class II diverged. The class II region also contains a group of genes which seem to be involved in antigen processing, the *TAPs* and *LMPs*. We have sequenced the entire *LMP-TAP* region to describe the exon-intron structure of these genes and the data show that *TAP1* and *2* probably arose by duplication. The *LMP* sequences are related to those of proteasome components and it has been proposed that the proteasome is involved in making peptides for feeding to MHC class I in the endoplasmic reticulum. The *LMP7* gene has a complex genomic arrangement with two different leader sequences that are cleaved off to make the mature protein. Transfection of deletion mutant cells with genes for the TAPs in the absence of the LMPs restores class I assembly and expression, indicating that the LMPs may not be essential for antigen processing but protein evidence shows that they are associated with the TAP complex. Antisera raised against all of the LMP and TAP products are being used to probe their associations and functions. Since a gene involved in class II maturation is also located in the MHC it is possible that the region has evolved as a cluster of loci with interconnected functions in the immune system. Analysis of variation in *TAP* and *LMP* genes provides little evidence for strong disequilibrium in the region in human populations in health and disease.

Late Abstract

PEPTIDE TRANSPORTERS AND SELECTIVITY, Jonathan C. Howard, Edward V. Deverson, Simon Powis and Geoffrey W. Butcher, Department of Immunology, Agricultural and Food Research Council Institute of Animal Physiology and Genetics, Cambridge Research Station, Babraham, Cambridge, U.K.

The MHC linked transporters are required for normal loading of peptides into class I MHC molecules in the endoplasmic reticulum. In their absence, the only peptides known to have access to class I are those derived from the activity of signal peptide peptidases. Allotypic variation *in primary sequence and three-dimensional structure of the peptide* binding domains of class I causes selectivity in loading of peptides which can be explained at least in part in terms of complementarity of peptide side chains with binding pockets associated with the peptide binding domains. A critical question is to what extent the accessory apparatus associated with class I peptide loading function is also restrictive either in determining the peptides available for loading, or in adapting the loading process so that only certain peptides or peptide types are permissible. At present, three classes of proteins are known or suspected to be involved in the provision or loading of peptides into class I molecules; namely, the MHC-linked proteasome copmponents, LMP-2 and LMP-7, the two ATP binding cassette transporter chains, TAP1 and TAP2, and p88 calnexin. Of these, a key role for the two proteasome chains in normal constitutive peptide generation for class I loading seems unlikely, since the homozygous deletion cell line, T2, can apparently be fully reconstituted to functional antigen processing by TAP chains alone, and no functional variation in peptide loading into class is known to be associated with the limited polymorphism reported for the LMP2 and LMP7 products. The significance of calnexin's association with newly formed class I molecules before peptide binding is not known, but no variation in peptide binding has yet been attributed to polymorphism or *other property of calnexin. In the rat, polymorphism has been found in* the TAP2 transporter chain, associated with the membrane-spanning N-terminal segment of the molecule. This polymorphism is functionally related to striking variation in the identity of peptides loaded into certain class I alleles, while other class I alleles are insensitive to transporter polymorphism. Thus there appears to be functional complementarity between transporter and class I polymorphism, presumably serving to optimise loading efficiency into a wide range of class I structures. The requirement for TAP protein for class I loading is normally assumed to relate to a peptide transport function across the ER membrane, but such a property remains to be shown in vitro. Further analysis of the realtionship between TAP structure and peptide loading awaits the resolution of this issue, although the identification of defined substitutions associated with differential peptide loading provides an approach to a functional dissection of the transporter molecule which is now in progress.

Class I Molecules

HZ 100 EXTERNAL MHC-I BINDING PEPTIDES ARE RECYCLED TO THE CELL SURFACE AFTER INTERNALIZATION, Ussama M Abdel Motal*, Xianzheng Zhou*, Annalena Joki*, Abdur Rehman Siddiqi*, Kristina Stenvall§, Jan Dahmén§ & Mikael Jondal*. *Department of Immunology, Karolinska Institute, Stockholm, §Symbicom AB, IDEON, Lund, Sweden.

Cytotoxic T lymphocytes (CTL) recognize target antigens as short, processed peptides bound to MHC-I heavy and light chains (B_2-M)[1,2]. The heavy chain, which comprise the actual peptide binding α-1 and α-2 domains, can exist at the cell surface in different forms, either free, bound to B_2-M or as a trimeric peptide bound complex [3,4]. MHC-I chains are also known to internalize, and recycle to the cell surface, and this has been suggested to be important in peptide presentation[5-10]. We have used both peptide transporter mutant RMA-S cells[11] and non-mutant EL-4 cells to investigate recycling of external D^b binding peptides by two different approaches. First, peptides were covalently linked with a galabiose (Galα4Gal) at the aminoterminus, a position which did not interfere with D^b binding or immunogenicity, and peptide recycling tested with Gal_2 specific monoclonal antibodies. By flow cytometry, a return of Gal_2 epitopes to the cell surface was found, after cellular internalization and membrane clearance by pronase treatment. Second, specific CTL:s were reacted with peptide loaded target cells, after complete removal of membrane D^b molecules by pronase, and after different times of incubation at 37°, to allow re-expression. By this procedure, re-appearance of target cell susceptibility was seen. As earlier described, external peptides also upregulated the membrane expression of D^b molecules,[12] and this upregulation was inhibited by chloroquine. The results are in accordance with a model for optimizing peptide presentation by MHC-1 recycling.

HZ 101 PEPTIDE LIGANDS INDUCE REVERSIBLE UPREGULATION OF MHC CLASS II MOLECULES ON CELL SURFACE, Babita Agrawal, Ester Fraga, Kevin Kane and Bhagirath Singh, Department of Immunology, University of Alberta, Edmonton , Canada T6G 2H7.

MHC class I and class II molecules present peptide antigens to T lymphocytes. Peptides are critical in class I heavy chain folding and/or stable association with β2m. A recent study suggests the role of peptide antigen binding for MHC class II α and β chains heterodimers to enter into a *compact* state and allow their transport to the cell surface. We have investigated the effect of peptide ligands on the expression of MHC class II I-A^d molecules on the B cell hybridoma, TA3. TA3 cells, when cultured *in vitro*, gradually lost the surface expression of I-A^d molecules. Incubation with peptides, having high affinity for binding to intact I-A^d molecules, significantly increased the surface expression of I-A^d in a dose dependent manner in less than 24 hrs. The ability of peptides to induce increased expression of I-A^d correlated with the affinity of peptide to intact I-A^d ie. an I-A^k restricted peptide did not have an effect on I-A^d expression. The effect could be reversed following the removal of the peptide antigen. Based upon our studies with inhibitors of protein synthesis and intracellular transport, the mechanism for upregulation of I-A^d expression by peptides seems to involve intracellular pathway but appears not to require new protein synthesis or transport from ER. Our results suggest that the decrease in surface expression of I-A^d on TA3 cells may result from their failure to be saturated with naturally processed peptide ligands. Thus peptide ligands are evidently important in regulating surface expression of MHC class II molecules and their recognition by T lymphocytes.

HZ 102 INTRACELLULAR TRANSPORT OF CLASS I MHC MOLECULES IN ANTIGEN PROCESSING MUTANT CELL LINES, Karen S. Anderson and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Intracellular transport and stability of class I MHC glycoproteins depends on assembly of heavy chain, β_2-microglobulin, and peptide. The antigen processing mutant cell lines T2 and RMA-S have corresponding defects in class I assembly and transport. The murine class I molecules in RMA-S can be detected at the cell surface at lower temperatures, suggesting that they are transported to the cell surface, but are unstable. However, most human class I molecules in T2 cannot be detected at the cell surface, even in the cold. To directly compare the transport of human and mouse alleles in RMA-S and T2, the human alleles HLA-A2, A3, and B27 were transfected into RMA-S with human β_2-microglobulin. Although surface expression of the alleles HLA-A3 and B27 could be induced 10-fold with lower temperature, the expression remained low. The mouse alleles H-2K^b and D^b in both cell lines, however, were expressed at 20-30% wild-type levels, and could be induced to wild-type levels with temperature and peptides. The selective detection of mouse alleles at the cell surface is not due to greater stability with human β2m, since mouse alleles dissociate more rapidly *in vitro* than human alleles. These results suggest that the difference in transport between human and mouse alleles in T2 and RMA-S reflects a fundamental property of the alleles themselves, and is not limited to human cell lines.

HZ 103 RECONSTITUTION OF HLA CLASS I TRANSPORT IN SEMI-PERMEABILIZED CELLS, Matthew J. Androlewicz and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

To elucidate the role of peptide in class I assembly and transport we developed a semipermeabilized cell system using the pore-forming bacterial toxin Streptolysin O. Our main objective was to test the effects of peptide, ATP, cytosol and other components on the reconstitution of class I transport. In semipermeabilized human melanoma cells the transport of endogenous HLA-A3 is inhibited as shown by sensitivity to Endoglycosidase H. Partial reconstitution of A3 transport is achieved after the addition of ATP, while full reconstitution of transport occurred upon addition of ATP and cytosol. The addition of an A3 binding peptide derived from the HIV nef protein had no effect on stimulating A3 transport. In addition, antibodies directed against the putative transporter of peptides (TAP) had no inhibitory effect on reconstituted transport. Finally, the reconstitution of transport we observe is not specific for class I molecules as transferrin receptor transport mimics that of class I under the conditions tested. We can conclude that cytosolic components are required for class I transport. Whether these components are peptides and/or cytosolic factors involved in vesicular transport remains to be determined.

HZ 104 DEFINING THE INTERACTION BETWEEN HLA-A2.1 AND THE ADENOVIRUS E3/19K PROTEIN. Dina C. Beier, Josephine H. Cox*, Peter Cresswell† and Victor H. Engelhard, Dept. of Microbiology and the Beirne Carter Center for Immunology Research, Univ. of Virginia, Charlottesville, VA 22908, *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892 and †Howard Hughes Medical Institute, Section on Immunobiology, Yale Univ. School of Medicine, New Haven, CT 06517.

We have analyzed a panel of HLA-A and B locus products, transfected into the human B cell line Hmy2.C1R, for their ability to associate with the adenovirus E3/19K (E19) protein. Following infection with a vaccinia expression vector containing the E19 gene, a monoclonal antibody specific for the class I heavy chain was used to detect co-immunoprecipitation with E19. Of the class I molecules tested, HLA-A2.1, A3 and B7 bind equally well to the viral protein. Compared to A2.1 however, 7-30 fold less E19 was associated with A1, Aw68, Aw69, B27 and Bw58 following immunoprecipitation. Digestion with endoglycosidase H indicated that both levels of association resulted in inhibition of intracellular transport and processing. In contrast to the human class I molecules analyzed, transport of the murine H-2D^d molecule is not inhibited in the presence of E19. Interspecies hybrid class I molecules, in which exons coding for homologous domains of A2.1 and H-2D^d have been exchanged, were used to define those regions of A2.1 that are required for association with E19. These studies indicated that the α1 and α2 domains of A2.1 contain the minimum residues necessary for both stable association with E19 and subsequent inhibition of tranport. The hybrid construct containing only the α1 domain of A2.1 was not inhibited by E19. However, the intracellular transport of the construct containing the α2 domain of A2.1 was inhibited in the presence of E19, although co-immunoprecipitation could not be detected. Taken together, these results suggest that residues in the α2 domain of A2.1 make contacts with E19 that are required for inhibition of intracellular transport of the complex, while residues in the α1 domain influence how well the class I molecule binds to the viral protein.

HZ 105 *Abstract Withdrawn*

HZ 106 MINIGENES ENCODING T CELL EPITOPES

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Cytotoxic T lymphocytes (CTL) provide a critical component of the protective immune response by eliminating virus infected cells early in infection. These effector cells recognize processed viral antigens in the form of peptides associated with class I molecules. The induction of CTL requires the presence of viral antigen in the cytosol of the antigen presenting cell. This can be achieved by designing recombinant virus vectors that encode individual CTL epitopes derived from heterologous pathogens. The expression of target CTL epitopes from minigenes instead of the full length gene has become an alternative strategy for vaccines designed to induce a protective CTL response.

To analyze the efficacy of this approach, two distinct viral CTL epitopes are studied in the murine system: one comprises a sequence from the gp160 protein of HIV-1, designated p18, the second is derived from the Murine Hepatitis Virus nucleocapsid (MHV N) protein, designated pN. The p18 epitope is presented by the murine class I Dd molecule, whereas the pN epitope is restricted to the Ld molecule. Minigenes encoding either the individual epitopes as single peptides or tandem epitopes were expressed using both recombinant vaccinia and Sindbis virus expression systems. To initially omit processing artefacts the epitopes were synthesized as polypeptides comprising appr. 80 amino acids. These cytosolic polypeptides were tested for their ability to be processed and presented to both gp160 specific and N specific CTL. Specific lysis of target cells expressing each of the minigenes was demonstrated using a standard ^{51}Chromium release assay. The p18 epitope is also efficiently recognized by gp160 specific CTL when expressed endogenously as a 10 amino acid peptide. Recognition of the individual epitopes from the tandem p18/N construct is currently under investigation. In addition, it will be determined if the minigene constructs are able to induce an epitope specific CTL response following immunization with the recombinant viruses.

HZ 107 PRESENTATION OF ENDOGENOUS PEPTIDES TO MHC CLASS I RESTRICTED CTL IN TRANSPORT DELETION MUTANT T2 CELLS.

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Mutant T2 cells, with a deletion of the two known TAP genes in the MHC and defective in presentation of cytoplasmic viral proteins to CTL, were used to study presentation of endogenously generated peptides made from minigenes. T2 cells that express minigenes encoding the influenza virus matrix peptide 58-66 (GILGFVFTL) and two HTLV I Tax peptides 11-19 (LLFGYPVYV) and 12-19 were lysed by HLA-A2 restricted peptide-specific CTL. Minigene expression of a HLA-A2 restricted HIV reverse transcriptase peptide 476-484 (ILKEPVHGV) with three charged residues sensitized T2 cells poorly for lysis by HIV-specific CTL unless the peptide was preceded by an ER translocation signal sequence. Expression of an influenza virus nucleoprotein peptide 383-391 (SRYWAIRTR) with three charged arginine residues did sensitize HLA-B27 positive T2 cells for lysis by peptide-specific CTL. These and other results with endogenously expressed peptide analogs in which hydrophobic and charged amino acids were interchanged demonstrate that antigenic peptides can be translocated from the cytoplasm into the class I antigen presentation pathway independent of MHC encoded transporters; and that peptide hydrophobicity appears not to be a major determinant in selecting peptides for this alternate pathway.

HZ 108 PEPTIDE BINDING TO THE MURINE MHC CLASS I H-2K^k ALLELE, Eugene L. Brown, Joseph L. Wooters, Catherine R. Ferenz, Rodney M. Hewick and Steven H. Herrmann, Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140

We have isolated and sequenced peptides bound by the murine major histocompatibility complex class I H-2K^k protein. The peptide-class I complex was isolated from RDM-4 and x5563 cells with a 11-4.1 monoclonal antibody affinity column. An acid denaturation of the complex followed by HPLC fractionation lead to the determination of ten individual sequences. All sequences characterized were 8-mers with glutamic acid and isoleucine the most common amino acid found in the P2 and P8 positions, respectively. Several of the peptides were derived from abundant intracellular proteins such as, actin, BiP/GRP78 and a mammalian analogue of the yeast S24 ribosomal protein.

To better understand how peptides interact with the H-2K^k molecule, a competitive binding assay utilizing immobilized MHC and a biotinylated peptide for signal generation was developed. A number of shorter peptides derived from the influenza virus nucleoprotein epitope, SDY EGR LIQ NSL TI, were tested to evaluate the assay; we conclude that the observed binding was cleft-specific. The complete set of single-alanine variants for one "self-peptide" was tested in the assay and the results showed that only substitutions at P2 and P8 significantly decreased the affinity for the class I protein. A comparison of the relative affinity of a number of "self-peptides" with several reported K^k-restricted epitopes within the influenza virus hemagglutinin is expected to help understand how peptides make their way to the cell surface complexed with the class I MHC molecule.

HZ 109 ASSEMBLY AND THERMOSTABILITY OF PURIFIED CLASS I COMPLEXES, Deborah N. Burshtyn and Brian H. Barber, Dept of Immunology, University of Toronto, Toronto, Canada M5S 1A8.

Recent reports have indicated that peptide antigens induce conformational changes of class I heavy chains, rendering them reactive in detergent lysates with α1 and α2 domain specific antibodies[1]. We have extended these findings to a purified system using isolated H-2D^b heavy chains adsorbed through the α3 domain to an antibody-bead matrix. In the presence of specific peptides or β_2m these molecules bind a radiolabelled antibody reactive with the α1 domain of the folded D^b heavy chain. By utilizing this purified solid phase system, we have examined the kinetics and temperature dependence of the generation and decay of the D^b α1 epitope. We have examined the various combinations of heavy chain with peptide and β_2m and the effect of peptide length on the thermostability of the complex. Reciprocally, we have characterized the association kinetics of radiolabelled peptide to *in vitro* assembled D^b-β_2m complexes and those derived from the RMA and RMA-S cell lines. We have observed rapid association of peptide to pre-formed complexes of D^b and β_2m as compared to the co-incubation of all three subunits. The combination direct peptide association kinetics and the α1 epitope configuration suggest that at physiologic temperatures, both β_2m and peptide are required to create a stable folded D^b complex. However, complexes of the heavy chain and optimal peptidesp produce a longer half-life for the α1 epitope than β_2m heavy chain complexes at all temperatures examined. Therefore, once formed, the rate of decay of the heavy chain α1 epitope is predominantly determined by the associated peptide.
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1. Elliott, T. et al., Eur. J. Immunol. (1992) **22**; 2085. Elliott, T. et al., Nature (1991) **351**; 402.

HZ 110 AMINO ACID SUBSTITUTIONS ON THE HLA-A2 MOLECULE CAN ABROGATE OR ENHANCE RECOGNITION OF INFLUENZA VIRUS M1 PEPTIDE PULSED CELLS BY CYTOTOXIC T LYMPHOCYTES, Ming Cao, Stephanie L. Brown and Kevin T. Hogan, Department of Microbiology, Medical College of Wisconsin, Milwaukee, WI 53226

The functional role of individual residues of the HLA-A2.1 molecule was examined by utilizing a panel of cells expressing variant HLA-A2 molecules that had been created by site-directed mutagenesis of the *HLA-A*0201* gene and transfection of the resulting mutant genes into the class I MHC-null cell line, HMY2.C1R. A total of 69 variant molecules encompassing 28 different positions were created, with each of the variants differing from the prototypic HLA-A2.1 by a single amino acid substitution at positions within either the α1 or α2 domains. The effects of these substitutions on HLA-A2.1-restricted, influenza virus matrix protein M1 (57-68) peptide specific CTL recognition was investigated by using peptide titration, cold target inhibition, and time course analysis. Studies on variants with amino acid substitutions that are predicted to interact with the TCR showed that most substitutions in the α1 domain do not have an important effect on recognition by the M1 peptide specific, HLA-A2.1-restricted CTL. In contrast, substitutions at positions 154, 162, and 163 in the α2 domain abolished recognition by the same CTL. Additionally, substitutions at positions 138 in the α2 domain and positions 107 and 127 on the loops connecting the β-strand in the α1 and α2 domains, were recognized in a more efficient, heteroclitic fashion. These results indicate that the "footprint" of the TCR may be more extensive than previously predicted and encompass a broad region that extends beyond the α2-helix and includes the loop regions of the underlying β-sheet. Similar studies on HLA-A2 variants, with amino acid substitutions within the peptide binding groove, showed that amino acid substitutions at positions 9, 66, 74, 99, 152, and 156 could abolish CTL recognition, while variants with amino acid substitutions at positions 95 and 171 enhanced M1 peptide presentation. These results, combined with those obtained from other laboratories, demonstrate the importance of each of the six defined "pockets" (A-F) in the HLA-A2 molecule for peptide presentation.

HZ 111 MANIPULATING MUCOSAL IMMUNE RESPONSE BY INTRADERMAL IMMUNIZATION

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Each year, production losses in the livestock industry amounting to millions of dollars, can be attributed to the activities of parasites and microbial pathogens. Many of these parasites and pathogens possess a predilection for the skin surface or the mucosal epithelia of the respiratory, digestive and reproductive tract. For example, while the aetiologic agent for scabby mouth (pox virus), fleecerot (*Pseudomonas aeruginosa*) and footrot (*Bacteroides nodosus*) as well as ecto-parasites such as itchmite and lice are primarily skin pathogens, microbial agents such as *Mycoplasma* spp., *Haemophilus* spp., respiratory synctial virus; *E. coli*, *Treponema* spp., *Salmonella* spp.; and *Brucella* spp., are considered important pathogens of the respiratory, digestive and reproductive tract respectively. Can a global strategy be devised for immunisation against both skin and mucosal pathogens? Traditionally, IgA antibodies against mucosal pathogens is best elicited by intraperitoneal priming with adjuvanted antigen followed by local stimulation. It is believed that Peyers patches play a pivotal and vital role in mucosal immunity. However, recent studies on the mechanism of cellular immune responses in the skin as well as the mucosal epithelia, suggest that there may be sufficient similarities in these two organ compartments to permit the use of the skin as a route for the manipulation of mucosal immune responses. Inflammatory responses in the skin arising from infection or infestation results in the mobilization of distinctive subsets of effector lymphocytes. Intradermal injection of antigen with an appropriate adjuvant can simulate such changes and provide a mechanism for the recruitment of memory T and B cells in the skin compartment. These may then relocate to distal mucosal sites. We now report a successful method for manipulating mucosal immune in the respiratory tract of sheep by vaccination via the skin route. IgG, IgA and IgM levels are stimulated and there is also a memory response.

HZ 112 B POCKET TRANSPLANT FROM HLA-A2 INTO B27 CHANGES REQUIREMENT FOR P-2 ANCHOR RESIDUE IN PEPTIDE PRESENTATION. Robert A. Colbert, Sarah Rowland-Jones*, Andrew J. McMichael*, and Jeffrey A. Frelinger, *Institute of Molecular Medicine, University of Oxford, Oxford, OX39DU UK, and Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC, 27599.

The peptide binding site in MHC class I molecules contains six pockets (A-F) formed by two adjacent α-helices and the underlying β-sheet. Most amino acids in pockets A and F are conserved and appear to interact with amino and carboxyl termini of bound peptides, while many other residues comprising pockets B-E are polymorphic, and may determine the allelic-specificity of bound peptides. To test this directly we mutated the HLA-B*2705 gene at six positions to encode a B27 molecule, designated B27A2.6, whose B pocket is identical to HLA-A*0201. Two other mutants with 4 and 5 substitutions were also produced. Transfection of these genes into C1R cells results in surface expression of mutants at levels comparable to B*2705, yet they are not recognized by alloreactive CTL, indicating that these mutants bind a distinct set of endogenous peptides. When treated with a known B27-restricted influenza A nucleoprotein epitope (NP 383-391), or infected with virus, these mutants are not killed by virus-specific CTL lines or clones. This is consistent with loss of peptide binding due to the A2-like B pocket no longer accommodating a peptide with Arg at P-2. Furthermore, NP 383-391 with Leu (the A-2 anchor residue) substituted for Arg at P-2 restores recognition by these same CTL. A complete A2-like B pocket appears to be required since the two other mutants are not recognized. The requirement for Arg at P-2 in the NP peptide is quite stringent, since even the conservative substitution of Lys for Arg abolishes recognition. In contrast, a Lys for Arg substitution at P-9, predicted to interact with the F pocket, is recognized normally. These functional data, combined with the refined crystal structure of HLA-B27, firmly establish the B pocket-P-2 side chain interaction, confirm the "left to right" orientation of peptide within the groove, and demonstrate the specificity of this pocket for Arg. Thus the B pocket appears to play a dominant role in the allelic specificity of HLA-B27 and HLA-A2.

HZ 113 SEQUENCE MOTIFS IMPORTANT FOR PEPTIDE BINDING TO HUMAN MHC CLASS I MOLECULES. John E. Coligan, Lisa K. Hull, Marianne DiBrino, Ursula Utz, William E. Biddison, and Kenneth C. Parker, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; Molecular Immunology Section, Neuroimmunology Branch, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD 20892

Previous studies have indicated that most HLA-A2 binding peptides are 9 amino acid residues (aa) long, with a LEU at position 2 (P2), and a VAL or LEU at P9. We compared the binding properties of different peptides by measuring the rate of dissociation of β_2m from peptide-specific HLA-A2 complexes. The simplest peptide that we identified that could form stable HLA-A2 had the sequence (in single letter aa code) GLFGGGGGV, indicating that three non-glycine aa are sufficient for binding to HLA-A2. To determine whether most nonapeptides that contained LEU at P2 and VAL or LEU at P9 could bind to HLA-A2, we tested the binding of nonapeptides selected from published HIV and melanoma protein sequences, and found that six of seven tested formed stable HLA-A2 complexes. We identified an optimal antigenic undecapeptide from the cytomegalovirus gB protein that could form stable HLA-A2 complexes that contained apparent anchor residues at P2 and P11 (sequence FIAGNSAYEYV), indicating that the spacing between anchor residues can be somewhat variable. We measured the stability of a series of single-site mutant HLA-A2 complexes containing each of three antigenic peptides (HTLV1 tax, influenza M1, and hCMV gB). We found that certain mutations caused all three antigenic peptides to bind less well than the wild type. Other mutations were nearly neutral for all three peptides, whereas still others enhanced binding for all three peptides. Even though these mutations were localized to certain pockets within the groove, most of these mutations did not alter the specificity of peptide binding; rather they changed the stability of peptide/MHC complexes. In addition, endogenous peptides isolated from a number of human class I molecules have been used to identify anchor residues important for the specificity of peptide binding to class I molecules.

HZ 114 β2-MICROGLOBULIN (β2-m) REGULATES MHC CLASS I H-CHAIN SURFACE EXPRESSION AND CONFORMATION, Ursula Danilczyk and Terry L. Delovitch, Banting and Best Department of Medical Research and Department of Immunology, University of Toronto, Toronto, Ontario, Canada M5G 1L6

We introduced the native HLA-A3 H-chain gene and a truncated (lacks an intron 3 downstream regulatory element of 450 bp) form of this gene into separate founder lines of transgenic mice. Although these mice transcribe and translate both forms of the HLA-A3 gene, respectively, they express different levels of the surface HLA-A3 antigen in thymocytes and splenic T cells. The level of surface expression of the HLA-A3 transgene product correlates inversely with that of endogenous K^d and D^d mouse class I molecules. The free HLA-A3 H-chain can be expressed at the cell surface in the absence of human β2-m. HLA–A3 H-chains associate intracellularly with mouse β2-m, and and are transported to the cell surface in association with mouse β2-m. Reactivity patterns of a panel of antibodies that react with either the free or β2-m-bound HLA-A3 H-chain demonstrate that a stable conformation of the HLA-A3 H-chain is induced by its association with mouse β2-m. This conformation can not be altered by exchange of associated mouse β2-m for human β2-m. These data indicate that the conformation of an MHC class I H-chain assumed during its intracellular association with β2-m is not altered appreciably after it reaches the cell surface. (Supported by MRC of Canada).

HZ 115INTRACELLULAR ASSOCIATION OF A SYNTHETIC PEPTIDE WITH A MHC CLASS I MOLECULE. Patricia M. Day, Jack Bennink, Jan Lukszo and Jonathan Yewdell. Viral Immunology Section, Laboratory of Viral Diseases. National Institute of Allergy and Infectitious Diseases. National Institutes of Health. Bethesda, Maryland 20892.

We synthesized a fluorescein deriviative of a peptide that represents the precise determinant recognized by CTL raised against a viral protein. Flow cytometric analysis demonstrated peptide-binding to live cells infected with a vaccinia virus recombinant expressing the known class I restriction element. No binding was evident to cells that were infected with recombinants expressing irrelevant class I molecules. Interestingly, when surface expression of the class I protein was prevented by brefeldin A treatment, peptide binding was still clearly detected. Therefore, it appears that specific association of this peptide with class I molecules can occur intracellularly. Fluorescence microscopy confirmed that the peptide was located within the cell. The accessory requirement of peptide transport and association with class I are being investigated.

HZ 116 A MUTATION IN THE "NONANCHOR" RESIDUE OF CLASS I RESTRICTED PEPTIDES THAT AFFECTS BOTH MHC BINDING AND T CELL RECOGNITION, R. Dyall and J. Nikolic-Zugic, Laboratory of T Cell Development, Sloan-Kettering Institute, New York, NY 10021

Three nonconservative mutations were introduced into a K^b-restricted $OVA_{257-264}$ peptide $S_1I_1I_3N_4F_5E_6K_7L_8$ to study the peptide *MHC:*interaction. Mutations 7K→E and 7K→Q did not affect K^b binding, while 2I→E decreased it by two orders of magnitude. Analysis with a panel of CTL lines and clones revealed a complete absence of CTL recognition of 7K→E, indicating that position 7 contacts the TcR. By contrast, 7K→Q and 2I→E were recognized about twofold worse than the wild type peptide. Analysis of presentation of mutant peptides by K^{bm} molecules revealed two points. First, when mutations in the peptide and in the MHC were in the vicinity of each other, recognition of the complex was often better than the recognition of mutant peptide + K^b or wild type peptide + K^{bm}. And second, this effect is very pronounced in the case of 2I→E + K^{bm8}. K^{bm8} is a poor presenter of wild type OVA. For example, clone B3 weakly recognizes the complex even if huge concentrations of OVA are used. However, excellent lysis is obtained when 2I→E is used to sensitize K^{bm8} target cells. In light of the binding data, we discuss the mechanism of action of the 2I→E mutation.

HZ 117 **TARGETED GENE DISRUPTION OF THE MURINE INVARIANT CHAIN GENE IN EMBRYONIC STEM CELLS**

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The invariant chain (Ii) noncovalently associates with the alpha and beta chains of the MHC class II molecule following their synthesis and entrance into the endoplasmic reticulum (ER). The Ii chain gene is coordinately regulated with class II gene transcription although it is not encoded within the MHC. A number of different polypeptides are encoded by a single Ii chain gene due to alternate splicing of a single exon and the variable usage of two translational start sites.

Recently, it has been suggested that MHC-encoded class I and class II molecules present different sets of antigenic peptides to their corresponding T cells. Class I molecules present endogenously synthesized processed peptides and class II molecules present exogenously synthesized-processed peptides. It has been proposed that the Ii chain may play a role in peptide discrimination. One hypothesis proposes that the Ii chain is involved in targeting and transport of class II molecules from the ER to the endosome where it meets peptide antigen. A second hypothesis suggests that the Ii chain functions as a blocking agent, by either binding to the class II molecule and shielding the binding groove or by potentiating a conformational change in the class II molecule which inhibits peptide binding.

Toward decíphering the function of the class II-associated Ii chain, we are attempting to generate Ii deficient mice by homologous recombination in embryonic stem cells (ES). We have successfully established both heterozygous and homozygous ES cell lines carrying deletion-insertions which remove the promoter and exon 1 of the Ii chain gene. We have generated a large number of chimeric mice and are currently backcrossing these mice to establish a germline transmitting breeding pair.

HZ 118 **PEPTIDES BOUND TO HLA-B7 DETERMINED BY MASS SPECTROMETRY**, Victor H. Engelhard, Eric L. Huczko, Wanda Bodner, David Benjamin, Kazuyasu Sakaguchi, Nian Zhu Zhou, Jeffrey Shabanowitz, Robert Henderson, Ettore Appella, and Donald F. Hunt, Beirne Carter Center and Department of Chemistry, University of Virginia, Charlottesville, VA 22901

Microcapillary HPLC/ electrospray ionization/ tandem mass spectrometry was used to sequence 18 peptides eluted from the human class I molecule HLA-B7. 16 of these were 9 residues long, while 2 were 10mers, and one was an 11mer. All peptides contained a small hydrophobic residue at the carboxyl terminus, 14 contained proline at position 2, and 11 contained a positive charge, usually arginine, at position 3. Alanine or arginine was found at position 1 in 16 sequences. Direct binding experiments using synthetic peptides indicated that these conserved features were important determinants for specific interaction with HLA-B7, and suggested a similar mode of interaction regardless of peptide length. Modeling of peptides in the binding cleft of a predicted HLA-B7 structure, derived from the known crystal structure of HLA-B27, suggests explanations for the observed residue preferences. The B, D, and F pockets are involved in selection of residues at position 2, 3, and 9. The size and nature of the F pocket is similar to that of some other class I molecules and allows for the observed range of several small hydrophobic residues at the carboxyl terminal position. Proline appears to be preferred at position 2 because the B pocket of B7 is drastically reduced in size and hydrophilicity compared to B27 due to the substitution of tyrosine for cysteine 67. Arginine at position 3 participates in extensive van der Waals interactions in the D pocket, and forms a salt link with aspartate 114. These are the first data to suggest a role for the D pocket in specific peptide binding. These results provide additional support for a general mode of peptide interaction common to several class I MHC molecules, but demonstrate additional constraints on peptide binding to HLA-B7 that impose additional restrictions on peptide heterogeneity.

HZ 119 ALTERNATIVE SPLICING AND POST-TRANSLATIONAL PROCESSING OF THE MHC-ENCODED PROTEASOME COMPONENT, LMP7. Richard J. Glynne, Lesley-Anne P. Kerr, C. Ian Mockridge, Adrian P. Kelly and John Trowsdale. Human Immunogenetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.

The *LMP7* gene maps to the MHC class II region between the *TAP1* and *TAP2* ABC transporter genes. Derived protein sequence from the second exon of the *LMP7* gene shares homology with N-terminal peptide sequence from proteasome subunits. Here we show that the *LMP7* locus encodes two messenger RNAs, *LMP7a* and *LMP7b*, which have alternative first exons but are otherwise identical. Both transcripts are expressed in a variety of cell lines and both are up-regulated by interferon-γ. The first exons of *LMP7a* and *LMP7b* encode protein sequences which are similar in molecular weight (about 5 kD) but which have different predicted pI values (3.3 and 10.5, respectively). The derived protein sequence of *LMP7b* has homology to the equivalent mouse and rat cDNAs throughout its length, whereas that of *LMP7a* has no homology to any of the published rodent cDNAs over the N-terminal 45 amino acids. Here we show biochemically that the LMP7 protein is incorporated into the proteasome complex. The LMP7 encoded subunit is ~5kD smaller than expected from the cDNA-derived protein sequence. Pulse-chase analysis shows that about 5 kD is cleaved off the N-terminus of the LMP7 protein prior to its incorporation into the proteasome as a 23 kD protein. The half-life for this process is approximately 2 hours. We propose that the alternative first exons of the *LMP7* gene encode two leader sequences with differing pI values. The resulting proteins may be directed to different intracellular compartments or may associate with different proteins.

HZ 120 A NOVEL GENETIC APPROACH TO THE IDENTIFICATION OF PROTEINS REQUIRED FOR THE PRESENTATION OF INTRACELLULAR ANTIGENS TO CTL, Keith Gould, Alison George, Catherine Hubbard*, Wen Chang*, H. Earl Ruley*, George G. Brownlee and Jasper Rees, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U. K. and *Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge MA 02139

Antigen processing and presentation to T cells is observable only in intact cells, and a genetic approach to the identification of the proteins involved in this pathway is therefore attractive. Already, antigen presentation mutants such as the RMA-S cell line, initially selected for low cell-surface expression of MHC class I molecules, have played a major part in providing the evidence for the current model of the class I presentation pathway. We are using a novel insertional mutagenic technique using a defective retrovirus in order to isolate new antigen presentation mutants. Initially, Chinese hamster ovary (CHO) cells, which are functionally haploid, were transfected with the murine ecotropic retrovirus receptor in order to make them susceptible to infection by the "promoter trap" retrovirus. This virus was designed such that only insertions ocurring just downstream of active cellular promoters are recovered as hygromycin resistant clones. Subsequently, the CHO cells were modified so that they expressed both the mouse MHC class I K^k molecule and the signal-minus influenza A/PR/8/34 hemagglutinin (HA). A CHO cell line was derived which was recognized and lysed by HA-specific, K^k-restricted CTL clones, giving ~60% specific lysis in a 5h chromium release assay. This cell line was infected with the "promoter trap" retrovirus and a library of hygromycin resistant clones was generated. Potential antigen presentation mutants were selected from this library by repeated exposure of the cells to HA-specific CTL. CTL-resistant clones were isolated by limiting dilution of the resulting polyclonal population of cells, and these clones were analysed for cell-surface expression of K^k by FACS. The majority of the clones were negative for surface K^k, but 5 out of 32 clones showed positive, but greatly reduced cell surface expression. These 5 clones were not recognized by HA-specific CTL in a chromium release assay, and the sites of integration of the retrovirus in these cell lines are being determined using PCR methods in order to characterize the disrupted genes.

HZ 121 SELECTIVE RELEASE OF PEPTIDES BY LYSOSOMES, Lois Isenman and J. Fred Dice, Department of Physiology, Tufts University School of Medicine, Boston, MA 02111

A 10,000 x g fraction prepared from human fibroblasts that had previously endocytosed [^{3}H]RNase A was incubated for 2 h, repelleted, and the repelleted material and supernatant analyzed by chromatography. In fibroblasts RNase A is degraded in lysosomes, and a 50 min wash preceding cell harvesting would have chased the label out of endosomes. Radiolabeled fragments with a molecular weight of 400 Da as well as several labeled fragments with molecular weights between 900 and 1900 Da were released by the in vitro lysosomes. This includes peptides in the approximate size range of antigens for T cell-mediated immunity. At 24° C, but not at 0° C, 400 Da fragments were formed within lysosomes and readily released by them. Release was selective since almost no intact RNase A and <10% of the major labeled fragments, peptides of 550 Da, were secreted. Human fibroblasts that had endocytosed [^{3}H]RNase A secreted similar peptides into chase media and likewise most of the radioactivity remaining in cells was in 550 Da fragments; this strongly suggests that the release observed in vitro was not due to leaky or damaged membranes. We confirmed the capacity of lysosomes for the selective secretion of small peptides by demonstrating a 4-to 5-fold range in the relative release in vitro of three biochemically similar peptide probes. These results suggest a pathway for the selective secretion of lysosomal degradation products such as peptide antigens and possibly the Alzheimer's ß-amyloid protein.

HZ 122 EXPRESSION CLONING OF ENDOGENOUS T-CELL ANTIGENS, Jaana Karttunen, Sarah Sanderson, and Nilabh Shastri, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

We have developed a novel expression cloning strategy for isolating cDNAs encoding Class I restricted T-cell peptide antigens. Our system consists of two parts: 1) The first is an extremely sensitive T-cell activation assay. Using a *lacZ* reporter construct, we can rapidly detect ligand-induced activation of single activated T-cells, even when they are present at very low frequencies (1 in 10^3 - 10^4 cells)[1]. This high level of sensitivity allows the detection of correspondingly low frequencies of antigen presenting cells (APC). 2) The second is our choice of APC. Instead of using lymphocytes or L-cells as APC, we use COS cells stably transfected with Class I murine major histocompatibility complex (MHC) molecules[2]. These cells support the replication of circular DNA containing the SV40 replication origin. When the MHC expressing COS cells are transfected with plasmid vectors containing the SV40 origin, high levels of transient protein and peptide/MHC ligand expression can be obtained. Thus, by using *lacZ* inducible T-cells as indicators to detect peptide/MHC expression on COS cells, cDNA libraries can be screened for sequences encoding peptide antigens. We describe here the *lacZ*/COS cell system and its application in identifying the H-Y antigen, an unknown histocompatibility antigen expressed on male tissue and defined only on the basis of T-cell reactivity.

1. Karttunen, J. & Shastri, N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3972-3976.
2. Karttunen, J., Sanderson, S. & Shastri, N. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6020-6024.

HZ 123 RESTORATION OF MUTANT PHENOTYPES BY TRANSFECTION OF TAP1 AND TAP2, Adrian Kelly and John Trowsdale, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

We have identified a cluster of four genes (TAP1, TAP2, LMP2 and LMP7), from within the MHC class II region, that display properties suggestive of roles in antigen processing. We have analysed gene function through complementation of mutant cells (BM36.1, LCL.174 and RMAS) that possess defects in antigen processing characterised by an inability to present intracellular antigens, and the possession of unstable class I molecules. A defective TAP2 gene was identified in BM36.1 and shown to be responsible for the mutant phenotype. Independent transfection of these cells with three full length TAP2 cDNA alleles resulted in restoration of wild-type phenotype. No differences in peptide presentation or in the profiles of peptide eluted from different TAP2 transfectants were observed. Complementation of the murine line RMAS by the human TAP2 gene demonstrated that despite cross species differences the TAP complex still functioned in antigen processing.

Cotransfection of TAP1 and TAP2 into the deletion mutant LCL.174 resulted in restoration of surface class I expression and presentation of cytoplasmic antigens to class I restricted T cells. These data have so far failed to identify a function for either LMP2 or LMP7, but we have preliminary protein data demonstrating a direct association between the TAP and LMP proteins.

HZ 124 TRANSPORT OF ANTIGENIC PEPTIDES INTO MICROSOMAL VESICLES

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The class I major histocompatibility complex (MHC) molecules are polymorphic glycoproteins that bind antigenic peptides as part of their biosynthetic assembly process and are expressed on the plasma membrane of nearly all nucleated cells. At the cell surface, the peptide-class I MHC complexes are recognized by T cell receptors on $CD8^+$ T lymphocytes 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. Additional evidence for this route of transport comes from the identification of two MHC encoded genes with sequence homology to the family of ATP-binding-casssette transporter molecules. In order to characterize this pathway, we have developed *in vitro* assays for the transport of antigenic peptides into the rough endoplasmic reticulum. Previously we have demonstrated transport of chemically synthesized peptides (13-21 amino acids) containing N-linked glycosylation acceptor sequences into microsomal vesicles derived from canine pancreas. The peptides served as glycosylation substrates and their transport resulted in the depletion of the pool of available dolichol high mannose oligosaccharides in the lumen of the microsomal vesicles. Presently we are using two additional biochemical assays to measure this process. Rapid filtration of radiolabeled peptides coincubated with microsomal vesicles should be able to reveal the kinetics of this transport process. Independently, the ability of labeled glycosylation acceptor peptides to bind to Conconavalin-A following incubation with microsomal vesicles has supported the assumption that the peptides have entered the lumen of the vesicles. We are currently purifying rough microsomal vesicles from human lymphoid cells in an effort to ascertain whether transport activity is higher in this tissue. Results will also be presented comparing the transport of nonameric versus larger size peptides.

HZ 125 **CLASS I MHC MOLECULES RETAINED IN THE ENDOPLASMIC RETICULUM BIND TO NATURALLY PROCESSED ANTIGENIC PEPTIDES,**

Cheryl K. Lapham, Igor Bacik, Jonathan W. Yewdell, Kevin Kane, and Jack R. Bennink, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD.

We isolated MHC-specific viral peptides from cells infected with influenza virus in the continuous presence of the drug brefeldin A (BFA), which blocks exocytosis of newly synthesized molecules. MHC specific peptides were also isolated from cells expressing an endoplasmic reticulum (ER)-retained form of the mouse K^d class I MHC molecules. Since the association of antigenic peptides with class I is necessary for their isolation from whole-cell extracts, this provides direct evidence that naturally processed peptides associate with class I MHC molecules in an early intracellular exocytic compartment.

HZ 126 ALTERNATIVE SPLICING OF HLA CLASS I TRANSCRIPTS INDUCED BY IFN-γ AND TNF IN FIBROBLASTS: RELEASE OF SOLUBLE HLA CLASS I HEAVY CHAIN AND AN ASSOCIATE PROTEIN. Junming Le and Xiao He. Department of Microbiology, New York University Medical Center, New York, NY 10016

FS-4 fibroblasts were found to secrete HLA class I heavy chain in response to IFN-γ or TNF. Monoclonal antibodies known to recognize nonpolymorphic epitopes on HLA class I heavy chains unequivocally precipitated the 37 kD soluble HLA class I heavy chain. Induction of soluble HLA class I molecules by IFN-γ or TNF is time- and dose-dependent, and a synergism between IFN-γ and TNF was observed. Immunoprecipitation of IFN-γ- or TNF-induced FS-4 cell culture supernatants by mAb A1.4 revealed an additional 33 kD protein in association with the 37 kD heavy chain. The 33 kD protein appeared to be expressed in a 38 kD form on the membrane of FS-4 cells induced by IFN-γ or TNF, as immunoprecipitation of the lysates of radioiodinated FS-4 cells by A1.4 resulted in the appearance of the 38 kD band in association with the 44 kD transmembrane HLA class I heavy chain. Protein fingerprinting studies showed different digestion patterns of the 37 kD heavy chain and the 33 kD protein by three different proteases, suggesting that the 33 kD molecule is structurally unrelated to HLA class I heavy chain. Treatment with endoglycosidase F resulted in band shifts of both proteins from 37 kD and 33 kD to 34 kD and 30 kD, respectively, indicating that both are N-linked glycoproteins. Secretion of the 37 kD heavy chain could well be due to an alternative RNA splicing with the deletion of exon 5 of HLA class I heavy chain gene, because exon 5 encodes the hydrophobic transmembrane region of membrane-anchored HLA class I heavy chain. Northern blot analysis and S1 nuclease protection assay using an antisense oligonucleotide probe (A4-6) consisting of the 3' boundary region of exon 4 and the 5' boundary region of exon 6 suggested the existence of HLA class I heavy chain mRNA lacking exon 5 in IFN-γ- or TNF-induced FS-4 cells. Further evidence was provided by Southern blot analysis on the products of reverse transcription-PCR amplification from cytoplasmic RNA of IFN-γ- or TNF-induced FS-4 cells. While an antisense oligonucleotide (A5) corresponding to a conservative region of exon 5 only hybridized with full-length HLA class I heavy chain cDNA, A4-6 probe detected the cDNA reverse-transcribed from the alternatively spliced heavy chain mRNA lacking exon 5. Soluble HLA class I molecules induced by IFN-γ and TNF may play important roles in the regulation of T cell interaction with antigen-presenting cells.

HZ 127 Vaccination to Immunodominant MHC Class I Binding Peptides: Quil A and Peptide Containing Liposomes Induce Specific CD8+ Cytotoxic T Cells, Grayson B. Lipford, Hermann Wagner and Klaus Heeg, Institute for Medical Microbiology, Technical University of Munich, Munich, Germany.

Antigen-specific cell-mediated immune responses are believed to be crucial for protection against viral and intra-cellular parasites. CTL recognize short peptides (8-10 amino acids) which are the product of processing intracellular proteins presented at the surface by major histocompatibility complex (MHC) class I molecules. In the past, in vivo induction of CTL usually required the infection of the host, which allows for antigen colocalization with cellular processing and presentation machinary of the MHC class I pathway. We have previously demonstrated that ISCOM (a form of Quil A containing liposome) could be used to shuttle soluble proteins into the class I pathway resulting in the in vivo induction of antigen specific CTL. In an effort to extend the usefulness of exact peptide CTL epitopes, we encapsulated class I binding peptides into Quil A containing liposomes followed by inoculation to induce primary CTL. Liposomes were formed by a dry down method followed by resuspension with an aqueous solution containing peptide and Quil A and an extrusion step. Mice were injected in the foot pad and four days later the draining lymph nodes were harvested. The cells were cultured four days in IL-2 containing media followed by a chromium release assay utilizing peptide pulsed cells or transfectants as targets. As a working model the ovalbumin (OVA) peptide 257-264 was used, which has been shown to be Kb dependent in C57Bl/6 mice. The elicited response was CD8+ T cell mediated and peptide dose dependent, while Quil A as well as intact liposome structure were necessary. The approach proved to be versatile. CTL responses were raised against an extended OVA peptide 254-276 yielding CTL responsive to OVA 257-264, which implied proper processing. A specific response could also be raised to vesticular stomatitis virus peptide 52-59, another Kb restricted epitope. In addition, in Balb/c mice, CTL were induced with listeriolysin peptide 91-99, which is Kd restricted, and CMV pp89 168-176, which is Ld restricted. These applications encompassed two mouse strains, three class I presenting structures and antigens from various sources. Currently under investigation are; protection by vaccination with peptide, antigenicity of immunodominant peptides which have been point substituted at various amino acid positions, and the influence of flanking sequences in processing. These data could rapidly enhance our understanding of subunit vaccine viability and the rules of processing and MHC class I binding.

HZ 128 EFFECT OF AMINO ACID SUBSTITUTIONS IN THE REGION OF SV40 T ANTIGEN CTL DETERMINANTS ON PROCESSING, PRESENTATION AND RECOGNITION BY SV40 SPECIFIC CTL CLONES, John D. Lippolis and Satvir S. Tevethia, Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, Hershey, PA 17033.

Simian Virus 40 (SV40) T antigen is a transforming protein and immortalizes cells in culture and induces neoplasia *in vivo*. Mice immunized with cells expressing T antigen induce the generation of cytotoxic T lymphocytes (CTL). T antigen specific CTL clones established from C57Bl/6 mice designated as Y-1, Y-2, Y-3, Y-4 and Y-5 recognized five discrete epitopes in T antigen. Site I is recognized by CTL clone Y-1 and site II/III is recognized by CTL clones Y-2 and Y-3; these sites are H-2D^b restricted. In order to study the structure function relationship, point mutants were generated in T antigen residues 200 through 239 spanning epitopes I, II/III and their flanking sequences. The mutant T antigens were expressed in primary C57Bl/6 mouse embryo fibroblast cells and the transformed cells were utilized as targets for lysis by CTL clones Y-1, Y-2 and Y-3. The results showed that amino acid substitutions outside the identified epitopes did not effect recognition of either CTL clones Y-1, Y-2 or Y-3, with the possible exception of a serine to proline substitution at 206. The 206 proline substitution partially inhibits recognition by CTL clone Y-1 but does not affect recognition by the CTL clones Y-2 or Y-3. Studies are is progress to determine whether residue 206 is a flanking residue or part of the endogenous peptide epitope. T antigen with amino acid substitutions at residues 207, 210, 212 and 214 in site I was not recognized by the CTL clone Y-1. The amino acid substitutions in residues 224, 226, 228, 229 and 230 in site II/III affected CTL recognition differentially by CTL clone Y-2 and Y-3. Amino acid substitutions which do not affect CTL recognition (208, 209 or 213) may not play a critical role in binding to either the MHC class I or the T cell receptor and may be spacer residues.

HZ 129 PRESENTATION OF ENDOGENOUS CYTOSOLIC ANTIGEN BY CLASS II MHC MOLECULES, Eric O. Long, Mauro S. Malnati, Timothy LaVaute, Stephanie Ceman, and Robert DeMars, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, and Department of Genetics, University of Wisconsin, Madison, WI 53706

Exogenous antigen endocytosed into acidic compartments is very efficiently presented to T cells by class II MHC molecules. In addition, endogenous antigen synthesized within the cytosol may also be presented by class II molecules. By use of recombinant vaccinia viruses encoding the naturally cytosolic matrix protein of influenza virus or a cytosolic form of the influenza virus hemagglutinin, we have demonstrated that expression of these antigens in B cells led to presentation to HLA-DR1-restricted T cell clones. The presentation of these 2 cytosolic antigens occurred in a mutant B-cell line deficient in TAP1, TAP2, LMP2 and LMP7 genes. Thus, neither TAP transporters, nor MHC-encoded proteasome subunits are required for class II-restricted presentation of cytosolic antigen. However, this endogenous pathway of antigen processing for presentation by class II was dependent on another function encoded within the class II region of the MHC.

HZ 130 DIRECT BINDING OF PEPTIDES TO MHC CLASS I MOLECULES ON LIVING CELLS: **Analysis at the Single Cell Level,** José Alejandro López, Immanuel F. Luescher, and Jean-Charles Cerottini. Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland.

To directly assess the binding of exogenous peptides to cell surface-associated MHC class I molecules at the single cell level, we examined the possibility to combine the use of biotinylated peptide derivatives with an immunofluorescence detection system based on flow cytometry. Various biotinylated derivatives of the adenovirus 5 early region 1A peptide 234-243, an antigenic peptide recognized by CTL in the context of H-2D^b, were first screened in functional assays for their ability to bind efficiently to D^b molecules on living cells. Suitable peptide derivatives were then tested for their ability to generate positive fluorescence signals upon addition of phycoerythrin-labeled streptavidin to peptide derivative-bearing cells. Strong fluorescent staining of D^b-expressing cells was achieved after incubation with a peptide derivative containing a biotin group at the C terminus. Competition experiments using the unmodified parental peptide as well as unrelated peptides known to bind to K^d, K^b or D^b, respectively, established that binding of the biotinylated peptide to living cells was D^b-specific. By using Con A blasts derived from different *H-2* congenic mouse strains, it could be shown that the biotinylated peptide bound only to D^b among > 20 class I alleles tested. Moreover, binding of the biotinylated peptide to cells expressing the D^{bm13} and D^{bm14} mutant molecules was drastically reduced compared to D^b. Binding of the biotinylated peptide to freshly isolated D^{b+} cells was readily detectable, allowing direct assessment of the relative amount of peptide bound to distinct lymphocyte subpopulations by three-color flow cytometry. While minor differences between peripheral T and B cells could be documented, thymocytes were found to differ widely in their peptide binding activity. In all cases, theses differences correlated positively with the differential expression of D^b at the cell surface. Finally, kinetic studies at different temperatures strongly suggested that the biotinylated peptide first associated with D^b molecules available constitutively at the cell surface and then with newly arrived D^b molecules.

HZ 131 STRUCTURE-FUNCTION RELATIONSHIPS BETWEEN AN IMMUNOGEN AND THE IMMUNE RESPONSE, Raphael J. Mannino,[1] Susan Gould-Fogerite,[1] Robert Woods,[2] Scott Koenig,[2] Michael D. Miller,[3] and Norman L. Letvin,[3] Dept. of[1] Pathology, UMDNJ-New Jersey Medical School, Newark, N.J.,[1] MedImmune, Inc., Gaithersberg, MD,[2] and Harvard Medical School, New England Regional Primate Center, Southborough, MA,[3]

In the course of natural infection the immune system often encounters antigenic determinants presented in the context of a lipid matrix. One approach to understanding the mechanisms of antigen processing in vivo is to create simple, well defined immunogens which mimic the structural motifs that the immune system has evolved to recognize.

Through reconstituting immunologically important, pathogen derived determinants into a lipid matrix we have been able to define minimal characteristics essential to the stimulation of either humoral or cell mediated responses.

For example, we have designed a novel viral subunit immunogen by encapsulating a previously defined synthetic peptide CTL epitope of the simian immunodeficiency virus (SIV) gag protein within a proteoliposome capable of attaching to and fusing with plasma membranes. Upon fusing, the encapsulated contents of this proteoliposome are delivered to the cytoplasm through which they can enter the MHC class I processing pathway. After a single intramuscular vaccination, rhesus monkeys develop a CD8$^+$ cell-mediated, MHC class I-restricted CTL response which recognizes the synthetic peptide epitope. Moreover, the induced CTL also demonstrate anti-viral immunity by recognizing SIV gag protein endogenously processed by target cells infected with SIV/vaccinia recombinant virus.

Combining the structural features common to our formulations with other subunit preparations which have been shown to induce CD8$^+$ CTL, the minimal components for such an immunogenic formulation can be proposed to be i) a peptide which represents an MHC class I epitope; ii) characteristics which enhance uptake by the MHC class I antigen presenting cells of the reticuloendothelial system, and iii) properties which can compromise the integrity of a lipid bilayer, facilitating delivery of the antigen into the cytoplasm.

HZ 132 The Molecular Mechanism of the Specific Binding of Peptides to Class I MHC Molecules

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To evaluate the contribution of each peptide sidechains to the binding to H-2K^b, we generated Ala substituted peptides of VSV-8 (RGYVYQGL) and OVA-8 (SIINFEKL). In vitro peptide binding assay using purified, empty K^b molecules (1) revealed that the *residues at position* 5 (Tyr^{P5} in VSV-8 and Phe^{P5} in OVA-8) are crucial for the efficient peptide binding. However, the substitutions of Leu^{P8} for Ala showed no effect on the binding affinity in the both peptides. The lack of preference for hydrophobic residues at the peptide C-terminus was also observed when the peptides eluted from K^b, which had been pre-incubated with randomly synthesized octapeptides, were analyzed (1). To further elucidate the minimum requirements of peptide residues for the binding to K^b, the octaserine peptide (Poly-S) were synthesized. Although the single introduction of either Tyr^{P5}, Phe^{P5} or Tyr^{P3} increased the binding affinity moderately, the Poly-S that has both Tyr^{P3} and Tyr^{P5} exhibited almost identical affinity to VSV-8, suggesting that only two Tyr^{P3} and Tyr^{P5} are sufficient to explain the high-affinity binding of VSV-8. As for OVA-8, the introduction of Ile^{P2}, Ile^{P3} and Phe^{P5} into the Poly-S fully restored the affinity. Interestingly, the introduction of both Ile^{P2} and Tyr^{P5} did not increase the affinity at all. These results, in conjunction with the structures of K^b pockets (2, 3), suggests that the tight packing of peptide sidechains in the binding groove would be the mechanism of high-affinity binding to class I molecules.

(1) Matsumura, M., Saito, Y., Jackson, M. J., Song, E. S. & Peterson, P. A. J. Biol. Chem. in press.
(2) Fremont, D. H., Matsumura, M., Stura, E. A., Peterson, P. A. & Wilson, I. A. Science **257**, 919-927.
(3) Matsumura, M., Fremont, D. H., Peterson, P. A. & Wilson, I. A. Science **257**, 927-934.

HZ 133 PRESENTATION OF S-LAYER CONJUGATES TO THE IMMUNE SYSTEM,

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Crystalline bacterial cell surface layers (S-layers) had been found as the outermost cell envelope component of archaeobacteria and eubacteria. They can form different lattices and were composed of identical protein or glycoprotein subunits. Their molecular weight varied strainspecifically between 40,000 and 200,000 (1).
We used purified, isolated S-layers for immobilization of weakly immunogenic haptens and antigens. In order to elicit cellular immune response against small carbohydrate haptens glutaraldehyde-fixed S-layer preparations were applied (2). Unfixed S-layer self-assembly products, however, elicited better humoral immune reactions. With capsular polysaccharides from *Streptococcus pneumoniae* or hydrolytically derived oligosaccharides we obtained good antibody titers and class switching from IgM to IgG subclasses. Even nasal/oral administration of S-layer conjugates elicited antigen-specific immune reactions (3).
The use of crystalline bacterial S-layers as a new carrier/adjuvant system in conjugate vaccine development will be discussed.

(1) P Messner, UB Sleytr, in AH Rose, DW Tempest (ed.) *Advances in Microbial Physiology*, Vol 33, Academic Press, London, 1992, pp 213-275. (2) RH Smith, P Messner, LR Lamontagne, UB Sleytr, FM Unger, *Vaccine*, in press. (3) AJ Malcolm, P Messner, UB Sleytr, RH Smith, FM Unger, in UB Sleytr, P Messner, D Pum, M Sára (ed.) Immobilised Macromolecules: Application Potentials, Springer, London, in press.

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HZ 134 INTRACELLULAR LOCALIZATION OF MHC CLASS II IN L CELLS EXPRESSING DISTINCT FORMS OF INVARIANT CHAIN,

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Invariant chain (Ii) is a nonpolymorphic protein which associates with class II during biosynthesis, and has been shown to have many functions during antigen presentation to T cells. Ii may facilitate transport of class II out of the ER, inhibit peptide binding to class II during transit through the ER and Golgi, direct the transport or retention of class II in an endocytic compartment where processed exogenous antigens are encountered, and enhance antigen presentation. Interestingly, our lab has shown that presentation of some class II-restricted antigens was facilitated only by the p41 form of Ii, and the p31 form had little or no effect [Nature 357:596]. One hypothesis to explain these data is that p41 may contain an intracellular transport signal that directs class II from an early to a late endosomal or lysosomal compartment. To test this hypothesis, we are investigating the transport of class II in L cells transfected with I-A^d in the absence and presence of cotransfected p31, p41, or genomic Ii (which encodes for both p31 and p41). Localization of class II is measured by two color immunofluorescence using a panel of monoclonal antibodies directed against class II and against proteins associated with ER, Golgi, endocytic, and lysosomal compartments. We found that class II in Ii-negative cells stained predominantly in an ER staining pattern; class II in Ii-positive cells stained in a more pronounced vesicular pattern that was most dramatic in p41-positive cells. These results are consistent with a role for Ii in facilitating class II egress from the ER. Studies to define the class II-positive vesicular compartments in p31- and p41-positive cells are in progress.

HZ 135 ALLELES AND HAPLOTYPES OF THE HUMAN MHC ENCODED ABC TRANSPORTERS *TAP1* AND *TAP2*,

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The class II region of the MHC contains two genes, *TAP1* and *TAP2*, whose products form a transporter complex involved in endogenous antigen processing. In the rat, polymorphism within *TAP2* changes the spectrum of peptides bound by the class I antigen RT1.A^a. Similar observations in humans might have important consequences for the study of HLA associated diseases. We have investigated polymorphism within *TAP2* by sequencing cDNA clones from different cell line libraries; others have used SSCP to analyse variation within both genes. These studies have identified 2 polymorphic residues within TAP1 and 3 within TAP2, combinations of which form potential alleles. We have used ARMS (Amplification Refractory Mutation System) PCR to characterise these alleles in a panel 115 homozygous typing cell lines (HTCs). Of four potential *TAP1* alleles, we observed three, and of eight potential *TAP2* alleles, we observed five. Amongst 88 HTCs homozygous at *HLA-DR*, *-DQ* and *-DP*, 80 were also homozygous at *TAP1* and *TAP2*. Of 27 HTCs homozygous at *HLA-DR* and *-DQ*, but heterozygous at *-DP*, 14 were homozygous at *TAP1* or *TAP2* and 13 heterozygous, consistent with recombination taking place either side of the *TAP* loci. Of the fifteen potential haplotypic combinations of *TAP1* and *TAP2*, we observed eleven, each at a frequency similar to that predicted from individual allele frequencies. In this ethnically heterogeneous panel, there is no indication that particular combinations of *TAP1* and *TAP2* have been maintained together.

HZ 136 Polymorphism at position nine of the MHC class I heavy chain controls the stability of association with β2-microglobulin as evaluated in a cell free protein assembly system. R.K.Ribaudo and D.H. Margulies, Molecular Biology Section, NIAID, NIH, Bethesda, MD. 20892.

Previous studies using an *in vitro* translation, processing, and assembly system have revealed differences in the stability of the class I heavy chain / β2-m complex related to the structure of the heavy chain. These observations extended previous biosynthetic studies that showed an apparently weaker interaction of the H-2L^d heavy chain with β2-m as compared to H-2D^d. The weak association of H-2L^d for β2-m is emphasized by the finding that an anti-α3 domain specific mAb 28-14-8, coprecipitates β2-m only when the H-2 molecules have been exposed to a high affinity antigenic peptide. In contrast, H-2D^d heavy chains, treated with the anti-α3 domain specific mAb 34-2-12, coprecipitate β2-m efficiently, even in the absence of antigenic peptide, and in the absence of the formation of a native α1α2 domain structure. To investigate further the structural basis for this apparent heterogeneity among class I molecules, we compared the amino acid sequences of two "low stability" class I molecules, H-2L^d and H-2D^b, with those of "high stability" H -2D^d, H-2K^b, and H-2K^d. We sought to identify residues that both segregated with the "stability" phenotype and also were, based on available x-ray crystallographic structures, likely to serve as contacts with β2-m. Despite numerous differences between these molecules, only the residue at position nine segregated consistently with the "stability" phenotype, and was likely to contribute directly or proximally to the β2-m interaction. H-2L^d and H-2D^b contain glutamic acid (E) at this position, while most other murine class I molecules have valine (V). Although the side chain at position nine is directed toward the peptide binding site, β2-m makes intimate contacts with the adjacent residues 8 and 10 from below, and β2-m contacts main chain atoms of position nine in HLA-A2. Using site directed mutagenesis, we converted the E at position nine of H-2L^d to V, and made the reciprocal mutation of V at position nine of H-2D^d to E. Using an *in vitro* translation/assembly system, we analyzed the coassembly of newly translated class I heavy chain with β2-m. The H-2L^{d}E9V mutant assembled from three to ten-fold more stably with β2-m than the parental H-2L^d, as assessed by immunoprecipitation, even without the addition of antigenic peptide. The H-2D^{d}V9E mutant assembled less well with β2-m by similar immunoprecipitation assays. These observations indicate that position nine, located at a focal position in the floor of the peptide binding groove, either directly or through proximal interactions with neighboring residues, is critically involved in the stabilization of class I heavy chains with β2-m.

HZ 137 PEPTIDE RELEASE MUTANTS: IDENTIFICATION OF INFLUENZA-DERIVED PEPTIDES BOUND TO HLA-A2 AND -A69, Russell D. Salter, Walter J. Storkus, Departments of Pathology and Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

A temperature sensitive mutant of HLA-A201 was previously characterized which does not bind β_2m and is not transported from inside cells when grown at 37°. At reduced temperatures, class I molecules assemble and transport peptides normally to the cell surface. By shifting the cells back to 37°, peptides as well as β_2m are released into the supernatant. This latter characteristic of the mutant has now been used to identify an epitope derived from influenza A strain infected CIR cells. Low molecular weight material released from the A2m242 mutant was fractionated by reverse phase HPLC and then pulsed back onto uninfected A2 targets. Lytic fractions co-migrated with a synthetic peptide, m1 58-66, shown previously to be a major epitope for recognition by flu-specific A2-restricted T cells. This suggests that m1 58-66 corresponds to the naturally processed matrix peptide. In contrast, fractionated material derived from cells expressing identically mutated HLA-A69 molecules also stimulated lysis by the A2-restricted T cells, but the peak lytic fraction eluted later on the HPLC gradient. This demonstrates that A2 expressing cells can be sensitized by a flu peptide derived from A69, but do not generate that peptide intracellularly. This suggests a active role for class I molecules in selecting or protecting peptides during antigen processing.

HZ 138 LacZ inducible, antigen /MHC specific T-cell hybrids.

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Activation of a single T-cell, induced by T-cell receptor (TCR) occupancy with its peptide/MHC ligand, can be measured by the lacZ assay. Applications of lacZ inducible T-cell hybrids have, however, remained limited due to the difficulties in generating individual T-cell transfectants with the NFAT-lacZ construct. We now describe a general method for obtaining lacZ inducible T-cell hybrids that makes use of two new fusion partners BWZ.36 and BWZ.36 CD8α which are derivatives of the α-β-BW5147 cell line in which the NFAT-lacZ construct is stably integrated. Using these fusion partners and normal T-cells from immunized mice we have generated lacZ inducible T-cell hybrids that are specific for antigenic peptides bound to either MHC Class I or MHC Class II molecules. Measurement of ligand induced T-cell activation by the lacZ assay offers both general and unique advantages over conventional IL-2 assays. The exquisite sensitivity of the single T-cell lacZ assay allows exploration of the mechanisms of endogenous antigen presentation and the development of strategies for expression cloning unknown T-cell antigen genes.

HZ 139 PEPTIDE-INDUCED CHANGES IN THE SOLUTION STRUCTURE OF A CLASS I MHC MOLECULE,

Jonathan Schneck†, Branimir Catipovic†, Joseph Oh, Taiyin Wei¥, Teit Johansen†, Michael Edidin¥, †Division of Clinical Immunology, Johns Hopkins University,, ¥,Department of Biology and Biophysics, Johns Hopkins University..

Serologically distinct forms of H-2K^b are stabilized by loading cells expressing "empty" class I MHC with different H-2K^b binding peptides. The H-2K^b epitope recognized by mAb 28.8.6 was stabilized by ovalbumin (257-264) and MCMV pp89 (168-176) peptides, but not by VSV NP (52-59) and Influenza NP (Y345-360) peptides. The H-2K^b epitope recognized by mAb 34.4.20 was stabilized by VSV NP (52-59) peptide but not by ovalbumin (257-264), MCMV pp89 (168-176), or Influenza NP (Y345-360) peptides. Using alanine-substituted derivatives of the VSV peptide, the 28.8.6 epitope was completely stabilized by substitution of the first residue and partially stabilized by substitution of the fifth residue in the peptide. To directly analyze the influence of peptides on the MHC molecule, the cell surface MHC molecules were analyzed using Fluorescence Resonance Energy Transfer (FRET). FRET can define the spatial relationship between different regions on the same molecule or the relationship of two different but physically close polypeptides. By this analysis, we were able to document that "empty" MHC molecules exist in floppy configuration. In contrast to the floppy conformation seen with "empty" H-2K^b molecules, very distinct peptide-specific energy transfer patterns were seen when H-2K^b was stabilized by H-2K^b binding peptides derived from either VSV (52-59) or Ova (257-264). Thus, peptide binding to H-2K^b molecules induces peptide-specific physically distinct conformations in the solution structure of a cell surface MHC molecule. The changes we detect influence surface areas available for contact with T cell receptors and may be important in determining T cell recognition.

HZ 140 C-TERMINAL FLANKING RESIDUE DETERMINES PRESENTATION OF OVALBUMIN PEPTIDE/K^b COMPLEX, Nilabh Shastri and Federico Gonzalez, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Class I MHC molecules present peptide fragments of cellular proteins on cell surface as ligands for the T-cell receptor. How flanking residues influence generation of these precisely defined peptides from their precursors is not known. Using ovalbumin octapeptide (OVA257-264, SL8)/K^b specific T-cells, expression of peptide/MHC ligand was measured in cells synthesizing Met-X-SL8 or Met-SL8-X precursors with X as a variable flanking residue. Presentation of OVA/K^b ligand was found to be independent of residues preceding the SL8 N-terminus, but was strongly inhibited when either Ile, Leu, Cys or Pro flanked the SL8 C-terminus. Significantly, Ile or Leu residues also define conserved C-termini of peptides bound by K^b as well as K^d and HLA-A2.1 MHC, suggesting that specific C-terminal residues play an important role in antigen processing. These results also bear upon algorithms for identifying peptides presented by class I MHC and upon optimal vaccine design.

HZ 141 UPREGULATION OF MHC CLASS II I-A^d SURFACE EXPRESSION BY SELECTED PEPTIDE LIGANDS ON TA3 HYBRIDOMAS, Bhagirath Singh, Beverley Rider, Qiang Yu, Ed Lee-Chan, Babita Agrawal, Ester Fraga, Department of Microbiology and Immunology, University of Western Ontario, Ontario N6A 5C1 and Department of Immunology, University of Alberta, Edmonton, Alberta T6G 2H7 CANADA.

Peptide antigens in association with MHC class I and class II are presented to CD8+ and CD4+ T lymphocytes respectively. Peptides are critical in class I heavy chain folding and/or stable association with β2-microglobulin. Recent evidence suggests that peptides also influence the compact state formation and the stability of MHC II α and β chain association leading to surface expression. We are studying the effect of various peptides on the MHC II I-A^d surface expression on TA3 B cell hybridomas. TA3 cells slowly lose their surface I-A^d expression in culture. The addition of selected I-A^d specific peptides can restore expression within 24 hrs in a dose- and affinity-dependent fashion. This decrease may therefore be due to the lack of peptide ligand saturation of TA3 cells. Neither ovalbumin antigen nor ovalbumin (323-339) peptide had an upregulatory effect on the I-A^d expression. We found that an I-A^d restricted synthetic peptide, EYK(EYA)$_4$, increased the level of surface I-A^d. Therefore, selected peptide ligands, but not all I-A^d restricted peptides are effective in the regulation of MHC class II surface expression and may have an effect on antigen presentation to T lymphocytes. The levels of RNA in high and low I-A^d expressing TA3 cells are currently being assayed and peptides with substituted amino acid residues are being used to identify specific residues involved in upregulation.

HZ 142 PROTEASE INHIBITORS BLOCK THE INTRACELLULAR TRANSPORT OF CLASS I MHC MOLECULES, Matthew Tector and Russell D. Salter, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Class I MHC molecules consist of heavy chains, β_2m, and peptides bound in a cleft formed by the heavy chain. How such peptides are generated and transported to the site of class I biosynthesis is the subject of intensive study. Based on genetic and biochemical analysis, it appears that proteasomes, including subunits encoded in the MHC, may provide the major source of peptides destined to bind class I molecules. Since stable assembly and transport of class I molecules depends on peptide binding early in biosynthesis, we asked whether protease inhibitors added to cells could shut off the supply of peptides and thereby block assembly and transport of several HLA class I proteins. Using the isocoumarin derivative 3,4-DCI, we found that assembly and transport of HLA-A69 and B7 was specifically blocked, as determined by lack of glycan processing and peptide binding assays. Under the same conditions, transferrin receptor was transported normally and protein synthesis was unaffected. Other serine protease inhibitors known to inhibit activities of the proteasome are also being studied. Our data provides evidence that protease activity is required for class I expression, and should allow identification of the specific activities involved.

HZ 143 EFFECTS OF MUTATIONS IN THE HLA-A2 MOLECULE ON PEPTIDE BINDING, Lynda G. Tussey[1], Masanori Matsui[2], Andrew McMichael[1] and Jeffrey Frelinger[2]. [1]Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK. [2]Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599.

Seventeen mutations lining the peptide binding groove of the HLA-A2.1 molecule have been tested for their effects on the ability of this molecule to bind three known HLA-A2.1 restricted peptide epitopes. Seven of the mutations were found to dramatically diminish the ability of A2 to bind these peptides and interestingly, two were found to substantially increase (relative to wild type) binding of the epitopes. Four of the nine mutations found to affect peptide binding could alter direct interactions with the peptides since they involve polymorphic residues with side chains exposed in the groove. The remaining five however, involve invariant residues with side chains pointing away from the groove and therefore would not be expected to interact with peptide directly. These mutations presumably alter the local conformation of the molecule in such a way that it can no longer bind these peptides. Finally, some mutations were found to specifically affect binding, in that binding of only one or two of the three peptides was affected. Taken together, the results suggest that most, but not all, of the residues which are critical for binding a given epitope are also critical for binding others.

HZ 144 A NEUTRALIZING B CELL EPITOPE WITHIN HCMV GB INDUCES CLASS I AND CLASS II RESTRICTED T CELL RESPONSES, Ursula Utz*, and William E. Biddison*, *Neuroimmunology Branch, NINDS, NIH, Bethesda, MD 20892.

Glycoprotein B (gB) is one component of the envelope of Human Cytomegalovirus (HCMV). Homologous proteins to gB have been found in all human and animal herpesviruses investigated so far. A high percentage of HCMV neutralizing Ab in patients are directed against gB. Recently, a neutralizing epitope on gB was mapped to amino acids 603-630[1]. We identified in the same region an epitope for HLA-A2[2]. An 11 amino acid long peptide FIAGNSAYEYV (gB 618-628) was found to be the optimal peptide for recognition by HLA-A2-restricted $CD8^+$ cytotoxic T cells. The shortest derivative that still can sensitize target cells, although much higher concentrations are required, is a 10mer (IAGNSAYEYV). We here report that the very same 11mer peptide that is the optimal peptide for binding to HLA-A2 is also presented by HLA-DR4. We have cloned $CD4^+$ DR4-restricted T cells from a HCMV positive donor that are specific for gB 618-628, but do not recognize shorter peptides. A 30 amino acid long region of gB therefore not only induces neutralizing antibodies, but also induces class I-restricted cytotoxic T cells and class II-restricted potential helper T cells. Moreover class I and class II epitopes for HLA-A2 and HLA-DR4 seem to be not only overlapping, but indistinguishable.

[1] Utz et al. (1989), J. Virol. 63:1995.

[2] Utz et al. (1992), J. Immunol. 149:214.

HZ 145 **THE FATE OF ISCOM BORNE ANTIGEN IN PERITONEAL MACROPHAGES,** **Maria Villacres-Eriksson and Bror Morein, Department of Veterinary Microbiology, Section of Virology, Swedish University of Agricultural Sciences, Uppsala, S-751 23 Sweden**

Resident peritoneal macrophages from Balb/c mice were allowed to internalize iscoms bearing biotinylated influenza virus envelope proteins for 5 min. The samples were fixed and processed for electron microscopy. The internalized antigen was detected by streptavidin-gold conjugate. Labelling was observed at the plasma membrane and in endocytotic vesicles. A significant level of labelling was scored in the intracellular space but not related to membranes, presumably in the cytosol. Some studies demonstrated that iscoms induce T cell responses under MHC Class I and II restriction. Recent experiments suggest that the presentation of iscom borne antigen taking place under Class II restriction occurs at least partially without involvement of the endocytotic-lyzosomic pathway. This indicates the possibility of an alternative pathway for binding of antigen to Class II molecules subsequent to processing in the cytosol. Our results support the theory of accessibility of iscom borne antigen for proteolytic processing in the cytosol. That explains why iscom borne antigens are presented by Class I molecules, and by Class II molecules in the absence of lyzosomic processing. It still remains to be experimentally shown how iscom borne antigens are transported to the cytosol.

HZ 146 A DEFINED VACCINE AGAINST AN INTRACELLULAR BACTERIAL INFECTION: IDENTIFICATION OF A NOVEL PROTECTIVE ANTIGEN FROM *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA), a chronic disease of sheep and goats characterised by necrotic lesions in peripheral lymph nodes and the lungs. *C. pseudotuberculosis* is a facultative intracellular pathogen, which multiplies within non-immune ovine macrophages, resulting in cell death and ultimately the formation of a necrotic lesion. Two major virulence determinants have been previously identified; a powerful phospholipase D exotoxin and a cell-wall associated lipid.

Through application of a novel strategy for the identification of protective antigens, we have identified a 40kD antigen from *C. pseudotuberculosis* that has not been previously described. The 40kD antigen was purified, incorporated into aluminium hydroxide adjuvant and used to vaccinate sheep. Following infectious challenge, CLA lesions were scored in lymph nodes and lung tissue. High levels of protection were obtained with the 40kD antigen, equivalent to that achieved with a commercially available complex vaccine. Protection of sheep against CLA by vaccination with a novel single antigen illustrates that defined subunit vaccines can be effective against intracellular bacterial pathogens.

HZ 147 FAILURE OF GPI-ANCHORED D^b MOLECULES TO PRESENT MoMuLV ANTIGEN TO CTL CORRELATES WITH DIFFERENCES IN INTRACELLULAR TRAFFICKING,

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Transfected R1.1 thymoma lines expressing glycosyl phosphatidylinositol (GPI)-anchored or transmembrane (TM) D^b molecules are lysed efficiently by H-2^b-allospecific CTL. Moloney virus (MoMuLV)-infected cells expressing TM-D^b are lysed efficiently by MoMuLV-specific D^b-restricted CTL; however, MoMuLV-infected cells expressing GPI-D^b molecules are not. The GPI-D^b molecules have native D^b domains ($\alpha1,\alpha2,\alpha3$) and are expressed at levels similar to the TM form; therefore, the defect in CTL recognition maps solely to the GPI anchor. Confocal fluorescence and electron microscopy reveal that this defect correlates with differences in membrane topology between GPI-D^b and TM-D^b molecules.

The GPI anchor is a strong apical sorting signal in polarized epithelial cells, and can affect protein intracellular trafficking. Retroviruses assemble and bud from the basolateral surface, where TM-MHC-I molecules are localized. The GPI-D^b and TM-D^b constructs were therefore transfected into the LLC-PK_1 renal cell line, in order to determine whether these D^b isoforms would be sorted intracellularly to different epithelial membrane domains. We found that TM-D^b molecules are sorted to the basolateral surface, as expected, whereas GPI-D^b molecules are sorted to the apical surface. Similar differences in intracellular sorting presumably exist in the thymoma cells, but cannot be visualized due to their spherical nature. We hypothesize that these differences may affect compartments for antigen processing and presentation by MHC-I.

HZ 148 CHARACTERIZATION OF THE INTERACTION BETWEEN THE CLASS I HEAVY CHAIN AND THE MOLECULAR CHAPERONE, p88, David B. Williams, Lisa Margolese, Navneet Ahluwalia, Eric Degen and Gerald L. Waneck, Dept. of Biochemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A8 and Dept. of Surgery, Massachusetts General Hosp., Charlestown, MA 02129.

In previous studies [Degen, E. et al. (1991) *J. Cell Biol.* 112, 1099 and (1992) *J. Exp. Med.* 175, 1653], we showed that a novel protein termed p88 binds rapidly and quantitatively to newly synthesized class I heavy chains and subsequently dissociates at a rate that closely resembles the characteristic rate of ER to Golgi transport observed for each class I allotype. In mutant cell lines that lack β_2m or are deficient in peptide ligands, class I molecules accumulate in an endo H-sensitive form and exhibit a corresponding prolonged association with p88. Since class I transport and dissociation of class I molecules from p88 both require the formation of the complete ternary complex we suggest that one function of p88 may be to retain incompletely assembled class I molecules intracellularly. In the present investigation, we have identified the main region of the class I heavy chain that interacts with p88. Deletion of both the α_1 and α_2 domains or deletion of the α_3 domain of the heavy chain did not affect the interaction with p88. Deletion of all but three amino acids of the cytoplasmic tail was also without effect. However, replacement of the transmembrane and cytosolic domains with a glycosyl-phosphatidylinositol anchor abolished the interaction. Conversely, fusion of the transmembrane domain plus three flanking residues to a non-interacting passenger protein conferred the ability to associate with p88 thereby mapping the interaction site to this segment of the heavy chain. We previously demonstrated that p88 is identical to calnexin, a type I integral membrane protein of the ER that binds calcium and shares significant sequence identity with calreticulin [Ahluwalia N. et al. (1992) *J. Biol. Chem.* 267, 10914]. Since p88 binds calcium we assessed the effect of calcium depletion on the class I-p88 interaction. Depletion of calcium in cell lysates disrupted the complex. Furthermore, reduction of ER calcium in the peptide-deficient RMA-S cell line resulted in accelerated dissociation of the H-2K^b-p88 complex and a corresponding dramatic increase in the rate of transport of the K^b molecule to the cell surface. This latter finding is consistent with a retention function for p88.

Class II Molecules

HZ 200 INFLUENCE OF THE ROUTE OF VACCINATION ON ANTIGEN PRESENTATION AND T-CELL MEDIATED LYMPHOKINE RESPONSE IN MURINE CUTANEOUS LEISHMANIASIS, Toni Aebischer and Emanuela Handman, Immunoparasitology Unit, The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia

We have investigated the cellular immune response of susceptible BALB/c mice to vaccination with irradiated *Leishmania major* before and after challenge with virulent parasites. In this model, protection has been shown to be critically dependent on the route of vaccination. Subcutaneous immunization will exacerbate the disease whereas intravenous vaccination will protect the mice from a subsequent challenge infection. Understanding the mechanism underlying this phenomenon will be essential for the design of an anti-*Leishmania* vaccine. We show by limiting dilution analysis that subcutaneous immunization activated primarily CD4+ T-cells secreting IL-4, a lymphokine implicated in disease exacerbation. Typically, 64% of the responding *Leishmania*-specific cells secreted IL-4 whereas only 21% secreted IFN-γ after immunization. In contrast, after intravenous immunization less than 1% of the proliferating cells secreted IL-4 and 47% secreted IFN-γ, a lymphokine associated with protection. However, precursor frequencies and absolute numbers of responding cells in the intravenously immunized animals were lower than in the subcutaneously treated group before and after challenge with virulent *L. major*. Therefore, prevention of IL-4 secretion without any concomitant increase in the number of IFN-γ secreting precursor cells seemed to be sufficient for protection. It has been proposed that the kind of antigen presenting cell (APC) involved could be responsible for the induction of a particular lymphokine profile secreted by the activated T-cell. Therefore, the role of different APCs in this system was investigated *in vitro* and *in vivo*. The lymphokine response by CD4+ T-cells primed by either route of immunization and restimulated *in vitro* by antigen presenting spleen cells, bone marrow derived macrophages or the B-cell lymphoma line A20 was determined. In addition, the role of APCs *in vivo* in determining the diverging lymphokine profiles with special emphasis on dendritic cells is currently being investigated.

HZ 201 ANTIGENIC VARIATION AND IDENTIFICATION OF PARASITE-DERIVED ANTIGENS ON THE *BABESIA BOVIS*-INFECTED ERYTHROCYTE SURFACE. David R. Allred, Rene Cinqué and Kim P. Ahrens, Department of Infectious Diseases, University of Florida, Gainesville, FL 32611.

Antibody-mediated mechanisms, including opsonic and ADCC activities, are thought to be important in immunity to *B. bovis*. One target of these mechanisms which we wish to exploit for vaccine development is the *B. bovis*-infected erythrocyte (IRBC) surface, which previous workers have suggested may undergo antigenic variation. To assess this critically, a spleen-intact calf was challenged with 5x 10^8 IRBCs carrying the C9.1 clone of *B. bovis*. *In vitro* cultures were established from peripheral blood on days 31, 41 and 61 post-challenge. Antisera were collected on days 0, 32, 59, 89 and 109 post-challenge. The surface antigenic reactivity of IRBCs was then observed using live immunofluorescence. Clone C9.1 was reactive with day 32 and each subsequent antiserum. Cultures established on days 31 and 41 were not reactive with day 32 antiserum, but became reactive with day 59 and subsequent antisera. Cultures established on day 61 were reactive only with antisera from days 89 and 109. These results were not due to down-regulation of the originally expressed antigen(s), and indicate that true antigenic variation does occur on the *B. bovis* IRBC surface. To identify parasite-derived antigens on the IRBC surface, antibodies eluted from intact IRBCs using a low pH wash were used in immunoprecipitations (IPs), with Protein G-Sepharose, of materials solubilized from [^{35}S]-methionine-labeled IRBCs with NP-40. SDS-PAGE and fluorography identified bands of 107, 125, and 185 kDa. Monospecific antibodies eluted from western blots, when used in IFAs, confirmed the association of the 185 kDa antigen with the IRBC membrane. Surface-specific IPs were performed by incubating intact IRBCs with antisera, washing to remove unbound antibodies, then solubilizing immune complexes with NP-40, or with NP-40 then 4M urea. These experiments confirmed the presence of the 107 and 125 kDa antigens and showed that they vary in size among clones C9.1 (parental), and C8 and H10 (derived from day 41 cultures), and identified an additional IRBC surface antigen of »200 kDa. We are now identifying which, if any, of these antigens participate in antigenic variation. Supported by USAID Cooperative Agreement #DAN-4178-A-00-7056-0.

HZ 202 EFFECTS OF AN INVARIANT CHAIN PEPTIDE ON THE ASSOCIATION AND PRESENTATION OF ANTIGENIC PEPTIDE BY MHC CLASS II MOLECULES, Ravi R. Avva and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Dominant peptides ranging in size from 22 to 24 amino acid residues and derived from the invariant chain (CLIP; AA 81-104) have been isolated and sequenced from the antigen processing mutant T2 transfected with MHC Class II genes (Riberdy, JR and P Cresswell, manuscript submitted). Experimental evidence suggests that antigenic peptides are exchanged for CLIP as part of the normal maturation process for MHC Class II molecules. Both fixed and mitomycin C treated T2 cells that have been transfected with DR4 (T2.DR4) present an influenza hemagglutinin peptide (HAp; AA 306-324) much more efficiently than a wild type $DR4^+$ B lymphoblastoid cell line (A2M). HAp is also presented much more efficiently when it is pulsed with fixed T2.DR4 at pH 5.0 than pH 7.3; the opposite is true for A2M. Competition studies *in vivo* have demonstrated that CLIP inhibits the binding of free HAp to fixed T2.DR4 and A2M at pH 5.0 or 7.3 as assayed by T-cell responses. The same is true for mitomycin C-treated cells at neutral pH. Furthermore, the presence of 200 μM CLIP during the infection of wild type B lymphoblastoid cell lines with influenza virus inhibits the presentation of newly synthesized viral epitopes. We suggest that the dominant presence of CLIP in T2 derived class II molecules is related to its MHC class II antigen processing defect and that its removal or displacement by antigenic peptide at low pH reflects a normal physiological process. Competitive *in vitro* binding studies between antigenic peptides and CLIP using purified mature MHC class II αβ dimers from both T2 transfectants and wild type cells will be discussed.

HZ 203 A_α CHAIN RESIDUES CONTROLLING A^d- vs. A^k- RESTRICTED PEPTIDE BINDING *N. Bangia, B. Ghumman, and T. H. Watts Dept of Immunology, University of Toronto, Toronto, Ontario Canada M5S 1A8*

MHC class II molecules are heterodimeric cell surface glycoproteins comprised of a 34 kd α chain and a 29 kd β chain. These molecules serve to present peptide Ag to $CD4^+$ T cells. A particular allelic MHC class II product can only bind a restricted set of peptides from the universe of peptide Ags available. For example, the mouse A^k molecule binds the peptide HEL46-61 well, but binds the OVA323-339 peptide poorly. The reciprocal association is observed with the mouse A^d molecule. We are examining what determines the selection of one peptide over the other by these two molecules. Previous work has shown that $A_\alpha{}^dA\beta^k$ chimeric MHC proteins prefer OVA323-339 over HEL46-61 suggesting that the α chain controls binding specificity for these two peptides. The α_1 domains of the A^k and A^d molecules differ by only 12 amino acids located in three hypervariable regions (E, F, G). To localize the MHC residues critical for OVA vs HEL peptide binding, we are testing cell lines expressing mutant $A_\alpha{}^k$ chains for which the k-allele hypervariable regions have been singly and multiply substituted by the corresponding d-allele regions. The cell line containing 'd-for-k' substitutions in region F still presents peptide to the A^k - HEL46-61 specific T hybrid A2.A2. However, cells expressing A^k with substitutions in region E, or regions F and G fail to stimulate A2.A2. Direct binding studies are in progress to determine which region(s) confer HEL vs. OVA peptide binding capacity.

* Supported by the National Cancer Institute of Canada.

HZ 204 MAPPING RESIDUES OF STAPHYLOCOCCAL ENTEROTOXIN A CRITICAL FOR IMMUNE RECOGNITION AND TOXICITY. Sina Bavari*, George Mayhew, Bradley Stiles, and Robert G. Ulrich. Department of Immunology and Molecular Biology, U.S.A.M.R.I.I.D., Frederick, Maryland 21702.

Staphylococcal enterotoxin A (SEA) belongs to a family of structurally related toxins produced by Staphylococcal and Streptococcal species. The potency of these superantigens in stimulating T cell proliferation and cytokine production is several orders of magnitude higher than that of conventional antigens. To better define the mechanisms by which these enterotoxins induce an immune response, we prepared several site-directed mutants of SEA. Critical amino acid side chains, conserved within all of the enterotoxins, were mutated. This approach was designed to locate the minimal essential amino acids required for MHC class II or TCR binding. These mutations altered relative binding affinities from 10 to >1000 fold. The changes in binding were further studied to distinguish effects on TCRVβ or MHC class II interactions. The relative affinity of SEA wild type varied with different molecules of class II alleles. This class II allelic difference was preserved for the majority of mutants. On the basis of these results, specific regions of the SEA molecule essential for TCR and class II binding were mapped.

*Supported by a National Research Council Research Fellowship.

HZ 205 DIFFERENTIAL LOSS OF B AND T CELL TOLERANCE TO CYTOCHROME c IN A MURINE MODEL OF AUTO-IMMUNITY. PR Blier*, R Joseph, and MJ Mamula, *Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, 06877, and Section of Rheumatology, Yale University School of Medicine, New Haven, CT, 06510.

A hallmark of systemic autoimmune disease is the loss of peripheral or "non-deletional" tolerance to intracellular self antigens. We have been studying the response to a self protein, cytochrome c (cyt c), in the autoimmune mouse strain MRL-+/+, in an effort to better understand the mechanisms by which non-deletional tolerance is lost. In one approach, normal T cell tolerance to self cyt c was broken by co-immunization with self and foreign proteins (human and murine cyt c). Primed cross-reactive B cells were shown to be responsible for activating normally silent auto-reactive T cells.

In a second approach, mice immunized with the C-terminal peptide of murine cyt c (Ms81-104) developed a strong T cell response to the peptide which did not respond to intact cyt c. In contrast, immunization with peptide 81-104 of human cyt c elicited T cells which responded to intact human cyt c as well as Hu81-104. The Ms81-104 peptide therefore represents a "cryptic" epitope to which tolerance was never attained.

We wondered whether B cells primed to Ms81-104 could activate an immune response to other regions of murine cyt c, similar to that seen in co-immunization. Mice were immunized with Ms81-104 and serial bleeds tested for reactivity to various regions of cyt c by Western blot against intact cyt c, synthetic peptides, and a CNBr digest, which generates fragments corresponding to aa 1-63, 1-80, 64-80, and 81-104. Sera were also tested by ELISA against intact cyt c and synthetic peptides. No antibody reactivity was seen to regions of cyt c other than aa 81-104. In contrast, T cell assays from immunized (but not naive) animals did demonstrate reactivity to the N-terminal 1-80 fragment of murine cyt c, despite their never having been immunized with exogenous cyt c. These results are consistent with a model in which activated cross-reactive B cells present endogenously derived self-antigens and activate autoreactive T cells. The lack of a corresponding antibody response suggests that additional factors are needed to extend this autoimmune response to the B cell arm of the immune system.

HZ 206 CRYSTAL STRUCTURE OF THE CLASS II HISTOCOMPATIBILITY ANTIGEN HLA-DR1, Jerry H. Brown, Theodore Jardetzky, Joan C. Gorga, Lawrence J. Stern, Jack L. Strominger, and Don C. Wiley, Department of Biochemistry and Molecular Biology and the Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138

Class II histocompatibility antigens present processed antigens to helper T cells. Comparison of the polymorphic and conserved residues of Class II and Class I molecules[1], along with functional, biochemical, spectroscopic and Patterson function studies, have provided evidence for an overall similarity in their three-dimensional structures. We have now proven this by X-ray crystallography.

Using crystals of DR1 purified from human L cells[2,3], we initially collected 4.2 Å data sets from native (presently past 3Å) and K_2HgI_4 derivatized crystals. Iterative averaging with similar data sets from DR1 in complex with the superantigen SEB revealed the expected peptide binding site, domain structures, disulfide bridges, carbohydrates, and a trace of the polypeptide chains.

A comparison of the structures of Class II and Class I MHC molecules in the context of the overall quaternary structure, the peptide binding site, and the shape and extent of the endogenous bound peptides will be presented. The predicted locations of the T-cell receptor, superantigen, and CD4 binding sites and an arrangement DR1 molecules might form when presenting foreign antigens to T cells will be discussed.

[1]Brown, J.H., et. al. (1988) Nature 332, 845-850
[2]Gorga, J.C., et. al. (1991) Res. Immunol. 142, 401-407
[3]Gorga, J.C., et. al. (1987) J. Biol. Chem. 262,16087-16094

HZ 207 CHARACTERIZATION OF NATURALLY PROCESSED SELF PEPTIDES BOUND TO HLA-DR ALLELES, Roman M. Chicz, Robert G. Urban, Joan C. Gorga, Dario A. A. Vignali, William S. Lane*, and Jack L. Strominger, Department of Biochemistry and Molecular Biology and the *Microchemistry Facility, Harvard University, Cambridge, MA 02138

Class II MHC molecules present a complex mixture of peptides derived from both endogenous and exogenous protein sources. Naturally processed peptides bound to six HLA-DR alleles purified from cultured B cell lines were characterized by high-performance liquid chromatography, mass spectrometry and Edman microsequencing analyses. Over 200 unique peptide masses were identified from each allele ranging between 1,200 and 4,000 daltons (10-34 amino acids in length). The most abundant M_r values were between 1,700 and 2,100 (average peptide length, 15-18 residues). At the present time, complete sequence data have been obtained for 150 peptides (derived from 62 different proteins) from the six alleles. These peptides were derived from either endogenous proteins (with a predominance of peptides from MHC-related proteins) or from exogenous bovine serum proteins that passed through the endocytic pathway. Most peptides represented sets nested at both the N- and C-terminal ends. Binding experiments using synthetic analogs confirmed that isolated peptides had high affinity for the groove of HLA-DR. Some peptides were allele specific. In addition, degenerate self peptides binding to multiple HLA-DR alleles were identified. Degenerate binding self-peptides may have a physiological role as immunomodulators of the cellular immune response.

HZ 208 ANTIGENS BOUND TO α_2-MACROGLOBULIN UNDERGO ENHANCED MACROPHAGE UPTAKE AND PRESENTATION TO T-CELLS, Charleen T. Chu and Salvatore V. Pizzo, Department of Pathology, Duke University, Durham, NC 27710

Macrophages and fibroblasts secrete the proteinase "inhibitor" α_2-macroglobulin (α_2M), which can spontaneously form covalent complexes with nonproteolytic proteins during a transient proteinase-activated state. Neutrophil elastase, pancreatic elastase, plasmin, trypsin, and potentially any proteinase that is inhibited by α_2M, can initiate complex formation. Since proteinase-treated α_2M is rapidly endocytosed through high affinity receptors, this may result in efficient delivery of potential antigens into macrophages (MØ). We have used MHC-restricted murine T–hybridoma clones specific for hen egg lysozyme (HEL) to probe the effect of HEL-α_2M complex formation on antigen uptake and processing. HEL was internalized by MØs more rapidly and to a greater extent when complexed with α_2M than when free. MØs that were pulsed for 2 h with α_2M-HEL-elastase complexes required less than 1/200 the concentration of antigen required by those pulsed with free HEL. MØs pulsed for only 15 min with nM levels of HEL–α_2M-elastase could stimulate T-cells added after extensive washing. Adding equimolar amounts of receptor-recognized α_2M to HEL had no effect on HEL presentation, indicating that the enhanced effects required physical attachment of HEL to α_2M rather than resulting simply from ligation of the α_2M receptor. Receptor-recognized α_2M, however, did compete specifically for both uptake and presentation of HEL-α_2M complexes. Since many inflammatory proteinases, such as neutrophil elastase, react with α_2M, we propose that "proteinase-activated" α_2M may mediate receptor-enhanced antigen uptake by macrophages in areas of inflammation, resulting in augmented antigen processing and presentation.

HZ 209 MEDULLARY THYMIC EPITHELIAL CELLS SELECT FOR MATURE CLASS I RESTRICTED T CELLS. Mogens H. Claesson, Thomas Tscherning and Carsten Ropke, Department of Anatomy A, The Panum Institute, Blegdamsvej 3, 2200N, Copenhagen, Denmark.

Cortical thymic epithelial cells (TEC) are belived to be the main responsible thymic stromal cell type for the positive selection of immature thymocytes. We have examined the capability of cultured medullary thymic epithelial cells to select - negatively or positively, for mature $CD4^-CD8^+$ female thymocytes carrying a transgenic TCR for the male H-Y antigen restricted by $H\text{-}2D^b$ (mice used in the present study are offsprings of transgenic mice generously provided by Dr. H. von Boehmer). The purified $CD8^+$ thymocytes were exposed to male or female TEC prior to culture at limiting dilution conditions with irradiated male splenic stimulator cells. One in 50 and less than 1:1000 thymocytes preexposed to male and female TEC respectively, formed cell colonies under these conditions. In contrast, the clonability of double negative female thymocytes was inhibited by both male and female medullary TEC. Fifty and 80% of the expanded $CD8^+$ transgenic T cell clones exhibited antigen specific proliferation and IL-3 secretion respectively, whereas none of the expanded clones developed into a cytotoxic phenotype. Our data clearly demonstrate that medullary TECs, in contrast to their cortical counterparts, positively select for antigen specific mature, MHC class I restricted T cells.

HZ 210 THE RECEPTOR-BINDING DOMAIN OF DIPHTHERIA TOXIN AS A POTENTIAL IMMUNOGEN AGAINST DIPHTHERIA, R. John Collier, Wei Hai Shen, and Haian Fu, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

Diphtheria toxin (DT) is secreted from *Corynebacterium diphtheriae* as a single polypeptide chain of 535 amino acids. Recently X-ray crystallographic analysis of DT has revealed three distinct structural domains, corresponding the its three biological functions: receptor-binding, membrane trnslocation, and ADP-ribosylation of elongation factor-2. We have expressed the receptor-binding domain (DTR) as an independent structural entity, with the goal of developing a safe and efficient alternative vaccine against diphtheria. Guided by the crystallographic domain boundaries, we placed a DNA fragment encoding DTR (residues 379 to 535 of DT) under the control of the T7 promoter and fused the protein to a hexa-Histidine tag to facilitate purification. The overexpressed DTR protein was soluble, and western blotting indicated that DTR conserved its reactivity with anti-DT antibodies. Crude extracts prepared from DTR-expressing *E. coli* protected Vero cells from the lethal action of DT, suggesting that DTR blocks the binding of DT to cells. The immunogenicity of DTR is currently under investigation. Because the toxic, catalytic fragment of DT is absent, there is no risk of reversion of the molecule to a toxic form. Thus DTR may be a good candidate for incorporation into live multivalent vaccines, as well as an alternative to diphtheria toxoid for use in conventional vaccines.

HZ 211 B CELLS PROCESS AND PRESENT LUPUS AUTOANTIGENS THAT INITIATE AUTOIMMUNE T CELL RESPONSES. Joe Craft, Saeed Fatehnejad, Mark Mamula. Dept. of Medicine, Section of Rheumatology, Yale University School of Medicine, New Haven, CT 06510

Antibodies against U small nuclear ribonucleoprotein (snRNP) particles are a common finding in the sera of humans with systemic lupus erythematosus and certain strains of mice with murine lupus. It is likely that helper T cells are important in amplifying this autoantibody response. The focus of this work was to investigate events that might initiate autoimmune B and T cell responses in non-autoimmune mice to native U snRNP particles. Mice (B10.BR) that were immunized and boosted with native mouse snRNPs, purified via anti-trimethyl guanosine affinity chromatography, failed to produce any detectable specific anti-snRNP antibody or T cell responses, suggesting that these autoreactive cells were deleted from the repertoire or were anergic to stimulation with this self antigen. In contrast, immunization with native foreign (human) snRNPs elicited both T cells and anti-snRNP antibodies; the latter predominantly were directed toward the A protein of the U1 snRNP. While this antibody response was cross-reactive with self (mouse) snRNPs, the T cells were specific for human snRNPs and were not cross-reactive. When mice were immunized with human and mouse snRNPs together in adjuvant (or immunized with recombinant human and murine A polypeptides), T cells specific for mouse snRNPs (or murine A) could be elicited. The results of these experiments suggested that the mechanism of breaking T cell tolerance to self snRNPs was dependent on the ability of cross-reactive B cells to process and present these autoantigens. To address this hypothesis, B cells purified from mice immunized with recombinant human A protein were transferred into naive mice. Upon boosting with native mouse snRNPs, autoreactive CD4+ T cells specific for mouse antigens, and not cross-reactive with human snRNPs, were observed. Transferred immune T cells or normal B or T cells failed to elicit this response. These studies support a model of molecular mimicry whereby autoantigen presenting B cells are generated by foreign cross-reactive determinants that can, in turn, prime an autoimmune T cell response.

HZ 212 MHC CLASS II MOLECULES MAY FUNCTION AS A TEMPLATE FOR THE PROCESSING OF A PARTIALLY PROCESSED PEPTIDE INTO A T CELL EPITOPE, Terry L. Delovitch and Ying Lang, Banting and Best Department of Medical Research and Department of Immunology, University of Toronto, Toronto, Ontario, Canada, M5G 1L6

It has been hypothesized that by binding to a partially processed peptide, MHC molecules may protect and direct the further processing of such a peptide into a T cell epitope. This hypothesis was tested by using pork insulin (PI) as a model antigen and TA3 (I-$A^{d/k}$, I-$E^{d/k}$) B lymphoma cell APCs to process and present PI. A TA3 cell lysate was used as a source of enzyme(s) to process either PI or its disulfide-linked peptide A1-A14/B1-B16 into a T cell epitope. Processing of PI or the A1-A14/B1-B16 PI peptide by the cell lysate at pH 5.5, but not pH 7.3, in the presence of fixed APCs (source of MHC class II molecules) elicited the activation of PI/I-A^d–specific T cells. Pre-incubation of the PI peptide with fixed Ia^d–positive M12.4.1 cells, but not the Ia–negative variant M12.C3 cells, removed the ability of this peptide to be presented by TA3 cells. Inhibition of T cell activation was also achieved by preincubation of fixed TA3 cells with the MK-D6 anti-I-A^d mAb but not the 14-4-4S anti-I-$E^{d/k}$ mAb. When PI or the PI peptide was pre-processed in the absence of APCs (i.e. no absence of MHC class II), no T cell activation was observed upon subsequent addition of MHC-class II bearing APCs. These findings demonstrate that I-A^d molecules are involved in the binding, further processing and presentation of a PI peptide. They also suggest that MHC class II molecules function as a template for guiding the processing of a partially processed peptide into a T cell epitope. (Supported by MRC of Canada).

HZ 213 IDENTIFICATION OF AN MHC CLASS II-BOUND ANTIGENIC PEPTIDE ON THYMIC EPITHELIAL CELLS THAT MAY MEDIATE NEGATIVE SELECTION, Frederique Forquet, Mirko Hadzija and Terry L. Delovitch. Banting and Best Department of Medical Research and Department of Immunology, University of Toronto, Toronto, Ontario, Canada M5G 1L6

To further understand the role of positive and negative selection of thymic cells in the acquisition of the T cell repertoire for a circulating extra-thymic antigen, it is important to identify peptide-MHC complexes present on different thymic APCs that mediate these selection events. Using biosynthetically labelled recombinant human insulin (rHI) as a model antigen, we previously demonstrated qualitative and quantitative differences in the plasma membrane (PM) -associated HI peptides processed by thymic stromal APCs that mediate positive (epithelial cells, EC) and negative (dendritic cells, DC) selection. The major EC PM-associated peptide that was acid eluted from MHC class II (I-A^d) is a heterodimeric disulfide-linked peptide composed of residues A6-A11/B7-B19. If the B-chain residues of this peptide bind MHC and the A-chain residues engage the TCR, as our previous data suggest, note that the length of the peptide (13 amino acids) that can bind MHC class II on thymic EC corresponds closely with that previously found on class II molecules of B lymphoma cells. Neither this heterodimeric peptide nor other rHI peptides could be eluted from DC class II molecules. More recently, we have shown that while both EC and DC APCs present rHI to HI/I-A^d-specific T cell hybridomas or polyclonal lymph node T cells, neither thymic EC nor thymic and splenic DC can efficiently present the synthetic A6-A11/B7-B19 peptide. We verified that this absence of presentation was not due to a lack of binding of this peptide to MHC class II molecules on the surface of the APCs by showing that this peptide competes for the presentation of rHI by B lymphoma cells and thymic APCs. These results suggest that this major HI A1-A6-A11/B7-B19 peptide bound to MHC class II on EC mediates negative selection, and that another as yet undetectable class II-bound HI peptide on DC may also regulate negative selection. Studies are underway to test these predictions. (Supported by MRC of Canada and Juvenile Diabetes Foundation International).

HZ 214 ANERGY INDUCED BY SYNTHETIC PEPTIDES OF MYELIN BASIC PROTEIN BLOCKS PROGRESSION OF AUTOIMMUNE ENCEPHALOMYELITIS, Amitabh Gaur,B. Wiers, J. Rothbard* and C. Garrison Fathman, Division of Immunology & Rheumatology, Department of Medicine, Stanford University Medical Center CA 94305 and *IPC,Palo Alto

Experimental autoimmune encephalomyelitis (EAE), a demyelinating disease of the central nervous system which can be induced by immunization with Myelin Basic Protein (MBP) or its immunodominant T cell determinants, serves as a mouse model of human multiple sclerosis. We have used synthetic peptides corresponding to immunodominant determinants of MBP to induce tolerance in adult animals to MBP. The ability of the peptides to induce tolerance had a direct relationship with the heirarchy of their immunodominance. This determinant induced tolerance was able to prevent MBP induced EAE in adult mice. Furthermore, tolerizing regimens of peptides given to mice once disease had begun (10 days following induction with MBP) blocked progression and decreased severity of ongoing disease. This peptide induced tolerance resulted from the induction of anergy in proliferative antigen specific T cells as addition of exogenous IL-2 to cultures restored proliferative response.

HZ 215 **LOCALISATION OF SELF ANTIGEN: CONSEQUENCES FOR ANTIGEN PRESENTATION.**
Catriona Grant and Brigitta Stockinger
Division of Molecular Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

The fifth component of complement (C5) is used as a model self antigen for studies of antigen presentation in the context of tolerance. Some mouse strains have a genetic defect which causes absence of C5 and consequently lack of tolerance to C5. This allowed the generation of C5-specific, class II restricted T cell clones. Macrophages taken directly from C5 expressing mice can activate these T cell clones without the need of C5 addition *in vitro*, which indicates that C5 is constitutively processed and presented *in vivo*. Although hepatocytes are the main producers of C5, macrophages synthesise C5 as shown by biosynthetic labelling and Western blot analysis. The question arises as to the source of C5 which is presented. Do they present C5 which they have taken up from the serum or C5 which they have synthesised themselves?.To determine if the C5 presented is endogenous or from an exogenous source, bone marrow chimeras were made in which macrophages were the only source of C5. These mice had no detectable hemolytic C5 in serum indicating that they are not secreting C5, although they do synthesise it. These chimeras were not tolerant of C5 and their macrophages failed to activate C5-specific T cells *in vitro* indicating that intracellular C5 does not get presented with class II MHC. So why do macrophages fail to present C5 peptides in association with class II MHC molecules to T cells? Are the amounts of antigen synthesised too low for T cell activation or does C5 localise in a compartment which does not have access to class II. Preliminary experiments indicate that the amounts of synthesised C5 if given exogenously induce T cell responses *in vivo* and *in vitro*, suggesting that quantity of C5 is not the factor limiting presentation. So the failure of macrophages to present their intracellular C5 in association with class II MHC seems to be due to compartmentalisation preventing access to the class II presentation pathway.

HZ 216 INTRACELLULAR PATHWAYS FOR TRANSPORT OF CLASS II MHC MOLECULES. Sandra Husbands, Derek Dial, Lynne Guagliardi and Frances Brodsky, Departments of Pharmacy and Pharmaceutical Chemistry, University of California, School of Pharmacy, San Francisco, California, 94143-0446.
Class II major histocompatibility molecules (MHC II) bind antigenic peptide fragments of internalized antigens for presentation to T helper cells. These antigenic fragments are likely to be generated in the degradative endosome-lysosome pathway. Immunoelectron microscopy studies were carried out to map the intracellular locations of internalized surface immunoglobulin, proteases and MHC II, in an effort ot locate the sites of antigen degradation and MHC II-peptide association. Endocytic compartments were labelled by uptake of anti-immunoglobulin, to mimic the antigen uptake pathway. Invariant chain-associated MHC II molecules, *en route* to the cell surface, were found throughout the endocytic pathway, as were mature MHC II molecules, i.e. dissociated from invariant chain, and so able to bind peptides. The proteases Cathepsins B and D also colocalized in these compartments. In spite of extensive colocalization of molecules involved in antigen processing and presentation,the precise location in which antigenic complexes form is unclear. This is likely to be in the later part of the pathway, since there is an accumulation of MHC II molecules towards the end of the pathway, where the compartments are more degradative. The localization of immature MHC II in the early stages of the endocytic pathway may reflect the route by which these molecules accumulate in later endocytic compartments, as recently demonstrated for the lysosomal protease, Cathepsin D.

Pulse-chase labelling studies have been performed to investigate the rate of invariant chain dissociation from MHC II. Differential rates of class II maturation were noted in different cell lines, which may account for the differing distribution of both mature and immature MHC II in these lines. Further pulse-chase studies are underway to determine how soon after synthesis MHC II/invariant chain complexes reach the early endosome and the time course of their traffic through the endocytic pathway. Results of these studies, together with the results of the maturation studies, should contribute to defining the intracellular location of peptide-MHC II complex formation.

HZ 217 MHC CLASS II BINDING CAPACITY OF AUTOIMMUNE DISEASE RELATED T CELL DETERMINANTS IN THE LEWIS RAT. L. Joosten*, M.H.M. Wauben*, M.C. Holewijn, E.J. Hensen* and S. Buus**. * Dept. Infectious Diseases and Immunology, Fac. Veterinary Medicine , University of Utrecht, The Netherlands. ** Institute for Medical Microbiology and Immunology, University of Copenhagen, Denmark.
New strategies in the treatment of autoimmune diseases involve blocking or modulation of major histocompatibility complex (MHC) - peptide - T cell receptor (TcR) interactions either at the MHC level or at the T cell level. For this strategy to be successful the ability to determine peptide - MHC interaction is of great importance. Several autoimmune disease models exist in the Lewis rat from which relevant disease related T cell responses have been found to be RT.B^{l} restricted. Recently we developed a novel non-radioactive biochemical MHC class II - peptide binding assay which is sensitive and specific. We employed this assay to test the binding capacity of a set of peptides comprising T cell determinants and analogues thereof, relevant to the adjuvant arthritis, experimental autoimmune encephalomyelitis and experimental autoimmune uveoretinitis models in the Lewis rat. We extended the panel by testing some non-related peptides with the objective to facilitate the determination of a possible RT1.B^{l} binding motif. All peptides were simultaneously tested in T cell functional competition assays using analogous and non-analogous systems. The combined results significantly increased the understanding of the nature and effectiveness of the in vitro competitor activity of the various peptides tested. For the in vivo manipulation of autoimmune disease models in the Lewis rat this information may prove essential.

HZ 218 STAPHYLOCOCCAL ENTEROTOXINS AND CLASS II-ASSOCIATED INVARIANT CHAIN BIND TO DISTINCT SITES ON HLA-DR, David R. Karp, Robert N. Jenkins, and Eric O. Long, Simmons Arthritis Research Center, UT Southwestern Med. Ctr. Dallas, TX 75235, and Laboratory of Immunogenetics, NIH, Bethesda, MD, 20852

During biosynthesis, class II molecules of the major histocompatibility complex (MHC) exist as complexes of the polymorphic α and β chains in association with trimers of the invariant chain (Ii). The non-polymorphic Ii contains sequences necessary for proper targeting of class II to endosomal compartments, where Ii is degraded. Ii also prevents the premature association of antigenic peptides with class II molecules. It is not known whether the effect of Ii on peptide binding extends to other ligands of class II, specifically exogenous superantigens. Cells that express a mutant Ii molecule stably associated with HLA-DR at the cell surface were tested for their ability to interact with staphylococcal toxins. Most toxins (SEA, SEB, SEC, SED, SEE, and exfoliative toxin) bound to cells expressing this αβIi complex with levels comparable to cells expressing only αβ chains at the cell surface. Cells expressing surface abIi complexes stimulated polyclonal populations of peripheral blood T cells in association with these toxins. Binding of toxic shock syndrome toxin (TSST) and subsequent stimulation of T cells was reduced by the presence of the surface invariant chain. This reduction was not due to an alteration in the repertoire of T cells responding to TSST in the presence of the invariant chain. Data with a T cell clone suggest that TcR/MHC interactions occur during SEA-mediated stimulation and that surface Ii does not interfere with such interactions.

HZ 219 LINEAGE-SPECIFIC T CELL RECOGNITION OF CLASS II ALLOANTIGENS, John F. Katz and Andrea J. Sant, University of Chicago, Chicago, IL 60637

Cells of different lineages can selectively activate distinct populations of T lymphocytes. It is clear that different resident thymic cells are responsible for positive and negative selection events in the thymus. In addition, cells of different lineages activate distinct T cell subsets in the immune response to pathogens. This disparity in antigen presentation may be secondary to differences 1) in the array of cell surface accessory molecules, 2) in their antigen processing machinery, or 3) in their repertoire of antigens processed and presented in context of MHC. Under the premise that some allorecognition is peptide dependent, we addressed the latter two hypotheses by probing for different MHC peptide complexes with a panel of alloreactive T cell hybridomas.

We have generated a panel of 32 I-A^d restricted alloreactive T cell hybridomas. Upon screening the panel on several established cell lines of different lineages, we observed cases of tissue-specific reactivity. Interestingly, their reactivity follows a hierachial pattern; some cell lines stimulate the preponderance of the panel, others stimulate only a few. Flow cytometric analysis suggests that this differential reactivity does not simply reflect accessory molecule discrepancies among the different antigen presenting cells or individual T cell hybridomas. These data suggest, first, that allorecognition of class II MHC is peptide specific, and second, that APCs of different lineages display distinct peptides. These differences may be due to lineage dependent processing events of the same protein, or tissue-specific protein expression. The panel was also screened on several cell lines transfected with the genomic DNA clone of murine invariant chain. Here, we observed several cases of Ii chain dependent reactivity. One can envision at least two possible mechanisms to account for this reactivity: the T cells are specific for an Ii chain-dependent antigen, or an Ii chain-derived peptide.

HZ 220 ALLELIC COMPETITION IN PEPTIDE/HLA CLASS II αß INTERACTIONS Susan Kovats[1], Janice S. Blum[1], Alessandro Sette[2], Kazuyasu Sakaguchi[3], Ettore Appella[3], William Kwok[1], and Gerald T. Nepom[1]; [1]Immunology Program, Virginia Mason Research Center, Seattle, WA 98101; [2]Cytel, 3525 John Hopkins Court, San Diego, CA 92121; [3]National Cancer Institute, NIH, Bldg. 37, Room 1B04, Bethesda, MD 20892.

Genetic differences that encode structural variation between HLA class II molecules are linked to relative susceptibility or resistance to several diseases. In autoimmune diseases such as insulin dependent diabetes mellitus or rheumatoid arthritis, the mechanisms accounting for genetic linkage with disease may directly involve presentation of peptide antigens by specific class II molecules. Since self peptide binding to particular class II alleles may be important for initiation and/or maintenance of autoimmunity, we would like to understand the molecular events that regulate antigen binding and presentation by HLA class II molecules. To this end, we have developed a model system for studying the formation of peptide/class II complexes in living cells. Defined class II transferent cell lines are being used to study peptide binding and presentation by different class II alleles (DR 4w4, 4w14, 5) present singly or in combination. Peptide/class II complex formation is assessed using antigen-specific T cell clones or directly visualized after incubation of cells with a model peptide and immunoprecipitation and electrophoresis of class II molecules. This system is being used to determine if competition between structurally different HLA class II molecules for peptide influences the number and kind of peptide/class II complexes formed and expressed on the cell surface.

HZ 221 THE FAILURE TO PROCESS AN AUTOANTIGENIC PEPTIDE DEFEATS T CELL TOLERANCE. Mark J. Mamula, Yale University School of Medicine, New Haven, CT 06510.

It is now clear that APCs do not present all the possible peptides of self proteins to the immune system. What then, is the fate of T cells specific for those self peptides that escape processing? In this study, the carboxy terminal peptide (residues 81-104) of self cytochrome *c* (cyt *c*) elicited strong autoimmune T cells, as well as auto-antibodies specific for this immunogen. These T cells did not respond to stimulation with the whole self cyt *c* molecule demonstrating that APCs cannot process and present this self 81-104 peptide. While mice were unresponsive to immunization with the whole mouse cyt *c* molecule, the mouse 81-104 fragment together with the whole self molecule induced and expanded the autoimmune T cell response to sites within the 1-80 peptide. It appears that B cells elicited with the self peptide 81-104 can bind the self cyt *c* protein and present distant peptides in the priming of autoreactive T cells. In studies with recombinant mouse cyt *c*, a single amino acid change can allow APCs to process and present the terminal 81-104 peptide. In addition, we have defined single amino acid residues in the self peptide that are critical in its binding by the T cell receptor. Therefore, T cells that never contact the relevant self peptide become functionally ignorant; they do not become tolerized or deleted, nor do they normally participate in immune responses since APCs cannot present this peptide.

HZ 222 PROTEOLYTIC PROCESSING OF THE HLA CLASS II ANTIGEN ASSOCIATED INVARIANT CHAIN, Maja A. Marić and Janice S. Blum, Immunology and Diabetes Programs, Virginia Mason Research Center and the University of Washington, Seattle, WA 98101.

HLA class II antigens are associated intracellularly with a chaparone-like protein the invariant chain. The function of the invariant chain remains unclear, although this protein may serve to prevent the premature binding of self antigens to class II molecules in early *biosynthetic* compartments as well as direct the transport of class II complexes to compartments containing foreign antigens. The invariant chain is proteolytically released in an acidic compartment prior to the surface expression of functional class II $\alpha\beta$ dimers. In human B-lymphoblastoid cell lines, invariant chain proteolysis has been localized to endosomes using subcellular fractionation techniques. A 21 kilodalton fragment of the invariant chain, termed LIP is generated in endosomes along with a novel series of 11-14 kilodalton fragments. These results support the hypothesis that invariant chain facilitates the sorting of class II $\alpha\beta$ to endosomes and that class II complexes in this compartment must be available for peptide binding. Thus, both self and foreign antigens in endosomes may compete for association with class II antigens. Two classes of proteases, aspartyl and cysteine, both found in endosomes and lysosomes appear to be required for invariant chain cleavage and release. These proteases have been implicated in antigen processing as well as invariant chain proteolysis.

HZ 223 THE *SALMONELLA g...* FAMILY FLAGELLAR ANTIGEN AS A CARRIER OF FOREIGN EPITOPES. B.J. Masten and T.M. Joys, Department of Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Salmonella flagella filaments are polymers of a protein termed, "flagellin". A single flagellum may be composed of several thousand flagellin molecules and there may be 10-30 flagella per cell. We are interested in using substituted flagella of a live attenuated oral *Salmonella* vaccine strain as carriers of medically important epitopes. This approach, which localizes epitope sequences to the cell surface, is achieved by substitution of small specific regions of the flagellin with explicit foreign epitopes. Preliminary work has utilized flagellin variants which are naturally stable. We have examined the *Salmonella g...* family flagellins, which although related, are antigentically different. Here we report the covalent structure for 10 members of this group (*S. berta [f,g,t], S. budapest [g,f], S. derby [f,g], S. dublin [g,p], S. enteritidis [g,m], S. montevideo [g,m,s], S. moscow [g,q], S. oranienberg [m,t], S. rostock [g,p,u]* and *S. senftenberg [g,s,t]*), the location of their individual antigenic determinants, the resulting selection of a suitable site for epitope substitution, and the initial results obtained when "foreign" epitopic regions are inserted in this site.

HZ 224 CLASS II EXPRESSION AND FUNCTION IN HUMAN T CELLS.
Moore, Marilyn CIML Case 906 Luminy, 13288 Marseille France.

Human T cells express class II antigens as late activation markers but it is unclear to what extent they can function as antigen presenting cells. Class II positive transformed T cell lines and mitogen activated peripheral blood T cells were assayed for their ability to present a panel of pre-processed peptides of Human papilloma virus to appropriate T cell clones. At various doses of peptide or presenting cell numbers, the T cells failed to stimulate the clones while DR matched EBV lines did.

We compared the biosynthetic route of class II in T and B cells using confocal microscopy, and found that $\alpha\beta$ complexes could be detected in the endocytic pathway in B cells but most of the T cell class II was found at the surface or in the ER. Furthermore, on T cells class II was not endocytosed and remained on the cell surface for at least 24hrs.

Co-immunoprecipitation studies indicated that class II does not bind to Invariant chain (Ii) during synthesis in T cells while early transcient association between these molecules can clearly be seen in B cells. Ii may protect the peptide binding site of class II, directing it to the endosomes where Ii is removed, allowing binding to exogenously derived antigenic peptides to occur.

These results indicate that class II may have a distinct intracellular trafficking pattern which could affect it's access to antigenic peptides, favouring the presentation of endogenously generated peptides such as those produced by viruses or parasites.

HZ 225 CHARACTERIZATION OF AMINO ACID SIDE CHAINS WITHIN ANTIGENIC PEPTIDE PERMISSIVE FOR INTERACTION WITH POLYMORPHIC DR1 β CHAIN POSITIONS 85 AND 86, Debra K. Newton-Nash and David D. Eckels, Immunogenetics Research, The Blood Research Institute, Milwaukee, WI 53233

Major histocompatibility complex molecules encoded within the DR region of the human leukocyte antigen (HLA) complex are polymorphic, cell-surface associated $\alpha\beta$ heterodimers that restrict the recognition of antigenic peptides by CD4+ T cells. We have demonstrated previously that polymorphism resulting in amino acid substitutions at HLA-DR1 β chain positions 85 and 86 ($V_{85}G_{86}$ or $A_{85}V_{86}$) affects binding and, thus, recognition of DR1-restricted antigenic peptides. Furthermore, we defined the corresponding contact residue within antigenic peptide as contact position 1 (CP1). To characterize the effect of amino acid substitutions at DR1 β chain positions 85 and 86 on the nature of amino acid side chains permitted at CP1, substitutions of amino acids bearing aromatic (F, Y or W) or aliphatic hydrocarbon (A, V, L or I) side chains were introduced at CP1 in two unrelated DR1-restricted antigenic peptides. Binding of biotinylated peptides to DR1-homozygous EBV-transformed B cells was determined by flow cytometric analysis. Peptides bearing A at CP1 were unable to bind to DR1 on cells expressing either $V_{85}G_{86}$ or $A_{85}V_{86}$ whereas peptides bearing substitutions of V, L or I at CP1 bound to DR1 on both. DR1 allelic variants differed from one another in that all amino acids bearing aromatic side chains at CP1 bound to $V_{85}G_{86}$-expressing cells whereas peptides bearing Y and W at CP1 did not bind to DR1 on $A_{85}V_{86}$-expressing cells. Standard T cell proliferative assays revealed that peptide binding was necessary but not sufficient for T cell stimulation. These results suggest that the CP1 side chain of DR1-restricted antigenic peptides extends into a hydrophobic pocket whose dimensions are determined by the side chains of amino acid residues at DR1 β chain positions 85 and 86. Our results also suggest that a positive contribution involving the CP1 side chain is required for DR1-restricted peptide binding and may influence the conformation adopted by peptide in the DR1 peptide binding groove.

HZ 226 THE ROLE OF INVARIANT CHAIN FOR TRANSPORT AND FUNCTION OF MHC CLASS II MOLECULES.
Marga Nijenhuis, Marcel de Haas, Karel C. Kuijpers, Oddmund Bakke, Jero Calafat, Jacques J. Neefjes, Hidde L. Ploegh, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Good evidence exists that the invariant or γ chain plays an important role in the sorting of MHC class II molecules from the trans Golgi reticulum to lysosomal structures, where they take up peptides. To determine the importance of the γ chain as well as the part of the endocytic route that is involved in peptide binding, we transfected kidney cells with only the MHC class II α and β chains or class II α and β together with wild type or mutant γ chains. The mutant γ chains either have deletions in the cytoplasmic tail, abolishing transport to the endocytic route, or the transmembrane and cytosolic domains of the transferrin receptor.
Analysis of these transfectants showed that the presence of the γ chains enhanced the rate of transport of the $\alpha\beta$ complex from the endoplasmic reticulum. Since the transferrin receptor/γ chain chimaera induced exit as well as the wild type γ chain, it is likely that the lumenal domains of the α,β and γ chains are responsible for transport from the endoplasmic reticulum, as is the case for MHC class I proteins. In addition the way of transport of the $\alpha\beta\gamma$ complexes to the cell surface was the same as predicted for the different γ chains alone. Thus the γ chains is both important for efficient transport of the $\alpha\beta$ complex from the endoplasmic reticulum and for sorting of these molecules after they leave the trans Golgi reticulum. Moreover, while the lumenal part of the γ chain is sufficient for the first phenomenon, are the cytoplasmic and transmembrane parts responsible for the second phenomenon. The effect of the different γ chains and thus different sorting pathways on peptide binding and antigen presentation is currently investigated and will be presented.

HZ 227 CLASS I DOMAIN ORGANIZATION DOES NOT IMPOSE A LIMITATION ON THE LENGTH OF PEPTIDES BOUND TO HLA-DR1 MOLECULES, Richard R. Olson and Jeff J. Reuter, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242.

We are interested in whether certain functional features unique to human class II molecules may be dependent upon their distinct domain organization compared with class I. In class II molecules, the two membrane distal domains forming the peptide binding and T cell recognition sites are contributed by different polypeptide chains. This feature may impart unique properties compared to class I molecules (wherein a single polypeptide chain provides both of these domains.) In order to address this question, we tested whether it was possible to express HLA-DR1 molecules on the cell surface in which the domain organization was altered to resemble class I. Using overlap extension PCR, cDNA constructs were made encoding a_2-TM-CY and $a_1\beta_1\beta_2$-TM-CY chains. These constructs, in the pCD expression vector, were transfected into L cells. Indirect immuno-fluorescence and FACS were used to isolate clones expressing the domain-shifted "3+1-DR1" molecule. We found that the 3+1-DR1 molecule was recognized by several, but not all, mAb reactive with conventional DR1 molecules expressed on L cells. L cells expressing 3+1-DR1 were able to stimulate antigen-specific T cell clones when pulsed with appropriate synthetic peptides of 13 and 14 amino acids, suggesting that the class I domain organization does not prevent peptides of this length from binding to, and being presented by, class II molecules.

HZ 228 COSTIMULATION THROUGH MURINE B7 MOLECULE RESTORES IMMUNOGENICITY OF AUTOLOGOUS TUMOR CELLS EXPRESSING TRUNCATED MHC CLASS II MOLECULES.
S. Ostrand-Rosenberg*, S. Baskar*, N. Nabavi#, G. Freeman+, L. Nadler+, and L. Glimcher%; *Dept. of Biology, U. of MD, Baltimore, MD 21228; #Hoffman-LaRoche, Nutley, NJ 07110; +Dana-Farber Cancer Inst., Boston, MA 02115; and %Depts. of Cancer Biology and Medicine, Harvard School of Public Health and Harvard Medical School, Boston, MA 02115.

Antigen presenting cells (APC) trigger T cell activation by presenting antigen via their MHC molecules to the responding T cell. Although several accessory molecules facilitate activation, costimulation via the B7 molecule of the APC is required for activation, in at least some cells. APC whose class II molecules have a truncated cytoplasmic domain are ineffective in presenting antigen to some T cells, and cannot transmit transmembrane signals, as measured by PKC translocation. These observations led to the hypothesis that the class II cytoplasmic domain transmits an intracellular signal that up-regulates B7 expression. Here we test this hypothesis using the mouse Sal sarcoma. Although Sal cells are malignant in autologous A/J mice, Sal transfected with syngeneic MHC class II genes (Sal/A^k cells) are immunologically rejected, and immunization with Sal/A^k cells confers potent immunity to challenges of wild type, class II^- Sal. We have suggested that Sal/A^k cells are efficient inducers of tumor immunity because they function directly as APC for endogenously synthesized tumor peptides, thereby stimulating specific T_h cells. Sal cells expressing MHC class II molecules with truncated cytoplasmic domains (Sal/A^ktr), however, are as malignant as class II^- wild type Sal. If the cytoplasmic domain is involved in stimulating B7 expression, then supertransfection of Sal/A^ktr cells with the B7 gene (Sal/A^ktr/B7 cells) should bypass the requirement for the class II cytoplasmic region. In the present study we show that Sal/A^ktr/B7 cells are rejected by A/J mice, and that immunization with these cells stimulates immunity as effectively as immunization with Sal/A^k cells. Constitutive expression of B7 therefore overcomes the absence of the cytoplasmic domain of the class II molecule, indicating that the cytoplasmic region plays a role in signal transduction. These results also demonstrate the utility of co-expression of B7 and MHC class II molecules for enhancing tumor immunity.

HZ 229 EXPRESSION AND CHARACTERIZATION OF MHC CLASS II MOLECULES IN INSECT CELLS,
Leslie Pond and Per A. Peterson, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Our goal is to establish an insect cell system to study the intracellular transport of class II molecules. The advantages of using insect cells are that they probably do not express the specialized molecules required for antigen presentation, and that mammalian protein targeting signals function in insect cells. *Drosophila melanogaster* cells transfected with MHC class II cDNA express cell surface class II molecules. Co-transfection with p31 invariant chain (Ii) cDNA allows co-localization of class II molecules and Ii in an intracellular vesicular compartment and on the cell surface. In contrast to mammalian class II and Ii molecules, these molecules remain associated at the surface of insect cells. Insect cells expressing class II molecules present exogenous peptide antigen to a T cell hybridoma two orders of magnitude better than cells expressing both class II and Ii molecules. Insect cells do not appear to process whole protein antigen for class II antigen presentation. Consistent with this result is the finding that the insect cell class II molecules are not SDS-stable at low temperature and thus appear to be devoid of peptides. We anticipate that these insect cell transfectants will be especially useful for determining the requirements for class II molecule and Ii transport, and for class II molecule peptide acquisition.

HZ 230 EXPRESSION OF A FUNCTIONAL α-MACROGLOBULIN RECEPTOR BINDING DOMAIN IN ESCHERICHIA COLI. Long T. Quan, Scott J. Snipas, Jan J. Enghild, Salvatore V. Pizzo and Guy S. Salvesen. Department of Pathology, Duke University, Durham, NC 27710

We have expressed receptor-binding domains of human α_2-macroglobulin and rat α_1-macroglobulin in E. coli. Expression levels of both recombinants were *quite high*, but the human one was insoluble, probably forming inclusion bodies. The rat domain, which lacks the human disulfide, was produced in a soluble form and readily purified by two simple chromatographic steps. Purified recombinant rat α_1-macroglobulin receptor binding domain was fully functional in binding to the α-macroglobulin receptor on human fibroblasts. It should be possible to ligate this 142 residue domain to the C-terminus of any of a number of proteins for internalization and processing by antigen-presenting cells.

HZ 231 **DEFINITION OF THE TRI-MOLECULAR COMPLEX OF HUMAN THYROID PEROXIDASE SPECIFIC AUTOREACTIVE T CELL CLONES.** **Sonia Quaratino, Colin Dayan, Marc Feldmann, Marco Londei.**
Kennedy Institute of Rheumatology, Sunley Building, 1 Lurgan Avenue, Hammersmith, London W6 8LW. U.K.

The key components of T cell recognition are now well understood. This involves the HLA molecule binding peptide epitopes which are recognized by the TCR. Thus a central task in autoimmunity is the definition of these 3 different components in truly autoantigen reactiveT cells. We addressed the problem by studying *in vivo* autoantigen reactive T cell clones established from IL2R+ cells in the absence of antigen from a patient affected by Graves' disease. The analysis of a panel of TPO-epitope specific T cell clones provided the most significant information. Three T cell clones specific for TPO residues 535-551 and sharing the same genetic restriction element DQ6 expressed different Vβ usage (Vβ1 and Vβ6.4). Another TPO epitope was defined, residues 632-645, which used Vβ2, as well as another genetic restriction element (DQ2). The sequence analysis of the β chain of the TCR confirmed that all the T cell clones possess a different CDR3 region.The Vα usage was heterogeneous as well in clones specific for the same epitope. Our data thus demonstrates the presence of considerable TCR heterogeneity, even for a single epitope of the TPO molecule, which puts reservations about the applicability of TCR directed immunotherapy in chronic autoimmune diseases in man, at the time patients need therapy.

HZ 232 EXPRESSION OF INVARIANT CHAIN GOVERNS INTRACELLULAR CONFORMATIONAL CHANGES OF I-A^k CLASS II MOLECULES ACCUMULATED IN VESICLES AND RESPONSIBLE FOR EFFICIENT ANTIGEN PRESENTATION. Chantal Rabourdin-Combe, Patrick Bertolino, Isabelle Chrétien, François Cretin, Cyrille Gimenez, Suzanne Lombard-Platet and Denis Gerlier. Laboratoire ENS-CNRS UMR 49, 69364 Lyon Cedex 07, France.

A new experimental method allowing modulation of endogenous Ii gene expression within a single APC expressing a constant level of MHC class II mRNA was carried out. I-A^k transfected L cells maintained strictly below confluence for several days in 10% FCS supplemented medium expressed undetectable Ii mRNA. A strong induction of endogenous Ii mRNA could be observed after serum starvation (0.5% FCS) for more than 24-30 hours. Accordingly, efficient presentation of HEL to 3A9 T cell hybridoma was only observed after Ii chain induction. In contrast, APC expressing or not Ii chain were as efficient in presenting RNase to TS12 T cell hybridoma. Immunoprecipitation and immunofluorescence studies revealed that, after Ii gene induction in I-A^k transfected L cells, there was a strong increase in the amount of detectable intra-cellular MHC class II molecules roughly proportional to the level of Ii chain expression. Most I-A^k molecules localized in vesicles. In contrast, cell surface I-A^k molecules expression was hardly affected upon Ii induction and most of them were SDS-resistant forms. Therefore there is a strict correlation between Ii chain expression, reactivity of MHC class II molecules with antibodies and their ability to bind some antigen derived peptides to be presented to specific T cells. This favours that Ii chain induces conformational changes of MHC class II molecules and their accumulation in some endosomal compartments required for efficient association with some antigen derived peptides.

HZ 233 MODULATION OF THE ANTIGEN PROCESSING CAPACITY OF BONE MARROW-DERIVED MACROPHAGES BY LYMPHOKINES, Angelika B. Reske-Kunz, Stefanie Frosch, and Ursula Bonifas. Institute for Immunology, Johannes Gutenberg University, W-6500 Mainz, FRG.

Bone marrow-derived macrophages (BMMph) grown for ten days in a liquid culture system in the presence of M-CSF exhibit antigen presentation function activating insulin-specific T clone cells and T hybridoma cells to proliferation and/or lymphokine production. This capacity is slightly enhanced after treatment of the BMMph with IFN-γ . In contrast, BMMph activated by GM-CSF are much more potent insulin presenting cells, although they express significantly fewer MHC class II molecules than IFN-γ -treated cells. To analyze whether a divergent capacity to process insulin in the two macrophage populations might be responsible for the different antigen presentation potential, the activity of several proteases involved in the degradation of protein antigens was inhibited by the addition of protease inhibitors. Inhibition of thiol/serine proteases resulted in a drastically augmented antigen presentation function of IFN-γ -activated BMMph, while GM-CSF-treated BMMph were not influenced. Weakly enhanced antigen presentation capacity was found with both macrophage populations, when metallo proteases were inhibited. Inhibition of acidic proteases had no influence on insulin presentation. Elevation of the endosomal/lysosomal pH by treatment of BMMph with chloroquine or NH_4Cl rendered IFN-γ -treated BMMph more efficient insulin presenting cells, while GM-CSF-induced BMMph were not affected. These findings suggest a differential regulation of the activity of thiol/serine proteases in BMMph by the two cytokines. A higher level of activity of thiol/serine proteases in IFN-γ -treated BMMph as compared with GM-CSF-induced cells appears to result in successive degradation of insulin and destruction of antigenic determinants.

HZ 234 HLA-DR MOLECULES FROM AN ANTIGEN PROCESSING MUTANT CELL LINE ARE ASSOCIATED WITH INVARIANT CHAIN PEPTIDES, Janice Riberdy and Peter Cresswell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

The invariant chain, which associates with MHC class II molecules in the endoplasmic reticulum (ER), serves two functions important in antigen processing. First, it prevents class II molecules from binding peptides in the early stages of intracellular transport. Second, it contains a cytoplasmic signal which targets the class II-invariant chain complex to an acidic endosomal compartment. Proteolytic cleavage and subsequent dissociation of the invariant chain then occurs, allowing peptides derived from endocytosed proteins to bind to released class II molecules prior to their cell surface expression. Certain human cell lines, mutant in one or more MHC-linked genes, are defective in class II-restricted antigen processing. Here we show that in one of these cell lines, T2, this deficiency results in the association of a nested set of invariant chain-derived peptides with a large proportion of class II molecules expressed from transfected genes. These *C*lass II-associated *I*nvariant chain *P*eptides (CLIP) may define the region of the invariant chain responsible for obstructing the class II binding site. We also show that HLA-DR3 molecules isolated from T2 transfectants can be efficiently loaded with antigenic peptides by exposure to a low pH which may reflect the normal in vivo conditions in which peptides associate with class II MHC molecules.

HZ 235 THE CYTOPLASMIC TAIL OF THE INVARIAN CHAIN MEDIATES RAPID ENDOCYTOSIS OF HLA-DR, Paul A. Roche, Christina L. Teletski, Oddmund Bakke, and Eric O. Long. Lab. of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852 and Dept. of Biology, Univ. of Oslo, Oslo, Norway

The MHC class II-associated invariant (Ii) chain inhibits immunogenic peptide binding to immature class II molecules and directs the intracellular trafficking of newly synthesized class II molecules to an acidic endosomal compartment. Although the presence of Ii on the surface of antigen presenting cells has been well documented, the function of surface Ii has remained a mystery. We now show that cell surface Ii is rapidly internalized in both class II-positive and class II-negative B-LCL with a $t_{1/2}$ of 4 min. In addition, fibroblasts containing mutant forms of Ii have been used to show that the rapid internalization of Ii can be prevented by the removal of fifteen amino acids from the cytoplasmic tail of Ii. This data suggests that the Ii internalization signal is related to the targetting of class II molecules to endosomes.

HZ 236 INVARIANT CHAIN MODIFIES ENDOSOME MORPHOLOGY AND TRANS-ENDOSOMAL TRANSPORT, Paola Romagnoli, Flora Castellino and Ronald N. Germain, Laboratory of Immunology NIAID, NIH, Bethesda, MD 20892

Invariant chain (Ii), which associates with MHC class II molecules in the endoplasmic reticulum, contains a targeting signal for transport to endosomes/lysosomes. We demonstrate here that in transiently transfected cos cells, complexes of uncleaved Ii and MHC class II molecules accumulate in transferrin-accessible endosomes. Ii expression alters endosomal morphology, giving rise to large lucent vesicles (Ii-macrosomes) with apparently normal transferrin recycling but significantly delayed export of endocytosed proteins to late endosomes/lysosomes. Lumenally cleaved Ii accumulates in a distinct cohort of vesicles with the characteristics of late endosomes/pre-lysosomes. Localization to this late compartment can be markedly enhanced by removal of 10 residues in the cytoplasmic tail of Ii. C-terminal truncation of Ii (Iip31(1-90)) affects Ii-macrosomes formations. This truncated form of Ii is still able to associate with MHC class II molecules and to mediate its localization in endocytic vesicles.Iip31(1-131) shows the same properties of the full length Ii. Further studies are in progress to understand the importance of the lumen of Ii in the proper assembly of Ii and Of Ii-MHC class II complexes.

HZ 237 DISTINCT KINETIC STATES OF MHC CLASS II-PEPTIDE INTERACTION CORRELATE WITH DISTINCT CLASS II STRUCTURES. Scheherazade Sadegh-Nasseri, Lawrence Stern, Donald Wiley & Ronald Germain, Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH, Bethesda MD 20892 and Department of Biochemistry, Harvard University, Cambridge MA 02138.

Previous studies have reported two distinct kinetic states for the specific interaction of peptide with $E\alpha E\beta^k$ molecules - a fast-binding, fast-dissociating state, and a slow-accumulating, slow-dissociating state. The occupancy of available class II binding sites in the fast-associating state occurs with a $t_{1/2}$ of about 2 minutes for pigeon cytochrome *c* (PCC)peptide 88-104 (25°C, pH 7.2). The dissociation of these complexes occurs with a $t_{1/2}$ of about 10 minutes. The slowly dissociating complexes involve the same class II molecules giving rise to the fast dissociating complexes at shorter incubation times, and raised the possibility that an infrequent event limited the capacity of interacting peptide and MHC molecules to generate the slowly dissociating form of complex.

Class II dimers exist in several structural states. A loosely associated dimer state is characteristic of molecules with unoccupied peptide binding sites. Occupancy with a suitable peptide converts these unstable dimers to more denaturation resistant forms, characterized by migration as associated $\alpha\beta$ complexes in SDS-PAGE performed without sample heating. We investigated the relationship between changes in the biochemical properties of class II and of two distinct kinetic states of peptide interaction.

Real forward rate measurements are complicated by occupancy of purified MHC. We have used soluble DR1 molecules produced in insect cells which are free of peptides. Addition of labelled HA peptide 306-318 to sDR1 led to rapid occupancy, ~100%, ($t_{1/2}$<5 minutes at 25°C, pH 7.2). Such complexes also dissociate rapidly ($t_{1/2}$ = 10-30 minutes). They were specific, with binding of labelled peptide inhibited proportionately by cold identical peptide, but not by PCC peptide 88-104. The DR1 molecules occupied with such rapidly dissociating peptides remained in the unstable state characteristic of the initial empty molecules. With extended incubation, slowly dissociating complexes ($t_{1/2}$ of >100 hr) accumulated. This accumulation was accompanied by a proportional accumulation of SDS-stable DR1 dimers. Thus, the observation that class II dimers show two kinetically distinct forms of specific peptide binding has been confirmed in a second model system, and a relationship between the kinetic state of binding and the structural properties of the class II molecules demonstrated.

HZ 238 MHC CLASS II MOLECULES EXPRESS A TARGETING SEQUENCE THAT DIRECTS LOCALIZATION TO THE ENDOCYTIC PATHWAY OF ANTIGEN PRESENTING CELLS

Andrea J. Sant and Alexander Chervonsky. University of Chicago, Chicago, IL 60637

MHC Class II molecules have the unique ability to present peptides derived from exogenous antigen. Although it is generally accepted that this ability reflects selective trafficking to endocytic compartments of antigen presenting cells, the structural features in the Class II complex *that* direct it into these compartments are a subject of some debate and include at least two possibilities: sequences in the Class II molecule itself or sequences in the associated invariant glycoprotein (Ii). The finding that cells lacking Ii are capable of presenting exogenous antigens suggested to us that Class II molecules access the endosomal compartments independently of Ii.

We have used site directed mutagenesis and transfection to identify sequences in the class II molecule that control this localization. Our studies have focussed on a conserved sequence in the class II β chain (80-84) that had been shown to be important in late class II transport events. We have mutated this region and transfected mutated or wild type (WT) genes with WT α chain genes into a variety of Ii negative cell types and analyzed the impact of the mutations on the subcellular localization of Class II. Through the use of immunofluorescence staining of permeabilized cells, we have found that single, conservative amino acid changes in this region can either eliminate or dramatically enhance expression in the late endosomal compartments of Ii negative APC. Co-transfection of genes encoding Ii have indicated that the ability of Ii to enhance localization of Class II in the late endosomal compartments is absolutely dependent on the Class II sequence in the β chain at position 80-84. Our findings demonstrate that the 80-84 sequence in β to be the dominant factor in class II localization and suggest the presence of a intra-lumenal transport protein that recognizes Class II β chain and directs Class II complex into the late endosomal compartments. Invariant chain may enhance localization either through its ability to facilatate folding of Class II in RER or by its ability to retain Class II in the endocytic pathway.

HZ 239 INDUCTION OF I-A EXPRESSION IN PERITONEAL MACROPHAGES BY PARASITIZED ERYTHROCYTES OF LETHAL AND NON-LETHAL RODENT MALARIA, Hannah L. Shear, Julie Dunne, and Edward Dimayuga, Div. of Hematology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, 10467.

The immune response to Plasmodium chabaudi adami is dependent on CD4+ T cells. Activation of these cells is via their interaction with peptides and Class II molecules expressed on antigen-presenting cells. In this study, we assayed the ability of parasitized erythrocytes to induce I-A expression on peritoneal macrophages. After incubation of macrophages from BALB/c mice with parasitized erythrocytes for 18 hrs, I-A expression was measured using the MK-D6 monoclonal antibody (anti $I\text{-}A^d$) followed by ^{125}I-labeled anti Ig Fab_2 fragments. P. chabaudi adami-infected RBC induced greater I-A expression in peritoneal macrophages than did normal erythrocytes. We next compared I-A induction after incubation of macrophages with cloned parasites from a mild strain compared with a more virulent strain of P. chabaudi. The mild strain induced a higher level of I-A expression. Passage of the mild strain of the parasite in mice led to an increase in its virulence and a concomitant decrease in I-A induction. In order to determine whether malarial peptides of P. chabaudi were expressed on the macrophage membranes, macrophages were incubated with moAbs against a P. chabaudi adami antigen, Pc96, which has been shown to be partially protective to mice. One moAb 8F2G9, bound to macrophages preincubated with the mild but not the virulent strain of P. chabaudi, whereas another moAb against the same antigen, 6C12, did not bind to macrophages incubated with either parasite. The data provide a model for studying antigen processing of a malarial antigen and relating the degree of I-A induction and epitope expression to protective immunity.

HZ 240 SYNOVIOCYTE-T CELL INTERACTIONS: ANTIGEN PRESENTATION BY SYNOVIOCYTES TO PEPTIDOGLYCAN-POLYSACCHARIDE-SPECIFIC T CELLS IN VITRO.

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A single injection of peptidoglycan-polysaccharide (PG-PS) from group A streptococci results in a chronic T cell-dependent arthritis. We have examined whether the fibroblast-like synoviocyte may contribute to the proliferation and expansion of PG-PS-specific T cells in the joint by directly presenting PG-PS to T cells. Three CD4+, TcR α,β+ PG-PS specific T cell lines were derived from lymph node cells of arthritic Lewis rats. One T cell line, SC(-), was derived from naive Lewis rats, indicating that PG-PS specific T cells pre-exist in the unimmunized rat. Fibroblast-like synoviocytes were derived from normal or arthritic joints and shown to be positive for MHC class I antigens and the rat fibroblast antigen Thy1.1, but negative for several other dendritic cell, macrophage/monocyte and lymphocyte cell surface markers including MHC class II. Co-culture of the synoviocytes with PG-PS specific T cell lines in the presence of PG-PS, but not control antigens, resulted in PG-PS dose-dependent proliferation of the T cell lines. T cells derived from arthritic versus naive animals responded similarly to PG-PS in the presence of synovial cells derived from arthritic or normal joints. This response was inhibited by antibodies to CD4 or MHC class II, but not CD8 or MHC class I antigens indicating MHC class II-restricted antigen presentation. MHC class II antigen expression was shown to increase in synoviocytes treated with IFN-gamma or by co-culture with T cells and PG-PS, but not with PG-PS alone. Therefore, PG-PS specific T cells activate synoviocytes *in vitro* to express MHC class II antigens, which in turn present PG-PS to T cells. We speculate that such an interplay between synoviocytes and T cells may be operative *in vivo* and plays a role in the exacerbation and chronicity of PG-PS induced polyarthritis in rats.

HZ 241 CLASS II I-A^k MOLECULES CAN BIND AND PRESENT ANTIGEN FOLLOWING INTERNALIZATION

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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 first model, recent biochemical measurements of class II MHC internalization in B cells have failed to reveal internalization patterns similar to other known recycling cell surface molecules. We have used a novel internalization assay to show that, in mouse Ltk^- cells transfected with class II genes, I-A^k molecules are internalized at a high rate, less than the internalization rate of endogenous transferrin receptor but significantly greater than that for endogenous class I MHC molecules. The internalization rate is not altered by the intracellular association of class II molecules with various forms of wild-type invariant chain nor by the cell surface association of class II with a mutant form of invariant chain lacking a cytoplasmic tail. In all transfectants, regardless of the form of invariant chain expressed, another molecule with an apparent molecular weight of 55 kd, detectable only by cell surface labeling, is coprecipitated with I-A^k α and β chains. This molecule is associated only with surface I-A^k molecules free of invariant chain and is cointernalized with I-A^k molecules. Thus, in cells expressing the mutant form of invariant chain, there are two populations of class II at the cell surface, those associated with invariant chain and those associated with p55; both are internalized. We have shown that invariant chain-dependent antigen presentation by these transfectants occurs following internalization of class II. Experiments are being done to determine whether or not class II molecules not associated with invariant chain also become accessible for peptide binding after internalization. The role of class II internalization will also be assessed in other cell types to determine if it is limited to L cell transfectants.

HZ 242 DRβ AMINO ACID SIDE CHAINS ENGINEERED TO STABILIZE INTERACTIONS WITH SUPERANTIGENS. Robert G. Ulrich, Theresa J. Smith, and Sina Bavari*, Department of Immunology and Molecular Biology, U.S.A.M.R.I.I.D., Frederick, Maryland 21702.
Site-directed mutations were introduced into DRα and DRβ genes at polymorphic, conserved, or semi-conserved structural positions. Mammalian cell lines were transfected with expression vectors of each mutation, and then used to detect changes in antigen binding affinities and T cell receptor interactions. Of these side chain alterations, key conserved residue replacements within DRβ1 were found to enhance the interaction of class II molecules with select staphylococcal enterotoxins. These new amino acid residues introduced no detectable changes in overall protein structure for the class II molecule, suggesting that these side chains directly interacted with some but not all superantigen/T cell receptor complexes. The nature of this interaction was further studied in terms of charge, hydrophobicity, and surface area.

*Supported by a National Research Council Research Fellowship

HZ 243 BOVINE T CELL RESPONSES TO CATHEPTIC FRAGMENTS OF FOOT AND MOUTH DISEASE VIRUS, Van Lierop M.J.C., Van Noort J.M., Meloen R.H., Hensen E.J., Inst. of Inf. Diseases and Imm., Vet. Fac., University of Utrecht, P.O. Box 80.165, 3808 TD Utrecht, The Netherlands.
In this study we first investigated whether the endosomal proteases cathepsin D and B and a reduction of disulfide bonds could play a role in processing of Foot and Mouth Disease virus (FMDV). After incubation of the virus with cathepsin D discrete fragments were released. The HPLC-patterns of reduced and non-reduced digests were almost identical. However, with cathepsin B no significant fragments were found. The cathepsin D digests (reduced and non-reduced) were compared with the intact virus with regard to the ability to induce a specific proliferation of PBLs of three vaccinated and MHC class II typed cattle. In all cases the digests stimulated to the same extent as the intact virus. Subsequently, a similar assay was performed with 70 HPLC-fractions of the catheptic (non-reduced) digest. Fractions that induced high responses and those that were recognized by PBLs of all animals were analyzed. Several of these fractions contained fragments that were derived from sequences within or flanking the immunogenic region VP1[140-160]. These findings indicate that cathepsin D plays an important role in the processing of FMDV and releases fragments that can be recognized by T cells. At the moment the capacity of synthesized fragments to prime for a virus-specific T cell response is under study.

HZ 244 REPLACEMENT OF NATURAL T-CELL EPITOPES OF SALMONELLAR FLAGELLIN. Ricardo A.Vanegas and Terence M.Joys, Department of Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX.79430.
Our research is focused on using the flagella of an oral vaccine strain of *Salmonella* as carriers of medically important epitopes. These organelles are polymers of a protein termed 'flagellin' .Placement of a variety of known B-cell epitopes in flagellin has resulted has resulted in chimeric flagella capable of eliciting specific antibody to the inserted epitope. Recently, the T-cell epitopes of a flagellin have been identified .We have manipulated the flagellin structural gene of *Salmonella muenchen* to allow removal of the normal T-cell epitopes, substitution of these by another known T-cell epitope, and placement of the natural epitopes in different areas of the molecule. Such engineering involve a highly conserved region of the molecule and results in the production of flagellins that are unable to polymerize into flagella. Consequently,we have utilized an expression system for production of the chimeric flagellins and are currently examining them immunologically.

HZ 245 INHIBITION OF ADJUVANT ARTHRITIS BY A DISEASE RELATED MHC BINDING COMPETITOR PEPTIDE: THE COMPETITOR-MODULATOR CONCEPT
Marca H.M. Wauben, Claire J.P. Boog, Ruurd van der Zee, Irma Joosten, Angelique Schlief and Willem van Eden, Institute of Infectious diseases and Immunology, Faculty of Veterinary Medicine, University of Utrecht, P.O. Box 80165, 3508TD Utrecht, The Netherlands
Immune intervention in experimental autoimmune diseases by competitor peptides is subject of intensive research. It appeared that if the competitor peptide is closely related to a disease associated T cell epitope MHC blockade is not necessarily the sole mechanism of immune intervention. To study the mechanism of disease inhibition by immunogenic disease related competitor peptides, we selected the MBP induced EAE, and the Mycobacteria induced AA model in Lewis rats. After *in vitro* definition of an EAE- and AA associated competitor peptide it appeared that, although the EAE analogue had a superior MHC binding affinity, the AA analogue was a better inhibitor of *in vitro* proliferation of arthritogenic T cells. Furthermore, although in MBP induced EAE the efficacy to inhibit disease development correlated with the MHC binding affinity of the competitor peptides, in the AA model the AA analogue was far more efficient than the EAE analogue. Moreover, the AA analogue also prevented AA upon preimmunization. Remarkably, in contrast to what was seen in the EAE model and what was expected of a MHC competitor peptide, the AA analogue induced T cell responses cross-reactive with the original disease associated epitope. This T cell population differed from the T cells triggered after immunization with Mycobacterium tuberculosis or with the original disease associated epitope in that they recognized the original epitope in a cryptic way. It is concluded that besides MHC blockade an antigen specific mechanism was involved in AA inhibition by the AA analogue. Whether this antigen specific effect is due to a direct effect of the analogue on the arthritogenic T cells or to T cells activated by the analogue inducing protective regulatory T cell responses is currently under investigation.

HZ 246 T CELL RECOGNITION OF 130 SYSTEMATICALLY SHORTENED ANALOGUES OF A DR2 RESTRICTED 13-MER PEPTIDE: INFLUENCE OF TERMINAL CHARGES

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An attempt was made to define the N- and C-terminal borders of a T cell determinant encompassed by the 49-61 region of the meningococcal P1 outer membrane protein (OMP). For this purpose, a series of systematically shortened peptide analogues was assembled and tested for recognition by a panel of OMP(49-61)-specific HLA-DR2 restricted T cell clones. All 65 possible analogues with a size between 3 and 12 residues were obtained. In order to study the influence of terminal charges on T cell recognition, we have prepared a series of peptides with free amino and carboxyl termini and a homologous series with acetylated N-termini and C-terminal carboxamides.

In proliferation experiments, unexpected results were obtained. Particular unilateral truncations were allowed. However, when the same truncations were made departing from an active peptide with one residue missing at the other terminus, complete unresponsiveness of the clones was observed in many cases. Apparently, certain C-terminal amino acid residues compensate for the truncation of an important N-terminal residue, and vice versa. Compared to the uncharged analogues, very few residues could be omitted from charged analogues. Finally, different fine specificity patterns were observed for each of the clones.

These findings are discussed in the context of the development of synthetic B and T epitope containing conjugates, a possible approach to a vaccine against meningococcal (and, eventually, gonococcal) disease.

HZ 247 *Abstract Withdrawn*

HZ 248 CHARACTERIZATION OF RECOMBINANT DR4Dw4 MOLECULES EXPRESSED IN BACULOVIRUS-INFECTED INSECT CELLS.

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We have used the baculovirus expression system to generate recombinant human DR4Dw4 molecules with truncated transmembrane domains. The α and β-chains were both expressed at very high levels, but the formation of stable dimers was relatively inefficient. The α/β dimers were recognized by most, but not all, DR-specific antibodies that were tested. The L227 monoclonal antibody (β1-specific), which binds to both nondenatured and denatured DR4Dw4 molecules, bound efficiently only to denatured recombinant DR4Dw4 molecules. Thus, conformational differences exist between native DR molecules derived from human B-cells and recombinant DR molecules derived from insect cells. The α/β dimers were secreted from infected insect cells and accumulated in the culture supernatant. Purified recombinant dimers bound DR-specific peptides very efficiently and with enhanced kinetics. Minigenes that encode DR-binding peptides are being co-expressed with recombinant DR molecules in an attempt to increase dimer formation and to generate homogeneous DR/peptide complexes.

Class I & II Molecules

HZ 300 PREFERENTIAL Vβ USAGE BY CYTOTOXIC T CELLS WITH THE UNUSUAL PHENOTYPE OF CROSSREACTIVE RECOGNITION OF TWO HIV-1 EPITOPES AND DEGENERACY OF PRESENTATION BY THREE CLASS I MHC MOLECULES, Jay A. Berzofsky*, Mutsunori Shirai*, Melanie S. Vacchio §, and Richard J. Hodes §, *Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, and §Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

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

HZ 301 CLONAL DELETION OF ID/MHC-SPECIFIC T CELLS IN THE THYMUS

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We have previously shown that both endogenous and exogenous Ig is processed and that generated Id-peptides are presented by MHC class II molecules to Id-specific T cells. These findings suggested the possibility that processed Id from self Ig could influence the T cell repertoire by deletion in the thymus. To investigate this, we generated $\lambda 2^{315}$ Ig L-chain gene transgenic mice as well as TCR transgenic mice with a receptor specific for Id.$\lambda 2^{315}$/I-E^{d} complexes. Analysis of ($\lambda 2^{315}$ transgenic x TCR transgenic)F_1 offspring shows that Id can induce negative selection of thymocytes at the $CD4^+CD8^+$ stage. Both Id produced *in situ* in the thymus, as well as soluble Id brought to the thymus from the periphery, cause deletion. However, based on the serum concentration requirement (>200µg/ml) for negative selection, it is doubtful if thymocytes which can react with peptides encoded by Ig V gene segments are deleted under physiological conditions. Individual CDR3 peptides created through V-D-J junctions are certainly of too low concentrations to delete T cells. Deletion caused by serum Ig is of short duration because the thymus has regained a normal appearance already after 7 days.

HZ 302 ANTIGEN PRESENTING CAPACITY OF SYNOVIAL FIBROBLASTS, Annemieke Boots, Astrid Bertens, and Ton Rijnders, Department of Immunology, Organon Int., P/O Box 20, 5340 BH Oss, The Netherlands.

Synovial fibroblasts normally do not express MHC class II molecules. However in inflamed joints of RA patients these cells have been shown to express HLA-class II. Moreover, in vitro studies have shown that MHC class II expression can be easily induced on synovial fibroblast cell lines by γIFN. These observations might suggest a role for these cells in antigen presentation to T-cells.

The aim of the present study was to investigate the antigen presenting capacity of synovial fibroblasts with γIFN induced class II expression. Human synovial biopsy tissue of a RA patient was minced and digested with collagenase. Cells were seeded into culture flasks in medium supplemented with 10% NHS. Overnight culture allowed the separation of adherent cells from the non-adherent population. The adherent cells were routinely passaged and were used as APC within 4-12 passages. The cells thus isolated morphologically resembled the type B or "fibroblast like" synoviocytes and were Leu M3 and HLA-DR negative. Forty-eight hours prior to use in the T-cell proliferative assay cells were cultured in the presence of 1000 U/ml γIFN. Maximal induction of HLA-DR expression was seen using a mAb against a monomorphic HLA-DR determinant.

The non-adherent cell population was stimulated with Tetanus toxoid (TT) and 3 days thereafter fed with IL2. CD4 positive cells were isolated with the use of anti-CD4 coated magnetic beads. Prior to use in the T-cell proliferative assay, cells were rested for 7-14 days after stimulation.

The data presented show that *synovial fibroblasts with induced class II* expression are able to process and present TT to TT-specific, CD4-positive T-cells. This observation might suggest a role for these cells in the maintenance of chronic inflammation.

HZ 303 THE INTERACTION OF CD4 AND CLASS II MHC PROTEINS, Jennifer Brogdon and Carolyn Doyle, Duke University Medical Center, Department of Immunology, Durham, NC 27710

A cell binding assay has been used to study the interaction of CD4 and class II major histocompatability complex (MHC) proteins. Class II^+ human B lymphocytes adhere to monolayers of Chinese hamster ovary cells transfected with human CD4 cDNA. Peptides of 15 amino acids in length and corresponding to overlapping sequences of the DRβ polypeptide were tested for the ability to inhibit the CD4-class II interaction. Addition of these peptides inhibited the cell-cell adhesion to varying degrees. Three peptides, DRβ41-55, DRβ121-135 and DRβ141-155, exhibited a concentration-dependent inhibitory effect; thus, implicating these regions of the class II β chain as the CD4-binding site.

Based on these results, a number of mutations have been engineered 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 and a class II^- Burkitt's lymphoma which have previously been transfected with an HLA-DRα cDNA. Transfected cells, expressing mutated class II proteins, have been analysed for surface expression of the introduced class II proteins. CD4 binding has been assessed using the cell binding assay. Finally, antigen-specific and alloreactive DR1-restricted T cell clones have been used to analyse T cell recognition of the altered gene products.

HZ 304 THE RELATED MHC-LINKED LMP AND PROTEASOME COMPLEXES REPRESENT STRUCTURALLY DISTINCT PROTEINS, Brown, M.G. and Monaco, J.J., Department of Microbiology and Immunology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23225

Most peptide antigens destined to associate with MHC class I molecules are produced in the cytoplasm and subsequently transported through the rough endoplasmic reticulum membrane into the ER lumen. Currently, the mechanism responsible for proteolytic processing of such cytoplasmic antigens remains unclear. Several *findings* suggest that the low molecular weight polypeptide (LMP) complex might be involved in this process. Firstly, LMP is structurally and serologically similar to proteasome, a cytoplasmic, multi-subunit protein complex which possesses multiple proteolytic activities. Secondly, LMP expression is induced by interferon-γ. Finally, two *Lmp* genes, *Lmp-2* and *Lmp-7*, map to the MHC class II region which encodes multiple proteins involved in the class I antigen processing pathway. Thus, current evidence supports the hypothesis that LMP/proteasome might be involved in MHC class I antigen processing. Here we report that "proteasome" is composed of multiple related, yet distinct, protein complexes with distinct polypeptide composition. These variable complex forms are related by common polypeptides, yet each complex possesses unique polypeptides. One of these, the LMP complex, may be distinguished structurally and serologically from the related proteasome complex. LMP and proteasome coexist *in-vivo* in most cell types. The polypeptide composition of LMP and proteasome and the ratio observed among the complexes is variable among cell lines and dramatically influenced by interferon-γ. LMP also has proteolytic activity, and LMP expression, but not proteasome expression, correlates with MHC class I surface expression in H6 cells. Thus, distinct "proteasome" complex forms may be required *in-vivo* for unique cellular function.

HZ 305 CHANGES AT PEPTIDE RESIDUES BURIED IN THE MHC CLASS I BINDING CLEFT INFLUENCE T CELL RECOGNITION: A POSSIBLE ROLE FOR INDIRECT CONFORMATIONAL ALTERATIONS IN THE THE MHC CLASS I OR BOUND PEPTIDE IN DETERMINING T CELL RECOGNITION, Weisan Chen[1], James McCluskey[1, 2] and Francis R. Carbone[3]. [1]Department of Clinical Immunology, Flinders Medical Centre, Bedford Park, South Australia, 5042. [2]Australian Red Cross Society, S.A. Division, Blood Transfusion Service, 301 Pirie St. Adelaide, South Australia, 5000. [3]Department of Pathology and Immunology, Monash Medical School, Commercial Rd. Prahran, Victoria, Australia 3181.

Recent crystallographic studies on two peptide complexes with the mouse K^b molecule have shown that peptide binding appears to alter the conformation of the class I α-helical regions that flank the antigen binding cleft. Given that this study also showed that much of the foreign peptide is buried within the class I binding cleft with only a small portion accessible for direct interaction with the components of the T cell receptor, this finding suggests that at least some component of T cell specificity may arise as a consequence of peptide-induced conformational changes in the class I structure. In order to assess this possibility, we have made systematic substitutions at residues within the K^b-restricted determinant from ovalbumin ($OVA_{257-264}$) that are thought to be buried on binding to the class I molecule. We have found that changes in this determinant at the completely buried second residue (P2) can influence T cell recognition without affecting binding to K^b, suggesting that the substitutions may indirectly determine T cell recognition by altering the conformation of the class I molecule or the bound peptide.

HZ 306 T CELL RECEPTOR Vβ GENE DELETION IN $V\beta^c$ MUTANT MICE AUGMENTS DEVELOPMENT OF AUTOIMMUNE MYASTHENIA GRAVIS. Premkumar Christadoss*, Chella David+, Elzbieta Goluszko* and Mohan Shenoy*, *Department of Microbiology, University of Texas Medical Branch, Galveston, TX. 77555. +Department of Immunology, Mayo Clinic, Rochester, MN 55904.

The effect of Vβ gene deletion in R111/SJ ($H\text{-}2^r$; $V\beta^c$) on the development of experimental autoimmune myasthenia gravis (MG) was evaluated. Only one of nine (12%) B10.R111 ($H\text{-}2^r$;$V\beta^b$) mice which has normal Vβ repertoire developed muscle weakness, after two immunizations with acetylcholine receptors (AChR) in CFA; while a dramatic augmentation in the incidence of clinical MG was seen in R111/SJ mice, with 8 of 10 (80%) mice developing muscle weakness. To link MG susceptibility to the deleted chromosomal segment bearing TCR-Vβ genes, and to rule out the possible role of non-H-2 genes other than the TCR genes in the R111/SJ background, TCR-$V\beta^{a,b,c}$ haplotype mice in the B10 background were screened for MG. Eight of ten (80%) B10.TCR^c ($H\text{-}2^b$; $V\beta^c$) mice developed muscle weakness, while only 4/14 (28%) of B10 ($H\text{-}2^b$; $V\beta^b$), and 1/7 (14%) of B10.TCR^a ($H\text{-}2^b$; $V\beta^a$) mice developed muscle weakness. Thus, Vβ gene deletion in $V\beta^c$ haplotype but not $V\beta^a$ haplotype strain in the context of $H\text{-}2^r$ or $H\text{-}2^b$ augmented clinical MG. Further, an augmented anti-AChR antibody response was evident in the serum of B10.TCR^c mice, when compared with the antibody response of B10 or B10.TCR^a mice. The primary genetic difference in the B10.TCR^a and the B10.TCR^c mice is the presence of Vβ6, Vβ15 and Vβ17 genes in the former. Therefore, the deletion of Vβ6, Vβ15 or Vβ17 gene in the $V\beta^c$ haplotype mice could have augmented MG susceptibility, thus tentatively linking resistance to the deleted chromosomal segment bearing Vβ6, Vβ15 or Vβ17 genes. On the other hand, over expression of non-deleted Vβ genes in the $V\beta^c$ haplotype might have contributed to the augmentation of the disease. It is quite plausible that T cells bearing Vβ6, Vβ15 or Vβ17 gene products could be involved in the regulation/suppression of an autoimmune response to AChR. Supported by the MDA, Sealy Smith Endowment Fund and NIH #CA 24473.

HZ 307 INTERSPECIES SPECIFIC INTERACTIONS BETWEEN HUMAN AND MURINE CD4 AND MHC CLASS II ISOTYPES AND ALLELES. Sylvain Fleury*, Gilbert Croteau*, Wayne A. Hendrickson# and Rafick-Pierre Sékaly*. Laboratory of Immunology, Clinical Research Institute of Montreal, 110 West Pine Avenue, Montreal, Quebec, Canada, H2W 1R7*. Columbia University, New-York, NY 10032#.

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 and a cytoplasmic regions. The two amino-terminal domains (D1 and D2) of the human CD4 have been shown to contain amino acids involved in binding to human MHC class II molecules.

Whether a species barrier exists in the interaction between CD4 or L3T4 and class II molecules remains controversial. In order to determine the existence of a such barrier, 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 3DT52.5.8 hybridoma, a murine $CD4^-CD8^-$ T cell and human or murine MHC class II alleles and isotypes were transfected in murine DAP-3 cell line. After a 18 h. co-culture between $CD4^+$ hybridomas and class II target cells, supernatant were tested for IL-2 production. Our results indicate that L3T4 or CD4 interact equally well with either murine or human class II molecules. Human class II that interact very well with CD4 are also able to interact very efficiently with L3T4. The same phenomenon with murine class II is observed with L3T4 and CD4. Furthermore, mutations on CD4 residues that abrogate the interaction with human class II affect to the same extent the interaction with murine class II molecules. These mutations are localized on the CDR1 and CDR3 loops of D1, on β strands A, B and G of D1, and on the F and G β strands of D2. All these residues are exposed to solvent and located on the same face of the CD4 molecule, which confirm our previous results.

HZ 308 HSP-65 REACTIVE γδ TCR$^+$ HYBRIDOMAS UNDERGO APOPTOSIS IN RESPONSE TO AUTOLOGOUS AND MYCOBACTERIA ANTIGEN.
Yang-Xin Fu, Kalataradi Hershan, Kent D. Heyborne, Rebecca L. O'Brien and Willi Born. Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. αβ T cells can undergo apoptosis after TCR/CD4/CD8-mediated interactions with their antigenic ligands. Most γδ T cells express already in the thymus high levels of TCR but only rarely CD4/8. Antigen-induced apotopsis of γδ T cells has not yet been studied in detail. We have previously reported that certain γδ T cell hybridomas, derived from thymus and spleen, expressing Vγ1 and mostly Vδ6, spontaneously release lymphokines without deliberate addition of antigen and can be stimulated to stronger lymphokine responses by the addition of PPD, HSP-60 or synthetic peptide corresponding to a portion of HSP-60 (e.g. amino acids 180-196 of mycobacterial HSP-65). The spontaneous response appears to be directed against an antigen expressed by the hybridomas themselves and both spontaneous and foreign antigen-induced reactivities are TCR-dependent. We have now found that both high spontaneous lymphokine production and peptide induced responses in these cells are correlated with high levels of ^{51}Cr release and decrease of ^{3}H thymidine uptake. Conversely, soluble anti-TCR antibody can block lymphokine production, and can increase ^{3}H thymidine uptake. Our data suggest that both autologus ligands and peptide antigen, engaging the same TCRs, can trigger apoptosis. Furthermore, the peptide-induced effect is dose-dependent. Consistent with the concept that apoptosis is mediated via the TCR, hybridomas expressing other TCR and TCR loss variants from HSP-60 reactive cells do not undergo apoptosis after exposure to HSP-60 peptide. Also, MHC class I deficient variants of HSP-60 reactive cells expressing high levels of TCR can not be triggered, suggesting that induction of apoptosis by the peptide requires recognition of peptide in context with MHC class I or related molecules. These observations suggest that apoptosis can be induced by autologous ligands and heterologous peptide antigens via the γδ T cell receptor. This mechanism may play a role in development and regulation of γδ T cells.

HZ 309 COINCIDENCE OF Vγ5 AND Vγ6 GENE REARRANGEMENTS IN A SUBSET OF γδ-TCR$^+$ HYBRIDOMAS: IMPLICATIONS FOR AN INTRAEPITHELIAL LYMPHOCYTE LINEAGE.
Kent Heyborne, Willi Born, Yang-Xin Fu, Christopher Reardon, Christina Roark, and Rebecca O'Brien. Dept. Ob/Gyn, Univ. of Colorado Health Sciences Center and Dept. Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, 80262
Intraepithelial lymphocytes of the reproductive tract and skin have similar characteristics, including epithelium-specific localization, germ-line junctional configuration, Vδ1 pairing, and synchronized thymic development, suggesting a common developmental lineage for these two populations. We used PCR amplification of RNA-derived cDNA clones to analyze a large panel of γδ-TCR$^+$ hybridomas derived from fetal and newborn thymus and adult placenta-decidua, spleen, liver, mammary gland and skin. Panels of Vγ6$^+$ and Vγ6$^-$ hybridomas were tested for Vγ5 message. We found that all Vγ6$^+$ hybridomas tested (n=30) contained Vγ5 message. Sequence analysis of a subset of these Vγ6$^+$/Vγ5$^+$ cells revealed primarily out-of-frame Vγ5 junctions, although two hybridomas from fetal and newborn thymus apparently have in-frame junctional rearrangements for both Vγ6 and Vγ5. All Vγ6$^-$ hybridomas tested (n=34) were negative for Vγ5 message. We also analyzed smaller panels of Vγ5$^+$ and Vγ5$^-$ hybridomas for Vγ6 message. Vγ6 message was found infrequently in Vγ5$^+$ hybridomas (1 of 9) and all Vγ5$^-$ cells (n=11) were negative for Vγ6 message. Our finding of coincidence of both Vγ5 and Vγ6 message in a defined subset of γδ-TCR$^+$ hybridomas from a variety of tissues supports the concept of a developmentally regulated intraepithelial lymphocyte lineage, and suggests that many cells of this lineage that rearrange Vγ5 out of frame go on to rearrange Vγ6 in frame and localize to the reproductive tract.

HZ 310 OUTER MEMBRANE PROTEIN PhoE OF E. coli AS A CARRIER FOR T-CELL EPITOPES,
Riny Janssen[1], Marca Wauben[1], Ruurd van der Zee[1], Jan Tommassen[1,*] Department of Molecular Cell Biology, Utrecht, The Netherlands ! Faculty of Veterinary Medicine, Utrecht, The Netherlands

PhoE is an abundant outer membrane protein of *E. coli*. In its native form the protein is a trimer, with each monomer traversing the membrane 16 times. We are investigating the possibilities of using this protein as a carrier for the transport of foreign antigenic determinants to the *E.coli* cell surface. The ultimate goal is to develop new live oral vaccines.
Previous work has proven the suitability of PhoE a carrier for B-cell epitopes.
To study the antigenicity of foreign T-cell epitopes inserted in PhoE, an epitope (amino acid 180-186) of HSP65 of *Mycobacterium tuberculosis* was chosen. This epitope is important in adjuvant arthritis, an experimental autoimmune model in rats. Synthetic oligonucleotides encoding this epitope were cloned in the DNA corresponding to the second, fourth, seventh and eighth cell surface exposed region of PhoE. The insertions did not affect the biogenesis of PhoE protein. PhoE trimers were isolated and tested for their ability to induce proliferation of a T-cell clone, specific for this HSP65 epitope. Some of the PhoE constructs were recognized by the T-cell clone. Induction of proliferation appeared to depend on the amino acids flanking the epitope. By changing the flanking amino acids, we are investigating the underlying mechanism.
Also the possibilities of inducing an *in vivo* T-cell response against the inserted epitope were investigated. Some constructs could induce a T-cell response *in vivo* and others could not. There appears to be a clear correlation between induction of a T-cell response *in vivo* and induction of proliferation of the T-cell clone *in vitro*.

HZ 311 EVIDENCE FOR A STRAIN SPECIFIC SELECTIVE MECHANISM IN Vγ1/Vδ6+ γδ T CELLS.
Harshan Kalatardi, Cassandra Eyster, Willi Born and Rebecca O'Brien, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine.
We have previously described a panel of γδ T cell hybridomas, from newborn thymus and adult spleen of C57BL/10 mice, bearing Vγ1-Jγ4-Cγ4-Vδ6 receptor, having the unusual property of spontaneous IL2 secretion, possibly in response to an unidentified autoantigen. All of these hybridomas were also found to react to a mycobacterial HSP60 peptide, L180-196. HSP60 reactivity among these cells correlated with the Vγ1Cγ4 expression. Most of these cells expressed Vγ1 in association with Vδ6.3. Recently we have isolated Vγ1Vδ6+ hybridomas from the spleen of newborn AKR mice. However, the majority (12/13) of these cells did not respond to the HSP60 peptide. Sequence analysis of the receptors of AKR derived hybridomas showed differences from the C57BL/10 in the Vδ6 genes that were expressed. Whereas the C57BL/10 Vδ6+ hybridomas predominantly expressed Vδ6.3, the Vδ6+ AKR hybridomas nearly all expressed Vδ6.2. This plus minor differences in the Vγ1 sequences could account for the differences in reactivity pattern among the AKR derived Vγ1Vδ6+ γδ subsets. Similar non HSP60 reactive Vγ1Vδ6.2 receptor bearing cells were also isolated from C3He/N mice newborn thymus (Ezquerra et al, and unpublished observation). Interestingly, Vδ6.3 and Vδ6.2 genes are present in both the C57BL/10 and AKR strains and no defects in their sequences have been found so far. Strain specific usage of different Vδ6 genes could indicate a difference in a selective mechanism for δγ T cells. Experiments examining the Vγ1Vδ6 cells in C57BL/10 X AKR F1 mice are now underway to test this idea.

HZ 312 CD8+ T CELL IMMUNITY TO TOXOPLASMA GONDII, Lloyd H. Kasper, Kenneth Ely and Imtiaz A. Khan, Departments of Medicine and Microbiology, Dartmouth Medical School Hanover, NH 03756.

The role of cell mediated immunity in the host response to the obligate intracellular parasite, *Toxoplasma gondii* is well recognized. Recently, we and others have shown the protective cellular immune response to be mediated by Class I, MHC restricted CD8+ T cells and IFN-γ. Utilizing the major surface protein of the parasite, P30, we have been able to induce near 100% protection against acute and chronic infection when administered with the appropriate adjuvant, Quil A. We have now developed a panel of P30 antigen specific murine T cell clones from Balb/C mice. These clones proliferate vigourously in vitro in response to parasite antigen. These clones express either CD4+ or CD8+ surface phenotype, but not the γ, δ heterodimer. Evaluation of cytokine mRNA by PCR amplification indicates these clones transcribe IFN-γ, TNF, and Il-2. Adoptive transfer experiments using these cloned cells show that only one of the CD8+ clones (C3) is able to induce >90% protection against acute challenge. Moreover, none of the surviving mice have evidence of intracerebral cysts containing bradyzoites. Although the CD4+ clones are P30 antigen specific and produce high quantities of IFN-γ, they are unable to induce protective immunity. MAb induced CD4+ depletion of naive mice, followed by adoptive transfer of clone C3 did not alter protection (>80% survival). However, depletion of IFN-γ, abrogated the protective effect of the C3 clone. Studies are currently underway to evaluate CD8+ mediated protection in β2-microglobulin knock-out mice. These observations suggest that neither CD8+ T cells nor IFN-γ alone are able to induce protective immunity in the experimental host. A combination of both antigen specific CD8+ T cells and IFN-γ are essential in protective immunity against parasite infection.

HZ 313 CELL SURFACE EXPRESSION AND ANTIGEN PRESENTATION BY A RECOMBINANT SINGLE CHAIN CLASS I MHC MOLECULE. Li Lee*, Louise McHugh*, Randall K. Ribaudo#, Steven Kozlowski#, David H. Margulies#, and Michael G. Mage*. *Laboratory of Biochemistry, NCI, and #Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

We have previously engineered a biologically active recombinant, single chain, soluble class I MHC molecule, SCβD^{d}s (Mage et al., PNAS in press 1992). This molecule consists of ß2 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. We have now engineered a recombinant single chain class I molecule with transmembrane and cytoplasmic domains, SCβD^{d}m. This molecule is expressed on the surface of transfected L cells grown at 37°, as measured by flow cytometry with antibodies to conformational epitopes on the α1 and α2α3 domains. Functional activity of cell surface SCβD^{d}m is indicated by the ability to present an H-2D^d restricted antigenic peptide derived from the HIV-1 gp160 envelope glycoprotein ("p18I10") to a peptide specific, H-2D^d restricted T cell hybridoma, an activity previously found for the soluble single chain molecule. Experiments are in progress to evaluate the potential of peptide-loaded single chain MHC molecules as [MHC+peptide]-specific T cell immunogens.

HZ 314 EVIDENCE FOR DISTINCT ACTIVATION MECHANISMS DURING T CELL RESPONSES TO A SPECIFIC PEPTIDE VERSUS THE NATIVE ANTIGEN, *PLASMODIUM BERGHEI* SPOROZOITES. **Heidi Link+, Katherine White+, Joseph Bressler* and Urszula Krzych+§. §Dept. Immunology, WRAIR, +Dept. of Biology, CUA Washington DC 20307; *Dept. Neurology, Kennedy-Krieger Research Institute, Johns Hopkins University, Baltimore MD.**

Immunization with radiation-attenuated *Plasmodium* sporozoites (SPZ) induces protection to a live SPZ challenge, yet the mechanism of protective immunity are still poorly understood. Adoptive transfer of T cells specific for the circumsporozoite (CS) protein, the major surface antigen of SPZ mediates, in part, protective immunity. Since SPZ are carried to the liver where they express liver-specific antigens, including CS protein, it is unknown whether the CS protein T cell specificity arises from SPZ- or liver-stage immunity. Moreover, the precise mechanism of T cell recognition of SPZ-associated CS protein has not been extensively investigated. We have previously demonstrated that *P. berghei* SPZ recall proliferative responses of SPZ-Induced CD4+T cells without the need for an intracellular processing and that these responses requires class II MHC compatible activated B cells as antigen presenting cells. In this study, we further investigated T cell reactivity to SPZ using cloned T cell lines induced by synthetic peptides from the *P. berghei* CS protein. T cells specific for the repeat motif of the CS protein, DPAPPNAN, (P14) responded *in vitro* not only to the priming peptide, P14, but also to SPZ presented by class II MHC compatible activated B cells. Lymphokine analysis showed that while P14-activated responses of P14-specific T cells yielded IL-4 production, activation with SPZ elicited proliferative activity without any detectable lymphokines (IL-2 and IL-4). Further analysis of SPZ-recognition by P14-T cells revealed that SPZ, unlike conventional protein antigens, induced proliferative responses without the need for processing. Although T cell activation by SPZ was MHC dependent, it did not require MHC antigen groove occupancy. Currently investigations are underway to determine the precise mechanism of SPZ-specific recognition/ activation of T cells. These analyses involve identification of costimulatory molecules and mechanism(s) of signal transduction during P14- and SPZ-induced responses of P14-specific T cells. Supported in part by WHO/World Bank TDR..

HZ 315 ENDOGENOUS PEPTIDES AND PEPTIDE-LOADING OF RECOMBINANT SOLUBLE SINGLE CHAIN CLASS I MHC MOLECULES. Michael G. Mage*, Marypat Corr#, Li Lee*, Louise McHugh*, Randall K. Ribaudo#, Steven Kozlowski#, and David H. Margulies#. *Laboratory of Biochemistry, NCI, and #Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892.

We have reported the engineering of a biologically active recombinant soluble single chain class I MHC molecule SCβD^{d}s (Mage et al. PNAS in press 1992). SCβD^{d}s consists of ß2 microglobulin linked at its carboxyl terminus to a peptide spacer linked in turn to the amino terminus of a soluble form of the H-2D^d heavy chain. A mutant variety of the same molecule with 3 broken salt bridges (D39N, R48W, and E166G) was also studied. The molecules were analysed for endogenous peptides by Edman degradation of fractions from reverse phase HPLC. Both molecules contained endogenous peptides with the H-2D^d motif of G and P at positions 2 and 3 respectively, with R or K predominant at position 5. The presence of endogenous peptides provides additional evidence for correct folding and assembly of the single chain class I MHC molecules in the endoplasmic reticulum. The average peptide length appears longer for the mutant than for the nonmutant single chain molecule. The longer peptides may be able to stably bind because of the mutations.

The nonmutant SCβD^{d}s could be loaded with the decapeptide P18I10 from the gp120 envelope glycoprotein of HIV-1 by dissolving the peptide in the alkaline elution buffer used in the affinity purification (0.15M NaCl, 0.15M Na_2CO_3, pH 11.5), which was rapidly neutralized after elution. This may be a useful way to prepare specifically loaded class I MHC molecules.

HZ 316 ALLOREACTIVE AND VIRAL-SPECIFIC T CELL RECOGNITION OF HYBRID L^d/D^d CLASS I MOLECULES. D. M. McKinney, P. Chen, J. M. Dadgari, W. E. Schulz, C. C. Killion, and M. McMillan. Norris Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033.

To understand the molecular basis of alloreactive and viral-restricted T cell recognition, we have constructed a series of cassettes corresponding to the α-1 and α-2 domains of the L^d class I molecule into which we introduced D^d-specific codons at polymorphic residues. These hybrid molecules have amino acids altered either singly or in groups on the α-1 α-helix , α-2 α-helix , or β-pleated sheet, and permit us to distinguish the amino acids involved in recognition by the T cell receptor (TCR), as well as those necessary for peptide binding. These molecules have been characterized using bulk alloreactive cytotoxic T lymphocytes (CTL), as well as a panel of alloreactive L^d-specific T cell hybridomas, which pinpoint the residues on the class I molecule critical for TCR recognition. Additionally, these molecules have been analyzed by JHM-restricted CTL clones, allowing the identification of the residues involved in viral peptide binding. We will discuss our results in terms of the role peptides play in allorecognition versus viral-restricted recognition of class I molecules by TCRs.

HZ 317 IN VIVO INTERACTIONS BETWEEN PEPTIDES AND CLASS II MHC: PREVENTION OF AUTOIMMUNE DIABETES IN NOD MICE. Zoltan A. Nagy and Ursula Hurtenbach, Department of Inflammation and Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, NJ 07110, and Preclinical Research, Sandoz Pharma Ltd., 4002 Basel, Switzerland

Susceptibility to autoimmune diseases is associated with certain class I or Class II MHC alleles. Since the peptide binding cleft of MHC molecules exhibits allele/isotype dependent binding specificity, it is reasonable to assume that MHC-disease association may reflect selective presentation of a disease inducing autoantigen by the particular allelic form of MHC molecule that confers susceptibility. However, this hypothesis is not directly testable, for the antigens involved in most autoimmune diseases are unknown. We have used the approach of in vivo MHC blockade to test this hypothesis experimentally. We demonstrate that a peptide capable of blocking antigen presentation by the NOD class II molecule ($I\text{-}A^{g7}$) in an allele/isotype specific manner, can also prevent the spontaneous development of type I diabetes linked to this molecule.

HZ 318 FUNCTIONAL IMPORTANCE OF INCREASED TARGET CELL MHC CLASS I ANTIGEN EXPRESSION ON T-CELL AND NK CELL MEDIATED CYTOTOXTICITY. Mogens H. Nissen and Mogens H. Claësson, Institute of Medical Anatomy, University of Copenhagen, DK-2200 Copenhagen, Denmark.

MHC class I antigens are known to be of importance in T-cell and NK-cell target recognition. Thus, the susceptibilty to NK-cell killing is inversely correlated to the amount of MHC class I antigen, whereas T-cell cytotoxticity is dependent upon the amount of MHC class I antigen expressed.

We studied the effect of MHC class I antigen expression on a number of murine cell lines after incubation with human ß-2-microglobulin. By incubation of RBL-5, EL-4, BW 5147, P 815 and L cells (10^6/ml) with human ß-2-microglobulin (5mg/ml) in 60 min, the expression of α-chain determinants was increased significantly from 1.4 10.7 fold. The increase was most pronounced on EL-4 cells.

In order to investigate if the increase of heavy chain determinants was of functional importance, the cells were subsequently examined for susceptibility to CTL and NK mediated killing. NK mediated cytotoxticity decreased by 80-90% following MHC class I upregulation, whereas the CTL mediated cytotoxticity was found to be unaltered.

These data indicate, that increased MHC class I antigen expression induced by xenogenic ß-2-microglobulin does not affect allo-recognition, whereas the NK mediated cytotoxticity is significantly downregulated. Additional data will be presented.

HZ 319 TARGETING RARE ANTIGENS ON APCs FOR mAb DEVELOPMENT: COMPARISON OF TWO TECHNIQUES OF TOLERIZING MICE TO SHARED HUMAN LEUKOCYTE ANTIGENS, Una O'Doherty, Yasunori Yamaguchi, William J. Swiggard, Micheal Peng, Kayo Inaba[1], Nina Bhardwaj, and Ralph M. Steinman, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021 USA, and [1]Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606, Japan

Despite several attempts to isolate a mAb specific for human dendritic cells, none currently exists. Recent attempts have yielded only mAbs to antigens shared by dendritic cells and other leukocytes. These antigens include MHC I and II, CD45, CD40, CD11a, CD11c, ICAM-1, CD44, LFA-3, and B7/BB1. Therefore, we are attempting to bias the immune response toward rarer, dendritic cell-specific B cell clones by tolerizing mice to shared human leukocyte antigens.

In one approach, mice were injected with tolerizing nondendritic cells together with cyclophosphamide to ablate responding "nonspecific" clones. To assess if tolerance was present 15 d after the last dose of CP, the mice were challenged with the tolerizing cells and bled 1 wk later for serum antibody titers. We found that cyclophosphamide led to nonspecific immunosuppression, i.e., mice given cyclophosphamide with or without tolerogen failed to make an antibody response when challenged.

In a second approach, neonatal mice were injected with tolerizing nondendritic cells at birth, followed by a second dose at 1 wk. In adulthood (5-8 wks), the mice were challenged with the tolerogen, or sheep red blood cells as control. Serum antibody titers were measured 1 wk later and compared to the responses of sham-injected mice. Neonatal tolerance did lead to selective nonresponsiveness to the tolerizing cells without any nonspecific impairment of the sheep red blood cell response.

HZ 320 T CELL RECEPTOR ANTAGONISM MEDIATED BY CDR 3 INTERACTIONS, David Ostrov[1,4], Jeffrey Krieger[2], Alessandro Sette[3], and Patrick Concannon[4,5]. 1. Dept. of Biological Structure, University of Washington School of Medicine, Seattle, WA, 98195. 2. Centocor Corporation, 244 Great Valley Parkway, Malvern, PA, 19355. 3. Cytel Corporation, 3525 John Hopkins Court, San Diego, CA, 92121. 4. Dept. of Immunology, Virginia Mason Research Center, 1000 Seneca Street, Seattle, WA, 98101. 5. Dept. of Immunology, University of Washington School of Medicine, Seattle, WA, 98195.

Recent advances in understanding antigen presentation and T cell recognition provide strategies for the inhibition of antigen specific T cell responses. A novel approach for the induction of antigen specific nonresponsiveness, TCR antagonism, has been described (De Magistris, D.T., *et al.*, Cell 68:625). Singly substituted peptide analogs of the influenza hemagglutinin peptide HA 307-319 inhibit recognition of native HA 307-319 by a DR1 restricted T cell clone. It is unclear how single positions of antagonizing peptides affect the interactions between TCR, peptide and MHC. To investigate the molecular requirements for receptor antagonism, we have utilized two DR5 restricted T cell clones which exhibit mutually exclusive specificities for HA peptides with or without substitutions at position 313, a position that is implicated in TCR antagonism. We demonstrate that: 1) receptor antagonism is an effect which can be induced by peptide analogs presented in the context of different DR restricting elements, 2) the stimulatory or inhibitory effect of a peptide analog depends on the structure of TCR utilized by the responding T cell, not on the nature of the peptide/MHC complex, and 3) structural differences between TCRs expressed by T cell clones that are antagonized and those that are stimulated by the same peptide/DR complex map to junctional regions of the TCR. Our results suggest that TCR antagonism is an antigen specific phenomenon in which T cells are inhibited by interactions involving TCR residues required for the recognition of conventional antigens and the altered residues in peptide analogs.

HZ 321 THE ROLE OF MHC-ENCODED LOW MOLECULAR WEIGHT PROTEINS IN ANTIGEN PROCESSING, Salil D. Patel* and Hugh O. McDevitt*#, Department of Microbiology and Immunology*, and Medicine#, Stanford School of Medicine, Stanford, CA 94305.

Several new genes found within the class II region of the MHC have recently been suggested to play a role in the processing of endogenous antigens. Two of these genes, TAP1 and TAP2, are postulated to form a transmembraneous heterodimer which functions to transport peptides across the ER for subsequent presentation by MHC molecules. The other two genes, LMP 2 and LMP7, encode subunits of the Large Multifunctional Protease (LMP) complex. In the present study we have expressed the LMP2 and LMP7 cDNAs in a bacterial system and have raised antibodies to the recombinant proteins. These antibodies were initially used to investigate the relationship of LMP to Proteasome, a similarly large complex proposed to play a role in intracellular protein turnover. Immunoprecipitation using anti-LMP2 antisera with metabolically labelled B-cells results in the precipitation of a complex which differs from the Proteasome complex by five subunits. Interestingly, one of the missing subunits is LMP7. Preclearing experiments (we have a monoclonal antibody against a Proteasome subunit) show the two complexes to be distinct. We are presently investigating the functional significance of this difference. In addition, our antisera are being utilized to study the cellular location of these complexes by western blotting of cell organellar fractions and by immuno-elecron microscopy.

HZ 322 THE PATHOGENIC ROLE OF ACETYLCHOLINE RECEPTOR ALPHA CHAIN T CELL EPITOPE WITHIN α146-162 IN THE INDUCTION OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS (EAMG) IN C57BL6 MICE, Mohan Shenoy, Elzbieta Goluszko, and Premkumar Christadoss, Department of Microbiology, University of Texas Medical Branch, Galveston, TX 77555

The immunodominant T cell epitopes for experimental autoimmune myasthenia gravis (EAMG) susceptible C57BL6 (B6) mice have been mapped. EAMG resistant IA beta chain mutant strain bml2 T cells failed to respond to the immunodominant epitope within *Torpedo* acetylcholine receptor (AChR) alpha chain peptide α146-162 (LGIWTYDGTKVSISPES). Previously, we have shown that neonatal tolerance of B6 mice with peptide α146-162 prevented EAMG upon subsequent immunizations with AChR in CFA. However, multiple immunizations with α146-162 in CFA failed to induce clinical EAMG in susceptible B6 mice, since the epitope within α146-162 is predominantly a T cell epitope. In this study EAMG susceptible B6 mice were primed with the whole AChR molecule in CFA, and subsequently immunized twice with either α146-162 or control peptide (KAIVEKLAFTYRSDSFEN) in CFA. Seven of ten (70%) mice boosted with α146-162 developed muscle weakness, while, none of the mice (0/9) boosted with the control peptide showed any signs of the disease. Animals with weakness showed positive decremental response to repetitive nerve stimulation. These findings strongly indicate that epitope within α146-162 is one of the critical disease inducing T cell epitopes. Most probably, the helper T cells reactive to α146-162 interact with the memory B cells induced by priming the B6 mice with AChR in CFA in order to produce pathogenic antibodies and EAMG. Supported by MDA and Sealy Smith Endowment.

HZ 323 SELECTIVE DEFECTS IN CLASS I ANTIGEN PROCESSING ARE RESTORED BY INTERFERON-GAMMA IN A MOUSE T-LYMPHOMA, Sibille C.[1], Gould K.[2], Willard-Gallo K.[3], Butcher G.[4] and De Baetselier P.[1], [1]Institute of Molecular Biology, Free University of Brussels, Paardenstraat 65, 1640 Sint Genesius Rode, Belgium, [2]Sir William Dunn School of Pathology, Oxford, U.K., [3]I.C.P., Catholic University of Louvain, Brussels, Belgium, [4]AFRC, Cambridge, U.K.

We have studied class I antigen presentation in a subclone of the mouse BW 5147 T-lymphoma, designated Sp3. In spite of a relatively high level of MHC class I H-2K^k and H-2D^k expression, Sp3 cells were found to have a selective defect in the presentation of two intracellularly generated K^k-restricted hemagglutinin peptides (HA). This functional deficiency is not due to insufficient infectivity of influenza virus since other K^k-restricted viral epitopes are well recognized by their specific CTL clones. However, we have observed that Sp3 cells can be efficiently killed when infected with vaccinia vectors that express minimal antigenic HA-peptides, but not with vectors expressing longer sequences. Furthermore, we have noticed that IFN-gamma treatment of Sp3 cells restores their capability to present the HA-epitopes even when they are derived from unprocessed, *de novo* expressed hemagglutinin. These results suggest there is a partial defect in antigenic proteolysis that is responsible for selective lack of antigen presentation by Sp3 cells. The recently identified MHC-encoded proteins, LMP2 and LMP7, are known to be IFN-gamma-inducible, and it has been suggested that they participate in processes that generate antigenic peptides for assembling MHC class I molecules. Therefore, Sp3 cell line provides an interesting model for studying the involvement of IFN-gamma regulated proteins in antigen processing and further characterization of the Sp3 defect is in progress.

HZ 324 MUTATIONS IN AND SURROUNDING THE CLASS I MHC PEPTIDE-BINDING GROOVE DEMARCATE THE BINDING-SITE FOR T CELL RECEPTORS

K.D. Smith, A. Valenzuela and C.T. Lutz. Dept. Pathology, U. of Iowa, Iowa City, Iowa, 52242.

Davis and Bjorkman (Nature 334:395,1988) hypothesized that the T cell receptor (TCR) contacts the major histocompatibility (MHC) α-helices and bound peptide. However, TCR contact sites on MHC molecules have not been systematically tested. As models, we have studied how 11 alloreactive cytolytic T lymphocyte (CTL) clones recognize a panel of 29 HLA-B*0702 α1 and α2 domain variants. To select for alloreactive CTL that are less sensitive to peptide, four of the CTL clones were generated against HLA-B*0702 and peptide-binding groove variants of B*0702. As predicted, these four CTL clones appear less sensitive to peptide; each clone is affected by 3 out of 12 to 5 out of 12 peptide-binding groove mutations. In contrast, the seven conventionally raised anti-B*0702 CTL clones are affected by 5 out of 11 to 11 out of 13 peptide-binding groove mutations. Thus we could study TCR-MHC interaction using a diverse set of CTL clones with varying degrees of peptide dependency. All clones are also affected by B*0702 mutations at some residues predicted to interact directly with the TCR. These results suggest that both the foreign MHC molecule and bound peptide contribute to allorecognition. Interestingly, of 20 mutations at solvent accessible residues, only changes from position 62 to 80 and 152 to 167 on the long α-helices of the B*0702 α1 and α2 domains affect the CTL clones Solvent accessible B*0702 mutations outside this range do not affect any of the CTL clones tested. This contrasts with anti-HLA antibodies that recognize diverse B*0702 sites potentially encompassing the entire surface of the HLA class I molecule. The TCR binding-site may be confined to MHC bound peptide and MHC residues surrounding the peptide-binding groove. These results support the Davis and Bjorkman hypothesis and demarcate limits of TCR binding.

HZ 325 NATURAL SOLUBLE VARIANT OF QA-2 ANTIGEN BINDS NONAMERIC PEPTIDES WITH UNIQUE ANCHOR RESIDUES,

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Class Ib Qa-2 proteins exist as ~40kd antigens attached to the cell surface by phosphatdylinositol (membrane-derived or md Qa-2) and as soluble ~39kd molecules (sQa-2). The md Qa-2 and sQa-2 products are encoded by the same gene and are synthesized from alternatively spliced transcripts differing in the usage of exon 5. The biosynthesis of md Qa-2 and the levels of circulating sQa-2 in serum are inducible in vivo by regimens leading to the activation of immune system. The cDNAs encoding the two variants of Qa-2 were cloned downstream from strong promoters and transfected into myeloma cells. We have examined the nature of the endogenously encoded peptides bound to sQa-2 using genetically engineered cell lines overexpressing Qa-2 molecules. Sequence analysis of the short nonameric peptides associated constitutively with sQa-2 revealed that histidine (H) at position 7 may serve as the major MHC binding anchor. Microsequencing of several abundant peptides purified by HPLC indicated that residues at position 1 and position 9 may also help to anchor the peptides to sQa-2 groove. The aminoacid composition of sQa-2 bound peptides could not be matched to any protein sequences in database searches. We suggest that Qa-2 molecules bind a restricted set of endogenous peptides. This is consistent with the hypothesis that nonpolymorphic Qa-2 molecules evolved to present a limited number of unique antigens.

HZ 326 TH1 AND TH2 CLONES THAT RECOGNIZE DIFFERENT LENGTHS OF AN ENCEPHALITOGENIC REGION,

Roel C. van der Veen[1], Judith A. Kapp[2], John L. Trotter[3], [1]Department of Neurology, University of Southern California School of Medicine Los Angeles, CA 90033 USA, [2]Harrington Cancer Center, Amarillo, TX, [3]Washington University School of Medicine, St. Louis, MO.

The lymphokine production of two T-cell clones, which both recognize epitopes within the encephalitogenic 139-151 sequence of myelin proteolipid protein, was examined after stimulation with immobilized antibodies to the CD3 moiety of the TCR complex. Clone SP39A1 produced IL-2 and IFN-γ, but no IL-4, while clone SP41D5 produced IL-4, but no IL-2 or IFN-γ. SP39A1 therefore belongs to the Th1 subset, while SP41D5 is a Th2 clone. In addition, the Th1 clone induced severe EAE, while the Th2 clone did not induce any signs of EAE. Synthetic peptides were used to demonstrate that these clones recognized slightly different epitopes within the 139-151 sequence. Histidine 139 was shown to be optimal for the stimulation of the Th2 clone, while on the contrary this residue completely inhibited the stimulation of the Th1 clone. These differences in stimulation between Th subsets with specificity for the same encephalitogenic region may indicate differential processing of epitopes for either Th subset. This characteristic may be useful in designing strategies for the regulation of encephalitogenic Th1 cells by Th2 cells with similar but not identical specificity.

HZ 327 T CELLS CAN ONLY RECOGNIZE A LIMITED REPERTOIRE OF CLASS II MHC BOUND PEPTIDES IN THE ABSENCE OF CD4.

Dario A.A. Vignali and Jack L. Strominger, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

A close association between CD4 and the T cell receptor (TCR) occurs during antigen presentation as a result of their interaction with the same class II major histocompatibility complex (MHC) molecule. The potential consequences of such an intimate interaction on TCR specificity was addressed using CD4 loss variants of three different murine T cell hybridomas specific for the hen egg lysozyme (HEL) peptide 52-61. While all the CD4+ and CD4- variants tested responded comparably to immobilized anti-TCR mAbs and naturally processed HEL, the response to synthetic peptide 46-61 was barely detectable. Responsiveness was restored upon transfection of CD4. These data suggested that T cells can only recognize a limited repertoire of peptides in the absence of CD4. In order to investigate this question directly, a panel of 53 peptides consisting of every possible 12 to 19-mer which contains the minimal MHC binding epitope, 52-61, was produced. The CD4+ hybridomas responded strongly to all peptides. In contrast, the CD4- variants strongly recognize only those peptides which had the C-terminal tryptophan at position 63 (eg. peptide 52-63). Surprisingly, peptides progressively lost their ability to stimulate with the addition of amino acids after position 63. Apparently the TCR can only efficiently recognize peptides which contain C-terminal tryptophan 63, and require CD4 in order to respond to all other peptides. We are currently testing a panel of alanine and phenylalanine analog peptides of the two tryptophanes 62 and 63. These data may have important implications for our understanding of the role of CD4 in class II MHC mediated immune responses.

HZ 328 INFLUENCE OF T CELL RECEPTOR CODING REGION POLYMORPHISMS ON THE EXPRESSED IMMUNE REPERTOIRE. Christine Vissinga, Patrick Charmley, Patrick Concannon, Virginia Mason Research Center, Seattle, WA 98101.

Recently, a number of coding region polymorphisms in humans have been described. While the effect on the peripheral T cell repertoire of polymorphisms resulting in null (non-expressed) alleles, such as in the case of Vβ20 are obvious, it remains unclear what effect more subtle polymorphisms involving one, or a small number of amino acids, may have. In the present report we have studied the usage of Vβ6.7 alleles to determine the influence of coding region polymorphism on the expressed TCR repertoire. The Vβ6.7 allelic system involves two expressed alleles 6.7a and 6.7b (Li, 1990). The Vβ6.7a and Vβ6.7b alleles differ at two amino acid positions, which are in linkage disequilibrium. Vβ6.7a encodes ser^{38} and gly^{72}, and Vβ6.7b encodes arg^{38} and glu^{72}. We have chosen to study this allelic system because positions 38 and 72 both are critical residues since they are expected to be exposed to solvent based on modeling of the TCR structure. Position 38 is thought to face the α chain V domain. Position 72 is located in CDR4, which is thought to be a superantigen or Ag/MHC binding site. We have assayed several families by PCR for expression levels of the two alleles. In heterozygotes, equal usage of each allele would indicate random and unselected usage of the allelic forms. However, we found in heterozygotes variable expression levels (30%-70%) for the two Vβ6.7 alleles that could not be attributed to inheritance of TCRβ haplotypes or to differences in the promotor region of the two alleles. These results constitute the first report of variation in usage of allelic forms of the TCR in humans. Based on the location of these nonconservative amino acid substitutions in the Vβ6.7 sequence we propose that the observed variation is functional relevant and is probably a result of differential thymic selection.

HZ 329 THE GENE STRUCTURE, PROCESSING AND ASSEMBLY OF MHC-ENCODED PROTEASOMAL SUBUNITS LMP2 AND LMP7. Young Yang, Klaus Früh, James B. Waters and Per A. Peterson. Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

The processing of cytoplasmic polypeptides for antigen presentation by major histocompatibility complex (MHC) class I molecules has not yet been fully elucidated. Due to their homology to proteasomal subunits (*LMP2* and *LMP7*) and members of the ATP-binding cassette family (*TAP1* and *TAP2*), four newly discovered genes in the class II MHC region are likely to be involved in the processing and transport of antigens. We and others have shown that two subunits of the proteasome, which is responsible for the extralysosomal ATP/ubiquitin proteolytic pathway in cells, are MHC-encoded. In addition, using antibodies to LMP2 and LMP7 we have shown that these two MHC-encoded proteasomal subunits are products of *LMP* genes. By analyzing the genomic organization of *LMP7*, we have identified two cDNA forms of *LMP7* which arise by alternative exon usage. Furthermore, we have shown that polypeptides encoded by *LMP* genes undergo proteolytic processing after their incorporation into the proteasomal complex and incorporation of LMP2 into the proteasomal complex is dependent on LMP7.

We have employed class I thermostability assays, reverse-phase high performance liquid chromatography and cytotoxic T lymphocyte assays to show that purified proteasomes can generate antigenic peptides that are presented by class I MHC molecules. Our findings are consistent with the common view that the proteasome is involved in antigen processing for MHC class I presentation.

HZ 330 PRIMARY SEQUENCE OF THE MOUSE PROTEASOME SUBUNIT LMP-7, Eric Zanelli, Paul Zhou, Chella S. David, Department of Immunology, Mayo Clinic, Rochester MN 55905.

MHC class I molecules bind peptides derived from cytosolic proteins for presentation to T cells. These peptides are presumably generated in the cytosol by a large multicatalytic complex composed of at least 15 different subunits with 3 or 4 distinct peptidase activities, called the proteasome. Recently, the genes of two of these subunits, *Lmp-2* and *Lmp-7*, have been located in the MHC class II region. The complete amino acid sequence has been established for both human and mouse LMP-2 but only for human LMP-7 (initially called RING 10). Here we report the complete amino acid sequence of LMP-7 in mouse. A BALB/c mouse thymus cDNA library was screened with a RING 10 cDNA probe generated by PCR of RNA from the human AKIBA cell line. One colony was isolated; the sequencing of its 1086 nucleotides revealed a polyadenylation site followed by a poly A^+ tail and an open reading frame of 830 nucleotides if one considers the position 34 as the initiation codon. Sequence comparison between this cDNA and the published sequence of RING 10 revealed a high degree of homology, as expected. However, this homology starts from nucleotide 168 (340 on RING 10 sequence) which highlights the position 238 (410 for RING 10) as the potential initiation codon. Consequently, LMP-7 is a 208 amino acid protein which differs for only 16 residues between both species. Similar to all the members of the proteasome gene family sequenced so far, LMP-7 also does not contain any known element related to protease function. We are currently analyzing the allelic polymorphism of LMP-7 in different strains of mice.

HZ 331 Molecular Basis of Allelism of *Lmp-2* Gene. Paul Zhou, Hong Cao, Michele K. Smart and Chella S. David Mayo Clinic, Rochester, MN 55905

Based upon the published sequence derived from a cell line WEHI-3 ($H\text{-}2^d$), *Lmp-2* cDNA's were PCR-amplified, cloned, and sequenced from twelve inbred or H-2 recombinant mouse strains (DBA/1, SWR, B10.Q, B10.AQR, DBA/2, BALB/c, CBA/J, C3H.RKK, B10, B6, RIIIs/j, NOD). The *Lmp-2* from these mice follow the pattern of H-2 haplotypes; i.e. mice with the same H-2 haplotype have identical *Lmp-2*. In addition, the *Lmp-2* sequences of $H\text{-}2^d$ and $H\text{-}2^k$ and of $H\text{-}2^q$ and $H\text{-}2^{g7}$ are identical. Among 806 nucleotides of the *Lmp-2* cDNA, there are overall eleven nucleotide substitutions. Between $H\text{-}2^d$ and $H\text{-}2^q$, six nucleotide substitutions were identified, resulting in one amino acid change (Asn to Asp) at position 177. Between $H\text{-}2^d$ and $H\text{-}2^b$, ten nucleotide changes were found, resulting in three amino acid changes (positions 60, 126, 177). All these changes involve charged residues (Arg to His, Arg to Cys, Asn to Asp). Between $H\text{-}2^b$ and $H\text{-}2^q$, there are four nucleotide substitions, resulting in two amino acid changes (His to Arg at position 60 and Cys to Arg at position 126). The *Lmp-2* gene of $H\text{-}2^r$ has the same nucleotide sequence as that of $H\text{-}2^q$ except for one position (G to A nt 96). Thus, at the protein level, three allelic variants $LMP2^d$, $LMP2^b$, and $LMP2^q$ were found among these mouse strains. These sequence results suggest that 1) *Lmp-2* gene and MHC may have coevolved to obtain optimal functional interaction, since different strains with the same H-2 haplotype have identical *Lmp-2*; 2) amino acid residue Asn at position 177 is involved in antigenic epitope of $LMP2^q$ allele recognized by BALB.B anti-BALB/c serum; 3) $LMP2^q$ allele may be involved in the disease susceptibility, since it is shared by mice with the $H\text{-}2^{q,r,g7}$ haplotypes known to be prone to autoimmune diseases. Currently, we are investigating the effect of this allelism on the generation of endogenous peptides.

HZ 400 CD1b IS AN ANTIGEN PRESENTING MOLECULE: PRELIMINARY CHARACTERIZATION OF A MYCOBACTERIAL ANTIGEN RESTRICTED BY CD1b TO A TCR $\alpha\beta^+$ $CD8^-/CD4^-$ T CELL LINE, Evan M. Beckman, Steven Porcelli and Michael B. Brenner, Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115

The human CD1 locus contains 5 nonpolymorphic genes located on chromosome 1. At least four of the genes (CD1A, CD1B, CD1C and CD1D) are expressed as surface polypeptides with specific tissue distributions.

The T cell line DN1 (TCR $\alpha\beta^+$, $CD8^-/CD4^-$) was derived by repeated stimulation of CD4/CD8/TCR$\gamma\delta$ depleted PBMC with a crude sonicate of Mycobacteria (M.) tuberculosis (tb.) and monocytes treated with GM-CSF/IL-4 to induce CD1a, b and c expression. The proliferative and cytotoxic response of the DN1 to the crude mycobacterial sonicate is restricted by CD1b as shown by antibody blocking experiments and transfection studies. The response requires time for processing of M. tb. antigen and is inhibited by drugs that block Class II antigen processing steps such as chloroquine and monensin (S. Porcelli, et al, Nature, in press).

The proliferative response of DN1 is greatest to crude sonicates of M. tb., but activity is also found in crude sonicates of M. leprae, and several rapid growing strains of mycobacteria, including M. fortuitum and M. smegmetis. The antigen is neither HSP65 nor HSP70. In contrast to the predominant $\gamma\delta$ mycobacterial reactivity, the antigenic material is >3kd and nondialyzable. Preliminary biochemical characterization of the antigen responsible for the DN1 mycobacterial reactivity will be presented.

HZ 401 BINDING AND PRESENTATION OF MYELIN BASIC PROTEIN PEPTIDE Ac1-11 BY MHC CLASS II MOLECULES $A\alpha^uA\beta^u$, Anand M. Gautam[$], Cecelia Pearson[$], Christopher Lock[$] & Hugh McDevitt[$*], [$]Department of Microbiology and Immunology; and *Medicine. Stanford University School of Medicine.

Myelin Basic protein (MBP) Ac1-11 is presented by antigen presenting cells expressing MHC class II molecules $A\alpha^uA\beta^u$. Critical amino acids in Ac1-11 for binding to $A\alpha^uA\beta^u$ and for presentation by specific T cells have previously been identified.The present study demonstrates that substitution of all but four amino acids with alanines in Ac1-11 results in a peptide which binds $A\alpha^uA\beta^u$, stimulates specific T cells and induces experimental autoimmune encephalomyelitis (Gautam et al. J. Exp. Med. 176:605.1992). Since Ac3.5.6 (Ac**AAQARPA** AAAA; native residues are shown in bold) is the minimum peptide required for stimulating Ac1-11 specific T cells in vitro, we have chosen this peptide to study its conformation within the binding groove of $A\alpha^uA\beta^u$ by introducing D-amino acids at various positions. Our data show that D-amino acids are tolerated only from C-terminus and that the peptide binds in an extended conformation with a "kink" in the middle. To elucidate peptide binding to MHC class II further, site-directed mutagenesis in $A\alpha^uA\beta^u$ at the conserved residues was employed. Results show that at least one conserved residue in $A\alpha^uA\beta^u$ interacts with an amino side chain of Ac1-11 presumably by making a hydrogen bond. These results also provide insight into the orientation of the peptide in the binding groove of $A\alpha^uA\beta^u$.

HZ 402 IDENTIFICATION OF PEPTIDE-CLASS II MHC COMPLEXES IN SUBCELLULAR FRACTIONS OF MACROPHAGES. Clifford V. Harding, Department of Pathology, Washington University, St. Louis, MO 63110.

Exogenous protein antigens are processed within endocytic compartments to produce peptides that bind to class II MHC (MHC-II) molecules for presentation to T cells. Using subcellular fractionation we have identified the subcellular compartment in which immunogenic peptides bind to MHC-II as a subset of high density vesicles with lysosome-like properties, herein termed "lysosomes". Pulse-chase biosynthetic labeling of macrophages followed by immunoprecipitation of MHC-II from dense fractions showed that MHC-II molecules targeted efficiently to lysosomes after biosynthesis. Moreover, lysosomal MHC-II molecules were rapidly loaded with immunogenic peptide (as detected by T cells) upon targeting of antigen to this compartment, and prior to expression of peptide-MHC-II complexes on the plasma membrane. This compartment may be an "early lysosome" or a "dense late endosome." We propose that nascent MHC-II molecules target to lysosomes and bind immunogenic peptides therein; the resulting peptide-MHC-II complexes are then transported to the plasma membrane.

HZ 403 THE ALPHA 2 DOMAIN OF COMMON HLA B ALLELES DETERMINES A FAST OR SLOW ASSEMBLY PHENOTYPE.

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We have compared in vivo assembly of HLA Class I B locus alleles using metabolic labelling, pulse chase and isoelectric focusing. Within a closely-related group of molecules, the B5/35 group, two extreme patterns were found. All newly synthesized heavy chain of B35 and B53 was fully assembled (stably associated with β2m) and glycoslyated within 30 minutes of labelling. In contrast, labelled free heavy chain from B51 and 78 slowly entered the glycosylated, assembled pool over a period of several hours; free, unglycosylated heavy chain was still detectable 4 hours later. As B53 and B78 are naturally occurring exon shuffles of B51 and B35, it was possible to localize the region controlling assembly efficiency to the α2 domain. We explored several possible mechanisms for the assembly rate difference. Firstly, β2m affinity, assessed by its ability to stabilize free heavy chain in the assembly-deficient mutant T2, was found to be the same for both assembly phenotypes. Secondly, pooled sequencing of eluted peptides showed that peptides binding to B35, B51 and B53 share a dominant anchor residue in their motif (proline at position 2). This is consistent with these moleucles having identical "B" pockets based on published crystallographic structures of the antigen binding cleft. Binding assays of predicted epitopes revealed a number of peptides which bound to both B51 and B53, often binding to B51 with higher affinity. Given this degree of similarity in peptide binding, it seems unlikely that a difference in availability of or affinity for suitable peptides explains the difference in assembly phenotype. We are investigating the possibility that fast and slow assemblers differ in their interaction with ER resident chaperone or chaperonin molecules.

HZ 404 ISOLATION, CHARACTERIZATION AND PRESENTATION OF TUMOR-SPECIFIC PEPTIDES ON DIFFERENT MHC CLASS I LOCI, Alex Y.C. Huang, Elizabeth M. Jaffee, M.D., and Drew Pardoll, M.D., Ph.D., Johns Hopkins Oncology Center, Baltimore, MD 21205

Recent studies suggest that cancer arise *de novo* because of the failure of the immune system to respond to tumor-specific antigens. We have shown that injection of tumor cells which were genetically engineered to secrete certain lymphokines (tumor vaccines) can activate helper T cells and tumor-specific cytotoxic T lymphocytes (CTL), and provide systemic immunity to both the tumor vaccines and the parental tumor. Since antibodies generally do not recognize tumor antigens, traditional methods such as immunoprecipitation cannot be used to isolate and characterize tumor antigens.

We have obtained a series of protein-A purified murine monoclonal anbitodies which recognize specific MHC class I loci. Using the methods of immunoprecipitation, acid elution, size-exclusion chromatography and reversed-phase high performance liquid chromatography (RP-HPLC), we recently isolated tumor-specific antigens from the purified MHC class I groove of a MHC class I$^-$ murine tumor, B78HI, which were transfected with genes which encode specific MHC class I loci such as K^b, D^b, or both. We were able to identify RP-HPLC fractions containing tumor-specific antigens by utilizing tumor-specific CTL's, which were generated *in vivo* with a tumor vaccine system. The tumor-specific RP-HPLC fractions were identified using a heterogeneous population of CTL initially primed *in vivo* with B78HI which was genetically engineered both to express MHC class I molecules on the cell surface, and to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF). Interestingly, using chromium-51 CTL assay we are able to show consistantly that only a few RP-HPLC peptide fractions elicit strong CTL responses. Sequence analysis of the isolated peptide fractions using mass spectroscopic technology is currently in progress, and the result will be presented during the 1993 Keystone Symposia.

HZ 405 ISOLATION AND CHARACTERIZATION OF A TUMOR-SPECIFIC PEPTIDE

Elizabeth M. Jaffee, M.D., Alex Huang, Gary Pasternack, M.D., Ph.D., Amina Woods, Robert Cotter, Ph.D., and Drew Pardoll, M.D., Ph.D., Johns Hopkins Oncology Center, Baltimore, MD 21205

One of the most critical questions in cancer immunology is why the immune system fails to eliminate tumors that arise *de novo*. Recent studies have provided strong evidence that this failure owes to an inability to respond to tumor-specific antigens. We have demonstrated that the injection of tumor cells genetically engineered to produce certain lymphokines can activate inadequately functioning helper T cells and generate tumor-specific cytotoxic T lymphocytes (CTL) that provide systemic immunity to the parental tumor. Tumor antigens to which CTL respond have been difficult to characterize because they are not generally recognized by antibodies and therefore cannot be isolated by immunoprecipitation.

Using the method of acid elution and reversed-phase high performance liquid chromatography (RP-HPLC), we recently isolated tumor-specific antigens from the MHC class I groove of a murine colon tumor, CT26. Tumor-specific CTL, which are used to identify RP-HPLC fractions that contain these peptides, were generated in vivo using our tumor-vaccine system. With this approach we were able to detect 2 tumor-specific peptides out of 40 total RP-HPLC fractions using a heterogeneous population of CTL initially primed in vivo with a tumor vaccine genetically engineered to secrete the cytokine granulocyte-macrophage colony-stimulating factor (GMCSF). Interestingly, these two peptides repeatedly elute from other CT26 tumors in fractions number 25 and 32. Furthermore, these same peptides are detected by different populations of CTL generated in different mice of the same MHC haplotype using the same tumor vaccine. Recently, the RP-HPLC fraction numbers 25 and 32 of the CT26 colon carcinoma were analyzed by several methods employing mass spectroscopy. The results revealed that each fraction contained at least one peptide with a molecular weight in the range of 1200 to 1500 daltons, which is approximately the molecular weight of an 8 or 9 amino acid peptide. Our data therefore suggests that there is only one or two immunodominant peptides presented by a population of tumor cells that have been grown *in vivo*.

HZ 406 CHAPERONIN HSP60: NOVEL ISOFORMS AND TISSUE SPECIFIC EXPRESSION, Satish Jindal, Mari Madsen, Erica Burke and Donard S. Dwyer, Procept Inc., 840 Memorial Drive, Cambridge, MA 02139

Major heat shock proteins (HSPs) much like MHC class II antigens have affinity for binding to various peptides. Moreover, heat shock proteins in general and hsp60 in particular are also major target antigens of immune responses during a variety of bacterial and parasitic infections. Hsp60 has also been implicated as an autoantigen in autoimmune diseases such as insulin-dependent diabetes mellitus and rheumatoid arthritis. Unlike other major hsp's (e.g., hsp70), only a single hsp60 protein, localized in the mitochondria, has been characterized so far. Southern blot analysis of mammalian genomic DNA suggests that there may be more than one gene for this protein. However, attempts to isolate and sequence multiple genes for mammalian hsp60 employing DNA probes have resulted in the cloning and sequencing of more than a dozen closely related genes having features of "pseudogenes". Recently, we have developed a panel of monoclonal antibodies against human hsp60 protein. Western blot analysis of a wide variety of cell lines and animal tissues employing different antibodies have led us to conclude that there are different isoforms of this protein. Interestingly, some isoforms seem to have a tissue-specific localization. Using a panel of selected antibodies as probes we have isolated genes from λgt11 c -DNA libraries and are currently characterizing them. We are investigating the possibility that these novel proteins may become target antigens during development of autoimmune reactions. Because some of these proteins may be present in organelles other than mitochondria, we are also investigating their function as "chaperonins" in peptide/protein translocation in these organelles.

HZ 407 CLASS II MHC-BINDING HAPTENATED PEPTIDES, ANTIGENIC FOR CD4+, TNP-SPECIFIC T CELLS.

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We have recently identified trintropenyl (TNP) residues attached to class I MHC-associating peptides as the prominent hapten determinants for cytotoxic T cells. These findings and the results of detailed structural analyses of these antigenic peptides have been discussed in their possible relevance to hapten-induced allergic reactions. However, it is widely believed that despite an undoubted participation of $CD8^+$ T cells in allergic responses, the induction of these reactions is most likely mediated by T cells of the $CD4^+$ compartment. We have, therefore, produced TNP-reactive, IA^b-restricted hybridomas and T cell lines of the $CD4^+$ phenotype. The TNP-specific cell lines were induced *in vivo* by skin painting with picryl chloride. After depletion of $CD8^+$ lymphocytes from the lymphnode cells, the cells were cultured *in vitro* on syngeneic, irradiated spleen stimulators (C57BL/6) which had been chemically modified with trinitrobenzene sulfonic acid (TNBS). The cells were then either fused to a TCR $\alpha^-\beta^-$ subline of the thymoma BW5147 or kept in continuous culture by weekly restimulation in the absence of added factors. In an approach similar to that used for the identification of class I MHC-restricted TNP-peptide antigens, we assayed the cell lines for antigen-specific proliferation and the hybridomas for TNP-specific IL-2 production. TNP-specific stimulation was carried out on irradiated C57BL/6 spleen cells, modified either by TNBS or tryptic digests of TNBS-modified soluble proteins. Several hybridomas and also some of the T cell lines exhibited reactivity to digests of TNP-BSA. HPLC-purification and attempts to identify the reactive peptide(s) are under way. It is evident, however, already now that these reactions are inhibitable by IA^b-specific antibodies and that the cells in question do not react to TNP-free digests or unmodified stimulator cells. We also found some of our hybridomas crossreactive to other TNP-peptides, indicating that most probably our finding of "sequence-independent" TNP peptide determinants for class I restricted cells will hold true also for the class II restricted compartment. We are presently attempting to identify and subsequently synthesize several IA^b-specific TNP-peptides and will then try to define their optimally antigenic configuration. Induction of TNP-specific T cell responses, using combinations of class I and II restricted TNP-peptides *in vitro* as well as *in vivo* hopefully will allow more and new insights into the basic mechanisms of chemical-induced allergies.

HZ 408 ROLE OF CLASS I MHC-ANCHORING PEPTIDES IN DEFINING HAPTEN EPITOPES FOR T CELLS.

Stefan Martin, Arne von Bonin and Hans Ulrich Weltzien, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, Germany.

Our analyses of TNP-peptide reactive cytotoxic T cells (CTL) revealed the existence of two principally different types of reactivities. A major group of CTL was identified to crossreactively recognize TNP on a great variety of different peptides, provided they possessed sufficient binding affinity for K^b, and carried the lysine-attached TNP in a specific position of their sequence (i.e. position 4 of the octapeptide motif). These T cells were clearly selected in bulk cultures *in vitro* upon repeated stimulation with syngeneic cells, chemically modified with trinitrobenzene sulfonic acid (TNBS). In fact, we could show that such CTL even reacted to octapeptides consisting only of glycine residues except for TNP-Lys in position 4 and the postulated K^b-anchors Phe and Leu in positions 5 and 8, respectively. Due to the crossreactive antigenicity of TNP_4 substituted, K^b-associated peptides, these epitopes are distinctly more repetitive than most others, which explains their immunodominance in TNP-responses. A second group of CTL exhibited reactivities to TNP-peptides revealing a great deal of dependence on both, the positioning of TNP and the amino acid sequence of the peptide carrier. Also in these cases, the use of oligo-glycine "designer peptides" turned out to be extremely useful to define the structural identity of the TNP- as well as of the sequence-specific antigenic epitopes. We found, in line with recent crystallographic data on the structure of K^b-associated peptides, that amino acids in positions 3,4 and 6 were the major contributors to peptide-specific determinants. TNP in position 4 would either "cover" these residues or react together with them as a "complex-determinant" with the corresponding T cell receptors. In other cases, two distinguishable and independent determinants for "TNP" and "peptide" could be defined. The latter ones, in contrast to the "sequence-independent" TNP epitopes, must be considered extremely rare on TNBS modified cells. CTL specific for such structures usually reacted well with the respective peptides, but hardly or not at all with TNBS-modified target cells. It thus appears that chemically modified cell surfaces contain at least two principally different types of hapten determinants: one which is extremely repetitive and one which rather compares to rare "conventional" antigenic peptides. We assume that the immune responses induced by those different epitopes may be qualitatively different.

HZ 409 IMMUNIZATION WITH SOLUBLE VIRAL PROTEINS ELICITS MHC CLASS I-RESTRICTED CD8+ CYTOTOXIC T LYMPHOCYTE RESPONSES *IN VIVO*.

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Immunization with soluble protein antigens usually stimulates CD4+ but not CD8+ T cells. Subcutaneous, intraperitoneal or intravenous immunization of mice with low doses of two viral proteins (*i.e.*, HBV-derived S-antigen [HBsAg] and SV40-derived T-Ag) primed CD8+ cytotoxic T lymphocytes (CTL) *in vivo*. **(A)** CTL primed *in vivo* to native, particulate HBsAg(without adjuvants) efficiently lysed syngeneic transfectants of different histotypes expressing HbsAg. In this response CD3+CD4-CD8+ CTL showed L^d-restricted recognition of the $HbsAg_{21-40}$ epitope. $H\text{-}2^d/L^{d+}$ mice (BALB/c) were responders, $H\text{-}2^d/L^{d-}$ (dm2) and $H\text{-}2^b$ (B6) mice were non-responders. $H\text{-}2^d$ stimulator/target cells pulsed *in vitro* with HBsAg particles stimulated proliferation and were specifically lysed by anti-HBsAg CD8+ CTL. *In vitro* uptake and processing in the 'endogenous' pathway of HBsAg by different APC types was characterized. **(B)** Sensitization of $H\text{-}2^b$ C57BL/6 (B6) mice with SV40 T-Ag specifically primed CD8+ CTL. CTL primed *in vivo* by the 708 amino acid viral protein lysed *in vitro* syngeneic cells transfected with the SV40 T-Ag or transformed by SV40 infection. T-Ag epitopes were not presented to anti-T-Ag CTL by $H\text{-}2^b$ RMA-S cells that efficiently expressed T-Ag after transfection. The magnitude of the anti-T-Ag CTL response of B6 mice stimulated by soluble T-Ag was comparable to the anti-T-Ag CTL response of SV40-infected B6 mice. Injections of denatured or native T-Ag protein primed CTL equally well, but immunization with an equal dose of antigen in adjuvants inefficiently stimulated CTL. The N-terminal 272 amino acid T-Ag fragment primed B6 CTL *in vivo* efficiently. This protein fragment hence contains structural determinants required for this protein to access the 'endogenous' processing pathway for H-2 class I-restricted antigen presentation to CD8+

HZ 410 Maturation of functional class II elements in DC-populations,

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Dendritic cells (DC) represent a heterogeneous class of professional accessory cells, that originate in the bone marrow. Lately we reported on the development of a procedure, that allows to generate DC in sizable numbers by in vitro culture of bone marrow cells in the presence of low doses of rGM-CSF. In contrast to co-induced bone marrow macrophages and granulocytes, in vitro grown DC express prominant levels of MHC class II at the cell surface. This marker was demonstrated to be usefull for purifying DC by mAb panning and/or magnetic bead selection. In vitro differentiated DC exhibited highly potent functional capabilities as evidenced by MLR and antigen presentation testing. In this respect they resembled short term-cultured epidermal Langerhans cells (LC). Both DC-populations were much more efficient than other accessory cells, like B-cells, stable class II transfectants, and macrophages. Discrete structural requirements of the class II dimers became apparent, mandatory to produce peptide acquiring compact (C) folded forms in the various accessory cell populations. In line with their unique functional capabilities, in vitro grown DC and LC had the capacity to generate class II C forms in unsurpassed levels.

HZ 411 **MAKING ANTIGEN PROCESSING SUPERFLUOUS.**

NP Restifo, I Bacik, JR Bennink, SA Rosenberg, and JW Yewdell, NCI and NIAID, NIH, Bethesda, MD 20892.

Using a recombinant vaccinia virus (Vac) to transiently express the K^d molecule, we have studied antigen processing in several human tumor cell lines. Three small cell lung carcinoma (SCLC) cell lines failed to process endogenously generated antigen based on functional data. Pulse-chase experiments showed that MHC class I molecules remained in an Endo H sensitive for a prolonged period, suggesting that peptides were not available for binding to nascent MHC molecules in the endoplasmic reticulum (ER). Northern blot analysis of these cells revealed low to non-detectable levels of mRNAs for MHC encoded proteasome components *LMP-7* and *LMP-2* as well as the putative peptide transporters TAP-1 and TAP-2. To directly test the hypothesis that SCLC lines were in fact deficient in antigen processing, we constructed a vaccinia virus, similar to a previously reported plasmid based system (Anderson, *et al*, J. Exp. Med. 174:489, 1991), capable of endogenously synthesizing a 9 amino acid long "minimal determinant" (NP147-155 from influenza A/PR/8/34) preceded by an ER insertion sequence from adenovirus type 5, E3/19-kD glycoprotein. NP147-155 was presented very efficiently by SCLC cell lines if it was preceded by the signal sequence, but was not presented without the signal sequence. Apparently, the construct described eliminated the need for both protease activity and *TAP* activity. Thus, while down regulation of antigen processing may be one of the strategies employed by tumors to escape immune surveillance, antigen processing can be enhanced at the level of gene regulation or superseded by providing pre-processed antigen delivered directly to the ER.

HZ 412 AN-TERMINAL DOUBLE-ARGININE MOTIF CONFERS RETENTION OF TYPE II MEMBRANE PROTEIN IN THE ENDOPLASMIC RETICULUM, Marie-Paule M. Schutze, Michael R. Jackson and Per P. Peterson, The Scripps Research Institute, 10666 N.Torrey Pines Road, La Jolla, CA 92037

In the present study, we have examined the putative existence of a structural motif for the retention in the ER of the invariant chain. Invariant chain is a type II membrane protein associated to class II molecules of the MHC. For type II membrane protein, the most likely location for an ER retention signal is in the cytoplasmically exposed aminoterminus. Two forms of Ii exist, Iip31 and Iip33. These two forms are produced by differential initiation of translation from the mRNA, i.e at the first codon for Iip33 or at the second codon for Iip31. These two forms differ in their N-terminal extremity, Iip33 contain a 45 residues extension which preceed the transmembrane segment, whereas it is of 30 residues for Iip31. By eliminating one of the two initiator codons by point mutation, either Iip33 or Iip31 is produced. In contrast to Iip31 which is exported out of the ER and localised in endosomal structures, Iip33 has been localised in the endoplasmic reticulum (ER) (Lotteau et al. 1990). As Iip33 differs from Iip31 by only a 15 aminoacid extension of the cytoplasmic tail, the retention signal could be contained in this region. To analyze this, a series of aminoterminal truncation mutants of Iip33 were prepared. The different cDNA clones were cloned in an expression vector and the subcellular localisation of these mutated forms of Iip33 were analysed after transfection of human HeLa cells by immunofluorescence microscopy and biosynthetic pulse chase experiments. We have found that deletions of more than four residues from the aminoterminus of Iip33 resulted in the transport of the molecules out of the ER. This clearly indicated that the first four residues contained information sufficient for the retention of Iip33 in the ER. Pulse chase experiments showed that the mutated forms which were retained in the ER were all endoH sensitive confirming that they did not reach the Golgi compartment. Further extensive-site directed mutagenesis studies showed the crucial requirement for arginine residues at the N-terminal extremity in the constitution of the ER retention motif. The identified motif can be transplanted onto the cytoplasmic tail of other type II transmembrane protein such as transferrin receptor and conferred ER residency.

HZ 413 CRYSTALLOGRAPHIC STUDIES OF THE COMPLEX OF HLA-DR1 WITH HA(306-318), Lawrence J. Stern, Jerry H. Brown, Theodore Jardetzky, and Don C. Wiley, Department of Biochemistry and Molecular Biology and Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138.

HLA-DR1 is a human class II major histocompatibility protein. We have previously produced soluble, recombinant HLA-DR1 in SF9 insect cells, and used this material to investigate the mechanism of antigenic peptide binding. We have crystallized the complex of HLA-DR1 with HA(306-318), an antigenic peptide derived from influenza haemagglutinin. The complex crystalizes as square plates, in the tetragonal space group $P4_32_12$. Unit cell parameters are a=b=95.7, c=245.0 angstroms. The crystal diffracts to 2.7 angstroms. We have collected a full data set from a single crystal frozen at -165°C, (93% complete, Rmerge=6.8%, for data 20.0 to 2.7 angstroms). We have obtained an initial model of the HLA-DR1 - HA (306-318) peptide complex by molecular replacement. We are currently refining this model.

HZ 414 The Last Role of Invariant Chain? Robert G. Urban, Roman M.Chicz, Dario A. A. Vignali and Jack L. Strominger. Harvard University, Cambridge, MA 02138.

Many roles have been described for invariant chain during class II antigen presentation. We have extracted and analyzed the naturally bound peptides from six different human HLA-DR alleles (accompanying abstract, R.M. Chicz, *et al.*). During the course of these analyses the same invariant chain peptide (96-120 and related molecules) was found naturally bound in four of the six alleles; although binding studies indicate that the fifth does bind and the sixth is under study. In addition, other groups have identified this same core region from the murine homologue bound to several mouse class II alleles. The observed degenerate binding capability of Ii peptides has been studied using synthetic peptide analogs. In this presentation we will discuss the possibility that invariant chain liberates a high affinity peptide during its proteolytic senescence to severe as a competitor during the loading of antigen peptides.

HZ 415 ENHANCEMENT OF PEPTIDE IMMUNOGENICITY FOR CTL INDUCTION BY LINKAGE TO A T HELPER PEPTIDE. A.Vitiello[1], J.Furze[1], P.Farness[1], R.Bartholomeusz[1], L.Yuan[1], T.Arrhenius[1], A.Maewal[1], S.Colón[1], F.Gaeta[1], C.Ferrari[3], F.Chisari[2], and R.Chesnut[1], [1]Cytel Corp., San Diego, California, [2]The Scripps Research Institute, La Jolla, California, [3]Cattedra di Malattie Infettive, Universita di Parma, Parma, Italy.

The importance of antigen-specific MHC class I-restricted cytotoxic T lymphocytes (CTL) in the clearance of viral infections is well established. In vivo induction of anti-viral CTL is usually achieved by injection of live viruses. This procedure, however, bears two major disadvantages: 1) risk of induction of some form of the disease, and 2) lack of direct control over CTL epitope specificity. Since the discovery that short synthetic peptides can mimic the endogenous antigens recognized by CTL, much effort has been devoted to the development of synthetic peptide immunogens, although success thus far has been limited. We have previously defined an optimal length HBc antigenic peptide, HBc 18-27, that is recognized in the context of HLA-A2.1 by CTL raised in A2.1 transgenic mice primed with HBV and is present in patients with acute HBV infection. Subcutaneous injection of this peptide into A2.1 transgenic mice induced a low CTL response that could be detected only after two in vitro restimulations using APC bearing HBc 18-27 as antigen. One possible explanation for this very low response is that, by priming with a "pure" CTL epitope, the initial response is not amplified by the concomitant presence of helper T cells. To address this point, we added a helper T cell epitope, HBc 128-140 (known to function in the context of the murine A2.1 transgenic's class II Ia^b) to the HBc 18-27. A single s.c. priming with this new peptide induced a strong CTL response specific for HBc 18-27. The therapeutic relevance of this immunogen was demonstrated by finding that these CTL kill HBc transfected target cells. These findings have been confirmed in a parallel system by priming Balb/c mice with a CTL epitope derived from influenza PR8 virus (NP 147-155, K^d-restricted) linked to an ovalbumin T cell helper epitope (OVA 323-336, Ia^d-restricted). Efforts are presently underway to define the cellular and molecular basis of this helper function in CTL induction.

HZ 416 IMMUNODOMINANT EPITOPES FOR TNP-SPECIFIC T CELLS ARE FORMED BY HAPTEN-CONJUGATED PEPTIDES.

Arne von Bonin, Bodo Ortmann and Hans U.Weltzien, Max-Planck-Institut für Immunbiologie, D-7800 Freiburg, Germany.

Protein-reactive haptens represent a multitude of T cell inducing antigenic structures, responsible for numerous allergic disorders. We have asked the question whether the antigenic determinants created by the modification of cellular surfaces with reactive haptens such as trinitrobenzene sulfonic acid (TNBS) are represented mainly by covalently TNP-conjugated MHC molecules or by TNP attached to MHC-associated peptides. Two models were employed to investigate this question: First, we used the mutant cell line RMA-S, and secondly affinity-purified K^b molecules to trigger TNP/Kb-specific receptors. RMA-S cells, due to defect peptide transporters, at reduced temperature express "empty" K^b molecules, free of endogeneously processed peptides. We occupied the binding grooves of these molecules by addition of the vesicular stomatitis virus (VSV) derived peptide N 53-59. This peptide does not contain lysine, and thus no reactive aminogroup to bind TNBS. The only K^b-restricted TNP-determinants to be presented by such cells could, therefore, be produced by covalent reaction of TNBS with the K^b molecules themselves. All CTL clones tested reacted poorly or not at all with TNBS-treated RMA-S cells, but strongly with the same cells treated with K^b-associating TNP-peptides. The second experimental system used purified, plastic-immobilized K^b molecules to stimulate IL-2 secretion from $CD8^+$ hybridomas, produced from our K^b/TNP-specific CTL. Again, modification of these preparations with TNP-peptides but not with TNBS resulted in strongly antigenic determinants for our T cells. These findings imply that covalently "haptenized" MHC molecules, at least in the TNP system, do not represent T cell antigenic determinants of major importance. The immunodominant TNP epitopes are clearly represented by hapten residues conjugated to MHC-associated peptides. Our data further indicate that different T cells specific for the same TNP/peptide/MHC-complex may be activated at significantly different limiting epitope densities. This supports the notion that low affinty T cell receptors require a higher surface-density of antigenic determinants than high affinity receptors.

Late Abstracts

RATIONAL DESIGN OF PEPTIDES CAPABLE OF ACTING AS ANTAGONISTS OF THE T CELL RECEPTOR, Jeff Alexander, Ken Snoke, Peggy Wentworth, Howard M. Grey and Alessandro Sette, Cytel, 3525 John Hopkins Court, San Diego, CA 92121

We have engaged in a detailed analysis of the interactions between peptide antigens and human class II major histocompatibility (MHC) molecules and T cell receptors, as a first step towards rational design of drugs interfering with T cell activation. In recent studies, it was found, using a DR1-restricted, HA 307-319 specific T cell clone system, that complexes formed between MHC and single amino acid analogs of peptide antigens function as potent specific inhibitors of T cell activation. Analysis of the correlation between structure and activity revealed that powerful TCR antagonists could be generated by changing any of the crucial T cell contact residues on the peptide molecule. Furthermore, as the analog similarity to the antigen increased, so did the antagonistic activity, up to the point when the analogs themselves became antigenic. We next wished to determine whether analogs were capable of interfering with a polyclonal response. As a model for a polyclonal response, we generated DR4w4-restricted T cell lines specific for HA 307-319. Five T cell lines thus far tested use different TCR and also demonstrate different antigen fine specificities. Two peptide analogs have been identified that have been shown to be effective as TCR antagonists for 4/5 of these T cell lines.

RECONSTITUTION OF SPECIFIC PEPTIDE BINDING AND T CELL RECOGNITION OF CLASS II I-E^k HETERODIMERS FROM SUBUNITS PRODUCED IN *E. COLI*. John D. Altman, Philip A. Reay, and Mark M. Davis, Department of Microbiology and Immunology and the Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

The interaction between T cell antigen receptors and cell surface MHC molecules, which present peptide antigens, plays a central role in T cell development and specific immune responses. The fate of an individual T cell clone must be strongly correlated with the specific affinity of this interaction. However, since the TCR and MHC are both transmembrane glycoproteins and are not normally available in soluble form, affinity measurements have been rare. To facilitate further protein chemical studies, we have produced truncated α and β chains of the murine class II molecule I-E^k in *E. coli* and have developed conditions to refold them in the presence of specific peptides in yields approaching 2%. Correct folding is assayed by capture ELISA with an immobilized monoclonal antibody specific for I-E^k complexes. Competition studies show this is specific since peptides which bind I-E^k reduce the signal in the ELISA assays, while peptides that bind to other isotypes or alleles have no effect. Complexes of the refolded heterodimer, which we refer to as *Ec*I-E^k, with two peptide antigens were affinity purified and showed the expected molecular weight by non-reducing SDS-PAGE. Following immobilization on plastic microtiter plates, the *Ec*I-E^k complexes stimulated lymphokine production by three distinct T cell clones in an antigen-specific manner with a dose-response comparable to previously described soluble I-E^k molecules produced in CHO cells. These results demonstrate unequivocally that the *in vitro* folded molecules have a biologically relevant conformation and that carbohydrate modifications to MHC are not necessary for recognition by all T cells tested.

IMMUNOSTIMULATING RECONSTITUTED INFLUENZA VIROSOMES (IRIVs) CONJUGATED WITH SMALL ANTIGENS, R. Glück and R. Mischler, Swiss Serum and Vaccine Institute Berne, P.O.Box, CH-3001 Berne, Switzerland

Synthetic peptides, purified subunits or inactivated small virus particles require immunopotentiation if they are to be effective vaccines. A large range of procedures to enhance immunogenicity has envolved over the last decades: Aluminium salts, proteosomes, immunostimulating complexes (iscoms), liposomes, conjugation with bacterial products or derivatives, combination with surface-active agents or application of cytokines have been the most described classes of adjuvants. We describe here the design of an inactivated hepatitis A vaccine adjuvanted with immunopotentiating reconstituted influenza virosomes (IRIVs). The formalin inactivated hepatitis A particles are attached to reconstituted proteinlipid complexes consisiting of a mixture of phospholipids and influenza virus glycoproteins. With this new vaccine design we combined different immunostimulating effects: The immunopotentiation by phospholipid vesicles, recognition of the hemagglutinin (HA) epitopes by the immune system, binding capacity of HA to sialic acid containing receptors of macrophages and immunocompetent cells and mediation of the entry into the cytoplasm of macrophages by a membrane-fusion event triggered by HA. Hepatitis A seronegative human volunteers received one intramuscular injection with this new vaccine. There were only few mild local reactions and 14 days after vaccination 100% of the subjects were seropositive. Among the individuals (control group) who received an alum adsorbed vaccine, 88% developed local reactions.

The seroconversion rate was 44%. We conclude from these results that the IRIVs provide a new approach to the design of future adjuvanted vaccines.

RESTAURATION OF DEFECTIVE PRESENTATION OF A VIRAL PEPTIDE BY INTERFERON GAMMA, Ulrich H. Koszinowski, Anton Buchner, Thomas Ruppert and Hartmut Hengel, Department of Virology, University of Ulm, P.O. Box 4066, D-7900 Ulm, Germany

The nonapeptide YPHFMPTNL is a naturally processed and presented peptide of pp89, an immediate early protein of murine cytomegalovirus (Del Val et al., Cell 66:1145, 1991). In the BALB/c strain this peptide represents the dominant viral antigen recognized by CTL. This is a paradox, because efficient presentation of this peptide in vitro requires the arrest of the viral transcription program and productively infected cells do not present (Del Val et al., Cell 58:305, 1989). The defect in antigen presentation is due to a viral factor which prevents the transport of peptide loaded MHC molecules (L^d) through the golgi (Del Val et al., J.Exp.Med. 176:729, 1992).

Because this peptide stimulates the generation of the dominant CTL population, presentation must occur in vivo. We found that antigens of MCMV fall into at least two sets: one susceptible and the other resistant to the activity of the inhibitory factor. We found that cytokines, namely IFN-gamma alter the presentation pattern. Biochemical analysis revealed that IFN gamma had no effect on synthesis and stability of the relevant viral proteins. There was a significant increase of L^d synthesis, although the relative concentrations of unassembled and ß2m associated L^d remained constant. As indicated by endo H sensitivity the majority of the L^d molecules still remained retained in the cis-Golgi. Thus, the inhibitory interaction is probably not directly and stochiometric as in the adenovirus model. Extraction of naturally processed peptides showed that the IFN gamma effect on MHC is reflected by a similar increase in the amount of processed peptide.

The data demonstrate that infection of a cell line in vitro may completely fail to reflect the antigen presentation conditions in vivo.

HETEROLOGOUS PROTECTIVE IMMUNITY TO INFLUENZA A BY DIRECT INTRAMUSCULAR INJECTION OF DNA ENCODING A CONSERVED VIRAL PROTEIN, M.A. Liu, J.B. Ulmer, A. Friedman, D. Martinez, C.M. DeWitt, K.R. Leander, L.A. Hawe, H.C. Perry, J.W. Shiver, R.R. Deck, D.L. Montgomery, and J.J. Donnelly; Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486.

Cytotoxic T lymphocytes (CTL) specific for conserved viral antigens can respond to different strains of virus, in contrast to antibodies which are generally strain-specific. The generation of such CTL *in vivo* usually requires endogenous expression of the antigen, as in the case of virus infection. Although different approaches have generated cytotoxic T lymphocytes demonstrable following specific *in vitro* restimulation, efforts to demonstrate *in vivo* protection have met with less success.

We have used the novel approach of immunization with a non-replicating DNA expression vector to present a viral antigen to the immune system. Specifically, plasmid DNA encoding influenza A nucleoprotein (NP DNA; the nucleoprotein gene was cloned from A/PR8/34, H1N1) was injected into the quadriceps of BALB/c mice. This resulted in the generation of nucleoprotein-specific CTL. NP DNA injection also resulted in primary CTL which were able to kill epitope-pulsed or virally-infected targets simply following exposure to Con A and IL2 without antigen-specific *in vitro* restimulation. High titer anti-nucleoprotein antibody was also generated but was shown by serum transfer *in vivo* to not provide protection against subsequent influenza challenge. Mice injected with NP DNA were shown by several criteria to be protected against subsequent challenge with a heterologous strain of influenza A virus (HK/68, H3N2). A three log decrease in viral lung titers occurred in NP DNA-immunized mice compared to nonimmunized or control plasmid-immunized mice. The immunized mice also lost less weight and had significantly increased survival compared to controls. This approach thus provided a simple and effective means of providing immunity against a heterologous viral infection.

IMPROVED CONJUGATE VACCINES, Andrew J. Malcolm, Michael W. Best, Roderick J. Szarka and Zina Mosleh, Immunology Research and Development, Biotechnology Department, Alberta Research Council, P. O. Box 8330, Edmonton, Alberta, Canada, T6H 5X2

Weak or impaired immune responses to polysaccharide antigens (thymus independent) have been widely observed in the elderly, in infants and other population groups. Several investigators are attempting to elicit thymus dependent responses to a variety of bacterial polysaccharides or oligosaccharides using protein carriers. We have developed new chemical coupling technologies to produce oligosaccharide hapten-toxoid carrier vaccine prototypes. Oligosaccharides hydrolysed from polysaccharides of *Streptococcus pneumoniae* strains have been used to produce conjugate vaccines capable of eliciting immunoprotective antibody responses to the pathogenic organism.

Novel conjugate vaccines have also been developed using crystalline bacterial surface layers (S-layers) as carriers. We obtain more reproducible and better coupling efficiency with S-layer carriers than with toxoids. We believe this may be due to the repeating crystalline glycoprotein nature of S-layers, with the amino, carboxyl or hydroxyl groups available for hapten binding occurring on each protomer in identical position and orientation. Multi-hapten-S-layer vaccines are being developed using different coupling methods. We have identified several different S-layer glycoproteins which are immunologically distinct. These S-layers elicit non-cross reactive antibody and cellular responses. Vaccines to a variety of diseases can be developed using S-layers isolated from various bacterial strains, thereby avoiding the carrier suppression phenomena observed with toxoid carriers. S-layer carriers can elicit immunoprotective responses without the use of extraneous adjuvants or lipid material. We feel that the aggregate nature of the S-layer carrier endows it with an intrinsic adjuvant property. Conjugate vaccines to several bacterial strains are being developed.

FOREIGN HEAT SHOCK PROTEINS ARE PRESENTED TO α/β T CELLS BY MULTIPLE HLA-DR MOLECULES.

Fredrik Oftung[1], Knut E. A. Lundin[2], Annemiek Geluk[3], Jelle Thole[3], Tom Ottenhoff[3] and Abu Salim Mustafa[4].

1) National Institute of Public Health, Geitmyrsv. 75, 0462 Oslo, Norway. 2) Institute for Transplantation Immunology, The National Hospital N0027 Oslo, Norway. 3) Dept. of Immunohematology and Blood Bank, University Hospital 1 E3-Q p.o. P.O. Box 9600, 2300 RC Leiden, The Netherlands. 4) Department of Microbiology, Faculty of Medicine, Kuwait University, Box 24923, Safat, Kuwait.

Heat shock protein (HSP) reactive human T cell populations was identified by screening mycobacteria induced T cell lines and clones against well defined recombinant mycobacterial HSPs of 18, 65 and 70 kD. The HLA molecules required for HSP presentation to T cells was identified by standard methods. The results show that mycobacterial HSPs are presented to alfa/beta T cells by multiple HLA-DR restriction elements: The M. leprae 18 kD antigen was recognized in the context of DR4Dw4 and DR1, the M. leprae 65 kD antigen in context of DR1, DR2, DR4 (Dw4 and Dw14), DR5 and DR7, wheras the M. tuberculosis 70 kD antigen was recognized in the context of DR1, DR2, DR3, DR5, DR7 as well as HLA-DRw53. HSP70 fulfills some of the criteria to subunit vaccine candidates, since it contains many epitopes presented by multiple HLA class II molecules. T cell epitopes from the M. leprae HSP70 antigen presented by these HLA molecules were mapped by synthetic peptides.

HIGH EFFICIENCY EXPRESSION AND SOLUBILIZATION OF FUNCTIONAL T CELL ANTIGEN RECEPTOR HETERODIMERS. Tom H.M. Ottenhoff, Isaac Engel and Richard D. Klausner. Cell Biology and Metabolism Branch, NICHD, National Institutes of Health, Bethesda, MD 20892, USA.

The production of large amounts of soluble T cell receptor (TCR) αβ heterodimer is required for the determination of its three dimensional structure. The TCR α and β however are transmembrane proteins that are only expressed in association with proteins of the CD3 and ζ families.

In order to express high levels of functional TCR αβ heterodimers, we attached the extracellular TCR α and β domains to the TCR ζ chain. TCR αζ and βζ chimeras were efficiently transported to the cell surface of the rat basophilic leukemia cell line RBL-2H3 and expressed uniquely as αζ/βζ heterodimers. αζ/αζ and βζ/βζ homodimers were retained in the endoplasmic reticulum.

The transfected cells were activated by specific peptide/MHC ligand, demonstrating that the expressed αβ heterodimer was functionally competent and could be activated by its ligand in the absence of CD3 chains.

TCR αζ/βζ chimeric molecules in which a thrombin cleavable site had been inserted efficiently released *soluble disulfide-linked αβ heterodimers upon protease* treatment. Production of soluble TCR αβ heterodimer on a larger scale shows consistent recovery of 100-300 μg heterodimer from $5x10^9$ cells. Three out of three αζ/βζ heterodimers have been successfully expressed so far.

In conclusion, ligation of the TCR ζ chain and a protease cleavable site to TCR α and β chains induces high efficiency expression of functionally competent αβ heterodimers that can readily be solubilized.

PEPTIDE BINDING PROTEIN 74 IMPLICATED IN ANTIGEN PROCESSING IS ENCODED BY A GENE HOMOLOGOUS TO THE HEAT SHOCK PROTEIN 70 FAMILY, Susan K. Pierce, Susan Z. Domanico, Diane C. DeNagel and Jeffrey N. Dahlseid, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208-3500

Helper T cells recognize peptide fragments of protein antigens bound to Major Histocompatibility Complex class II molecules presented on the surface of antigen presenting cells. We have previously described peptide binding proteins of 72/74 kD (PBP72/74), which have been implicated to play a role in *antigen processing*, and are serologically related to the 70kD heat shock protein (hsp70) family. Here we report the cloning and sequencing of the cDNA encoding PBP74, accomplished using amino acid sequence information obtained from the purified protein. We show that PBP74 is highly homologous to members of the hsp70 family, but, significantly, is not identical to any known member of this family. Inspection of the cDNA nucleotide sequence indicates that it encodes a 46 residue N-terminal peptide which is not present in the mature PBP74 protein. Transcription and translation *in vitro* of the PBP74 cDNA verifies that it encodes a form of PBP74 which is larger than the mature protein. The presequence does not conform to known motifs for common organelle targeting sequences and, at present, its function is not known. By RNA *filter hybridization* analysis, two PBP74 mRNAs are detected in all cell types tested and both of these mRNAs contain the presequence coding region. Exposure of cells to heat shock does not result in an increase in the mRNA levels of PBP74 as compared to the dramatic increase observed for the stress-inducible hsp70 mRNA. Thus, PBP74 appears to be a constitutive member of the hsp70 family.

EFFECT OF TCR ANTAGONISM ON APC-T CELL INTERACTION AND T CELL SIGNALLING EVENTS, Jörg Ruppert*, Jeff Alexander*, Ken Snoke*, Mark Coggeshall~, Elizabeth Herbert~, Howard M. Grey* and Alessandro Sette*, *Cytel, San Diego, CA 92121; and ~La Jolla Institute of Allergy and Immunology, La Jolla, CA 92037

T cell receptor antagonism induced by antigen-analog/major histocompatibility complexes has been demonstrated to result in efficient inhibition of antigen dependent T cell responses. In the present study we investigated some of the possible mechanisms by which T cell receptor (TCR) antagonists bound to the MHC of antigen presenting cells (APC) inhibit T cell activation. Using a nonstimulatory analog of the antigenic peptide influenza hemagglutinin (HA) 307-319 we could show that MHC/antagonist complexes inhibit very early intracellular events of antigen dependent T cell activation, such as Inositol 1,4,5-triphosphate (IP) turnover and Ca^{2+} influx. In a parallel series of experiments, the effect of TCR antagonist on membrane related activation events was also investigated. It was found that antagonistic peptide-DR1 complexes on the surface of APC did not induce stable conjugates with T cells and most interestingly did not inhibit antigen-induced conjugate-formation. Thus, our data suggest that antagonistic peptides do not interfere with the cellular events that are required for stable APC:T cell conjugate formation but do inhibit early biochemical events required for T cell proliferation. This effect on T cells might be explained by TCRs engaging antagonists and antigen thus forming ineffective non-signalling clusters upon binding to the APC or by lack of clustering relevant for signalling.

CONSERVED AND ALLELE SPECIFIC ANCHORS IN HLA-DR BINDING PEPTIDES, Francesco Sinigaglia, David Bolin, Paola Valsasnini and Juergen Hammer, Roche Milano Ricerche, via Olgettina 58, 20132 Milan, Italy and Department of Inflammation and Autoimmune Diseases, F. Hoffmann-La Roche Ltd., Nutley, NJ 07110-1199, USA.

The major histocompatibility complex (MHC) class II molecules are highly polymorphic membrane glycoproteins that bind peptide fragments of proteins and display these peptides for recognition by CD4+ T cells. Most of the amino acid differences that distinguish MHC allelic variants lie in the peptide binding cleft, and different allelic forms of MHC molecules bind distinct peptides.

We have recently demonstrated that affinity purified MHC class II molecules can specifically bind to peptide sequences displayed by modified protein III on the surface of M13 phages. This allowed us to use M13 peptide libraries to screen for phages that bind to MHC class II HLA-DR1 alleles. The comparison of the peptide pool binding to DR1 with the original peptide pool of the M13 library revealed new insights in peptide-DR1 interaction (Hammer, J. et al., J. Exp. Med., 176, 1992).

To understand the principles of allele specificity for HLA-DR binding peptides we extended our analysis to HLA-DR4Dw4 and DRw11 class II alleles. We have isolated large M13 peptide pools selected by those alleles. Sequence analysis of the peptide encoding region of the selected phages led to the identification of general and allele specific anchor residues. The results provide a simple biophysical basis for the promiscuity and the specificity of peptide recognition by HLA-DR molecules. Furthermore, the knowledge of these anchor positions should prove useful for the design of MHC-specific antagonists.

ALLOREACTIVE T CELL RECOGNITION OF SDS-STABLE AND SDS-UNSTABLE CONFORMERS OF CLASS II MHC MOLECULES. Dominique A. Weber, Ned S. Braunstein, Nancy K. Terrell, John Martin, Charles A. Janeway, Jr.*, and Gail Strindberg, Department of Medicine, College of P&S, Columbia University, New York, NY 10032 and *Howard Hughes Medical Institute, Yale University Medical School, New Haven, CT 06510

During biosynthesis, class II MHC molecules mature from an SDS-unstable to an SDS-stable conformer. The acquisition of the SDS-stable phenotype correlates with the loading of peptide into class II MHC. Mutant cells that do not process antigen for presentation by class II MHC express only SDS-unstable class II. Nonetheless, as much as 25-30% of cell surface class II MHC expressed by normal cells are in the SDS-unstable conformer. To investigate the ability of alloreactive T cells to recognize and/or discriminate between these two conformers, we took advantage of one antigen processing mutant cell line, (.174 x CEM)T2. Mouse class II MHC (I-A) genes were transfected into T2, its phenotypically normal counterpart, T1, and mouse L cells. Immunoprecipitation and SDS-PAGE demonstrated that, as expected, the T2 transfectants expressed only SDS-unstable I-A; the other cells expressed both I-A conformers. Limiting dilution analyses of alloreactive T cell responses to these cells showed that CD4+ T cells that respond to SDS-unstable I-A were 1/6 as frequent as those that respond to SDS-stable I-A. Priming with normal allogeneic spleen cells predominantly expanded the response to stable and not unstable class II MHC. Priming with T2 transfectants, on the other hand, expanded CD4+ cells capable of responding to the SDS-unstable I-A conformer. Although T2 transfectants constitutively express only SDS-unstable I-A, the addition of peptide to T2 transfectants generated SDS-stable I-A-peptide complexes on the cell surface as determined by immunoprecipitation with an antibody specific for the MHC-peptide complex. Consistent with other reports, these complexes were recognized by MHC-restricted, antigen-specific T cells. Peptide-loaded T2 cells, therefore, appear to express monomorphic SDS-stable I-A-peptide complexes. Such cells are being used to explore the ability of alloreactive T cells to recognize individual I-A-peptide complexes in either a peptide-specific or non-specific way.

Targeted Delivery of an Exogenous Protein for Presentation by Class I MHC. I.B. Ulmer, J.J. Donnelly, L.A. Hawe, A. Friedman, X.-P. Shi, D. Montgomery, K.R. Leander, J.W. Shiver, and M.A. Liu. Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486.

Cytotoxic T-lymphocytes (CTL) recognize peptides derived from endogenously expressed proteins in association with nascent class I MHC. In contrast, exogenous antigens associate with class II MHC following endocytosis to an endosomal compartment. We utilized the binding and translocating domains of *Pseudomonas* exotoxin A (PE) fused with the CTL epitopes of influenza matrix protein and nucleoprotein as a means of delivering exogenous antigens to intracellular class I MHC. These recombinant fusion proteins (PEMa, PENP) were capable of sensitizing class I MHC+ target cells for lysis by the appropriate CTL. A point mutant of PEMa, corresponding to an endosomal proteolytic processing mutant of PE, had a substantially reduced ability to sensitize target cells for lysis, suggesting that presentation of the CTL epitope by class I MHC required internalization and processing of PEMa similar to that of the toxin. However, unlike PE, PEMa did not require translocation to the cytoplasm to exert its effect. First, two inhibitors of PE had no effect on PEMa. NH_4Cl, which raises endosomal pH and inhibits PE at a step subsequent to processing, and brefeldin A, which presumably inhibits PE by disrupting the Golgi complex, did not inhibit sensitization of target cells by PEMa. Second, PEMa was capable of sensitizing for lysis T2 mutant cells, which are defective in transport of peptides from the cytosol to the lumen of the ER for presentation by class I MHC. These results suggest that: i) PEMa is proteolytically processed in an endosomal compartment whereupon the epitope becomes available for association with class I MHC, perhaps by internalization of class I MHC and recycling to the cell surface, and ii) fusion proteins such as PEMa may be useful as vaccines intended to elicit a CTL response.