MOLECULAR IMMUNOLOGY OF VIRUS INFECTIONS

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Keynote Address (Joint)

N 001 IMMUNOLOGY BY VIRUSES, Rolf M. Zinkernagel, Peter Aichele, Manuel Battagay, Martin Bachmann, Daniel Brändle, Marie-Anne Bründler, Etienne Bucher, Christoph Burkhart, Giulia Freer, Urs Hoffmann Rohrer, David Kägi, Ulrich Kalinke, Thomas Kündig, Diego Kyburz, Demetrios Moskophidis, Ulrich Steinhoff, Hanspeter Pircher, Hans Hengartner; Institute of Experimental Immunology, University of Zurich (Switzerland)

Different viruses have coevolved with vertebrate hosts and their immune systems to reach evolutionarily balanced states; therefore many of these virus-host arrangements reveal limiting facets of the immune system. For example immunological unresponsiveness to viruses may be established by infection of the thymus by sequestration of antigens or presentation in an antigenic but not immunogenic form which are ignored by T cells, or by exhaustive differentiation of T cells in the periphery. Induction of antiviral T cells depends on proper antigen presentation and an environment with sufficient concentrations of cytokines; in their absence T cells are apparently neither induced nor anergized. T cell memory seems to be strictly dependent upon antigen persistence and so far no special memory T cell could be found. Mechanisms of T cell mediated antiviral protection also varies with the virus. For some either CD8+ or CD4+ T cell dependent cytokines may provide protection, dependent upon the site of viral replication. For others CD8+ T cells contact seems to be mandatory for virus elimination. The respective role of perforin, or of granzymes is still unclear. The implied cytolytic T cell effector pathway is always antivirally effective against cytopathogenic viruses. In contrast, immunopathological consequences of protective antiviral immune responses are seen whenever non- or poorly cytopathic viruses spread too widely before they can be stopped by antiviral cytotoxic T cells (CTLs), antibodies or other mechanisms.
Aggressive hepatitis by Hepatitis B virus in man or lymphocytic choriomeningitis virus (LCMV) in mice illustrate such a pathogenesis. Similarly the immunosuppression caused by LCMV, and possibly by HIV, may be caused by CTLs destroying antigen-presenting cells infected by LCMV. All these examples may easily impress as autoimmune disease if the infectious agents were not known. This conclusion is illustrated in a transgenic mouse model where the LCMV-glycoprotein (GP) is expressed as a new-self antigen in ß-islet cells of the Langerhans islets in the pancreas. Such mice do not develop diabetes sponteanously, but do so after infection with LCMV within 7-9 days. The immune system of these mice ignores apparently the new self antigen; these mice are therefore not immunologically tolerant to this new self antigen, but normaly no response is induced either. LCMV infection of antigen presenting cells causes prompt induction of CTLs and of diabetes. Again, did one not know the antigen or virus involved, this disease would be called an autoimmune disease. Accordingly, we speculate that possibly many so-called autoimmune diseases may actually reflect immunopathologies triggered by the unrecognized or unrecognizable trivial or special viruses or other infectious agents. Viruses also reveal fundamental aspects of antibody responses: B cells may be unresponsive to viral antigen because of lack of T help or because of anergy to a special form and organisation of the antigen. Also so-called affinity maturation, B cell memory, and the role of antibodies in protection against viruses reveal limitations of humoral responses. Collectively, the various balances between viruses and immune system, yield captivating and accurate data on role, efficiency and limitations of immune effector mechanisms in a biologically relevant setting.

Cell Biology of Antigen Processing and Presentation (Joint)

N 002 PRESENTATION OF VIRAL ANTIGENS TO CYTOTOXIC T LYMPHOCYTES, Jack R. Bennink[1], Igor Bacik[1], Cheryl Lapham[1], Daniele Arnold[2], Thomas Spies[2], Josephine Cox[1], Yuping Deng[1], Patricia Day[1], Robert Anderson[1], Nicholas Restifo[3], Laurence Eisenlohr[4], Fernando Esquivel[1], Jonathan W. Yewdell[1], [1]National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, [2]Dana Farber Cancer Institute, Boston, [3]National Cancer Institute, Bethesda, [4]Thomas Jefferson Cancer Institute, Philadelphia.

$CD8^+$ T-cells(T_{CD8+}) play an important role in controlling viral infections. T_{CD8+} recognize peptides of 8 to 10 residues derived from viral proteins located in the cytosol of infected cells. These peptides are recognized in a complex with class I molecules encoded by the major histocompatibility complex (MHC). Since the processing pathway begins in the cytosol and association with class I molecules occurs in an exocytic compartment proteolysis and peptide transport are thought to play key roles in presentation to T_{CD8+}. Two MHC encoded molecules termed Tap1 and Tap2 seem to specifically transport peptides from the cytosol into the intracellular compartment that contains class I molecules. To characterize the structure and function of the Tap genes we have inserted them into vaccinia virus. We have been studying the biochemical, immunocytochemical, and antigen specificity of the transporters in mutant cells failing to express one or both Tap genes. In addition, the endoplasmic reticulum location of peptide association with class I molecules in the exocytic pathway has been demonstrated using a vaccinia recombinant encoding an endoplasmic reticulum retained class I molecule.

N 003 MECHANISMS OF CLASS II MHC-RESTRICTED ANTIGEN PROCESSING, 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 αβ-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 αβ dimers are released to bind peptides derived from endocytosed proteins. The precise mechanisms involved in peptide generation and binding, and the route by which αβ-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 αβ-peptide complexes. We have determined that many αβ dimers from one such cell line, T2.DR3, are associated with a nested set of invariant chain-derived peptides, and that such αβ 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.

N 004 THE USE OF CRYSTAL STRUCTURE ANALYSIS OF RECOMBINANT CLASS I MHC-PEPTIDE COMPLEXES TO ANALYZE THE MOLECULAR BASIS OF PEPTIDE/MHC/TCR INTERACTIONS. Stanley G. Nathenson, Weiguo Zhang, Aideen C.M. Young and James C. Sacchettini. Departments of Microbiology and Immunology, and Cell Biology and Biochemistry, Albert Einstein College of Medicine, Bx 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.

N 005 PROCESSING AND PRESENTATION OF THE VESICULAR STOMATITIS VIRUS GLYCOPROTEIN IN ASSOCIATION WITH CLASS II MHC ANTIGENS. Carol S. Reiss[1], Lara M. Palevitz[1], Anna Wilson[1], and Stephanie Diment[2]. [1]Biology Department, New York University, New York, NY 10003, and [2]Pathology Department, New York University School of Medicine, New York, NY 10016.

Vesicular stomatitis virus (VSV) glycoprotein (G) has been shown to be a major target of both the humoral and cell mediated immune response of mice and other species to this pathogen. The cell mediated immune response is principally class II MHC restricted and includes both proliferative and cytolytic effector cells. We have studied the processing and presentation of this protein by murine B cells by both exogenous and endogenous routes. Published studies have demonstrated 1) an absolute dependence on newly synthesized Ia molecules, as emetine treatment completely prevents sensitization. 2) Acidification of the endosomal compartment is required, whether infectious virus or purified protein antigen is employed, as chloroquine, methylamine, and ammonium chloride pretreatment prevent uptake and degradation of purified protein, inactivated virions, and infectious virus. 3) Integrity of the vesicular compartments of the cell and their normal fluidity is necessary, as Brefeldin A treatment reversibly blocks sensitization of cells for CTL clone recognition and there is a reversible "cold block" in cells treated at 15°C. 4) A late post-Golgi block was observed in cells treated with acidification inhibitors, which presumably interfere with degradation of the invariant chain of Class II MHC. We have continued these studies by examining the role of the cytoskeleton in antigen processing and presentation both for effects on the processing cell and on the CTL clone recognizing Ia^d-peptide complexes on the surface of A20 cells. This has been probed with inhibitors of both microtubules (taxol, colchicine) and microfilaments (cytochalasian). As the cold treatment of cells had previously been shown to interfere with presentation, we expected microtubule-dissociation or -polymerization to substantially inhibit presentation. This was an inconsistent finding, even when the inhibitors were incorporated in assay medium to prevent reversible changes in the absence of drug; the tendency was to greater sensitivity to CTL effectors, not diminished recognition. Treatment of processing and presenting cells with microfilament-interfering drugs did not alter their recognition. However, cytochalasian treatment of T cells had profound inhibitory effects. This was not seen with either taxol or colchicine treatment of our clones, however, thus implying a central role for microfilaments (but not microtubules) in T cell cytoskeleton and lethal hit delivery. We have also been examining alternative sites (to late endosomes/early lysosomes) of protein degradation using 2 forms of the glycoprotein synthesized in the presenting cells and limited, due to either temperature sensitive mutation (tsO45 virus) or to genetic manipulation (vaccinia expressing a "poison tail" construct, generously provided by JK Rose, Yale). These protein variants are degraded within the lumen of the pre-Golgi endoplasmic reticulum, and peptides generated readily sensitize A20 cells for T cell recognition. The site of peptide acquisition and the maturation of the Ia complex are under intense study.

N 006 MANIPULATING THE ANTIGEN PROCESSING MACHINERY AND TUMOR IMMUNOLOGY.
NP Restifo, JW Yewdell, JR Bennink, I Bacik, Y Kawakami, F Esquivel, and SA Rosenberg.
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Some tumor cells in mouse and man clearly present peptide/MHC class I complexes recognized by CD8$^+$ CTL (T_{CD8+}). However, most cancers are not cured by T cell based immunotherapy. We have recently shown that the nonimmunogenic murine tumor, 101.WT, presented endogenously generated viral antigens in the context of MHC class I poorly, despite the presence of these antigens intracellularly in high quantities. Hypothesizing that this tumor might evade recognition by T_{CD8+} by failing to process and present tumor antigens in the context of class I molecules, we transduced 101.WT, with interferon-γ (IFN-γ) cDNA to create 101.NAT. Gene-modification increase class I expression by > 100-fold and reversed the viral antigen presentation deficit of 101.WT. Significantly, 101.NAT could be used to generate CD8$^+$ TIL that were therapeutically active *in vivo* against established pulmonary metastases from the wild-type tumor.

To examine whether or not such antigen processing deficiencies were present in *human* tumor cells, we used a recombinant vaccinia virus expressing mouse the K^d molecule. Because lysis by mouse T_{CD8+} was our read out, our assay independent of both the HLA type of the tumor, and the presence, or absence, of specific cellular proteins. Human small cell lung carcinoma (SCLC) cell lines, amongst others, processed endogenous antigens poorly. Pulse-chase experiments showed that MHC class I molecules were not transported by SCLC from the ER to the cell surface, suggesting that peptides were not available for binding to nascent MHC and β_2-microglobulin molecules. Consistent with this interpretation, northern blot analysis 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. Treatment of cells with IFN-γ enhanced expression of these mRNAs, and completely reversed the observed functional and biochemical deficits. In order to bypass the need for the processing of intracellular antigens, we constructed a vaccinia virus capable of endogenously synthesizing a 9 amino acid long "minimal determinant" from the nucleoprotein gene of influenza A/PR/8/34 preceded by an ER insertion signal sequence, (Anderson *et al*, J Exp Med 174:489, 1991) thus eliminating the need for both protease activity and transporter activity. The NP 9-mer preceeded by a signal sequence was very efficiently presented by the SCLC lines to T_{CD8+}. Thus, antigens, of precisely the right size, targeted to the endoplasmic reticulum, can bypass much of the antigen processing machinery.

Control of Viral Infection: T Cell Receptor Utilization

N 007 $\alpha\beta$ AND $\gamma\delta$ T CELLS IN INFLUENZA PNEUMONIA, Peter C. Doherty[1], Maryna Eichelberger[1], William Allan[1], David Woodland[1], Marcia Blackman[1], Alison Deckhut[1], Kieran Daly[1], Simon Carding[2], Peter Mombaerts[3], and Susumu Tonegawa[3], [1]St. Jude Children's Research Hospital and University of Tennessee, Memphis, College, Memphis, TN 38105, [2]University of Pennsylvania School of Medicine, Philadelphia, [3]Massachusetts Institute of Technology Center for Cancer Research, Cambridge.

Normal (H-2^b) mice given the HKx31 influenza A virus clear the infection from the respiratory tract within 7-10 days. Absence of either the $CD4^+$ or the $CD8^+$ populations throughout the course of the host response does not greatly modify the pattern of recovery, though depletion of both T cell subsets leads to persistence of the virus in lung and greater incidence of mortality. The $CD8^+$, H-$2D^b$-restricted virus-immune T cells are predominantly $V\beta 8.3^+$, combined with a range of $V\alpha$ phenotypes. Virus elimination proceeds, however, with apparently normal kinetics when all $CD4^+$ and $V\beta 8^+$ T cells are removed by mAb treatment. Mice (H-2^k) transgenic for a $V\beta 8.1$ gene also clear influenza virus by a $CD8^+$ T cell-mediated mechanism, even though $V\beta 8.1$ does not define the major $CD8^+$ T cell responder phenotype for influenza virus peptide in this haplotype. There is apparently considerable redundancy in the spectrum of possible TCR usage in the influenza-virus-specific $CD8^+$ T cell response. The mechanism whereby $CD4^+$ T cells clear virus is currently not understood, and could reflect either T cell help for antibody (local?) production or recognition of virus-infected type II alveolar cells which express MHC class II molecules as a consequence of exposure to cytokines. The role of the minority $\gamma\delta$ T cell population that is found in the lungs of influenza-infected mice has been much more difficult to assess. Analysis of TCR mRNA profiles, by both *in situ* hybridization and PCR, has shown that a limited range of $TCR\gamma$ and $TCR\delta$ phenotypes predominates in primarily and secondarily infected H-2^b mice. However, although these lymphocytes are activated to express mRNA for a variety of cytokine genes and are proliferating, their function (if any) is not understood. Mice that are homozygous for a $C\delta$ mutant gene have no problems in eliminating the virus, so this T cell subset is not essential for recovery. Even so, $C\alpha$ and $C\beta$ mutant mice, though more susceptible, show a variable capacity for delayed (day 20) clearance of influenza virus from the respiratory tract. This is complicated in the $C\alpha$ mutants set by the presence of a substantial population of $TCR\beta^+\delta^-$ lymphocytes in the inflammatory exudate. Hybridomas derived from these cells have not been shown to be virus-specific, though they do respond to the appropriate superantigens. Evidence of a virus-specific response, which discriminates cells infected with influenza A and influenza B viruses has, however, been found for both lymph node populations and a hybridoma derived from the $C\beta$ mutant mice.

N 008 **SPECIFICITY AND EFFECTOR MECHANISMS OF THE CELL MEDIATED ANTIVIRAL IMMUNE RESPONSE**

Hans Hengartner, Hanspeter Pircher, Daniel Brändle, David Kägi, Toni Aebischer, Demetrius Moskophidis, *Kurt Bürki, Rolf M. Zinkernagel

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The specific recognition of virus infected cells by cytotoxic T lymphocytes (CTL) is a prerequisit for the elimination of virus. We have analyzed the fine specificity of independently derived lymphocytic choriomeningitis virus (LCMV) specific CTL clones and compared the primary structures of their T cell receptor (TCR) α and β chains with the TCR used by T cells in LCMV infected H-2^b mice. The data indicated a preferential usage of the $V\alpha 4$ and $V\beta 10$ TCR-gene segments.

Another specificity analysis was carried out with our $V\alpha 2/V\beta 8.1$. TCR of CTL clone 14 expressing transgenic animals with H-$2D^b$ restricted anti-LCMV-glycoprotein peptide 33 to 41 (LCMV-GP 33-41) specificity. The $TCR\alpha$ and β chain usage was analyzed in TCR (P14) $V\beta 8.1$ - and $V\alpha$ 2 single chain transgenic animals respectively. Only upon LCMV-infection of these single transgenic mice the TCR $V\alpha$ and $V\beta$ - gene segment usage was strongly biassed towards the original TCR chain used. The infection of the TCR double transgenic animals with high doses of LCMV led to the selection of virus escape mutants with T cell epitope point mutations in the LCMV-GP 33-41 at three distinct positions. Such transgenic animals developed into mutant virus carriers.

Analogously to this in vivo selection, we also developed an in vitro method to select virus escape mutants. LCMV infection of fibroblasts in the presence of individual LCMV specific CTL clones or even of LCMV specific CTL from a secondary MLC resulted in the selection of virus escape mutants with individual amino acid exchanges at the relevant T cell epitope sites seen by the CTL used for the selection.

The specific recognition of the virus antigens as a first step of the complex effector mechanisms of CTL leads to the cytolysis of the virus infected cells. In extensive in situ hybridization and immunohistochemical studies we demonstrated the presence of perforin and granzyme expression in $CD8^+$ lymphozytes at the site of LCMV infected cells in the brain and liver. Further experiments supported the importance of perforin to cope with virus infections in vivo.

N 009 MMTV AND T CELL IMMUNITY, Brigitte T. Huber, Ulrich Beutner, Meena Subramanyam, Natesan Mohan and David Mottershead, Tufts University School of Medicine, Boston, MA.

Superantigens (SAG) represent a new class of T cell stimulating antigens. Mls-1, encoded by the *Mtv-7 sag* gene, is the prototype of endogenous retroviral SAGs. Although Mls-1 has been functionally defined for over two decades, the biochemical nature of this molecule has remained elusive until recently. Mls-1 induces a strong *in vitro* proliferative response of T cells expressing the Vβ6 or 8.1 chains. *In vivo*, Mls-1 causes the deletion of immature T cells bearing these particular T cell receptors, as well as the Vβ7 or 9 chains. We have cloned and sequenced the *Mtv-7 sag* gene. Comparison of the deduced amino acid sequence with that of other MMTV *sag* genes suggests that the polymorphic 3' end encodes the T cell receptor Vβ specificity. A 14 amino acid peptide from the carboxy terminus was, therefore, used to generate an Mls-1 specific anti-peptide mAb. This mAb reacts with a recombinant baculovirus product of *Mtv-7 sag* and blocks proliferation of T cells in response to Mls-1 specific stimulation. In addition, it recognizes the Mls-1 protein on the surface of LBB.A and *Mtv-7 sag* transfected cell lines. We have demonstrated that Mls-1 is efficiently presented by the human HLA-DR1 molecule to murine T cells. Similar to the murine system, a hierarchy has been observed in the ability of various HLA class II molecules to present Mls-1. We have generated a soluble Mls-1 molecule which will be used for binding studies to class II and T cell receptor molecules.

N 010 HEMAGGLUTININ SPECIFIC T CELL RECEPTOR REPERTOIRE IN NATURAL INFECTION; PARADOX OR PARADIGM? D. Brian Thomas, Sarah, A. Butcher, Christine M. Graham, and Claire A. Smith, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

A majority of $CD4^+$ T cell clones elicited by natural infection with influenza A virus (H3 subtype) recognise variable regions of the hemagglutinin HA1 subunit that have featured in antigenic drift, and a single Class II restriction element (A^k or A^d) can present a variety of synthetic peptides corresponding to major antigenic rites. Despite the diversity of antigenic recognition, the TCR repertoire was skewed to exclusive $V_\beta 1$ gene usage with conservation of junctional region residues. TCR specific for HA1 48-67, 56-76, 120-139, 186-205, or 177-199 used $V_\beta 1$ in association with four distinct J_β elements and different $V_\alpha J_\alpha$ elements. Moreover, junctional regions of β chains from different donors contained the conserved consensus sequence Q-X-G. Since hemagglutinin does not exhibit features of a superantigen, we propose that a natural route of infection elicits a novel TCR response to viral envelope glycoproteins.

Structural Aspects of Viral Interaction with Receptor and AB

N 011 INTERACTION OF ANTIBODIES WITH INFLUENZA VIRUS NEURAMINIDASE, Gillian M. Air, Jacqueline M. Nuss, Patricia Bossart Whitaker, Pamela S. Pruett, Department of Microbiology, University of Alabama at Birmingham.

An antibody known as NC41, made against influenza virus neuraminidase of the N9 subtype, has been extensively characterized antigenically (1) and by X-ray crystal structure analysis of the NC41 Fab in complex with neuraminidase (2). The crystal structure shows that the antibody contacts 19 amino acid residues on the NA surface which are localized on five polypeptide loops surrounding the enzyme active site. Since the coordinates of the structures were not available, we determined the crystal structure of the N9 influenza neuraminidase *a priori*, and used this structure as a starting point to design experiments to determine the relative importance of each of the contacting amino acids in the interaction with antibody and their role in virus escape from the antibody. Mutant NA genes were constructed and the proteins expressed from these were tested for effects of the replacement on NC41 binding. Our data revealed that NAs with changes at 368, 400, and 434 completely lost NC41 recognition. NAs with side chains replaced at residues 346 and 373 exhibited binding reduced to less than 50% of wildtype binding. Changes in seven other contacting residues, including substituted side chains which differed considerably from wildtype NA in size and charge, had no significant effect on NC41 binding.

These results indicate that only a few of the many residues which make up an epitope are crucial for interaction and provide the critical contacts required for antibody recognition. This implies that antibody escape mutants are only selected if they contain changes at these crucial sites.(3)

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3. Nuss, J., P. Whitaker, and G. Air. 1993. Identification of critical contact residues in the NC41 epitope of a subtype N9 influenza virus neuraminidase. Proteins: Structure, Function and Genetics in press.

New Strategies for the Molecular Understanding of Viral Pathogenesis

N 012 COORDINATION OF ACUTE NATURAL KILLER (NK) AND T CELL RESPONSES TO VIRAL INFECTIONS. Christine A. Biron, Rika Ishikawa, Helen C. Su, and Jordan Orange. Division of Biology and Medicine, Brown University, Providence, RI 02912.

NK cells respond early whereas T cells respond late during viral infections. Our laboratory has been studying the regulation of these populations during experimental infections of mice with either lymphocytic choriomeningitis virus (LCMV) or murine cytomegalovirus (MCMV). The endogenous production of cytokines and their biological functions for regulating acute immune responses are being characterized. Although interleukin-2 (IL-2) is capable of inducing NK cell proliferation, it does not appear to be a major mediator of the NK cell responses to viral infection as the peak IL-2 production during infection correlates with late T cell responses and as NK cell activation and proliferation are resistant to cyclosporin A. In vivo NK cell proliferation does correlate with the production of virus-induced interferons (IFNs) and the NK cell response induced during LCMV infection can be blocked by treatment with antibodies neutralizing IFNs α/β. NK and T cells are both induced to express high levels of IFN-γ at times of peak activation during infection. Biologically active transforming growth factors-β (TGF-β) are also produced at early times coinciding with the decline of NK cell activation and the increase in T cell activation during infection. Experiments evaluating the sensitivity of the virus-induced lymphocyte responses to TGF-β-mediated inhibition have demonstrated that NK cell proliferation is 100-fold more sensitive than is T cell proliferation. Taken together, these studies demonstrate that a variety of cytokines are induced during infection and that these cytokines can act to coordinate acute NK and T cell proliferative responses.

Cellular interactions regulating these lymphocytes and their regional localization were characterized in tissue sections prepared from infected mice. Histological examination revealed dramatic changes in splenic architecture during LCMV infection. At times coinciding with IFN production and NK cell activation and proliferation, white pulp areas were increased and leukocyte concentrations in red pulp areas were decreased. To evaluate IFN expression and the contribution of IFN to the induction of the observed morphological changes, mice were treated with the chemical inducer of IFN, poly inosinic-cytidylic acid (poly I:C). As early as 3 h after poly I:C administration, biologically active IFN was present in spleen and serum. Neutralization studies and Northern blot analyses demonstrated that IFN-β was preferentially induced. Histological examination revealed poly I:C-induced splenic changes comparable to those observed during infection. Spleen weights and splenic leukocyte yields remained relatively constant. Cell transfer experiments with DiI-labelled cells, demonstrated that IFNs, poly I:C, and/or LCMV-infection enhanced the accumulation of cells migrating from blood to white pulp regions. The changes in splenic leukocyte distribution were shown to be a result of IFN induction as treatment with anti-IFN antibodies inhibited the changes. In situ hybridization and immunohistochemical staining localized the cells expressing high levels of IFNs first to red pulp regions and later to white pulp regions. Cells expressing NK cell surface markers were primarily localized in red pulp regions. Splenocytes from SCID mice lacking T and B cell did not contain populations of cells capable of migrating into normal white pulp regions. The results indicate that migration of lymphocytes into white pulp regions of the spleen is a major IFN-induced change occuring at early times during infections. Experiments are underway to determine the mechanisms responsible for the observed leukocyte re-distribution and the role re-distribution might play in controlling immune responses to infection.

N 013 IMMUNE RESPONSES OF β_2-MICROGLOBULIN DEFICIENT MICE, Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 25599

Mice with a neo^r insertion in their β2 microglobulin genes lack β2 microglobulin, and hence cannot produce normal Major Histocompatibility Complex class I proteins on their cell surfaces. This lack of class I expression during T cell ontogeny results in the failure to positively select substantial numbers of αβ T cell receptor, CD8+ T cells in peripheral lymphoid organs. Normal mice are susceptible to lymphocytic choriomeningitis virus when challenged intracranially because they produce CD8+ cytotoxic T lymphocytes (CTL) which can react with virus infected cell in the central nervous system, and ultimately cause encephalitis and death. In contrast, β2 microglobulin deficient mice which do not produce significant number of CD8+ CTL, were expected to be resistant to LCMV challenge as are acutely CD8 depleted mice. Surprisingly, these mice died from LCMV disease, although more slowly than normal mice. We further showed that these mice produced CD4+ CTL able to lyse LCMV infected targets only when the target cells expressed class II as well as class I on their cell surface. We extend these results to examine the kinetics of induction of CTL and of NK activity. In accord with the kinetics of LCMV disease induction, we have found that CD4+ CTL are induced in β2 microglobulin deficient mice more slowly than CD8+ CTL in normal mice. Antigen presentation in these animals may not be via the classical endogenous pathway. We have shown that class II+ target cells can be sensitized for lysis by incubation with inactivated LCMV and that the presentation is sensitive to chloroquine, suggesting that shed virus is important in providing targets for CTL and presentation may occur via the exogenous pathway.

We have also studied the response to *Listeria monocytogenes*. β2 microglobulin deficient mice can respond to Listeria, and are resistant to lethal infection *in vivo*, although these mice do have higher levels of bacteremia early.

These experiments together argue for considerable plasticity of the immune system. CD4+ T cells which are only infrequently found in the cytotoxic population are recruited for CTL activity in mice where very few competent CD8+ T cells are present. This indicates that in the absence of CD8+ T cells, CD4+ clones, which would normally be displaced by higher affinity CD8+, cytotoxic T cells, can expand. These mice are thus extremely interesting for the determination of the ultimate functional repertoire of CD4+ cells.

Immune Suppression and Anergy (Joint)

N 014 THE ROLE OF CELL DIVISION IN THE INDUCTION OF T CELL ANERGY, Marc K. Jenkins, Julia Johnson, Besty Kearney, Dimuthu R. DeSilva, University of Minnesota, Minneapolis.

Secretion of maximal amounts of IL-2 by CD4+ Th1 clones depends not only on T cell antigen receptor (TCR) signaling but also on non-specific costimulatory signals from antigen-presenting cells (APC). When both signals are provided, for example when dendritic cells present antigen, the T cells undergo multiple rounds of cell division in response to the IL-2 that they produce, returning eventually to a resting state in which they are ready to respond to antigenic stimulation once again. We and others have shown that the T cell surface molecule CD28 transduces a costimulatory signal either when occupied by monoclonal antibodies or by its ligand, the APC surface molecule B7. CD28 crosslinking greatly enhances the amount of IL-2 and other lymphokines produced by T cells stimulated through their TCRs. CD28 signal transduction involves a cyclosporin A-resistant biochemical pathway distinct from the hydrolysis of inositol phospholipids.

If Th1 cells do not proliferate following TCR signaling they become unable to produce IL-2 in response to subsequent antigenic stimulation. Unresponsiveness (also called anergy) results when the TCR is occupied and CD28 signaling does not occur, for example when B7-negative APCs present antigen or when the CD28/B7 interaction is blocked by an uncrosslinked anti-CD28 antibody. Anergy can also be induced when the TCR is occupied and subsequent cell division is inhibited. This occurs when Th1 cells are stimulated with peptide antigen and APC in the presence of anti-IL-2 and anti-IL-2 receptor antibody or agents that inhibit IL-2 responsiveness such as rapamycin, genistein, or PGE_2. Chronic stimulation of Th1 cells with anti-CD3 antibody when APC are present results in IL-2 production, however the capacity of the T cells to proliferate is inhibited by an unknown mechanism. Anergy is also induced under these conditions. Therefore, based on these results the optimal situation for lymphokine production, clonal expansion, and retention of subsequent responsiveness by Th1 cells is transient antigen-presentation by a B7-expressing APC.

Using a differential screening approach we have recently shown that although T cells stimulated with anti-CD3 antibody in the absence of APC produce very little IL-2 mRNA, they do produce large numbers of transcripts that encode macrophage inflammatory protein-1α and another unknown member of the small cytokine family. The accumulation of neither of these transcripts is enhanced by CD28 costimulation. Although the functional significance of these findings is presently unclear it is possible that these cytokines may be involved in the unresponsive state either by preventing T cell responsiveness to IL-2 or by modifying the costimulatory properties of APC.

N 015 ROLE OF T CELL TOLERANCE IN THE PERSISTENCE OF HEPATITIS B VIRUS INFECTION, David R. Milich, Toshiyuki Maruyama, Joyce Jones, and Janice Hughes, The Scripps Research Institute, La Jolla.

Infants born to HBeAg-positive HBV carrier mothers invariably become persistently infected. To investigate the role of immunologic tolerance mechanisms in chronic infection of the newborn, we have generated HBeAg-expressing transgenic mice (B10.S/e31). These mice were tolerant to both HBeAg and the nonsecreted HBcAg at the T-cell level. Furthermore, nontransgenic littermates born to HBeAg-expressing mothers showed reduced T-cell responses to HBc/HBe antigens, suggesting that tolerogenic HBeAg may transverse the placenta. Tg mice did not produce antibody to HBeAg but did produce IgM antibodies to HBcAg via a T cell-independent pathway. The coexistence of tolerance to HBc/HBe T cell determinants and production of antibody to HBcAg *in vivo* parallel the immunologic status of neonates born to carrier mothers. The maintenance of T cell tolerance to HBc/HBe antigens required the continued presence of the tolerogen and in the absence of HBeAg persisted for less than 16 weeks. The reversibility of T cell tolerance to HBc/HBe antigens may explain the inverse correlation between age of infection and rates of viral persistence. These observations suggest that a function of the HBeAg may be to induce immunologic tolerance *in utero*. Expression of HBeAg may represent a viral strategy to guarantee persistence subsequent to perinatal infection. Further studies in F_1 hybrid Tg mice (B10 x B10.S/e31) illustrated that "self" tolerance to HBeAg is variable depending on the MHC genotype. A dominant T cell site on HBeAg (p120-131 and I-A^s-restricted) is tolerogenic, whereas a proportion of T cells recognizing p129-140 in the context of I-A^b evade induction of tolerance, persist in the periphery, and can be activated *in vivo* by a single injection of the 12 residue T cell self-peptide. Furthermore, the self-reactive T cells can cooperate with self-reactive, HBeAg-specific B cells to mediate *in vivo* production of autoantibody sufficient to neutralize detection of the autoantigen in serum. This model illustrates that T cells specific for an immunnogenic T-cell site on a nonsequestered autoantigen can escape induction of tolerance (i.e., are not deleted or anergic) and, more importantly, can mediate autoreactivity *in vivo*. These murine studies suggest that chronic HBV (CH-B) carriers may also possess "quiescent" T cells that have evaded tolerance induction. In order to examine the relevance of this murine model, 200 HBeAg-positive CH-B carriers with varying degrees of liver disease (ie. CAH, CPH, ASC) were analyzed with novel and sensitive serological assays capable of detecting serum anti-HBe and anti-HBs antibodies regardless of the simultaneous presence of their respective antigens. All 200 patients were seronegative for anti-HBs and anti-HBe by commercial assay. This analysis revealed: (1) virtually all CH-B patients with liver disease and approximately 50% of CH-B patients without liver disease demonstrated ongoing humoral immune responses specific for HBeAg, HBsAg, and pre-SAg in addition to variable responses to HBcAg; (2) three serologic profiles of CH-B infection were identified; (3) these "silent" immune responses may occur for a number of years prior to liver disease or viral clearance; (4) the quantitative or qualitative characteristics of the immune responses correlate with clinical status; and (5) this array of humoral immune responses appears to be mediated exclusively by HBeAg-specific T helper cells. These results redefine the serology of CH-B infection. The serological data is also consistent with the hypothesis that T cell tolerance to HBeAg plays a role in chronicity, and that HBeAg-specific T cells emerging from the tolerant state mediate liver injury and HBV clearance in CH-B infection.

N 016 IMMUNE REGULATION BY T CELL CYTOKINES, Tim Mosmann, Department of Immunology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The immune system can respond to infectious agents by a variety of effector mechanisms, each of which is appropriate for different types of pathogen. The decision between these responses is under stringent regulation, and part of this control is mediated by the cross-regulation of T cell subsets secreting different cytokine patterns. Two major T helper subsets are TH1 and TH2 cells, which differ markedly in the patterns of cytokines that they secrete after antigen stimulation. These patterns are mainly responsible for the different functions of the two subtypes. TH1 cells induce an inflammatory response, including activation of granulocytes and macrophages. In contrast, TH2 cells are excellent helpers for B cell antibody production, and in the absence of a significant Interferon γ response, TH2 cells induce a strong allergic response due to secretion of IL4, IL5 and IL10. Other T cell subtypes also exist, but the functions of these cells, and the interrelationships between them, are less well understood. The functions of TH1 and TH2 cells are often reciprocal, especially in strong immune responses such as occur during many parasite infections. Subset-specific cytokines are involved in this regulation: IL10 inhibits TH1 responses at the level of macrophage activation and indirectly, by inhibiting TH1 cytokine production; IL4 enhances differentiation of TH2 but not TH1 cells; and IFNγ inhibits the proliferation of TH2 cells. We have recently been studying the functions of another TH2-specific cytokine, P600, which was initially characterized as an induction-specific cDNA clone isolated from TH2 cells. We have now expressed recombinant P600 protein and characterized the functions of this cytokine. P600 does not have activity in many of the assays in which TH2-specific cytokines are active. However, P600 enhances the production of an adherent cell population from bone marrow precursors. The resulting cells have high expression of the macrophage markers MAC1 and F4/80. Although these cells are not phagocytic for antibody-coated erythrocytes, they are effective antigen-presenting cells for T cell clones specific for particulate, soluble or alloantigens.

Recovery from Infection by Viral Clearance: Different Strategies

N 017 CONTROL OF VIRUS INFECTIONS BY CYTOTOXIC LYMPHOCYTES. Raymond M. Welsh, Hugh I. McFarland, Sharon R. Nahill, Enal S. Razvi, and Liisa K. Selin. Univ. Massachusetts Medical Ctr, Worcester, MA 01655.

The repertoire of the cytotoxic lymphocyte response to infection was studied in mice infected with lymphocytic choriomeningitis virus (LCMV). At early stages of infection, LCMV stimulated activated NK cells and non-MHC-restricted cytotoxic T cells (CTL), whereas at later stages both virus-specific and allospecific CTL were induced. Antiviral activity of the NK cells and of non-MHC-restricted CTL was shown in vivo against murine cytomegalovirus but not against LCMV, whose replication in vivo was controlled by virus-specific CTL. Concomitant with the induction of the virus-specific CTL was a 10-40-fold increase in the number of $CD8^+$ T cells per spleen and a deficiency in the ability of the T cells to proliferate in response to mitogens. The T cell number subsquently declined, and responsiveness to mitogens returned. Limiting dilution analyses of CTL generated in vivo revealed a high frequency of virus-specific CTL cross-reactive with alloantigens. Activated CTL and subsequent memory CTL expressed CD11b and IL-2 receptors. The activated CTL isolated from mice during acute infection were triggered to undergo apoptosis upon TcR/CD3 cross-linking. Culture of activated CTL or resting immune CTL in vitro with IL-2 greatly enhanced apoptosis upon subsequent TcR/CD3 cross-linking. This suggests that infection-induced IL-2 may sensitize memory T cells directed against other antigens to die upon exposure to their specific antigen, thereby providing a mechanism for virus-induced immune suppression to unrelated antigens. Upon challenge of LCMV-immune mice with Pichinde virus or vaccinia virus, LCMV-specific memory CTL were reactivated, with some CTL clones derived in limiting dilution assays surprisingly showing cross-reactivity between the viruses. Mice receiving only one virus generated very few if any clones cross-reactive with two viruses, suggesting that the specificity of the CTL response to a virus is influenced by previous infections. We propose that memory CTL with remote cross-reactivities with the inducing antigen are commonly elicited during virus infection and that T cell memory to one virus may be either preserved or deleted by infection with a subsequent virus bearing T cell peptides only remotely cross-reactive with the first virus.

Pathogenesis and Persistence

N 018 THE HLA CLASS I RESTRICTED CYTOTOXIC T LYMPHOCYTE RESPONSE TO THE HEPATITIS B VIRUS, Francis V. Chisari[1], Patricia Fowler[1], Ramin Nayersina[1], Andreas Cerny[1], Gabriele Missale[1,2] and Carlo Ferrari[2]. [1]The Scripps Research Institute, La Jolla, CA 92037, [2]University of Parma, Parma, Italy.

The cytotoxic T lymphocyte (CTL) response to HBV encoded antigens is thought to be responsible for liver cell injury and viral clearance during HBV infection. Using a strategy involving an initial stimulation of peripheral blood mononuclear cells (PBMC) with HBV-derived synthetic peptides, followed by restimulation with HLA class I matched stable HBV transfectants, we have recently demonstrated, at the clonal level, that most patients with acute viral hepatitis develop a polyclonal HLA class I restricted CTL response to multiple HBV encoded antigens. In contrast, the response is not detectable in patients with chronic HBV infection. Our results may be summarized as follows. First, we have identified a nucleocapsid epitope (STLPETTVVRR) that is dually restricted by the HLA-A31 and Aw68 alleles. We have shown that both of these class I alleles recognize precisely the same sequence and that they both bind to the same agretopic residues, while the T cell receptors of independently derived CTL clones recognize different epitopic residues. Second, we have identified ten HLA-A2 restricted CTL epitopes within the viral envelope, nucleocapsid, polymerase and X proteins, and we have shown that these epitopes contain the HLA-A2.1 allele specific binding motif described by Rammensee's group. Third, we showed that the HBV specific CTL response in individual patients with acute viral hepatitis extends to multiple viral epitopes and different restriction elements; i.e., it is polyclonal, multispecific and polymorphically restricted, thereby minimizing the chances that CTL "escape mutants" will emerge in this setting. Fourth, we showed that there is a hierarchy of responsiveness to the HLA-A2 restricted epitopes in infected patients; ie two of the epitopes (core FLPSDFFPSV and envelope WLSLLVPFV) are recognized by the majority of infected patients while the remainder are seen by a minority of the patients we have studied thus far. This hierarchy does not correspond to the HLA-A2.1 binding affinity of the peptides, and therefore is probably determined at the level of virus sequence, antigen processing or the T cell repertoire. Fifth, we demonstrated that CTL nonresponse to a particular peptide sequence in an otherwise responsive patient may reflect infection by a virus that expresses a variant sequence in the region of the epitope. For example, several patients who failed to respond to the immunogenic envelope peptide GLSPTVWLSV were shown to be infected by a variant in which the C-terminal valine anchor residue was substituted by an alanine. Interestingly, the variant peptide was found to bind as well to HLA-A2.1 as the prototype peptide, and the variant peptide itself was immunogenic in at least one patient who was infected by the variant virus. Thus, it would appear that the conservative substitution of an alanine for a valine at the C-terminal anchor residue may have paradoxically affected the ability of the peptide to be recognized by the CTL receptor more than it affected its binding to the HLA-A2 molecule. Finally, several of these epitopes overlap with functionally and immunologically important domains within the viral nucleocapsid and envelope proteins. For example, the HLA-A2 restricted envelope epitope FLLTRILTI contains the core residues (RILTI) of an HLA DPw4 restricted helper T cell epitope previously described by Celis, et al. Additionally, 2 other envelope epitopes overlap with an important topogenic sequence within the transmembrane orientation of HBsAg, and the dually restricted nucleocapsid epitope described above includes sequences involved in the nuclear localization and genome encapsidation functions of the nucleocapsid protein. The generation of a CTL response to such epitopes might yield a double benefit to the host: first, by destroying the infected cells and, second, by precluding the emergence of viable mutants. A possible by-product of this research may be the rational design of CTL-based therapeutic strategies to terminate persistent HBV infection. The same strategy can be applied to study the CTL response to any human pathogen whose genome has been cloned and sequenced.

N 019 EVASION MECHANISMS OF CYTOMEGALOVIRUS FROM IMMUNE CONTROL, Ulrich H. Koszinowski[1], Hartmut Hengel[1], Margarita del Val[2], Thomas Ruppert[1], Pero Lucin[1], Stipan Jonjic[3], Maren Eggers[1], Gernot Geginat[1], and Maria Rapp[1], [1]University of Ulm, FRG, [2]Centro Nacional de Microbiologia, Virologia e Inmulogia Sanitarias, Madrid, Spain, [3]University of Rijeka, Croatia.

Cytomegalovirus (CMV) causes lethal disease in the immunocompromised host whereas infection of the immunocompetent is usually asymptomatic. Yet, even in presence of normal immune control, episodes of productive infection do occur and cause the horizontal transmission of the virus. Thus, the physiological antiviral immune response protects vital tissues but executes a less stringent control over others. By studying the infection of the mouse with the murine CMV (MCMV) we wish to unravel the relative contribution of the specific immune effector mechanisms involved.
Cell transfer experiments showed that only CD8$^+$ T lymphocytes dominate in the protection against lethal infection. This subset appears not to require CD4$^+$ T lymphocytes for activation because CD4$^+$ cell deficient mice generate a comparable CD8$^+$ response and control the infection. The productive infection in the salivary gland, however, remained completely refractory to the activity of the CD8$^+$ T lymphocyte subset. Despite of high titers in this organ the CD8$^+$ T lymphocytes prevent the spread to other tissues. Cell transfer studies and experiments with CD4$^+$ deficient mice showed that in the salivary gland CD4$^+$ T lymphocytes are essential effector cells, and that they can operate in absence of the CD8$^+$ subset. Thus, in the normal host, clearance of vital tissues and control of horizontal spread is under control of different T lymphocyte subsets.
CD4$^+$ subset control of salivary gland infection requires the activity of TH1 cells. This became evident from the blockade of effector function after administration of antibodies against IFN gamma. IFN gamma, in concert with TNF, represents a powerful inhibitor, active in the late phase of the viral replication cycle. The molecular identification of the major targets for the CD4$^+$ subset are under study.
Analysis of the specificity of the CD8$^+$ subset led to the definition of the naturally processed peptide derived from the dominant antigen, an immediate early protein. This nonameric peptide, when flanked by appropriate sequences in a carrier protein can serve as a vaccine. Remarkably, viral functions of the early phase prevent presentation of this peptide. This inhibition is due to the retention of the nascent peptide loaded MHC complex at the ER/cis Golgi level. The progress of the immune response counteracts this evasion mechanism because antigen presentation by cells pretreated with IFN gamma are capable to export the peptide/MHC complex.

N 020 IMMUNOPATHOGENESIS OF OCULAR HERPETIC, Barry T. Rouse, Department of Microbiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996-0845

Infection of the eye with Herpes simplex virus is a major cause of blindness. Its cause appears to be an immunopathological response to viral antigens since lesions persist beyond the time when virus can be recovered from corneal tissues, the disease can be managed with immunosuppressive drugs and in experimental animal models herpetic stromal keratitis (HSK) fails to occur in athymic mice[1]. Results of experiments using antisera to suppress T cell subset function *in vivo* indicate a principal role for CD4$^+$ T cells in mediating the pathology with CD8$^+$ T cells perhaps acting to clear infected cells and mediating recovery. Analysis of the inflammatory ocular exudates indicate that only T cells of the CD4$^+$ subset can be demonstrated and that on the basis of their induced cytokine profile, the cells appear to be mainly CD4$^+$ cells of the Thl phenotype. Whereas, HSK fails to occur in SCID mice, reconstitution with immune T cells provides a disease essentially identical to that occurring in intact mice. Again CD4$^+$ T cells with the cytokine profile of Thl cells appear principally responsible for lesions. Cells taken from draining lymphoid tissue of mice with ocular infection appeared more active at reconstituting HSK. The role of some adhesion molecules in localizing CD4$^+$ T cells to the corneal strome will be described as will the results of experiments to modulate the stromal reaction with cytokines, anticytokines, cytokine receptors and other approaches. Evidence will be presented to support the hypothesis that T cells cause pathology by releasing cytokines, such as gamma interferon, that in turn activate cells such as macrophages to cause tissue damage by nitric acid radical production as well as by the release of cytotoxic cytokines.

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Pathogenesis of Lentivirus Infections

N 021 NEUROPATHOGENESIS OF SIMIAN IMMUNODEFICIENCY VIRAL INFECTION, O. Narayan, S. Joag, M.C. Zink, J.E. Clements, R.J. Adams, Johns Hopkins University School of Medicine, Baltimore, MD 21205

SIV_{mac} is a primate lentivirus that causes persistent infection and clinical and pathological syndromes in macaques very similar to those caused by HIV in humans. However, factors responsible for the type of disease, the length of the incubation period and the severity of the diseases are not understood. We explored these questions in macaques infected with molecularly cloned SIV_{mac}239. This virus is tropic mainly for CD4+ T-cells and not macaque macrophages. Inoculation of the virus into animals resulted in massive productive infection in CD4+ T cells in lymphoid tissues but no infection in cells of the macrophage lineage. Animals developed lymphadenopathy and AIDS. Inoculation of this virus I.C., failed to result in infection in the CNS. This provided evidence that virus which is purely lymphocyte-tropic is capable of causing AIDS but not neurological disease. Passage of the virus in animals sequentially, using bone marrow cells to inoculate brain, resulted in development of a highly neurovirulent variant which is highly macrophage-tropic.
Analysis of virus-cell interactions during infection with lymphocyte-tropic 239 and the macrophage-tropic neurovirulent variant R71 showed that both viruses caused intense activation of T cells during the first 4 weeks of infection. Between 1% and 5% of the PBM expressed IL2-R during this period. This was accompanied by viremia and appearance of infected cells, but not cell-free virus, in the CSF during the first week of infection. Infected cells in the CSF were no longer detectable after PBM ceased expressing IL2-R. These data suggest that increased trafficking of infected T cells is the basis of neuroinvasion by the lentiviruses, irrespective whether the agents can replicate in the brain. The correct viral genome type and macrophage-tropism of the virus are however not the sole determinants of neurovirulence. Our data suggest that, similar to the requirement for activation of T cells before L-tropic virus can replicate permissively, macrophages in brain need to be activated before they will support productive infection with a potentially neurovirulent virus. Encephalopathy results only when both requirements are fulfilled.

N 022 TH-1 AND TH-2 TYPES OF IMMUNE RESPONSES IN SUSCEPTIBILITY TO HIV INFECTION AND PROGRESSION TO AIDS,
Mario Clerici[1], Jay A. Berzofsky[2], Robert L. Coffman[3], and Gene M. Shearer[1], [1]Experimental Immunolgy and [2]Metabolism Branches, National Cancer Institute, Bethesda, and [3]DNAX Research Institute, Palo Alto.

The most dramatic immunologic effect of infection by the human immunodeficiency virus (HIV) is the severe depletion of CD4+ T helper cells (TH). However, a complex pattern of TH dysunction occurs before CD4+ cells fall to critical levels, such that TH function, assessed by interleukin 2 (IL-2) production, is first lost to recall antigens (including to HIV antigens), followed by loss of response to HLA alloantigens, and finally by lack of IL-2 production in response to PHA. The loss of recall antigen-stimulated IL-2 production occurs concomitant with the loss of PHA-stimulated IFN-γ production, suggesting a lack of TH-1 function. We also noted sequential increases in PHA-stimulated IL-4 and IL-10 production, suggesting an increase in TH2 function. Because these events are predictive for the onset of AIDS, it appears that there is a TH-1→ TH-2 switch in the progression toward AIDS. Furthermore, we independently observed that PBMC from a high proportion (45-75%) of seronegative, HIV-exposed individuals exhibit strong TH 1 activity to HIV *env*-synthetic pepides. The seronegative, HIV-exposed groups studied included: gay men; IV drug users; health care workers; and newborn infants of HIV+ mothers. Only 2% of presumed unexposed individuals exhibit similar TH activity. Considered together, these two seemingly unrelated sets of data suggest that immunoregulatory cytokines contribute to TH dysregulation after HIV infection, and that HIV-specific TH-1 type immunity may be protective against HIV infection.

Vaccine Design and Strategies (Joint)

N 023 SYNTHETIC PEPTIDE STRATEGIES IN THE INDUCTION AND ANALYSIS OF T-CELL RESPONSES TO HIV AND TUMORS,
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Recent advances in the understanding of antigen processing and presentation facilitate the identification of antigenic sites recognized by CD4+ helper T cells and by CD8+ cytotoxic T lymphocytes (CTL), and suggest methods to optimize the activity of these for construction of synthetic peptide vaccines aimed at eliciting T-cell immunity. We have characterized the functional role of each residue in an HIV envelope peptide recognized by helper T cells from mice and humans. Only a few of the amino acid residues were necessary for binding to class II major histocompatibility complex (MHC) molecules and immunogenicity, as shown using a peptide consisting of only four of the original residues, with the rest replace by Ala. The other residues were more remarkable for the negative impact they could have. Several of the non-critical residues could play a deleterious role if replaced with the wrong substitution, and removal of one negative charge in the natural sequence led to a peptide 100-fold more potent for stimulating specific T cells and for binding to MHC molecules, emphasizing the importance of adverse interactions in peptide-MHC binding. Similarly for a CTL site presented by multiple murine and human class I MHC molecules, CTL specificity was shown to focus on the distinction between aromatic and aliphatic side chains at one position. This result, in turn, allowed the construction of chimeric peptides that induced broadly crossreactive CTL responses for multiple strains of HIV-1, and that may reduce the risk of outgrowth of escape mutants. Thus, understanding the molecular basis for MHC binding and T-cell recognition can facilitate improvements in synthetic vaccines beyond the use of just natural peptide sequences from the pathogen.

Another problem in the design of a synthetic vaccine is the polymorphism of the MHC, leading to differential recognition of different antigenic determinants of the same protein by individuals of different MHC types. One approach to overcome this problem that we have used for helper T-cells specific for the HIV-1 envelope protein was to identify multideterminant regions encompassing several overlapping determinants presented by different MHC molecules. Synthetic peptides spanning such multideterminant regions elicited T cell responses in multiple strains of mice and in humans of multiple HLA types. An alternative approach is to identify truly promiscuously recognized antigenic determinants. We have found one HIV CTL determinant to be presented by four different class I MHC molecules in mice and by at least two different class I molecules in humans. At least in mice, the same minimal core 10-residue sequence is presented by all four class I molecules. These antigenic sequences can then be coupled to produce a synthetic vaccine eliciting neutralizing antibodies and CTL in animals (or humans) of multiple MHC types. Thus, analysis of the T-cell responses to viral proteins can contribute to the rational design of new vaccines. Similar approaches can be applied to tumor antigens recognized by T cells, including mutant oncogene products and viral oncogene products as potential targets for vaccine prophylaxis and immunotherapy of cancer.

N 024 POOL SEQUENCING FOR DETERMINING MHC PEPTIDE MOTIFS
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MHC molecules are peptide receptors of particular specificity. Many MHC class I molecules, for example, require nonapeptides with two positions conserved, whereas the other positions can be occupied by various amino acid residues. This information can relatively easily be obtained by immunoprecipitation of the MHC molecule of interest, elution of the associated peptides, and sequencing of those peptides all together as a pool. This laboratory has used that approach for several MHC molecules, including H-2 K^d, L^d, D^d, K^b, D^b, K^k, K^{km1}; Qa-2; HLA-A2 (*0201 and *0205), A11, A31, B35, B7, B37, B27 (*2705; *2702), Cw7, Cw4, Cw6, DR 1, and DR 5. The results indicate the use of pool sequencing for the purpose of obtaining information on MHC-peptide motifs, including the analysis of class II ligands. Several interesting aspects have been noted and will be discussed; for example, Qa-2 molecules are peptide receptors of higher stringency than ordinary class I molecules, and A11 molecules obviously can accommodate peptides from 8 to 11 residues, always with a charged C-terminus. For determining class II peptide motifs, a useful way appears to be to use both pool sequences as well as a few individual peptides. Participation in this work of the following persons is acknowledged: M. Takiguchi, D. Schendel, J. Strominger, M. Soloski, E. Weiss (contribution of cell lines); S. Stevanovic, G. Jung (peptide sequencing).

Effector Mechanisms in Tumor Virus Systems

N 025 **SENSITIZATION OF CELLS TO TNF-MEDIATED CYTOLYSIS BY ADENOVIRUS E1A REQUIRES TWO E1A FUNCTIONS,** Linda R. Gooding, Penelope Duerksen-Hughes, and Joanna L. Shisler, Emory University School of Medicine, Atlanta, GA 30322

Normal mouse fibroblasts are resistant to the cytolytic effects of tumor necrosis factor (TNF). However, expression of the adenovirus E1A protein renders these normally resistant cells sensitive to TNF lysis. TNF sensitivity is observed both when E1A is introduced into cells by acute virus infection as well as by transfection and production of stable cell lines. A variety of activities has been ascribed to E1A, including transactivation of viral and some cellular genes and repression of expression of other cellular genes. E1A transforms cells in cooperation with activated ras. The ability of E1A to effect transformation appears to require its binding to several cellular proteins including retinoblastoma (pRB). Sequence analysis of multiple adenovirus serotypes has identified three conserved regions of the molecule (termed *CR1, CR2* and *CR3*). Mutational analysis has located several E1A functions to one or more of these regions. Hence, identification of E1A regions responsible for induction of TNF sensitivity could provide information about the mechanism by which E1A influences the complex series of intracellular events leading to TNF cytolysis. Previous studies using acute infection with a panel of adenoviruses containing deletion mutants within E1A had implicated only residues within CR1 in induction of TNF sensitivity. Surprisingly, when these same mutants were employed as plasmids and transfected to produce stable cell lines, deletion of either the CR1 or CR2 region resulted in loss of TNF sensitivity, indicating that both regions are required. This finding suggests that two E1A functions are necessary for TNF sensitivity, one of which is provided by CR1, and the other by either CR2 or another gene in adenovirus. Evidence that another gene(s) in adenovirus can function to alter cellular susceptibility to TNF comes from the following observation: Although neither SV40 T antigen nor the bovine papilloma virus (BPV) early region alone alters cellular susceptibility to TNF, both complement adenovirus mutants lacking all of E1A in inducing TNF sensitivity. Thus, T antigen, BPV, and a non-E1A adenovirus gene can all contribute to the cellular phenotype, with the suggestion that T antigen and BPV provide one function (the "CR1" function) that complements the "CR2" function elsewhere in adenovirus. Furthermore, it appears that the two regions of E1A can function independently to induce TNF sensitivity. This suggestion comes from transfection studies in which mutant proteins lacking either CR1 or CR2 were introduced separately or together into mouse fibroblasts and stable cell lines developed. Although cells expressing either mutant alone remained as resistant to TNF as the parent NIH3T3 cell line, cells expressing both mutants become nearly as sensitive to TNF as cells transfected with the intact E1A gene. This complementation again suggests that two separate functions, acting in *trans*, are responsible for induction of cellular sensitivity to TNF lysis. Dyson *et al.* (J. Virol. 66, 4606 (1992)) have recently shown that E1A makes two distinct contacts with pRB, one involving residues within CR1 and one within CR2, and that both regions are necessary for the highest affinity binding between E1A and pRB to occur. Furthermore, synthetic peptides derived from CR1 and CR2 were found to bind independently to pRB. Thus, the ability of E1A to occupy two independent binding sites on pRB correlates with its ability to create the TNF-sensitive cellular phenotype.

N 026 T CELLS DIRECTED AGAINST ADENOVIRUS E1 TRANSFORMED TUMOR CELLS RECOGNIZE BOTH VIRAL PEPTIDES AND VIRUS-INDUCED CELLULAR PEPTIDES, Cornelis J.M. Melief[1], R. Toes[1], R. Offringa[1], R. Blom[1], I. Meyer[2], A.J. v/d Eb[2], and W.M. Kast[1], [1]Dept. of Immunohematology and Blood Bank, University Hospital Leiden, [2]Dept of Medical Biochemistry, University of Leiden, The Netherlands.

In C57BL/6 (B6,H-2^b) nu/nu mice large tumors arise after subcutaneous implantation of syngeneic Adenovirus (Ad) E1 transformed embryo cells. In previous studies we showed that large tumor masses can be eradicated by i.v. infusion of cloned CTL directed against an immunodominant Ad5E1A encoded peptide in combination with interleukin-2 (1,2). The E1A peptide recognized is a 10 mer comprising amino acid sequence 234-243, SGPSNTPPEI (1,2). This peptide is presented by the H-2D^b MHC class I molecule and has a characteristic H-2D^b binding motif with N and I anchor amino acids at positions 5 and 10, respectively (2,3). Cell lines transformed by mutant E1A constructs were generated with T → I, T → K and position 8 P → S mutations in the immunodominant CTL epitope. Ten-mer peptides containing these mutations all bound equally well to the H-2D^b molecule. CTL recognition and MHC binding studies with cell lines carrying these mutations in addition to amino acid replacement studies with the Ad 234-243 peptide confirm the notion that position 5(N) and 10(I) are crucially important for D^b binding, whereas CTL recognition is abolished by most alterations at positions 6-9. CTL clones generated against the cell lines with E1 transformed lines carrying the afore mentioned mutations in the immunodominant CTL epitope did not recognize E1 encoded peptides. Instead these CTL's appear to recognize an E1A induced peptide encoded by a normal cellular gene. The expression of this CTL epitope in contrast to the E1A endoced epitope 234-243, is shut off by introduction of an activated H-ras gene. Interestingly *ras* transfected E1 transformed cells in contrast to parental E1 transformed cell without activated ras, are tumorigenic in immunocompetent B6 mice. This could be the result of loss of E1A induced but not E1A encoded CTL epitope expression, combined with TGF beta production by the E1 + ras transfected cell lines (see also abstract of R. Offringa et al.).

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N 027 PATHOGENESIS OF RETROVIRUS-INDUCED IMMUNODEFICIENCY IN THE MOUSE, Herbert C. Morse III[1], Ambros Hügin[1], Masahiko Makino[1], Yao Tang[1], Linda Selvey[2], Richard Hodes[2], Janet W. Hartley[1], NIAID[1] and NCI[2], NIH, Bethesda, MD.

Infection of mice of susceptible inbred strains with LP-BM5 murine leukemia viruses induces a syndrome, termed MAIDS, characterized by progressive lymphoproliferation and severe immunodeficiency. Development of disease is dependent on expression of a defective virus genome, BM5def, that encodes a highly protease resistant gag polyprotein as its only product. Also required are as yet incompletely characterized interactions between CD4$^+$ T cells and B cells, both being targets for infection by BM5def. Mice devoid of CD4$^+$ T cells or of B cells do not develop disease even though they express high levels of the defective virus. Induced expression of cytokines also contributes importantly to disease as mice treated with cyclosporin A exhibit a much delayed course of MAIDS. The characteristics of normal T cell responses to virus infected B cell tumors in vitro and the responses to infection in vivo bear a number of similarities to T cell responses to superantigens (SAG). Most striking is the preferential activation of T cells bearing restricted Vβ specificities including Vβ5, 11 and 12. In vivo, proliferation of Vβ5+ T cells is associated with expression of activation markers CD69 and CD44. Genetic analyses demonstrate that other Vβ can participate equally in the development of MAIDS, suggesting that if the BM5def gag is a superantigen, it is not a "conventional" SAG. Established genetic determinants of resistance and susceptibility to MAIDS include genes of the MHC with CD8$^+$ T cells mediating resistance by killing cells expressing the BM5def gag.

N 028 CYTOTOXIC T CELL CONTROL OF EPSTEIN-BARR VIRUS-POSITIVE MALIGNANCIES, Alan Rickinson[1], Jill Brooks[1], Steve Lee[1], Mike Kurilla[2] and Martin Rowe[1], [1]CRC Laboratories, Department of Cancer Studies, University of Birmingham, Birmingham, B15 2TJ, U.K. and [2]Department of Pathology, University of Virginia Health Sciences Center, Charolottesville, Virginia 22908, U.S.A.

Epstein-Barr virus (EBV) transforms human B cells in vitro to give lymphoblastoid cell lines (LCLs) which constitutively express a limited number of viral proteins; these include the nuclear antigens EBNAs 1, 2, 3A, 3B, 3C and -LP and the latent membrane proteins LMPs 1, 2. Such LCL-like cells also arise in vivo but their outgrowth is contained by the cytotoxic T lymphocyte (CTL) response. Thus healthy virus-carrying individuals possess strong CTL memory to EBV which can be reactivated in vitro by stimulating with autologous LCL cells; these reactivated cells recognise LCL targets specifically and in a HLA class I-restricted manner.

Using recombinant vaccinia viruses expressing the individual EBV latent genes as a means of sensitising target cells in CTL assays, the dominant target antigens presented by a variety of different HLA class I alleles have been mapped. Interestingly, the EBNA3A, 3B, 3C family of nuclear antigens provide the dominant epitopes on a number of HLA backgrounds and several of the relevant peptide epitopes (for instance, those presented by HLA-A11 and by several HLA-B27 subtypes) have been precisely defined.

Many of the EBNA proteins, including the EBNA3 family, are not expressed in some of the more important EBV-associated malignancies. In particular Burkitt's lymphoma cells only express EBNA1, whilst nasopharyngeal carcinoma cells and the malignant cells of EBV-positive Hodgkin's Disease express EBNA1, LMP1 and LMP2. Current efforts therefore concentrate on CTL epitopes present in this subset of latent proteins. Of particular interest here is an epitope of LMP1, now defined at the peptide level, which is conserved in all EBV-isolates studied to date and which is presented by HLA-A2.1, a common allele in many human populations. The frequency of CTL memory to this epitope in A2.1-positive individuals is now being assessed, as are means of selectively reactivating this memory population in vitro.

Mucosal and Skin Immunity

N 029 THE IMMUNOBIOLOGY OF RSV INFECTION AND IMMUNIZATION, Brian R. Murphy[1], Arun Kulkarni[1], Peter L. Collins[1], Herbert Morse III[2], and Mark Connors[1], [1]Laboratory of Infectious Diseases and [2]Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD 20892.

Respiratory syncytial virus (RSV) is the most serious viral respiratory tract pathogen of infancy and early childhood. A hallmark of RSV infection in humans is its ability to cause repeat infections. Three major mediators of immunity to RSV have been identified, namely serum IgG antibodies to the RSV fusion (F) and attachment (G) glycoproteins, nasal wash IgA antibodies, and $CD8^+$ cytotoxic T-cells. Reinfection with RSV results from inadequacies in one or more of these arms of the immune response. These inadequacies reflect one or more of the following: immunological immaturity at the time of first infection, antibody-mediated suppression of the antibody response to first infection by transplacentally transferred maternal IgG serum antibodies, or waning IgA or $CD8^+$ T-cell immunity. For example, the $CD8^+$ T-cell immune response is highly effective at restricting replication of RSV challenge virus on days 6-9 following immunization of mice with an immunogen inducing mainly $CD8^+$ T-cell immunity, but is ineffective at restricting replication or at accelerating clearance of virus 45 days after immunization. Repeat infection with RSV also reflects the inherent high infectivity and virulence of this virus as well as the existence of two antigenic subgroups that are about 25% related by cross-neutralization analysis.

A central problem with RSV immunization has been the observation that immunization of infants and young children with formalin-inactivated RSV (FI-RSV) not only failed to protect against RSV infection but actually potentiated the illness in those vaccinees subsequently infected with RSV. An analysis of the immunological basis of disease potentiation revealed that FI-RSV vaccine created a series of specific immunological imbalances including induction of antibodies with decreased ability to neutralize RSV, augmentation of $CD4^+$ T-cell response, and a decreased or absent $CD8^+$ T-cell response. Furthermore, depletion of $CD4^+$, but not $CD8^+$, T-cells in FI-RSV immunized mice at the time of RSV challenge abrogated inflammatory cell infiltration in the lungs of mice. Considered together, our findings suggest that the potentiated disease in humans is a delayed type hypersensitivity reaction at the sites of pulmonary virus replication. Immunization with RSV subunit and vaccinia virus vectored vaccines will be discussed in this context.

Late Abstracts

HERPES SIMPLEX VIRUS, THE END RUN. G. Campadelli-Fiume[1], E. Avitabile[1], J. Baines[2], R. Brandimarti[1], T. Huang[1], C. Di Lazzaro[3], M.R. Torrisi[3], P. Ward[2]. [1]Section of Microbiology and Virology, Department of Experimental Pathology, University of Bologna, Bologna, Italy; [2]M.B. Kovler Viral Oncology Laboratories, University of Chicago; [3]Department of Experimental Medicine, University la Sapienza, Rome, Italy.

The site of envelopment at the inner nuclear membrane determines for herpes simplex virus (HSV) the need to be transported vectorially to the extracellular compartment. During this transit the envelope glycoproteins are processed to the mature forms. In addition, HSV has evolved a specific function at the plasma membrane that prevents reinfection of infected cells by progeny virus, a phenomenon designated as restriction to superinfection.

In the past few years it emerged that HSV transport can be dissected in two major steps that require viral and cellular functions. The first step consists in the virion sorting from the space between the inner and outer nuclear membranes to the cytoplasm and occurs by pinching off the virions in vacuoles derived from the outer nuclear membrane. This step requires the viral protein U_L20, in that a HSV mutant deleted in the ORF U_L20 remains blocked at the space between the inner and outer nuclear membranes. As the block only takes place in some cell lines, some cells can complement the ORF U_L20 deletion in the viral genome. U_L20 is a multiple transmembrane protein that appears to be located in the virion itself. The results support the view that U_L20 belongs to a family of viral and cellular membrane proteins involved in intracellular transport. For the subsequent steps in the vectorial transport, HSV exploits the exocytic pathway. Studies with mutant cells defective in Golgi enzymes or with a variety of Golgi inhibitors indicate that virion particles interact with the Golgi apparatus, and this interaction is crucial for virus exit out of the cell and concomitantly for processing of the virion envelope glycoproteins to mature forms. Immunocytochemical location of Golgi-resident proteins coupled with electron microscopic examinations of HSV-infected cells reveals a fragmentation of Golgi apparatus and a dispersal of the fragmented elements throughout the cytoplasm. This occurs at the time of virus maturation and is peculiar to some cell lines.

Restriction to superinfection is mediated by the presence of the viral glycoprotein D (gD) at the plasma membrane. In gD-cells the superinfecting virus is taken up by endocytosis and degraded. Surprisingly, HSV mutants able to overcome the gD-mediated restriction and to infect productively the gD-expressing cell lines were found to be mutated in gD itself. These findings raise the possibility that the block to superinfection observed in gD-expressing cells is exerted through an interaction between cellular gD and virion gD.

PATHOGENESIS OF CD4 T CELL DEPLETION IN HU-PBL-SCID MICE,
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Isolates of HIV-1 or HIV-2 differ in their biologic behavior in cell culture with regard to cell tropism, replication rate, ability to induce cytopathic changes, and modulation of CD4 expression. We have compared the following series of molecularly cloned HIV-1 isolates for their ability to cause deletion of CD4 T cells in hu-PBL-SCID mice: HIV-1_{SF33} - T-cell tropic, high replication, cytopathic, CD4 modulation; HIV-1_{SF162} - MØ-tropic, low replication, non-cytopathic, no CD4 modulation; HIV-1_{SF2} - T-cell tropic, medium replication, cytopathic, CD4 modulation; and HIV-1_{SF13} (a later isolate from the same patient as SF2) - T-cell and MØ-tropic, high replication, cytopathic, CD4 modulation. We extended these studies to two HIV-2 isolates: HIV-2_{KR} - T-cell tropi, high replication; and HIV-2_{UC1} - MØ-tropic, low replication, non-cytopathic, no CD4 modulation. Hu-PBL-SCID mice were infected with 100 tissue culture infectious doses of each of these HIV, and CD4-, CD8-, CD3-, and CD45-positive human cells were enumerated by flow cytometry at 2 and 4 weeks after infection. In addition, viral load was determined by quantitative PCR analysis of proviral copy number and by plasma p24 core antigen levels. The macrophage-tropic, non-cytopathic HIV-1_{SF162} isolate induced the most rapid and profound CD4 T cell depletion, and the most highly cytopathic strain *in vitro*, HIV-1_{SF33}, induced the least and slowest CD4 T cell depletion. The relative ability of HIV-1 isolates to induce CD4 T cell death was SF162>SF13>SF2>SF33. This ability was not correlated with viral replication, as the relative viral burden at 4 wk after infection was SF33>SF162>SF13=SF2. These results were confirmed when we examined the HIV-2 isolates. Infection with HIV-2_{UC1} led to rapid and extensive CD4 depletion (comparable to HIV-1_{SF162}), whereas infection with HIV-2_{KR} did not result in a detectable decline in CD4 T cell numbers. We conclude that the behavior of HIV isolates in tissue culture may not predict their pathogenic potential *in vivo*, and suggest that macrophage-tropism may be a marker for a more highly cytopathic effect (either directly or indirectly mediated) for human CD4 T cells in HIV-infected individuals. In addition, viral load may not be an accurate predictor of disease progression. (Supported by NIH grants AI29182 and AI30238).

GENETIC MANIPULATION OF NEGATIVE STRAND RNA VIRUSES: INFLUENZA VIRUS

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Negative strand RNA viruses are a group of viruses whose genomes consist of single-stranded RNA of negative polarity. Since the purified RNA of these viruses is not in itself infectious when introduced into cells, novel methods have had to be developed to allow the genetic manipulation of these viruses. Recently, a ribonucleoprotein transfection protocol was designed which allows the rescue of influenza virus cDNA-derived RNA into infectious influenza virus. Thus, taking advantage of DNA technologies, it is now possible to alter the genome of at least one negative strand RNA virus and obtain viruses made from synthetic RNA.

Specifically, we have modified the noncoding sequences of different influenza virus genes and succeeded in further defining the sequences required for transcription and replication of influenza viral RNAs. The introduction of mutations into the noncoding sequences allows us to up- or downregulate the expression of specific influenza virus genes. Some of these constructs have resulted in viruses with novel growth characteristics. For example, downregulation of the expression of the neuraminidase gene leads to the formation of virus particles lacking an essential gene and thus to preparations containing a high level of defective particles. Such novel transfectant viruses are attenuated in animal models.

Introducing mutations into the coding region of influenza virus genes allows a detailed structure function analysis of influenza virus proteins. This approach is at times limited due to the fact that lethal mutations do not provide insight into the mechanism of the inactivation. However, it can help us to learn whether a feature is essential for infectious virus formation. For example, studies involving a mutational analysis of the cytoplasmic tail of the hemagglutinin have shown that palmitoylation is a posttranslational modification essential for viral morphogenesis. When the mutation is not lethal, the approach may help us to correlate specific biological properties with specific sequences in proteins. For example, modifications of sequences in the hemagglutinin or the neuraminidase have allowed us to obtain novel influenza viruses with changes affecting host range, virulence and/or antigenicity.

PATHOGENESIS OF THE AIDS DEMENTIA COMPLEX AND CNS HIV-1 INFECTION,
Richard W. Price, University of Minnesota, Minneapolis, MN 55455

The clinical syndrome of the AIDS dementia complex (ADC) was recognized early in the AIDS epidemic as both clinically important and biologically intriguing. However, despite the insight gained from the clinic and the laboratory, its pathogenesis remains puzzling in a number of fundamental aspects, including particularly its relation to HIV-1 infection of the central nervous system (CNS) and how the characteristic brain injury is mediated. While a number of considerations favor the hypothesis that ADC is caused by HIV-1 infection of the CNS, certain other observations suggest that the relationship between HIV-1 infection and ADC is not straightforward and may involve other processes. These observations include: 1) while productive infection and accompanying multinucleated-cell encephalitis generally correlate with more severe ADC, such correlation is not universal; 2) productive CNS infection is often either anatomically restricted or surprisingly sparse (even absent) in relation to clinical severity; 3) productive CNS infection *in vivo* is largely, if not exclusively, confined to cells of bone marrow origin rather than to the neuroectodermally-derived functional cellular elements of the brain (neurons, oligodendrocytes and astrocytes); 4) immune activation, reflected in elevated cytokines or surrogate markers of cytokine activation in cerebrospinal fluid and brain, correlates with ADC severity. Taking these considerations into account, our current (hypothetical) view of the pathogenesis of ADC envisions two principal pathogenic agonists (HIV-1 itself and immune system) which undergo an evolving, two-way interaction during the course of infection (with early 'control' of replication and late immunopathology, with the latter involving disturbance of negative feedback processes) with both of these agonists capable of effecting injury of CNS targets by indirect mechanisms. Two simple models of indirect injury are offered: (A) a 2-cell model in which the **infected cell** (a macrophage or microglial cell) releases either virus-coded (e.g., gp120, *tat* or *nef*) or cell-coded (e.g., quinolinic acid, TNF-alpha interferons activated in the infected cell by the viral genome and its products) neurotoxins which then injure surrounding uninfected **target cells**; or (B) a 3-cell model in which the **infected cell** releases signals (either virus-coded as above or cell-coded, in this case either activated by the viral genome and its products or less specifically stimulated by cell injury) which act on an intermediate **amplifier cell** (a macrophage/microgliocyte or astrocyte) which in turn releases cell-coded neurotoxins that alter the **target cell**. These indirect models, particularly the 3-cell model, are consistent with both *in vivo* human and more recent *in vitro* laboratory observations, but require confirmation and refinement with respect to the participating cells, the intermediary and toxic signals and the mechanisms of cell interactions and toxicity. They point to HIV-1 infection as still the principal focus of therapy, since the virus is the driving force of both viral and immunological neurotoxicity, but raise the possibility that some of the intermediate pathways of indirect neurotoxicity might also be amenable to therapeutic manipulation.

NEUTRALIZATION OF PICORNAVIRUSES BY MONOCLONAL ANTIBODIES,

Roland Rueckert, Donna Leippe, Anne Mosser, Wai Ming Lee and Thomas Smith[1], Institute of Molecular Virology, University of Wisconsin, Madison WI [1]Department of Biological Sciences, Purdue University, West Lafayette IN

Picornaviruses are attractive experimental models for basic studies on neutralization because the shell has a particularly simple architecture, 60 subunits organized as 12 pentamers. Crystallographic analysis has provided knowledge of shell structures to atomic resolution. In 1956 it was proposed that a single antibody molecule, binding to a "critical" site, is sufficient to neutralize poliovirus (1). We sought such a "single-hit" antibody from a panel of 46 IgG monoclonal antibodies (mAb): 32 against human rhinovirus 14 (HRV14) and 14 against type 1 poliovirus (PV1). Most of these antibodies neutralized by simple aggregation; 8, however, were very poor precipitators, producing no aggregates or clumps of two or three virions which did not further aggregate with time suggesting that both arms of the IgG molecule were stably attached to the same virion. Saturation binding levels, 30 molecules/virion, supported this picture, which implies that the anchoring arm of non-aggregating IgGs binds in an orientation favorable for rapid binding of the second arm to an adjacent epitope probably across 2-fold symmetry joints, thereby linking adjacent pentamers (2). This picture has now been visually confirmed by cryoelectron microscopy which shows that bound Fab fragments from one of these antibodies mAb17, directed against epitope IA of HRV14, point directly across a pentamer joint toward its partner epitope (3).

The intact mAb17 molecule bridges the canyon at the point where the cellular receptor molecular ICAM-1 and, as predicted, mAb17 interfered with viral attachment; about 6 molecules of the IgG were required to inhibit binding to HeLa cells by 50%.

Do picornaviruses have tails? The 8 non-precipitating monoclonal antibodies in our collection were possible candidates for one-hit neutralization. To test this possibility, the specific infectivity of stable antibody-monomeric virus complexes, separated from small amounts of viral aggregates by sucrose gradient sedimentation, was plotted against the number of bound IgG molecules. The number of IgG molecules required to neutralize initial infectivity (zero antibodies bound) to 37% (1 hit) ranged from 6 to 20; none exhibited one-molecule neutralization (4).

The neutralization stoichiometry of the 6-molecule antibody can be reconciled with Dulbecco's single hit model if the critical site were one of the 12 pentamers, rather than a single subunit (protomer). In this case only five of the 30 pairs of sites would involve the critical pentamer; hence, on average, 6 antibodies would be required to bind one IgG to the critical pentamer. The critical pentamer model is compatible with the idea that emergence of the RNA genome during uncoating involves a specific location, analogous to a tail. These speculations point to the need of a more direct means of testing the validity of tail model for uncoating.

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Epitopes and Processing

N 100 ANTIGENIC ANALYSIS OF THE IMMUNODOMINANT ANTIBODY RECOGNITION SITE (AD-1) OF HUMAN CYTOMEGALOVIRUS GP55-116(GB). W.J. Britt, University of Alabama at Birmingham, Birmingham, AL, M. Mach, B. Wagner, B. Kropff, Universität Erlangen-Nürnberg, Erlangen, Germany
The vast majority of anti-envelope antibodies produced after infection with human cytomegalovirus(HCMV) are directed against the major envelope glycoprotein complex,gp55-116(gB). This response includes virus neutralizing, non-neutralizing and infected cell surface reactive antibodies. We have previously shown that the immunodominant antibody binding site on gB consisted of a 70 a.a. continuous domain(AD-1), as defined with both murine and human monoclonal antibodies. We have further investigated the antigenic properties of this domain utilizing an expanded panel of murine monoclonal antibodies and human convalescent serum. In agreement with previous results, human antibody recognition of this region of gB required the intact 70aa domain. Deletions of either the amino-or carboxy- terminus of AD-1, as well as internal aa replacements abolished antibody recognition of the domain. In addition, we have shown that high affinity, AD-1 specific antibodies produced in patients undergoing primary infection with HCMV were restricted to the IgG1 subclass. Mice immunized with the intact AD-1 produced antibodies which were reactive with the full length gB but exhibited differing affinities and biological activities. A panel of monoclonal antibodies was derived following immunization with AD-1 and utilized to further characterize the antigenic structure of AD-1. Together with AD-1 mutants, this panel of monoclonal antibodies should provide additional insight into the antigenic structure of this unique region of gB.

N 101 ISOTYPIC DISTRIBUTION OF ANTI-LACTATE DEHYDROGENASE-ELEVATING VIRUS ANTIBODIES ELICITED BY INFECTION AND IMMUNIZATION WITH INACTIVATED VIRUS, J.-P. Coutelier, International Institute for Cellular Pathology, Catholic University of Louvain, Brussels, Belgium.

Like other antiviral responses, antibodies elicited in mice by infection with lactate dehydrogenase-elevating virus (LDV) predominantly belong to the IgG2a subclass. This isotypic bias could be due to modulation of immune mechanisms by the live infectious agent, via for example secretion of soluble factors or a particular type of antigen presentation, or to specific properties of the viral antigenic determinants, such as the presence of repetitive motives. To test the latter hypothesis, mice were immunized with whole inactivated virus and anti-LDV antibody isotypes were assayed in spleen cell supernatants after a second challenge with the same antigenic preparation. A large proportion of IgG1 was found in these antibodies which therefore shared their isotypic pattern with responses elicited by immunization with a soluble protein antigen such as keyhole limpet haemocyanin. In addition, LDV infection concomitant with immunization inhibited the IgG1 response triggered by inactivated virus. These results suggest that IgG2a restriction of anti-LDV antibodies elicited by live virus is a consequence of the infectious process. As various immunoglobulin isotypes display different functional properties, this observation could have important implications in vaccination procedures.

N 102 USE OF ANTIGEN PRESENTING CELLS EXPRESSING HLA-DR MOLECULES WITH POINT MUTATIONS IN THE ALPHA OR BETA CHAIN TO ANALYZE THE TRIMOLECULAR COMPLEX. Julie M. Curtsinger, Yung-Nan Liu, and Richard C. Gehrz, Biomedical Research Center of Children's Hospital of St. Paul, St. Paul, MN 55102.
Two helper cell clones both restricted by HLA-DR7 and reactive with the peptide gB(420-430) of HCMV glycoprotein B were assayed for their proliferative responses to peptide presented by a panel of transfected mouse L cells expressing HLA-DR7 heterodimers with single point mutations in either the DR alpha molecule or the DR7 beta molecule. Mutations in the region of the DR beta chain which forms the base of the peptide binding pocket had little effect on antigen presentation, while mutations in the region of DR beta which forms one side of the pocket could decrease the effectiveness of antigen presentation to one or both of the T cell clones. In contrast, mutations in the region of DR alpha forming the base of the binding pocket had the greatest effect on antigen presentation to both clones. Use of a peptide which was slightly longer at the amino terminus overcame the effects of some of the DR alpha mutants, but none of the DR beta mutants. Binding studies indicate that failure to bind peptide cannot account for all of the negative effects of mutations in DR molecules. T cell receptor sequences are being determined for these two clones, and for additional clones with the same peptide and HLA-DR specificities, to search for correlation between reactivity to the panel of mutant Class II molecules expressed by L cells and the T cell receptor sequence.

N 103 PRESENTATION OF HUMAN CYTOMEGALOVIRUS IMMEDIATE-EARLY (IE1) PROTEIN BY ASTROCYTOMA CELLS TO CD4+ T CELL CLONES.
Jean-Luc Davignon, Susan Michelson,* and Christian Davrinche, INSERM U 100, Hôpital Purpan, 31052 Toulouse Cedex, France, and *Pasteur Institute, Unité d'Immunologie Virale, Paris France.
The human cytomegalovirus immediate-early 72 kDa major protein (CMV-IE1) is a regulatory nuclear phosphoprotein important for the expression of the viral genome. A proliferative response specific for IE1 is found in CD4+ T cells from normal CMV seropositive blood donors. To better understand the anti-CMV immune response, we have developed CD4+ T cell clones specific for the IE1 protein. Astrocytoma cells (U 373 MG, named A0) were transfected with the 20.8 kb genomic Hind-III fragment containing the IE region of CMV Towne. The resulting cell line, named A2, was found to express substantial levels of the 72 kDa IE1 protein. Lysates from A2 cells were used as a source of antigen (Ag) to develop CD4+ T cell clones from the peripheral blood mononuclear cells (PBMC) of different donors. The specificity of the clones was confirmed using a recombinant IE1 protein produced by baculovirus. Astrocytoma cells are permissive for CMV in vitro and have been described as functional antigen presenting cells (APC). We first showed that interferon-gamma (IFN-γ) induced the expression of HLA-DR at the surface of A0 and A2 cells. These cells were typed as HLA-DR3 by oligotyping. T cell clones from a HLA-DR3 donor proliferated in the presence of IE1 and either IFN-γ treated A0 cells or HLA-DR matched PBMC, EBV transformed B cells. In addition, they proliferated when incubated with IFN-γ treated A2 cells in the absence of exogenously added IE1. The amount of Ag found in the supernatant of A2 cells was lower than the minimal amount of exogenous Ag required by A0 cells to induce a detectable T cell response. Comparison of presentation by A0 or A2 cells showed that the efficiencies of exogenous and endogenous presentation were of similar magnitude. However, bystander macrophages from a HLA-DR8 donor cocultured with A2 cells were able to present IE1 to a HLA-DR8 restricted T cell clone. Our data suggest that: 1) Similar epitopes can be presented by astrocytes and professional APCs. 2) Astrocytes can present Ag through endogenous and exogenous pathways. 3) Macrophages can efficiently present Ag produced endogenously by non professional APCs.

N 104 EPSTEIN-BARR VIRUS ISOLATES FROM A HIGHLY HLA A11 POSITIVE POPULATION LACK THE DOMINANT A11 RESTRICTED T CELL EPITOPE. de Campos-Lima P.O., Gavioli R., Zhang Q.J., Wallace L.E., Dolcetti R., Rowe M, Rickinson A.B. and Masucci M.G. Department of Tumor Biology, Karolinska Institute, Box 60 400, 104 01 Stockholm, Sweden

Cytotoxic T lymphocytes (CTL) mediate immune control over viral infections by recognizing viral peptides selected for presentation by MHC class I antigens. To what extent such responses have influenced virus evolution is difficult to discern, especially for genetically stable agents with a long association with their host species. Here we report findings which may bear upon this issue from the study of Epstein-Barr virus (EBV), a widespread human herpes virus. Amongst Caucasian the MHC class I antigen HLA A11 mediates potent EBV specific CTL responses through presentation of one immunodominant epitope, residue 416-424 of the EBV nuclear antigen-4 (EBNA4). This epitope appears to be conserved amongst type A EBV strains in Caucasian populations, where A11 is a relatively infrequent allele, and in Central African populations, where A11 is virtually absent. However, isolates from areas of New Guinea where HLA A11 is unusually prevalent carry a lysine-threonine substitution in residue 424; this not only abrogates recognition by the above CTLs but also renders the altered 416-424 peptide unable to bind nascent HLA A11 molecules. Thus, in a population with limited HLA polymorphism, viral strains lacking the dominant CTL epitope may have enjoyed a selective advantage

N 105 **FINE ANALYSIS AND COMPARISON OF THE OPTIMAL MHC BINDING SEQUENCE OF THE H-2D^b EPITOPES OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS.** **Jean Edouard Gairin and Michael B. A. Oldstone. Laboratoire de Pharmacologie et de Toxicologie Fondamentales, CNRS, Toulouse, France (J. E. G.) and The Scripps Research Institute, La Jolla, California, USA (M. B. A. O).**

Viral antigens are presented to cytotoxic T lymphocytes by H-2-restricted MHC glycoproteins. Binding of the endogenously processed viral peptides to MHC is an early intracellular event in the recognition process and is a prerequisite for subsequent killing of virus infected cells by virus-specific CTLs. It is now well established that interaction between viral antigenic peptides and MHC is greatly dependent on the length of the antigens. On the basis of CTL lysis, three D^b-restricted epitopes of LCMV have been mapped in the viral glycoproteins GP1 and GP2 and nucleoprotein NP and the sequences recognized by CTLs characterized. The goal of this study was to define the optimal MHC binding sequence of each epitope. Synthetic peptides truncated at the N- or C- terminus flanking the crucial D^b anchoring residue Asn of the viral antigens were tested in binding assays specific for D^b MHC on viable cells (the murine lymphoma cell line RMAS). The results allow a precise delineation of the optimal binding sequences: the GP1 and NP epitopes appear as 9 a.a. peptides: GP33-41 (KAVYNFATCG) and NP396-404 (FQPQNGQFI), while the GP2 antigen is defined as a 11 a.a. peptide: GP276-286 (SGVENPGGYCL). Interestingly, dramatic alterations of the binding properties of the epitopes are observed after C-, rather than N-, terminus modifications (shortening or lengthening). The peptide sequences optimal for binding to MHC and for recognition by CTL will be compared.

N 106 HUMORAL IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS VIRUS INFECTIONS, Denise A. Galloway, Joseph Carter, Mary Taflin and Michael Hagensee, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Attempts to study the immune response to HPV infection have been hampered by the inability to propagate HPVs in culture, the lack of an animal model system and an inadequate understanding of the natural history of HPV infections. We have developed a number of antigen targets to detect antibodies in human sera including: bacterially expressed fusion proteins for use in Western blot assays, yeast expressed proteins for use in ELISAs and HPV capsids derived from recombinant vaccinia viruses for use in ELISAs, radioimmunoprecipitations and Western blotting. Serum samples have been obtained from several ongoing studies in Seattle including a population based case-control study of cervical cancer (J. Daling), a study of sexually inexperienced college women (L. Koutsky), a pregnancy study (H. Watts, K. Holmes) and several cohorts attending the STD clinic (K. Holmes).

The results showed that the predominant antibody reactivity was to the capsid antigens and less frequently to early region genes. The Western blot assays detected antibodies to linear epitopes on the L2 protein that were masked in the capsids, or not sufficiently represented. The capsid ELISA assay detected antibodies to the L1 protein that were primarily to conformational epitopes. Antibodies to E7 were strongly associated with cervical cancer patients and were detected by both ELISA and Western blot assays. The association of these HPV antibodies with various risk factors will be presented.

N 107 **SCREENING AN MHC RESTRICTED PEPTIDE EXPRESSION LIBRARY WITH CYTOTOXIC T LYMPHOCYTES.** Marc A. Gavin and Michael J. Bevan. Department of Immunology, University of Washington, Seattle, WA 98195

Making use of a prokaryotic protein expression system, we have devised a method for screening peptide libraries with cytotoxic T lymphocytes (CTL). Our lab and others have previously shown that for H-2 K^b restricted octamers, residues in positions 3, 5 and 8 are important MHC binding anchor residues, while positions 1, 2, 4, 6 and 7 may contact peptide specific CTL. Oligonucleotides degenerate at codons 2, 4, 6 and 7 and encoding S-1 and anchor residues I-3, F-5 and L-8 (SXIXFXXL) were cloned downstream of the *malE* gene (encoding maltose binding protein, MBP) in an IPTG inducible expression vector (pMAL-c, New England BioLabs). Polyclonal pools of fusion protein were purified by affinity chromatography, and the peptides, released from MBP via factor X_a restriction protease digestion, were fractionated on a RP-HPLC gradient. A library of 190,000 clones contained several peptides that mimicked the H-2 K^b restricted epitopes OVA 257-264 and VSV-N 52-59, as well as self-peptides antigenic for alloreactive CTL. Such MHC allele specific libraries may have many applications in identifying natural epitopes and in characterizing the pre-immune repertoire.

N 108 RECOGNITION OF THE EBV ENCODED NUCLEAR ANTIGENS EBNA4 AND EBNA6 BY HLA A11 RESTRICTED CTLs AND MAPPING OF A11-RESTRICTED EBNA4 EPITOPES. Gavioli R., de Campos-Lima P.O., Kurilla M.G., Kieff E., Klein G., Rickinson A.B., Masucci M.G.. Department of Tumor Biology Karolinska Institute, Box 60 400, 104 01 Stockholm, Sweden

HLA class I restricted cytotoxic T-lymphocytes (CTL) play an important role in controlling the proliferative potential of Epstein-Barr virus (EBV) transformed B-lymphocytes. Processed products of one or more of the viral antigens expressed in transformed cells serve as their targets. Six EBV encoded nuclear proteins (EBNA1 to -6) and two membrane proteins (LMP1 and -2) have been identified in lymphoblastoid cell lines (LCL) of normal B-cell origin. We have investigated the target specificity of HLA A11 restricted CTLs derived by stimulation of lymphocytes from EBV seropositive individuals with autologous EBV transformed LCLs. Recombinant vaccinia viruses carrying the coding sequences for EBNA1, -2A, -2B, -5, -3, -4, -6 and LMP1 were used to induce high levels of expression of the relevant EBV antigen in fibroblasts derived from appropriately HLA class I matched individuals. EBNA4 expressing fibroblasts were the predominant target of HLA A11 restricted CTLs in three out of three donors tested. A less pronounced and less regular EBNA6 specific cytotoxic component was found in two of the donors. Recombinant vaccinia virus carrying deletion mutants of the EBNA4 gene and synthetic peptides covering relevant regions of the protein were used to identify the HLA A11 restricted EBNA4 epitopes recognized in different donors. A 9 aa long synthetic peptide corresponding to one of such epitopes induces EBV specific lysis of HLA A11 positive targets at a concentration of 10^{-14}M and promotes assembly and surface expression of HLA A11 polypeptides in HLA A11 transfected peptide transporter mutant cell lines.

N 109 $CD8^+$ T CELL RECOGNITION OF AN ENDOGENOUSLY PROCESSED EPITOPE IS REGULATED PRIMARILY BY RESIDUES WITHIN THE EPITOPE, **Young S. Hahn[1,3], Chang S. Hahn[2,3], Vivian L. Braciale[1,3], Thomas J. Braciale[1,3], Charles M. Rice[2],**
1. Department of Pathology, 2. Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO,3. Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, VA

Cytotoxic T lymphocytes (CTL) recognize short antigenic peptides associated with cell surface class I MHC molecules. This association presumably occurs between newly synthesized class I MHC molecules and peptide fragments in a pre-golgi compartment. Little is known about the factors which regulate the formation of these antigenic peptide fragments within the cell. To examine the role of residues within a core epitope and in the flanking sequences for the generation and presentation of the newly synthesized peptide fragment recognized by $CD8^+$ CTL we have mutagenized the coding sequence for the CTL epitope spanning residues 202-221 in the influenza A/Japan/57 HA. In this study over 60 substitution mutations in the epitope were tested for their effects on target cell sensitization using a cytoplasmic viral expression system. The HA202-221 site contains two overlapping subsites defined by CTL clones 11-1 and 40-2. Mutations in HA residues 204-213 or residues 210-219 often abolished target cell lysis by CTL clones 11-1 and 40-2, respectively. Although residues outside the core epitope did not usually affect the ability to be lysed by CTL clones, substitution of a Gly residue for Val-214 abolished lysis by clone 11-1. These data suggest that residues within a site which affect MHC binding and T cell receptor recognition appear to play the predominant role in dictating the formation of the antigenic complex recognized by $CD8^+$ CTL and therefore the antigenicity of the protein antigen presented to $CD8^+$ T cells. Most alterations in residues flanking the endogenously expressed epitope do not appreciably affect the generation and recognition of the site.

N 110 **ANALYSIS OF THE T-CELL RECEPTORS FROM VIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTES THAT RECOGNIZE AN IMMUNODOMINANT NINE AMINO ACID VIRAL EPITOPE**, Marc S. Horwitz, Beatrice A. Cubitt, Yusuke Yanagi, Hana Lewicki, and Michael B.A. Oldstone, Department of Neuropharmocology, The Scripps Research Institute, La Jolla CA 92037

T lymphocytes play an important role in the pathogenesis of both autoimmune disease and viral infection. The T-cell receptor (TCR) composed of an α and β chain is responsible for recognizing antigen in the context of the MHC molecules. Restricted usage of either the V_α or V_β chains of the TCR which has been observed in many immune mediated diseases and infections allows TCR-specific antibodies (Abs) to be used as a therapeutic approach. To assay for TCR α and β chain restriction, the structure of the TCR in LCMV specific cytotoxic lymphocytes (CTL) in Balb/c (H-2^d) mice was investigated. Greater than 95% of the LCMV specific CTL response at the clonal level is restricted to a single nine amino acid peptide from the viral nucleoprotein presented by L^d in H-2^d mice infected with LCMV. Three CTL clones with specificity for this unique NP epitope presented by L^d were randomly selected for study of their TCR structure. Using "Anchor PCR", all three clones were found to have unique V_α and V_β chains relative to each other and lack restriction to any particular variable chain. Additionally, none of the three CTL clones possess the $V_\alpha4$ chain previously reported to be restricted to the H-2^b (D^b) GP2 epitope in the LCMV model. Molecular analysis of two different MHC backgrounds (H-2^d, H-2^b) which present four major epitopes on two different MHC molecules(L^d, D^b), shows no restriction to one or two specific variable chains across these backgrounds. These results indicate that in this virus system, TCR-specific Abs directed at restricted variable chains will not be an effective therapy, and suggests that in situations with more complex patterns of MHC presentation like humans, TCR-specific immunotherapy is less likely to be useful.

N 111 THE IDENTIFICATION OF A MAJOR B CELL EPITOPE ON THE BeAn STRAIN OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS, Lisa J. Lovelidge, Helen O'Shea, David Brown and Tony A. Nash, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge, CB2 1QP U.K.
Intracerebral injection of the BeAn strain of Theiler's murine encephalomyelitis virus (TMEV) into CBA mice, results in a biphasic disease of the central nervous system (CNS). The first phase resembles poliomyelitis with some of the mice exhibiting paralysis. The virus is cleared from the CNS at about 4 weeks post-infection, and this clearance correlates with the development of a neutralising antibody response. The chronic phase of the disease occurs from 6 weeks post-infection and primary demyelinating lesions can be seen in the spinal cord. In order to define the B cell epitopes of TMEV, neutralising monoclonal antibodies were raised against BeAn in Balb/c mice. Two of these antibodies, which react with the capsid protein VP1 in a Western blot, will neutralise the demyelinating strains of TMEV as well as a virulent strain which causes acute encephalitis. The monoclonal antibodies were used to select two neutralisation-resistant variants of BeAn. These viruses can not be neutralised by the monoclonal antibodies, nor by hyperimmune sera raised against BeAn in rabbits and mice. However, sera raised against the two variants will neutralise themselves and the wild type virus. The B cell epitope has been located to the carboxyl region of VP1 by peptides and by sequencing the variants.

These results indicate that this B cell epitope, which is conserved through the different subgroups of TMEV, is immunodominant, but that other neutralising subsidiary sites also exist.

N 112 AN HLA A11-SPECIFIC MOTIF IN NONAMER PEPTIDES DERIVED FROM VIRAL AND CELLULAR PROTEINS. Masucci M.G., Zhang Q-J, Gavioli R., Klein G. Department of Tumor Biology, Karolinska Institute, Box 60 400, 104 01 Stockholm, Sweden

T lymphocytes recognize their antigenic targets as peptides associated with MHC molecules. The HLA A11 allele, a preferred restriction element for Epstein-Barr virus (EBV) specific cytotoxic T lymphocyte (CTL) responses, presents an immunodominant epitope derived from the EBV nuclear antigen-4 (EBNA4). Subpicomolar concentrations of a synthetic nonamer peptide, IVTDFSVIK, corresponding to aminoacids 416-424 of the EBNA4 sequence, can sensitize PHA blasts to lysis by EBV specific HLA A11 restricted CTLs. We show that µM concentrations of this peptide induce assembly and surface expression of HLA A11 in A11-transfected sublines of the peptide transporter mutant cell lines T2 and BM36.1. Using the IVTDFSVIK peptide and a series of synthetic nonamer peptides, differing from the original sequence by single aminoacid substitutions, we have defined a motif for HLA A11-binding peptides. This predicts the presence of an hydrophobic aminoacid in position 2, aminoacids with small side chains in positions 3 and 6 and a lysine in position 9. Using this motif we have identified a peptide in the carboxyterminal end of wild type p53, ELNEALELK, which is able to induce HLA A11 assembly as efficiently as the IVTDFSVIK viral peptide

N 113 SELECTION OF EPITOPES FOR RECOGNITION BY CLASS I MHC-RESTRICTED CYTOTOXIC T LYMPHOCYTES, Gregg N. Milligan[1] and Thomas J. Braciale[2], [1]James N. Gamble Institute of Medical Research, Cincinnati, OH 45219 and [2]Bierne Carter Center for Immunology, Univ. of Virginia, Charlottsville, Virginia 22908.

T cell repsonses to antigenic proteins are generally limited to recognition of a very small number of epitopes. The basis of this restriction was examined using the cytotoxic T lymphocyte (CTL) response to A/Japan/57 influenza hemagglutinin (HA) as a model. Previous studies have shown that the class I MHC restricted response in H-2^d haplotype mice is limited to recognition of two regions of the HA molecule. Additional regions of the HA molecule were found which bind to H-$2K^d$ but do not serve as class I MHC-restricted T cell epitopes. To determine if intracellular competition among antigenic peptides for processing may prevent presentation of cryptic epitopes, an HA gene construct deleted of the two dominant H-2 K^d restricted epitopes was inserted into vaccinia virus and used to prime mice. Even in the absence of dominant epitopes, CTL recognizing cryptic HA epitopes were not detected. Because potential epitopes may be destroyed or may not be accessible for processing, a non-recognized H-$2K^d$ binding synthetic HA peptide (HA 177-188) was used as a pre-processed antigen to elicit CTL in vitro. Peptide-specific CTL were not demonstrable, suggesting HA 177-188 specific CTL might be deleted during ontogeny. Forty-two variant peptides were therefore synthesized with single or double amino acid substitutions or deletions within the HA 177-188 sequence and were tested for their ability to elicit peptide-specific CTL in vitro. All of the variant peptides elicited peptide-specific CTL capable of lysing target cells sensitized with exogenous peptide, but only one peptide elicited CTL which could recognize endogenously expressed peptide. These data suggest antigenic peptides must bind MHC molecules such that peptide conformation within the Ag-binding groove of the MHC permits the high affinity interaction with TCR necessary for recognition of an enodgenously produced epitope.

N 114 ANTIGENIC VARIATION IN FOOT-AND-MOUTH DISEASE VIRUS: STRUCTURAL AND SEROLOGICAL ANALYSIS, Patricia G. Piatti, L. Lee France, John F.E. Newman and Fred Brown, Plum Island Animal Disease Center, USDA/ARS, Greenport, NY 11944.

The immunodominant epitope of FMDV, which is located on a loop comprising residues 132-159 of VP1, one of the four capsid proteins, also contains the putative cell attachment site Arg Gly Asp at aa 145-147. By growth in tissue culture, seven antigenic variants have been isolated from the tongue epithelium of a cow infected with a virus of serotype A12 and additional ones have been obtained by growing the virus in the presence of monoclonal neutralizing antibodies. These variants differ only at residues 148 and/or 153 of VP1.

148	Ser	Leu	Phe	Phe	Phe	Phe	Leu
153	Leu	Pro	Pro	Leu	Ser	Gln	Leu

The serological relationship of seven of these variants have been studied by indirect ELISA, radioimmunopreciptation and seroneutralization using the viruses and peptides corresponding to the 141-160 region of the immunodominant site.The structural basis of the antigenic variation was investigated by circular dichroism (CD) spectroscopy and molecular modeling of peptides corresponding to residues 132-159 of VP1. The results show that it is possible to place the variants into two groups: those with and those without Pro at position 153. Nevertheless, there is considerable cross-reactivity among the variants that can be accounted for by the presence of the highly conserved sequence Arg Gly Asp that corresponds to the cell attachment site on the virus particle.

N 115 A RADIOSENSITIVE APC SIGNAL FOR ACTIVATION OF SELF-REACTIVE T CELL HYBRIDOMAS. Shiv A. Prasad§, Steven P. Fling§, and Dale S. Gregerson§*. Departments of §Microbiology and *Ophthalmology, University of Minnesota, Minneapolis, MN.

Experimental Autoimmune Uveoretinitis (EAU) is a T cell mediated disease induced in LEW rats by immunization with bovine retinal S-antigen (S-Ag), or pathogenic peptides. Self-reactive T cell hybridomas recognize sequences on S-Ag that are conserved between both species whereas non-self reactive hybridomas recognize only bovine sequences. These hybridomas are also distinguished by their activation requirements. Ag presentation to the self-reactive hybridomas requires a radiosensitive APC signal. Ag presented by non-irradiated splenic APC stimulates the self-reactive hybridomas, however low doses of γ-radiation reduce the ability of the APC to present Ag to the hybridomas. This radio-sensitive APC signal is protected by treatment with PMA or LPS and IL-4. Neither the radio-sensitivity of this signal nor the protection corresponds to changes in MHC class II expression. The autoreactive hybridomas also do not respond to Ag presented by thymic APC. In contrast to the autoreactive hybridomas, a nonself-reactive hybridoma does not require a radiosensitive APC signal, and responds to Ag presented by splenic or thymic APC. These data suggest a radio-sensitive, tissue-specific co-signal required to activate autoreactive T cell hybridomas, and may aid in understanding peripheral tolerance of autoreactive T cells.

N 116 MULTIFUNCTIONAL SYNTHETIC PEPTIDES: BLOCKAGE OF HIV INFECTION, SYNCYTIA FORMATION AND INDUCTION OF HIV-SPECIFIC CTL RESPONSES, K.J. Sastry, K. Casement, P. Nehete, R.B. Arlinghaus, Department of Molecular Pathology, M.D. Anderson Cancer Center, Houston, TX. 77030

Several reports in literature described identification of peptides representing CTL epitopes in a number of human immunodeficiency virus type 1 (HIV-1) proteins. We have developed a new screening method that allowed us to identify those peptide epitopes that actually induce viral specific CTLs. Using this method we have identified V3 loop peptides from several HIV-1 strains capable of inducing HIV-specific CTLs. Because of the known role of V3 loop peptides in inducing HIV neutralizing antibodies, we also tested these peptides for inhibition of HIV infection of human T cells. We observed that V3 loop peptides from several HIV-1 strains when used at nanogram quantities exhibit efficient inhibition of virus infection of several human T cell lines as well as freshly isolated normal human T cells. Further, our studies revealed that these V3 loop peptides were capable of inhibiting syncytia formation between HIV-1 infected cells and normal CD4-expressing cells indicating that these peptides can prevent cell-to-cell spread of virus, a phenomenon known to play a major role in HIV-induced pathology in AIDS patients. Even though the V3 loop region is known to be highly variable among different virus isolates, a comparison of several HIV-1 strains revealed that most strains can be grouped into five or less categories. We therefore propose that V3 loop peptides as a mixture will provide a potent immunotherapeutic formulation for: (1) inducing HIV-specific CTLs which can kill virus-infected cells; (2) preventing infection of normal cells by infectious virus; and (3) reducing the spread of virus infection in HIV-infected individuals.

N 117 **A NOVEL ANTIGEN PRESENTATION MECHANISM DEFINED BY A $\gamma\delta$ T CELL CLONE,** Roger Sclammas, R.M. Johnson*, A.I. Sperling, W. Brady**, P.S. Linsley**, J.A. Bluestone; Ben May Institute, Dept. of Pathology, U. of Chicago; Chicago, IL; *Dept. of Medicine; Washington U.; St. Louis, Mo.; Bristol-Myers Squibb Pharmaceutical Research Institute; Seattle, Wa.

$\gamma\delta$ T cells have been shown to recognize several diverse antigens; however, their mechanism of recognition remains unclear. We studied the recognition of a Herpes Simplex Virus (HSV) Type 1 glycoprotein I (gI) specific $\gamma\delta$ T cell clone (TgI4.4). Molecular cloning and immunoprecipitation experiments showed that TgI4.4 expresses a $V\alpha 2$-$C\delta$ and a $V\gamma 1.2$-$C\gamma 2$ heterodimer. Previous data indicated that antigen recognition was MHC independent. In order to examine how the gI antigen is presented, molecular mutants of gI were constructed. A truncated form of gI, that has both the transmembrane and cytoplasmic regions deleted (gIt), was transfected into L cells. gIt was not expressed on the cell surface and was not recognized by TgI4.4. In contrast, a chimeric form of gI, consisting of the extracellular portion of gI and the intracellular portion of CD8 (gI:CD8) reconstituted cell surface expression of gI and was recognized by TgI4.4. This demonstrated that the presentation of gI was dependent on cell surface expression. In addition, the peptide transporter mutant cell, RMA-S, transfected with gI, presented antigen efficiently. These results suggest that the gI protein does not utilize the cytoplasmic protease machinery for antigen presentation and may be recognized as an unprocessed antigen. In support of this latter possibility, anti-gI antibodies blocked specific lysis by TgI4.4. Finally, and most strikingly, TgI4.4 recognized a soluble form of gI when immobilized on plastic. These findings indicate a novel form of antigen recognition since this T cell recognizes unprocessed antigen in an MHC independent manner and unlike superantigens, is antigen specific. In light of HSV's ability to colonize MHC deficient CNS cells, T cells that recognize unprocessed antigen might be an important effector in HSV protection.

N 118 IMMUNODOMINANCE IN THE NEUTRALISING ANTIBODY RESPONSE TO INFLUENZA INFECTION, Claire A. Smith, Christine M. Graham, Andriani C. Patera, Fernando Temoltzin-Palacios, Barbara C. Barnett and D. Brian Thomas, Division of Virology, National Institute for Medical Research, London NW7 1AA, U.K.

Sequence analysis of monoclonal antibody selected laboratory variants of influenza have previously identified five major antigenic sites on the 3D structure of the hemagglutinin molecule. However, there has been no attempt hitherto to investigate the influence of host genetic background and/or natural route of infection on the protective antibody repertoire. We have found that, following natural infection of inbred mice (CBA/Ca, or BALB/k or BALB/c) with X31 virus (H3N2 subtype) the neutralising antibody response is highly restricted and focused on HA1 158, or HA1 198 or HA1 63. The structural basis for such restriction is being investigated by sequence analysis of IgG heavy and light chain gene usage.

N 119 **AN ASSAY FOR THE IDENTIFICATION OF ANTIGENS RECOGNIZED BY CYTOTOXIC T CELLS, BASED ON THE TRANSIENT TRANSFECTION OF COS CELLS,** **Philip Toye[+], Peter Wijngaard[o], Niall MacHugh[+] and Hans Clevers[o], [+]International Laboratory for Research on Animal Diseases, Nairobi, Kenya and [o]Department of Immunology, University Hospital, Utrecht, Holland.**

The transient expression of cDNA libraries in COS cells has enabled the rapid isolation of genes encoding mammalian cell surface antigens. These procedures have relied on the use of monoclonal antibodies to isolate COS cells expressing the desired antigen. The significant advantage of the COS cell system is that plasmids replicate episomally to high copy number after transfection into the cells. This results in high levels of expression of introduced genes, and allows rapid and easy recovery of the gene of interest from identified cells. To date, the COS cell system has not been employed to identify antigens recognised by other components of the immune system, such as cytotoxic T lymphocytes.

Our research interests lie in the identification of those antigens of the protozoan parasite, *Theileria parva*, which are recognised by CTL from immune cattle. We wished to take advantage of the COS cell system to establish a procedure to enable screening of a library of parasite genes directly with CTL. In initial, model experiments, we have used bovine alloreactive CTL and COS cells transiently transfected with a plasmid comtaining cDNA encoding the corresponding bovine class I MHC molecule. We have demonstrated that, in a standard microcytotoxicity assay, bovine alloreactive CTL can detect a bovine MHC molecule transiently expressed in a COS cell population. The sensitivity of the assay is such that the alloreactive CTL can detect those cells expressing the bovine class I MHC molecule in a population of cells transfected with the plasmid containing the corresponding gene, plus 200 fold as many plasmids containing an irrelevant gene. To enable presentation of antigens expressed by transfected parasite genes, we have produced a COS cell line permanently expressing a bovine class I MHC molecule. Thus, these results indicate that transiently transfected COS cells could be used for the direct screening of an antigen gene library by immune CTL. The procedure is currently being used to screen a *T. parva* cDNA library.

N 120 *Abstract Withdrawn*

N 121 KINETICS OF ANTIGEN PRESENTATION TO INFLUENZA SPECIFIC HUMAN T-CELL CLONES BY DIFFERENT ANTIGEN PRESENTING CELLS. F.J. van Kemenade, K.C. Kuijpers, B. Hooibrink, R.A.W. van Lier and F. Miedema.
Dep. of Clinical Viro-Immunology, Central lab. of the Netherlands Red Cross Blood Transf. Serv. and Lab. of Exp. & Clin. Immunol. of the Univ.of Amsterdam, the Netherlands.

The time between virus infection of antigen presenting cells (APC) and presentation of peptide associated MHC molecules to T-cells might vary among different APC, such as monocytes and EBV-transformed B-cells (B-LCL). We investigated the kinetics of antigen presentation by measuring $[Ca^{2+}]_i$ changes in influenza (FLU) specific T-cell clones induced by FLU infected monocytes or B-LCL.

B-LCL presented class-II restricted peptides 2 hours after infection, reaching plateau values after 4 hours. Monocytes, however, presented these peptides much more rapidly as they reached plateau values within 1 hour. Like, B-LCL, monocytes were inhibited in class II presentation by chloroquine and presentation in both cells was unaffected in case of UV irradiated FLU.

Monocytes also presented class I restricted peptide of FLU in accelerated fashion, in comparison to B-LCL. Both APC types were inhibited in class I presentation in case of UV irradiated FLU. In conclusion: monocytes presented class I and II FLU-derived peptides more rapidly than B-LCL. Since both class I and II presentation was accelerated, it seems that both rapidity of antigeen processing and factors involved in viral entry (adherence, entry or uncoating) determine the kinetics of antigen presentation.

N 122 **ANTIGEN PROCESSING MUTANT T2 CELLS PRESENT VIRAL ANTIGEN RESTRICTED THROUGH H-2K^{b}.** Xianzheng Zhou*, Rickard Glas†, Hans-Gustaf Ljunggren† and Mikael Jondal*, Departments of Immunology* and Tumor Biology†, Karolinska Institutet, Box 60400, S-104 01 Stockholm.

T2 cells, derived from the EBV transformed B lymphoblastoid cell line .174, has a profound defect in the class I mediated antigen presentation pathway due to a chromosomal deletion encompassing ~1 megabase in the MHC class II region. This deletion includs peptide transporter genes associated with antigen presentation (TAP1 and TAP2) and genes coding for proteasome subunits. Surprisingly, we demonstrated that T2 cells, after infection with Sendai virus, were readily killed by H-2K^{b} restricted CD8^{+} T cells, whereas T2 or T2D^{b} were not killed. Control erperiments revealed that P815A2 was not killed whereas killing against P815K^{b} was readily observed. Killing of Sendai virus infected T2K^{b} was dependent of CD8^{+} TCR$\alpha\beta^{+}$ T cells. Treatment of effector cells with anti-CD4, anti-NK1.1 and complement (C') or C' alone gave significant lysis levels, while lysis levels were markedly reduced after treatment with anti-CD8 or anti-TCR$\alpha\beta$ and C'. In accordance with previous findings, T2K^{b} was not able to present vesicular stomatitis virus (VSV) antigens to VSV-specific CTL in the present study. Furthermore, presentation of influenza virus A/PR/8/34 and A/NT/60/68 by T2D^{b} after virus infection was greatly impaired. In addition, the RMA-S mutant line also presented Sendai virus specific antigen after virus infection. Interestingly, Brefeldin A (BFA) treatment completely blocked presentation of Sendai virus antigen in P815K^{b} control cells. Unexpectedly, presentation of Sendai virus antigen in T2K^{b} was not blocked by BFA, even when a high concentration of BFA was used. The present finding suggested that a non-classical antigen presentation pathway may function in T2 cells.

TCR Utilization/SuperAgs

N 123 CHARACTERIZATION OF THE *IN VIVO* IMMUNE RESPONSE TO Mls-1^{a}. Mikael Andersson and Hans Acha-Orbea, Ludwig Institute for Cancer Research, Lausanne Branch, CH-1066 Epalinges, Switzerland.

Superantigens encoded by endogenous and exogenous forms of the mouse mammary tumour virus (MMTV) have been shown to be capable of inducing an *in vivo* immune response involving expansion and effector function as well as anergy and deletion of responsive T-cells. We are studying the *in vivo* immune response to the endogenous Mtv-7 superantigen, also known as Mls-1^{a}, which in vivo stimulates almost exclusively CD4^{+} T-cells expressing Vß6. Spleen cells from BALB.D2 (Mls-1^{a}) are injected either locally (in the hind foot pad) or systemically (i.v.) into BALB/c (Mls-1^{b}) mice. The changes in the Vß6^{+}CD4^{+} population are recorded using flow cytometry. Lymphokine secretion patterns are anlysed using the ELISPOT technique as well as PCR analysis of lymphokine mRNA expression. Ig-production is analysed using an isotype-specific ELISPOT assay. Furthermore, the appearance of anergy to Mls-1^{a} is followed.
Local and systemic injection of Mls-1^{a}-expressing cells both induce a vigorous B cell response as well as an initial expansion of the Vß6^{+}CD4^{+} population. We are now correlating the isotype distribution of the B cell response with the lymphokine secretion pattern and the development of anergy and deletion of the responsive T-cells.

N 124 EVIDENCE FOR AN ENDOGENOUS SUPERANTIGEN DELETING THE HUMAN Vβ2 FAMILY OF T CELL ANTIGEN RECEPTORS, A.W. Boylston, G.R. Clark, F.C. Lancaster, Department of Clinical Medicine, Leeds University, Leeds, England.

Using a new mAb specific for T cells expressing the human Vβ2 gene segment we have discovered that Vβ2 gene use is bimodal in a normal human population. About 80% of normal individuals have 8.2±1.1% Vβ2$^+$CD4$^+$ T cells. The other 20% have 1.2±0.6% Vβ2$^+$CD4$^+$ T cells. Both Vβ2 high and Vβ2 low expression are stable over time. Vβ2 low expression is inherited, probably as an autosomal dominant trait, and is not linked to the TCR locus. Vβ2 low individuals have Vβ2 genes and the T cells which express them can be expanded by the Vβ2 selective toxin TSST-1 and by the Vβ2 specific mAb indicating that these cells are not anergic.

N 125 PHENOTYPIC FINGERPRINTING OF INFLUENZA VIRUS SPECIFIC T CELLS IN TRANSGENIC MICE WITH RESTRICTED T CELL RECEPTOR Vβ USAGE, Kieran Daly, William Allan, Anthony McMickle, David Woodland, Peter C. Doherty and Marcia Blackman, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105

We have shown that T cell receptor (TcR) beta chain transgenic mice, expressing Vβ8.1 on essentially all T cells, respond immunologically to influenza viral challenge, clearing virus from the lungs with kinetics comparable to the non-transgenic (CBA/Ca, H-2^k) counterpart. Here we characterize the TcR repertoire of, and the ligands recognized by, influenza specific T cells from transgenic and wild type mice. To determine the MHC restriction pattern, antigen specificity and TcR variable region gene usage in influenza specific T cells in wild type and TcRβ transgenic mice, we have generated a large panel of hybridomas from CD4$^+$ and CD8$^+$ T cells derived from the mediastinal lymph nodes of these strains. Most of the Class I restricted hybrids derived from the transgenic and wild type mice recognize nucleoprotein (NP) epitopes. Furthermore, these hybrids are restricted by K^k, indicating that the same epitope of NP is likely recognized in both strains of mice. The specificities of Class II restricted hybrids from transgenic and wild type mice will be compared to determine if similar TcR ligands are generated and recognized. Phenotypic analysis of the TcRVβ usage has shown that while all of the transgenic hybrids express Vβ8.1, cells bearing this Vβ element are poorly represented in the non-transgenic repertoire (20%). This finding suggests that while Class I restricted T cells in transgenic and non-transgenic mice may recognize identical ligands, they do so by using different TcR repertoires. This has implications for the plasticity of the T cell receptor repertoire.

N 126 RESTRICTED TCR USAGE AMONG INFLUENZA A VIRUS-SPECIFIC T CELLS. A. M. Deckhut, W. Allan, A. McMickle, M. Eichelberger, M. A. Blackman, P. C. Doherty, and D. L. Woodland. Department of Immunology, St. Jude Children's Research Hospital, Memphis TN 38105.

Clearance of influenza A virus infected cells is predominantly accomplished by CD8$^+$-virus specific cytotoxic T cells. In order to characterize this effector population, we have analysed the TcR usage in lymphocytes isolated from mediastinal lymph nodes (MLN) and bronchoaveolar lavage (BAL) of A/HKx31-infected C57Bl/6 (H-2^b) mice. We identified a strong enrichment of Vβ8.3$^+$ CD8$^+$ T cells in both MLN and BAL of secondarily-infected mice, and BAL taken from mice after primary infection. Depletion of Vβ8.3$^+$ T cells *in vivo* did not compromise the ability of mice to clear the virus, suggesting that other TcR elements are also involved in viral clearance. The Vβ8.3$^+$ CD8$^+$ T cells are directed to an immunodominant epitope of flu nucleoprotein (NP) since expansion of these cells was also seen in mice infected with a recombinant vaccinia virus construct containing the NP epitope 366-380. Analysis of T cell hybridomas, generated from freshly isolated BAL of infected mice, confirmed and extended these observations. The majority of flu-specific T cell hybridomas were Vβ8.3$^+$, though other TcR Vβ elements were represented. The Vβ8.3$^+$ hybridomas recognized the core H-2D^b-restricted NP epitope, 366-374. These hybridomas expressed similar Dβ and Jβ elements of the TcR whereas Vα usage was more variable. It was concluded that 1) the preferential expansion of Vβ8.3$^+$ CD8$^+$ T cells in response to influenza infection is due to recognition of an immunodominant epitope within NP, and 2) the response to influenza A virus infection is dominated by, but not exclusively, Vβ8.3$^+$ T cells.

N 127 PROCESSING OF AN EXOGENOUS PROTEIN FOR PRESENTATION BY CLASS I MHC. J.J. Donnelly, J.B. Ulmer, L.A. Hawe, A. Friedman, X.-P. Shi, D. Montgomery, K.R. Leander, J.W. Shiver, and M.A. Liu. Dept. of Cancer Research, Merck Research Laboratories, West Point, PA 19486.

Cytotoxic T-lymphocytes (CTL) recognize peptides derived from endogenously expressed proteins in association with class I MHC. In contrast, exogenous antigens associate with class II MHC following endocytosis to an endosomal compartment. We used the binding and translocating domains of *Pseudomonas* exotoxin A (PE) fused with CTL epitopes from 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. In contrast, an irrelevant protein, glutathione-s-transferase, fused to the matrix epitope did not sensitize target cells. A point mutant of PEMa, corresponding to an endosomal proteolytic processing mutant of PE, had a substantially reduced ability to sensitize target cells. Thus the 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 may not require translocation to the cytoplasm to exert its effect. Two inhibitors of PE intoxication, NH_4Cl, which raises endosomal pH and inhibits PE at a step subsequent to proteolysis, and brefeldin A, which may inhibit PE by disrupting the Golgi complex, did not inhibit sensitization of target cells by PEMa. Also, PEMa was capable of sensitizing T2 cells for lysis. The T2 mutant cells 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 PEMa is proteolytically processed in an endosomal compartment leading to association of the epitope with class I MHC, possibly by internalization of class I MHC and recycling to the cell surface. Fusion proteins such as PEMa may be useful as vaccines intended to elicit a CTL response.

N 128 A $\gamma\delta$ T CELL HYBRIDOMA DERIVED FROM A A/HK(X31) INFECTED $C\beta$-MUTANT MOUSE IS VIRUS SPECIFIC. M.C. Eichelberger, A. McMickle and P.C. Doherty. Department of Immunulogy, St Jude Children's Research Hospital, Memphis TN 38105.

When the murine respiratory tract is infected with influenza virus, there is an influx of $\gamma\delta$ T cells into the lung. In order to understand the role of these cells, it has been important to establish whether they have specificity for viral proteins. We have used $C\beta$-mutant mice kindly provided to us by Drs Mombaerts and Tonegawa (MIT, Boston MA) in order to work in a system deficient of virus-specific $\alpha\beta$ T cells. Cell suspensions from the mediastinal lymph nodes of A/HK (X31) infected mice were cultured with conA for 3 days before fusion with BWα-β- cells. The $\gamma\delta$ T cell hybridomas were screened for virus specificity by measuring IL2 released from cells in the presence of influenza-infected cells. A selected hybridoma could be stimulated by macrophages presenting the infecting virus A/HK (H3N2), as well as A/PR8 (H1N1) but not B/HK. H-2 compatible or H-2 different virus-infected macrophage cell lines could be used to stimulate the hybridoma. This suggests that the $\gamma\delta$ T cell hybridoma recognizes a conserved internal viral protein which is not presented by classical class I or II molecules.

N 129 T CELL RECEPTOR GENE USAGE IN HIV-1 SPECIFIC CYTOTOXIC T LYMPHOCYTES, Spyros Kalams*, R.P. Johnson*, A. Trocha*, M.J. Dynan*, J.T. Kurnick**, B.D. Walker*, *Infectious Disease Unit, Massachusetts General Hospital, Charlestown, MA 02129, ** Dept. of Pathology, Mass. General Hospital.

Recognition of virus infected cells by cytotoxic T lymphocytes (CTL) occurs through the interaction of the T cell receptor (TCR) with processed viral antigen. We wished to assess the spectrum of TCR gene usage in HIV-1 specific CTL isolated from infected individuals.

Peripheral blood mononuclear cells (PBMC) obtained from a seropositive individual were cloned at limiting dilution in the presence of IL-2, feeder cells, and a CD3-specific monoclonal antibody or PHA as a stimulus to T cell proliferation. CTL epitopes were defined using autologous EBV lymphoblasts incubated with synthetic HIV-1 peptides. cDNA was prepared from approximately $5x10^6$ cloned T cells. PCR was then performed with 5' specific oligonucleotides from the variable regions of the 29 known Vα genes and 24 known Vβ genes in conjunction with a 3' primer from the constant region of the respective TCR α or β gene. PCR products were then sequenced directly using the dideoxy chain termination technique.

Envelope specific CTL clones have been isolated from an HIV-1 infected individual over a 29 month span. All clones are HLA-B14 restricted and recognize the same nine amino acid minimal epitope. All clones thus far analyzed have utilized Vα14 and Vβ4 T cell receptor (TCR) genes. Sequencing of these genes has revealed identical diversity and joining regions, indicating a restricted TCR usage for this epitope which is maintained over time. Another HLA-B14 restricted clone isolated from this same patient, which is specific for an epitope in the reverse transcriptase (RT) molecule, utilizes the Vα 21 and Vβ 14 genes. Studies are in progress to determine whether CTL clones specific for other HIV-1 epitopes from this individual show limited TCR gene usage.

This study demonstrates that HIV-1 specific CTL of a given epitope specificity and HLA restriction can exhibit limited T cell receptor gene usage in a given individual which is maintained over time. Analysis of clones with the same epitope specificity and HLA restriction from different individuals will aid in the understanding of the role TCR gene rearrangement plays in CTL recognition of HIV-1 epitopes.

N 130 HUMAN T CELL RESPONSE TO MTV7 SUPERANTIGEN. Nathalie Labrecque, Helen McGrath, Meena Subramanyam, Brigitte T. Huber and Rafick-Pierre Sékaly. Laboratoire d'Immunologie, Institut de Recherches Cliniques de Montréal, Montréal, Canada, H2W 1R7 and Department of Pathology, Tufts University School of Medicine, Boston, MA 02111.

In the mouse, self superantigen (Mls) which influence the T cell repertoire and stimulate strong MLR responses have been described. These superantigens (sAg) are encoded by the ORF in the 3'LTR of MMTV virus integrated in the mouse genome. They are responsible for clonal deletion and inactivation of T cells expressing specific Vβs. No such self-sAgs have been described in man. Although human T cells respond to bacterial SAG, it is not known whether human T cells or human MHC class II molecules can interact with MMTV sAg.

In order to determine if human T cells are able to recognize murine endogenous sAg, we transfected the mtv7 sAg in DAP DR1 cells. These transfectants have been used to stimulate human T cells and we characterized this T cell response. The human T cell response to mtv7 was inhibited by anti-CD4 and anti-MHC class II, but not by anti-class I and anti-CD8 mAbs. Moreover, T cells responding to mtv7 sAg expressed a restricted number of Vβs. Cells expressing Vβ12, 13, 14 15 and 23 were amplified following mtv7 stimulation. These Vβs are the most homologous to the mouse Vβs that recognize mtv7.

These results clearly indicate that human T cells are able to recognize endogenous retroviral sAg and suggest that such endogenous sAg exist in man. We are presently investigating if human T cells are able to respond to other MMTV sAgs and we are characterizing their interactions with human MHC class II molecules.

N 131 ANALYSIS OF T CELL RECEPTOR (TCR) Vβ REPERTOIRE RESPONDING TO ANTERIOR CHAMBER (AC) INOCULATION OF HERPES SIMPLEX VIRUS (HSV) IN A MURINE MODEL OF HERPES RETINITIS (HR), **Soon Jin Lee, Zhengzhi Li, Victor Arrunategui-Correa and C. Stephen Foster. Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02114**

Genetic factors influencing the host immune response, including T cells, contribute to the generation of HR. T cell response was studied in BALB/c congenic mice that have different susceptibilities to HR. These congenic strains differ only at the Igh-1 locus, which influences T cell repertoire. In draining lymph nodes T cell subsets were analyzed by the polymerase chain reaction using oligonucleotide primers specific for 18 T cell subsets, defined by their Vβ gene usage of TCR, following AC inoculation with KOS strain of HSV-1. TCR Vβ gene usage in submaxilliary lymph node T cells was comparable in these congenic strains prior to inoculation. However, the kinetics of each T cell subset responding to herpes antigen differed between susceptible (BALB/c) and resistant (C.B-17) mice. Vβ 4, 6, and 14 were the major subsets responding to AC inoculation in C.B 17, while Vβ 9 and 13 were the major subsets in BALB/c. Higher levels of TCR Vβ 1, 4, 13, and 18 expression were observed in C.B 17 compared to BALB/c mice at earlier time points post inoculation. These results suggest that TCR Vβ gene usage was influenced by Igh-1-linked gene products and may contribute to the HR susceptibility patterns observed in BALB/c Igh-1 disparate congenic mice.

N 132 SUSCEPTIBILITY TO POLYOMA VIRUS-INDUCED TUMORS CORRELATES WITH Mtv-7- DELETION OF Vβ 6(7)-T LYMPHOCYTES,

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Determination of the tumor responses in a series of inbred mouse strains inoculated with mouse polyoma virus revealed two $H-2^k$ strains with completely opposite polyoma tumor susceptibility phenotypes. Virtually all C3H/BiDa mice inoculated with wild type polyoma virus developed a wide variety of tumors; polyoma-infected C57BR/cdJ mice failed to develop tumors. Of note, the (C3H/BiDa x C57BR/cdJ)F1 mice were as susceptible to polyoma-induced tumors as the C3H/BiDa parent. Genetic segregation analysis indicated that a single autosomal dominant gene in C3H/BiDa mice conferred susceptibility to polyoma-induced tumors (Benjamin et. al., manuscript in preparation). Because C3H/BiDa mice exhibit the Mls^1a Mls^2a phenotype (and carry Mtv-6, -7, -8, -14, -17) while C57BR/cdJ mice are Mls^1b Mls^2b (and carry Mtv-8, -9, -14, as well as deleting germline Vβ 5, 8, 9, 11, 12, 13 exons), we reasoned that either the Mls^1a or Mls^2a antigen mediates negative selection of Vβ 3 or Vβ 6(7)–expressing anti-polyoma T cells, respectively, resulting in development of polyoma-induced tumors in C3H/BiDa mice. FACS analysis of splenic T cells from polyoma-infected (C3H/BiDa x C57BR/cdJ)F1 x C57BR/cdJ backcross mice revealed very high correlation between the appearance of tumors and absence of Vβ6-positive T cells; in contrast, presence of Vβ 3-expressing T cells showed no correlation with tumor development. Segregation analysis of Mtv-7 in these backcross mice by Southern hybridization of genomic DNA with a Mtv-7 3' flanking cellular DNA probe showed perfect concordance with Vβ 6 usage. These studies suggest that the Mls^1a antigen encoded by the Mtv-7 open reading frame confers susceptibility to polyoma-induced tumors in C3H/BiDa mice by deleting Vβ 6 and/or Vβ 7-expressing T cells required for clearance of polyoma-infected cells/-induced tumors in vivo.

N 133 T CELL RECEPTOR USAGE OF INFLUENZA SPECIFIC HLA-A2.1 RESTRICTED CYTOTOXIC T LYMPHOCYTES FROM HLA-A2.1 TRANSGENIC MICE,

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In a previous study it was shown that HLA-A2.1 restricted influenza-specific cytotoxic T lymphocytes (CTL) derived from HLA-A2.1 transgenic mice recognized the same M1 peptide as HLA-A2.1 restricted human CTL (J.Imm 146:1226(1991)). Analysis of 10 independently derived murine CTL lines using a panel of mouse T cell receptor (TCR) Vß specific monoclonal antibodies demonstrated that Vß5.1, Vß6 or Vß8.1 dominated this response. TCR from representative clones expressing either Vß5.1, 6 or 8.1 were sequenced using VB specific and degenerate Vα primers to amplify cDNA by PCR. Both the Vß5.1 and Vß8.1 CTL were found to utilize the Jß2.6 segment whereas the Vß6 utilized Jß1.1. Three different Vα:Jα combinations were found among the clones and no consensus in amino acid sequence or length was seen for junctional regions of either α or β chains. Thus at least 3 different murine TCR are used to recognize the same human MHC:peptide complex. Comparison of these murine TCR sequences with sequences of human HLA-A2.1 restricted M1 peptide specific CTL revealed no common features of α or ß junctional regions. Interestingly, however murine Vß6 has striking homology to human Vß17 which is overrepresented in the human CTL response. Human Vα sequences that are the most homologous to the murine Vα sequences were also represented in the human T cell response, suggesting some conservation of TCR components recognizing the same MHC:peptide complex.

N 134 VIRAL SUPERANTIGEN PROCESSING AND PRESENTATION TO T-CELLS INVESTIGATED THROUGH MUTATIONAL ANALYSIS,

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Endogenous superantigens, encoded by mouse mammary tumor virus (MMTV) proviral integrants, have a profound effect on the shaping of the T-cell repertoire during tolerance induction. Viral superantigen (vSAG) forms a complex with MHC class II products on presenting cells, and is then able to stimulate T-cells bearing a particular Vβ element on their T-cell receptor, in a manner distinct from normal antigen presentation. MTV-encoded viral superantigens are type II membrane glycoproteins; however it is not known whether the superantigen is presented to T-cells in this form. Recent evidence (Winslow, G. M., *et al. Cell.* In press.) indicates that the Mtv-7 superantigen is found on the cell surface as a proteolytic fragment. To investigate whether this proteolytic cleavage is a requirement for viral superantigen presentation, protease recognition motifs in the vSAG-6 amino acid sequence were destroyed using site directed mutagenesis. These constructs were then expressed in a B-cell line and tested for their ability to stimulate $V\beta3^+$ T-cell hybrids *in vitro*. To the same end, the effects of specific protease inhibitors on vSAG processing are also being explored. In addition, vSAG-6 chimeric constructs are being generated using trans-membrane regions of Invariant chain that result in secreted or plasma membrane-bound type II proteins. Expression of these constructs should elucidate whether a vSAG-6 proteolytic fragment remains associated with the trans-membrane region of the protein, or whether the fragment alone can associate with MHC class II molecules with high affinity. Further mutational analysis of vSAG-6 should delineate regions of the superantigen that bind MHC class II products.

N 135 A MURINE MODEL FOR NEGATIVE SELECTION OF SELF-REACTIVE T LYMPHOCYTES: THYMIC EXPRESSION OF VIRAL SUPERANTIGENS-1 AND -6,

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Endogenous viral superantigens (vSAG) are encoded by proviral integrants of Mouse Mammary Tumor Virus (MMTV) and interact with T cells via the Vβ region of the T cell receptor, in an MHC unrestricted fashion. During thymic maturation, recognition of certain antigen/MHC complexes may sufficiently stimulate a developing thymocyte such that it undergoes negative selection. Thus, exposure to viral superantigens in the thymus results in the deletion of reactive Vβ-bearing thymocytes. Two lines of congenic mice have been derived in which the genes encoding vSAG-1 or vSAG-6 are expressed singly on a B10.BR background. Interestingly, the amino acid sequences of vSAG-1 and vSAG-6 are identical, yet deletion of Vβ3 bearing thymocytes varies between the two strains. This phenomenon could be explained by differences in: expression levels of vSAG on presenting cells, ability of presenting cells to efficiently present antigen and/or induce deletion, and/or susceptibility of reactive thymocytes to deletion. Data will be presented from our current study of the mRNA expression levels of vSAGs 1 and 6 within thymic microenvironments, thymocyte subsets, and in the periphery of these mice, using RNase protection assays. Concomitantly, surface vSAGs will be detected by their ability to stimulate Vβ3 bearing T cell hybridomas and their effect on thymocyte deletion will be analyzed by fluorescent antibody staining. These experiments will expand our understanding of the location, timing and cell types involved in the negative selection of "self"-reactive thymocytes.

N 136 ROLE OF A SUPERANTIGEN IN MAIDS: DELETION OF Vβ5.2-BEARING CD4 CELLS IN VIVO,

Girija Muralidhar, Susanne Koch, Robert S. Balderas, Dwight H. Kono, Argyrios N. Theofilopoulos and Susan L. Swain, University of California, San Diego and The Scripps Research Institute, La Jolla, CA

The BM5 mixture of replication competent and defective retroviruses causes a dramatic and fatal immunodeficiency disease (MAIDS) in susceptible strains of mice. The disease is characterized by lymphadenopathy and splenomegaly including an early increase in CD4 T cell number, but loss of CD4 T cell function. Importantly, both the initiation and the progression of MAIDS is dependent on CD4 T cells. Recently, Hügin et al. suggested that the BM5 retroviral mixture encodes a product which stimulates CD4 T cells bearing several Vβs in vitro. Here we report that the mRNA synthesis of Vβ5.2, but not other Vβs, is dramatically and selectively depressed in the thymus and in peripheral CD4 T cells of C57BL/6 mice with MAIDS as early as four weeks after infection. These results indicate that the BM5 mixture of retroviruses most likely encodes a functional superantigen or stimulates the expression of an endogenous superantigen specific for Vβ5.2.

To further evaluate the importance of this deletion in disease induction and progression, we infected C57L/J mice which lack a panel of Vβ genes including Vβ5.2. The progression of the disease was strikingly slower in C57L/J as compared to age matched C57BL/6 mice which develop the characteristics of MAIDS within the first few weeks of infection. The extent of splenomegaly, lymphadenopathy and function of CD4 T cells were compared between the two strains of mice during the course of the disease. At eight weeks of infection, splenomegaly and lymphadenopathy of C57L/J mice were less pronounced and the CD4 T cells still responded to mitogenic stimuli by proliferation and IL-2 production, whereas the CD4 cells from infected C57BL/6 mice failed to do so.

These results indicate that Vb5.2 deletion in BM5 induced MAIDS could play a role in disease progression and induction of anergy.

N 137 A NEW EXOGENOUS MMTV INDUCING AN Mls-1a CLONAL DELETION IN Mls-1b MICE

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We have previously described an Mls-1a-like clonal deletion of mature CD4+ T cells which express Vβ6 and Vβ8.1 chains of the T cell receptor in half of the mice of a BALB/c, Mls-1b colony (BALB/c, IC). This occurs in the absence of the Mtv-7 provirus which is responsible for the clonal deletion in Mls-1a mice (1). Using a PCR assay, we showed that Mtv-7 homologous transcripts were present in the mammary glands of lactating BALB/c, IC mice and in the thymuses and/or spleens of BALB/c, IC virgin mice with deletion of Vβ6+ lymph node T cells, and not in BALB/c, IC with normal Vβ6 expression (2). These results indicate that this BALB/c colony is infected with an exogenous MMTV retrovirus whose vSAG is similar to that of Mtv-7 provirus, as recently reported. Transmission of this virus is achieved through the milk from the lactating mother to the suckling pups. Mother forstering with mice of different H-2 backgrounds showed that the requirement for I-E expression is more stringent when the clonal deletion is induced by the exogenous than the endogenous virus. Thymectomies performed at 4-5 weeks of age (at least 4 weeks before detection of clonal deletion), did not affect the occurrence of clonal deletion in peripheral lymph nodes when tested twenty weeks later. This suggests that clonal deletion can be achieved without further intrathymic contact with the antigen. Since MMTV is transmitted through milk and is likely to be present in the gut, we evaluated the percentage of Vβ6+CD4+ T cells within the gut intraepithelial lymphocyte (IEL) population. Mice with normal Vβ6 expression in lymph nodes may show partial deletion of Vβ6+CD4+ IEL. This is explained by exclusive localisation of Mtv-7-like RNA in the gut of some mice. Finally, despite the similarity of the vSAG encoded by the endogenous Mtv-7 with the exogenous MMTV in BALB/c, IC mice, the latter has a much lower stimulatory capacity when compared to the former.

(1) Papiernik M, Pontoux C, Gisselbrecht S (1992). J.Exp.Med. 175: 453-460.
(2) Desaymard C, Tucek C, Rocha B, Korman A, Papiernik M (1992). Int.Immunol. (in press).

N 138 T-LYMPHOCYTE SUBSETS IN LUNG INFECTIONS : *γδ* T-CELLS ARE INDUCED BY NON-VIRAL PATHOGENS

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To compare the phenotype of T-cells responding to various pathogens, BALB/c mice were infected intranasally with *M.bovis* BCG, *Listeria monocytogenes*, *Chlamydia trachomatis*, *Cryptococcus neoformans*, influenza virus, or respiratory syncytial (RS) virus. Lung cells were collected by bronchoalveolar lavage (BAL) and analyzed by flow microfluorimetry. All the pathogens provoked a significant T-cell response compared with BAL cells from sham infected animals, but the relative abundance of alveolar macrophages, granulocytes and lymphocytes varied substantially between infections. After infection with influenza virus, RS virus, *C.trachomatis* or *C.neoformans* T-cells expressing *αβ* T-cell receptor (TCR) comprised >95% of total BAL T-cells. Significant recruitment of *γδ* T-cells to the lung was detected only in mice infected with *M.bovis* (*γδ* TCR$^+$ cells 8-58% of BAL T-cells, mean = 32.2%) or *L.monocytogenes* (*γδ* TCR$^+$ cells 1-22% of BAL T-cells, mean = 9.4%). In each case, T-cells expressing *γδ* TCR were predominantly of the CD4$^-$8$^-$ phenotype whilst CD4$^+$ and CD8$^+$ T-cells in these and other infections were exclusively *αβ* TCR$^+$.
The pathogens which induce *γδ* T-cell responses therefore seem to be those which are known to multiply within alveolar macrophages. Respiratory viral infections do not induce a strong *γδ* T cell response, as determined by surface TCR expression.

N 139 REACTIVITY OF RAT T CELLS TO MOUSE ENDOGENOUS MMTV-ENCODED SUPERANTIGENS

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Through the use of xenogeneic rat -> mouse chimeras and a polymerase chain reaction method to quantitate rat T cell receptor Vß mRNA composition we demonstrate in two ways that rat T cells can recognize mouse Mls antigens. The rat -> mouse chimeras possessing *de nove* generated rat T cells were established by transplanting Lewis rat fetal liver cells into neonatal C.B-17 SCID (H-2^d, Mls^c) mice. Firstly, the lymph nodes from the rat -> mouse chimeras contained considerably reduced levels of rat Vß 3, 5, 12 and 16-positive T cells - these Vß$^+$ T cells are easily detectable in normal Lewis rats although Vß11$^+$ T cells are inherently very low. (Note that rat TCR Vß chains are numbered according to the homologous mouse conterparts). The deletion of rat Vß16$^+$ T cells in the chimeras was confirmed with a recently produced rat Vß16-specific mAb HIS 42. Secondly, the T cells from the rat -> mouse chimeras proliferated very strongly to H-2 matched Mls-positive DBA/2 (H-2^d, Mls^a) stimulator cells in mixed lymphocyte culture, although these T cells showed complete tolerance to syngeneic BALB/c (H-2^d, Mls^{a-}) stimulator cells. The rat T cell population responding to DBA/2 stimulator cells was highly enriched for rat Vß6$^+$ and 8.2$^+$ T cells. Similar results were obtained with chimeras established by tranplanting fetal liver from another rat strain (Fisher 344) into C.B-17 SCID mice. These data suggest that rat T cells are able to react to mouse Mls antigens, and that rat T cells are be subjected to MMTV antigen-mediated clonal deletion during T cells differentiation in the rat -> mouse chimeras.

N 140 SUPERANTIGEN AND *NAF* ACTIVITIES OF MOUSE MAMMARY TUMOUR VIRUS CAN BE SEPARATED

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Mouse Mammary Tumour Virus (MMTV) has long been known to contain an open reading frame (ORF) within its unusually long U3 region. This ORF has the potential to encode a set of proteins of varying sizes. Recently we have shown that the ORF encodes a negative transcriptional regulatory factor (*naf*) which functions *in trans* to decrease the rate of transcription from an MMTV based indicator construct [J.Virol.,1990;64,6355]. Downregulatory *naf* effects can also be seen on homologous and heterologous promoters linked to the luciferase gene. It has also been shown that the MMTV ORF encodes a superantigen (*sag*), the expression of which during ontogeny, results in the Vß specific deletion of T-lymphocytes. This appears to occur by a specific interaction between the *sag* and the ß chain of the T-cell receptor that is common among a given class of T-cells. Although *sag* and *naf* are both products of the ORF, it is not clear how these two activities are related. However using a series of constructs we have been able to distinguish between the required coding regions for *naf* and *sag*. Additionally, we have identified a novel promoter located within the U3 part of the long terminal repeat region (LTR). Both the previously described promoter and the novel U3 promoter are active in mammary cells and B-lymphocytes. However the U3 promoter is preferentially active in LPS stimulated B-lymphocytes and is able to direct expression of the *sag* product, making it a candidate for the lyphocyte specific expression of this gene in the mouse.

N 141 REVERSIBLE VS. IRREVERSIBLE FORMS OF T CELL UNRESPONSIVENESS TO Mls-1a ARE TWO SEPARATE MECHANISMS OF T CELL CLONAL ANERGY.

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T cell clonal anergy is a form of tolerance in which mature T cells are physically present but are not able to mount an immune response. We induced T cell unresponsiveness to Mls-1^a in mice transgenic for T cell receptor Vβ8.1 by injection of Mls-1^a spleen cells, by mating with Mls-1^a mice, and by generating bone marrow chimeras in which Mls-1^a is present only on non-hematopoietic cells. $CD4^+8^-V\beta8.1^+$ cells from all these groups did not proliferate in response to irradiated spleen cells from Mls-1^a mice. However, the mechanisms underlying the unresponsiveness appear to differ. $CD4^+8^-V\beta8.1^+$ cells from Mls-1^a spleen cell injected Vβ8.1-transgenic mice mobilized cytoplasmic Ca^{2+} but proliferated at a reduced level in response to crosslinking with anti-TCR mAb. However, these cells formed conjugates, mobilized Ca^{2+} and proliferated in response to Mls-1^a when activated B cells were used as stimulators. In Mls-$1^{a/b}$ Vβ8.1-transgenic mice, a subpopulation of $CD4^+8^-V\beta8.1^+$ cells did not mobilize cytoplasmic Ca^{2+} after TCR crosslinking. They did not form conjugates, mobilize Ca^{2+} or proliferate in response to Mls-1^a on activated B cells. Finally, $CD4^+8^-V\beta8.1^+$ cells from the bone marrow chimeras proliferated to TCR crosslinking at a partially reduced level; and formed conjugates, mobilized Ca^{2+}, and proliferated in response to Mls-1^a on activated B cells. These features indicate that there are at least two distinct mechanisms underlying T cell clonal anergy *in vivo*, and that some form(s) of clonal anergy can be reversed when antigen is presented by an appropriate antigen presenting cells.

Evasion

N 200 RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION RESULTS IN A T CELL- DEPENDENT INCREASE IN HLA-DR, CD11B, CD11C, AND ICAM-1 ON MONOCYTES BUT A DECREASE IN RSV SPECIFIC T CELL PROLIFERATION.

Susanne Becker and Joleen Soukup. TCR Environmental Corporation and EPA Human Studies Division, Chapel Hill, NC .

The role of the immune response in RSV infection is not well understood. Re infection is common in both children and adults despite the presence of RSV specific antibodies and a T cell response to RSV antigens. It is intriguing that inactivated RSV (IRSV), but not infectious RSV (Inf-RSV) stimulates the T cell proliferative response in vitro. We have analyzed whether stimulation of blood mononuclear cells with IRSV and Inf-RSV results in differences in expression of surface markers and cytokines important in the immune response. HLA-DR, ICAM-1, CD11a, CD11b, CD11c, CD14, CD4 and CD8 expression was determined by flow cytometry on unexposed and RSV-exposed monocytes and lymphocytes 24 and 48 hours after stimulation. A dramatic effect on monocyte surface markers was found with both Inf-RSV and IRSV: a 4-8 fold increase in HLA-DR, a 2-4 fold increase in ICAM-1, a 2-4 fold increase in CD11c, a 1.5- 2 fold increase in CD11b, and a 2-4 fold decrease in CD14; Inf-RSV was generally twice as effective as IRSV. There was no change in CD11a expression. The only effect of RSV on the lymphocytes was an increase in CD11b positive, HLA-DR negative cells found with Inf-RSV but not with IRSV. Exposure of the monocytes to virus in the absence of T cells gave none of the changes in surface antigen expression. Various cytokines such as IFN-γ, IL-2, IL-4, IL-10, and GM-CSF have each been shown to induce some of the changes seen in monocytes after RSV stimulation. These cytokines are being analyzed in the supernatants of IRSV and Inf-RSV stimulated cells. The high HLA-DR and ICAM-1 expression on RSV infected monocytes would suggest effective antigen presentation, but these cells do not support RSV-specific T cell proliferation, as measured by ^{3}Hthymidine incorporation. Instead their state of activation may result in increased production of mediators which counteract immune responses, as well as influence T cell functions other than proliferation.

This abstract of a proposed presentation does not necessarily reflect EPA policy.

N 201 DO VACCINIA VIRUS-ENCODED SERPINS INHIBIT ANTIGEN PRESENTATION TO CLASS I-RESTRICTED CTL?

Neil W. Blake, Susan Kettle, Keith Gould, Judy Bastin*, Alain Townsend* and Geoffrey L. Smith, Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. *Institute for Molecular Medicine, University of Oxford.

Vaccinia virus, a member of the poxvirus family of large cytoplasmic DNA viruses, has been used extensively in studying the mechanism of antigen processing and presentation. However, as with some other viruses, vaccinia encodes a number of gene products which are involved in interfering with the host cell immune response. These include an interleukin-1β receptor, complement control proteins and serine protease inhibitors (serpins). Previous data from recombinant vaccinia viruses expressing the influenza virus haemagglutinin (HA) or nucleoprotein (NP) have shown that vaccinia infection interferes with the presentation of some epitopes from these proteins to class I-restricted cytotoxic T lymphocytes (CTL). Recombinant vaccinia viruses expressing the influenza HA or NP have been constructed in which either or both vaccinia serpins (B13R and B22R) have been deleted. The presentation of influenza epitopes from cells infected with influenza or vaccinia virus recombinants with or without the serpins was monitored by ^{51}Cr release after incubation with epitope-specific CTL. An epitope presented from influenza virus infected cells but not from serpin positive vaccinia virus infected cells was re-presented after serpin deletion. This restoration was epitope and serpin specific and occurred at early but not late times during vaccinia infection. Further experiments to address this block at late times are being undertaken.

N 202 The effect of the Adenovirus E3/19k protein on antigen presentation in vivo. Josephine H. Cox, Jack R. Bennink, Jonathan W. Yewdell, R. Mark L. Buller and Gunasegaran Karupiah. Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD 20892.

The Adenovirus E3/19k glycoprotein (E3/19k) is a resident glycoprotein of the endoplasmic reticulum(ER) that binds to some class I antigens. We have previously shown that mouse cells infected with a vaccinia (vac) recombinant that expresses the E3/19k protein blocks the ability to present antigens to MHC class I K^d restricted T cells. In order to assess the possible effect of the E3/19k on the pathogenesis of infection, we immunized mice of the relevant H-2 background with the E3/19k recombinant (E19-vac) or a recombinant that expresses the E3/19k protein lacking the ER retention sequence (ΔE19-vac). The ΔE19 protein still binds to class I but does not inhibit the presentation of antigens to T cells.

Vaccinia virus titres recovered from the spleen, lung, liver and ovaries of mice infected with E19-vac or ΔE19-vac were compared. In the same mice, we also examined whether E19-vac or ΔE19-vac could immunize mice for a primary or secondary cytotoxic T lymphocyte (CTL) response. The expression of the E3/19k protein did not substantially affect the recovery of vac virus from the various organs examined or the induction of CTL.

N 203 CHARACTERIZATION OF A VACCINIA VIRUS 42 KD ENVELOPE GLYCOPROTEIN WITH HOMOLOGY TO COMPLEMENT CONTROL PROTEINS, Maiken Engelstad and Geoffrey L. Smith, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, ENGLAND

The extracellular enveloped form of vaccinia virus (EEV) plays a major role in virus dissemination *in vitro* (comet formation) and *in vivo* and is thus important for virus pathogenesis. Ten proteins have been shown to be specific to the EEV outer envelope. One of these, a 42 kD constitutively expressed glycoprotein, is encoded by gene B5R. (Engelstad et al, Virology 188, 801-810, 1992) Western blot analysis locates the 42 kD protein as well as two higher MW complexes to the EEV (but not the intracellular naked virus (INV)) and anti-B5R sera inhibits comet formation.
The B5R ORF is highly conserved in four different strains of vaccinia virus as well as in variola major virus Harvey strain. The protein is predicted to be a class I transmembrane protein and contains four short consensus repeat regions characteristic of the regulators of complement activation superfamily. The protein also shows homology to another secretory vaccinia virus protein (C21L) which has been shown to interfere with the complement system.
The B5R gene product has been characterized to evaluate its role in vaccinia virus biology and to establish whether it contributes to virulence. By constructing a deletion mutant the gene is shown to be nonessential for virus growth in tissue culture. However, the deletion mutant has a reduced plaque size and is attenuated when inoculated intranasally into mice. Electron microscopy indicates that very little EEV form of the virus is produced or released by the deletion mutant. Consistent with this observation, when infected cells are labeled with [^{3}H]-thymidine and the INV and EEV particles released from the cells 24 hrs p.i. are separated by CsCl density gradient centrifugation, EEV production by the deletion mutant is reduced by 85% compared to WT levels.
These results suggest that the B5R gene product is important for EEV formation. Additionally, being located on the outer envelope, the protein might be involved in binding cell surface receptors to initiate infection and/or to inhibit complement-mediated lysis of EEV particles or infected cells.

N 204 VIRUS-INDUCED IMMUNOSUPPRESSION: DIFFERENCES IN SPLENIC DESTRUCTION CAUSED BY AN IMMUNOSUPPRESSIVE VARIANT (CLONE 13) OF LCMV COMPARED TO THE NON-IMMUNOSUPPRESSIVE PARENTAL VIRUS (LCMV ARM). Claire F. Evans, Persephone Borrow, and Michael B.A. Oldstone, Department of Neuropharmacology,The Scripps Research Institute, La Jolla, CA 92037
Lymphocytic choriomeningitis virus (LCMV) is a bisegmented RNA virus that can cause either an acute or persistent infection. Intravenous (i.v.) inoculation of 2X10^6 pfu of LCMV strain Armstong (ARM) into adult mice generates a vigorous cytotoxic T cell (CTL) response that clears virus by 7 to 14 days. In contrast, i.v. inoculation of 2X10^6 pfu of a variant of LCMV ARM, Clone 13, leads to a poor CTL response resulting in persistent infection. Those mice infected as adults with Clone 13 show a generalized immunosuppression, with low to negligible CTL responses generated in response to infection with other viruses such as vaccinia, influenza, and herpes simplex. Genetic and sequence studies map the immunosuppressive phenotype of Clone 13 to a single amino acid change at position 260 in the glycoprotein. To determine how Clone 13 causes persistent infection and generalized immunosuppression, the kinetics of infection by ARM or Clone 13 of lymphoid tissues in adult mice were studied. Histological examination revealed that after infection by ARM and Clone 13 a marked loss of splenic architecture occurred by day 5 post-infection. Areas of red and white pulp were no longer distinguishable as the normal follicular structures were lost. By day 14 post-infection, spleens from ARM-infected mice returned to normal and displayed a clear demarcation between red and white pulp. In contrast, spleens from Clone 13-infected mice continued to be disorganized. By day 30 post-infection, the spleens of mice infected with Clone 13 showed the reappearance of follicles, but with a layer of fibrosis around the white pulp regions and a marked loss of red pulp when compared to normal or ARM infected spleens. Immunohistochemical studies suggest a loss in the macrophage (antigen presenting cell) region. The T cell dependence of the macrophage layer loss is currently being investigated by studying infection of T cell deficient mice. In addition, unique cell populations are being isolated and analysed by in situ hybridization using virus-specific RNA probes to determine whether ARM and Clone 13 have a different tropism for various cells of the spleen.

N 205 DETECTION OF CYTOTOXIC T LYMPHOCYTES IN MICE INFECTED WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS. Chen Even, Raymond R. R. Rowland, and Peter G. W. Plagemann, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455.
Lactate dehydrogenase-elevating virus (LDV) establishes a lifelong viremic infection in all strains of mice. The persistent infection is maintained by a cytocidal replication of LDV in a nonessential subpopulation of macrophages. The infection is asymptomatic, except in certain strains (C58, AKR) in which LDV can infect spinal cord motor neurons and result in poliomyelitis. LDV causes a transient suppression of cellular immune responses during the acute phase of infection, but a marked permanent polyclonal activation of B cells resulting in the elevation of plasma IgG2a levels up to 10 mg/ml. Mice generate anti-LDV antibodies but these fail to protect mice from LDV infection or inhibit LDV replication during the persistent phase. Cytotoxic T cells (CTLs) also do not control LDV replication because LDV viremia is similar in nude and T cell depleted mice as in immunocompetent mice. The present study was designed to examine whether mice mount a cellular immune response to LDV and if so, why it fails to control LDV replication. Specifically we examined CTLs and the production of γIFN. As an alternative to the standard ^{51}Cr assay a colorimetric method was used to measure cell lysis by CTLs. Cells from lymph nodes (LN) of infected mice were incubated with infected macrophage targets and macrophage survival at the end of the assay was estimated by staining viable macrophages with the dye neutral red. Between 10-15% of the LDV-infected macrophages were lysed when incubated with LN cells from LDV-infected mice, whereas mock-infected macrophages were not significantly lysed. Since only 5-15% of macrophages from adult mice are LDV-permissive, these results suggest that all LDV-infected macrophages were lysed and that the colorimetric method is suitable for detecting cell lysis in our system. Production of γIFN, measured by both ELISA and Northern analysis of the mRNA, indicated increases in γIFN synthesis following LDV infection beginning about 4 days p.i. This correlated well with increased plasma levels of IgG2a. However, addition of IFN to macrophage cultures failed to inhibit virus replication. LDV infection did not alter the clinical course of the lethal disease caused by lymphocytic choriomeningitis virus in adult mice which is mediated by CTLs, indicating that LDV infection *per se* does not abolish the generation of CTLs. Taken together, it seems that strong anti-LDV cellular immunity is generated following LDV infection but with little or no effect on viral replication in the periphery. Future experiments are targeted at the mechanisms by which anti-LDV CTLs fail to control viral replication in *in vitro* and *in vivo* experimental systems.

N 206 T CELL IMMUNE RESPONSES IN SV40 T ANTIGEN TRANSGENIC MICE: TOLERANCE INDUCTION VERSUS AUTOIMMUNITY AND TUMOR IMMUNITY, Irmgard Förster, Philippe Hartl and Douglas Hanahan, Hormone Research Institute, UCSF, San Francisco, CA 94143
Transgenic mice expressing the SV40 large T antigen (Tag) under the control of the rat insulin promoter (RIP) are either tolerant toward Tag or develop an autoimmune reaction against their pancreatic ß cells depending on the onset of Tag-expression in ontogeny. Furthermore, both the tolerant and the autoimmune type of mice reproducibly develop pancreatic ß cell tumors that evade recognition by the immune system. We have isolated $CD4^+$ and $CD8^+$ Tag-specific T cell lines from the pancreas of an autoimmune RIP1-Tag5 mouse. The $CD8^+$ clone 2.6ß.4 was shown to mediate Tag-specific cytotoxicity and is presently being used to study the relative inaccessibility of pancreatic ß tumor cells to T cell recognition. In addition, we have cloned the T cell receptor (TCR) variable region genes expressed in one of the $CD4^+$ T cell lines (2.5T) by RT-PCR techniques and have subsequently isolated the genomic Vß8.3-Dß2.1-Jß2.4 and Vα2-JαMT1-27 V gene rearrangements encoding a MHC Cl.II restricted Tag-specific TCR. Clonotype-specific as well as Vß8.3-specific antibodies were generated by immunization of mice with a hybridoma line expressing this TCR. The genomic Vß8.3 and Vα2 gene rearrangements were inserted into cosmid vectors already containing the TCR α and ß constant region genes (provided by D. Loh) and are presently used to generate TCR transgenic mice which will then be bred to either the tolerant or the autoimmune RIP-Tag mice. With the availability of these reagents we are planning to further dissect the various pathways of T cell responsiveness toward the ß cells in RIP-Tag mice.

N 207 SELECTIVE INTERFERENCE IN CELLS INFECTED WITH HUMAN CYTOMEGALOVIRUS (CMV) WITH THE PRESENTATION OF IMMEDIATE-EARLY PROTEIN (IE1) VIA THE CLASS I PATHWAY , Mark Gilbert, Stan Riddell, Cheng-rong Li, and Philip Greenberg, Fred Hutchinson Cancer Research Center and Univ. of Washington, Seattle, WA 98104
IE1, an abundantly expressed viral transcription factor, is essential for CMV replication and has been suggested to serve as one of the main target antigens for Tc. However, IE1-specific Tc responses were not detected among nearly 500 CMV-specific Tc clones that were isolated by in vitro stimulation of PBMC from 10 individuals with CMV-infected autologous fibroblasts. Isolation of IE-specific Tc clones required stimulation in vitro with autologous cells that selectively expressed IE1 and no other CMV proteins. Even with this approach, only 6 CD8+, IE-specific Tc clones were isolated from over 7680 wells seeded at limiting dilution (0.3 cells/well). Each IE-specific Tc clone demonstrated greater than 32% Class I-restricted lysis (E:T=10) of cells infected with an IE1-recombinant vaccinia virus (vacIE), but less than 5% specific lysis of CMV-infected targets despite readily detectable IE1 expression. Pretreatment of CMV-infected target cells with IFN-gamma (100U/ml), which increases expression of Class I but not IE1, increased lysis of CMV-infected targets by IE-specific Tc clones (E:T=10) to between 15 and 34%. To determine if other viral antigens were also inefficiently presented in CMV-infected cells, lysis of these targets by Tc clones specific for CMV matrix protein pp65, and CMV envelope protein gB were analyzed and compared to IE-specific Tc responses. CMV-infected target cells were recognized and lysed by Tc specific for pp65 from early phase (<2hrs p.i.) (11% lysis) through late phase (48hrs p.i.) (50% lysis), and were efficiently lysed (25% lysis) by gB-specific Tc during late phase infection. As before, IE-specific Tc demonstrated less than 5% specific lysis of these targets during all phases of CMV infection (E:T=10). To determine if other CMV proteins were responsible for restricting IE1 presentation, we compared target cells infected with vacIE alone with those coinfected with vacIE and CMV for lysis by IE-specific Tc. IE-specific Tc demonstrated 45% specific lysis of vacIE-infected targets but less than 5% lysis of coinfected targets (E:T=10), even though both targets expressed equal levels of IE1. Our data demonstrate that CMV has evolved a mechanism that restricts the presentation of a specific viral protein, IE1. Such mechanisms may limit immune detection during acute infection and latency. Moreover, the results suggest that IE-specific Tc, if present, would have limited efficacy in providing protective immunity.

N 208 RESISTANCE TO TGF-β1 INDUCED SUPPRESSION IN HTLV-I IN VIVO INFECTED T CELLS, David A. Hafler, Per Hollsberg, Lara J. Ausubel, Center for Neurologic Diseases, Brigham and Woman's Hospital, and Harvard Medical School, Boston MA 02115
The HTLV-I regulatory protein tax transactivates the promoter for transforming growth factor ß (TGF-ß), a cytokine that potently suppresses T cell proliferation. Nevertheless, HTLV-I infected T cell clones display a prolonged state of T cell activation. T cell proliferation occurs after pRb phosphorylation, inactivating the suppressive effect of pRb. In these experiments, we investigated whether HTLV-I infected T cells from patients with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) conferred resistance to immune suppression by TGF-ß. T cell clones were generated from peripheral blood of HAM/TSP patients by direct single cell cloning and characterized by PCR and Southern blot analysis for the presence of HTLV-I infection. Response to TGF-ß was measured by ^{3}H-TdR incorporation. Phosphorylation of pRb was identified by immunoprecipitation of ^{35}S-methionine labelled T cells. We found that HTLV-I infected but not noninfected T cell clones were impaired in their growth suppression by TGF-ß1 and this correlated with the failure of TGF-ß1 to inhibit phosphorylation of pRb. These findings suggest that HTLV-I has evolved a defense against immune suppression of its host cell. Resistance to TGF-β1 induced suppression in HTLV-I infected T cells may have important implications for the altered immune regulation in HAM/TSP patients.

N 209 AN ADENOVIRUS 14.7K PROTEIN WHICH INHIBITS CYTOLYSIS BY TNF INCREASES THE PATHOGENICITY OF VACCINIA VIRUS IN A MURINE PNEUMONIA MODEL. JoAnn M. Tufariello[1], Sangho Cho[3] and Marshall S. Horwitz[1,2], Departments of [1]Microbiology and Immunology, [2]Pediatrics and [3]Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

Human adenoviruses (Ads) encode proteins with the potential to alter the host inflammatory response to infection. Ad immune modulators include the early region 3 (E3) protein, gp19k, which downregulates cell surface expression of class I MHC. Other E3 proteins have been shown to protect cells from TNF-α induced cytolysis in vitro (Horton et al. J Virol 65:2629-2639, 1991); these include the 14.7K and the combination of the 10.4/14.5K proteins. In addition, the 19K product of the E1B transcription unit has similar effects on TNF. The mechanism of inhibition of TNF-induced cytolysis and its significance in vivo are largely unknown.
To assess the ability of 14.7K from Ad2 to affect disease in mice, we constructed vaccinia virus (VV) recombinants expressing this protein. VV was chosen as a vector both to synthesize individual Ad proteins and because human Ads replicate poorly in mice. TNF expression in VV has been shown to have an attenuating effect on murine infections (Sambhi et al, Proc Natl Acad Sci USA 88:4025-4029, 1991). In order to ensure high local concentrations of both the agonist (TNF) and its antagonist (14.7K) at the site of infection, we cloned the gene expressing murine TNF-α into the VV HindIII F region and the Ad2 14.7K coding sequence into the VV thymidine kinase gene in both expressing and nonexpressing orientations. Balb/c mice were infected intranasally with each of the VV recombinants [TNF(+)/14.7(+) or TNF(+)/14.7(-)] and examined for clinical illness, mortality (LD_{50}), histopathology of lung and other tissues and viral replication. The expression of 14.7K by the TNF producing VV enhanced its pathogenicity as evidenced by increased severity of disease and lethality, increased pulmonary viral titers, delayed viral clearance from lungs, and a more pronounced peribronchial and perivascular pulmonary inflammatory response. Further study of this pneumonia model is underway to determine the extent to which the cytokine, cell mediated and humoral responses of the immune system are altered by 14.7K expression.

N 210 THE EFFECT OF SELECTIVE ABLATION OF IL-2 PRODUCING CELLS IN THE RESPONSE TO KLH

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IL-2 is well known as an autocrine growth factor in T cell clonal expansion, which is necessary for protection against infection. Recently, it has also been reported that the consequences of an infectious disease are symptomatic on whether Th1 or Th2 cells are activated. Cytokines are thought to account for the different results that follow the activation of these two types of cells. Th1 cells produce IL-2, gamma IFN and Th2 cells produce IL-4,-5 and -10. To study the function of IL2 in T cells, we have generated transgenic mice which express the herpes simplex virus-1 thymidine kinase (HSV-TK) gene under the transcriptional control of the murine IL-2 promoter which renders IL-2 producing cells sensitive to the cytotoxic effects of the anti viral drug gancyclovir. HSV-TK activity was specifically expressed in activated T cells from transgenic mice. We immunized these transgenic mice with KLH in vivo, purified CD4+T cells from drainage lymph nodes and observed the recall response to KLH in vitro in the presence or absence of gancyclovir. Cytokines from the supernatants and production of KLH specific antibodies were also measured . We found that KLH dependent proliferation was inhibited 80-90% and also that IL-4 and gammma IFN product was suppressed 70-80 % by gancyclovir. In the subsets of KLH specific antibodies, IgG1 showed partial suppression, and IgG2a, and IgE showed almost complete suppression. These results suggest that IL-2 is an important factor in Ag specific clonal expansion. In addition, we now suspect that IL-2 producing cells are precursors of IL-4 and IFN producing cells.

N 211 *IN VIVO* VIRUS-SPECIFIC TOLERANCE INDUCTION RESULTS IN THE DOWN REGULATION OF ANTIGEN-SPECIFIC CD4$^+$ TH1, BUT NOT TH2, LYMPHOKINE RESPONSES.

William J. Karpus and Stephen D. Miller, Department of Microbiology and Immunology, Northwestern University Medical School, Chicago, IL 60611.

We have previously demonstrated that tolerance induced via the i.v. injection of syngeneic splenocytes coupled with intact, UV-inactivated Theiler's murine encephalomyelitis virus virions (TMEV-SP) using ethylenecarbodiimide (ECDI) results in 'split tolerance.' Both TMEV-specific delayed type hypersensitivity (DTH) responses and anti-TMEV IgG2a levels were inhibited, whereas anti-TMEV IgG1 levels were greatly increased (by 20-100 fold) when compared to sham-tolerized controls. We now demonstrate that i.v. TMEV-SP-induced tolerance results in a dose-dependent decrease of virus-specific T cell proliferation, and reduced IL-2 and IFN-γ production. Additionally, tolerance induction results in the highly significant downregulation of Th1-derived IL-2, IFN-γ, and LT/TNF-β mRNA expression in response to *in vitro* stimulation with UV-inactivated virus as determined by a sensitive RT-PCR assay. In contrast, no change was observed in expression of Th2-derived IL-4 and IL-6 mRNA levels. A time course study confirmed that Th1-specific mRNA expression could not be detected up to 48 h after *in vitro* stimulation with virus. Tolerance induction appears to function directly at the level of CD4$^+$ Th1 cells since depletion of CD8$^+$ T cells had no effect on the inhibition of expression of Th1-specific lymphokine mRNA levels. The mechanism of *in vivo* tolerance of Th1 cells appears to be anergy induction because IL-2, IFN-γ, and LT/TNF-β mRNA expression could be restored by the addition of rIL-2 to *in vitro* cultures of TMEV-SP tolerant, but not control CD4$^+$ Th1 populations. These results suggest that 'split tolerance' induction involves the down regulation of virus-specific, CD4$^+$ Th1 cells and the priming of virus-specific, CD4$^+$ Th2 cells. (Supported in part by NIH Grant NS-23349, NRSA Grant NS-09131, and NMSS Grant FG-934-A-1)

N 212 INTERFERON-γ IS A POTENT INHIBITOR OF POXVIRUS REPLICATION IN CELLS OF MACROPHAGE/MONOCYTIC LINEAGE,

Gunasegaran Karupiah and R. Mark L. Buller, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Poxviruses like ectromelia virus (EV) and vaccinia virus (VV) are resistant to the antiviral effects of interferons (IFN) in certain cells of fibroblastic lineage. This phenomenon is due in part to the presence of the K3L and E3L ORFs. The K3L ORF has homology with eIF-2α and is believed to protect this molecule from inactivation by IFN-induced P_1/eIF-2 kinase. ORF E3L encodes a double-stranded RNA-binding protein that inhibits the activation of the P_1/eIF-2 kinase. This resistance to the IFN effects mediated by these two genes is not absolute, since replication of both viruses in fibroblast cells is partially sensitive to higher concentrations of IFN-β but not IFN-α or IFN-γ. In sharp contrast, EV and VV replication in a macrophage line is highly sensitive to IFN-γ, only partially sensitive to IFN-α but completely insensitive to IFN-β. These findings suggest (i) the K3L and E3L gene products cannot overcome the IFN-γ-induced antiviral effects in macrophages and, (ii) the antiviral effects of IFN-γ may be mediated via a different mechanism than the P_1/eIF-2 kinase or 2, 5-oligo A synthetase. These possibilities have been investigated and the in vitro and in vivo (mouse) relevance of our findings will be presented.

N 213 **IN VIVO SELECTION OF LYMPHOCYTE-TROPIC AND MACROPHAGE-TROPIC VARIANTS OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS**,

Chwan-Chuen King [1,2], Mehrdad Matloubian[1], T. Somasundaram[1], and Rafi Ahmed[1], [1]Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024-1747 and [2]Institute of Public Health, National Taiwan University, Taipei, Taiwan.

Our previous studies have demonstrated cell-specific selection of viral variants during persistent lymphocytic choriomeningitis virus (LCMV) infection in its natural host. We have analyzed viral isolates obtained from CD4$^+$ T cells and macrophages of congenitally infected carrier mice and found that three types of variants are present in individual carrier mice: (i) macrophage tropic, (ii) lymphotropic, and (iii) amphotropic. In this study we have examined the kinetics of the emergence of different variants and the associated genetic changes. Our results show that macrophage-tropic variants are selected first followed by the lymphotropic ones. Detailed genetic and sequence analysis of these variants has shown that LCMV exhibits minimal genetic drift during chronic infection and that a single or few mutations can result in the cell-specific selection of variants that are markedly different from the parental virus.

N 214 RAPID ANTIGENIC DRIFT DRIVEN BY IMMUNE SERUM FROM MICE IMMUNIZED WITH INACTIVATED TYPE A INFLUENZA.

R. Lambkin, L. McLain and N.J. Dimmock, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL. United Kingdom.

Antigenic drift in the haemagglutinin of influenza allows the virus to reinfect individuals who have been infected with the previously circulating strain of the same sub-type. In the laboratory antigenic drift can be mimicked with antibody escape mutants which can be selected in the presence of a single monoclonal antibody. However no selection occurs in the presence of two monoclonal antibodies as the probability of the relevant double mutation (about 10^{-10}) occurring is vanishingly small.

We have demonstrated that the serum from 10% of mice previously immunized intravenously with beta-propiolactone-inactivated type A virus selected virus that was predominately escape mutant, as judged by its reaction with monoclonal antibodies, in a single passage. We have sequenced these escape mutants and shown that half of them have an amino acid substitution at the same site recognised by the relevant monoclonal antibody.

Further we have constructed "pseudo immune sera" with mixtures of two monoclonal antibodies and shown that, in particular proportions, these can efficiently select mutants which have escaped inhibition by the more concentrated monoclonal antibody.

We suggest that escape mutant-selecting immune sera have an antibody response biased, although not necessarily exclusive, to a single antigenic site and that this may be relevant to the generation of antigenic drift in human infections.

N 215 POLYCLONAL ACTIVATION OF T CELLS, APOPTOSIS, AND MEMORY IN VIRAL INFECTION. Lisa Lau, Marisa Galvan, Rita Concepcion, T. Somasundarum, Mehrdad Matloubian, and Rafi Ahmed. Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

We have used lymphocytic choriomeningitis virus (LCMV) to study cytolytic T lymphocyte (CTL) activation and memory. LCMV induces a potent $CD8^+$ CTL response in adult mice and the infection resolves within two weeks. We have quantitated the virus-specific CTL response using limiting dilution assays, and T cell activation using the CD44 (Pgp-1) marker. The frequency of LCMV-specific CTL increases both in the spleen and blood from <1/10^5 at day 0 to ~1/50 at day 8, the peak of the primary CTL response. During this period, the entire $CD8^+$ subset expands polyclonally. Virus-specific CTL comprise a minor fraction (5%) of the amplified $CD8^+$ population. All of the expanded $CD8^+$ T cells are activated ($CD44^{hi}$), and, consistent with a polyclonal response, display broad TcR Vß usage. After day 8, there is a sharp decline in total $CD8^+$ cells and virus-specific CTL (98% loss). These cells are refractory to anti-CD3 stimulation and display DNA fragmentation and *in vivo* cell damage characteristic of apoptosis. This primary CTL population (day 8) is also unable to eliminate a chronic infection which requires prolonged survival of the T cells. This period of anergy marks a transition to the development of memory CTL. By day 30, LCMV-specific CTL drop to about 1/1000 in the blood and spleen, and then persist at this frequency for the life span of the mouse. They maintain the $CD44^{hi}$ activation/ memory phenotype, and, in contrast to the primary CTL population, can eliminate virus when transferred into LCMV-carrier mice. These CTL can also persist long-term in an antigen-free environment. Sorted, virus-specific CTL that are free of any detectable viral genetic material (PCR negative) have been recovered at all timepoints tested (up to 1 year), with little loss in cell number, after transfer into normal, uninfected recipients. Taken together, these findings suggest that memory T cells that are not dependent upon continuous stimulation by antigen, and are qualitatively different from primary CTL, develop in this system.

N 216 EFFECT OF IN SITU EXPRESSION OF INTERLEUKIN -10 ON VIRAL ANTIGEN RECOGNITION AND ALLOGRAFT REJECTION, Myung-Shik Lee, Lise Wogensen, Michael B.A. Oldstone and Nora Sarvetnick, Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

Interleukin (IL)-10, a homologue of Epstein-Barr virus gene BCRF1, has immunosuppresive functions such as inhibition of antigen presentation and down-regulation of class II MHC expression on macrophages. The possible inhibitory activity of IL-10 on in vivo viral antigen recognition and allograft rejection were studied using transgenic mice expressing IL-10 in pancreatic β-cells directed by human insulin promoter (Ins-IL-10 mice). Ins-IL-10 mice show peri-islet inflammatory cell infiltration without diabetes. The effect of in situ IL-10 on the response to local islet (viral) antigens was studied in lymphocytic choriomeningitis virus (LCMV)-infected double transgenic mice expressing both IL-10 and LCMV NP antigen in pancreatic β-cells. In our preliminary data, splenocytes from the infected double transgenic mice showed cytotoxic T cell response against virus-infected target cells. Two months after infection, double transgenic mice developed diabetes before single transgenic mice develop diabetes, indicating that local production of IL-10 does not inhibit recognition of islet (viral) antigens. The effect of IL-10 on allograft rejection was studied by transplantation of fetal pancreata expressing IL-10 into MHC-incompatible hosts. The graft tissue was rejected with a similar time frame as nontransgenic pancreata suggesting that IL-10 may not entirely inhibit local antigen presentation. These results imply that in situ expression of IL-10 may not prevent (viral) antigen presentation and recognition in the periphery.

N 217 CD8+ T CELL MEDIATED HEMATOPOIETIC DYSFUNCTION IN CHRONIC VIRAL INFECTION, Mehrdad Matloubian, Lisa Lau, Chwan-Chuen King, Betty Wu-Hsieh, and Rafi Ahmed, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

We have previously shown that infection of adult mice with macrophage-tropic variants of lymphocytic choriomeningitis virus (LCMV) results in a chronic infection that is associated with suppressed T cell responses and susceptibility to opportunistic infections. A hallmark of this chronic viral infection is a prolonged pancytopenia and atrophy of lymphoid tissues. We asked whether this immune deficiency is due to a central defect in hematopoiesis. Analysis of bone marrow from these mice showed impaired production of both lymphocytes and macrophages. To elucidate the mechanism of this suppressed hematopoiesis we examined infection of bone marrow and characterized the infiltrates within this compartment. Infection of bone marrow cells peaks at day 5 post-infection with ~60,000 cells per million being infected and decreases to ~300 cells per million by day 14. This drop in the number of infected cells is preceded by infiltration of activated CD8+ T cells. By 8 days post-infection ~90% of CD8+ T cells in bone marrow are activated as measured by increased CD44 expression. However, limiting dilution analysis shows that only a minor fraction (~1/1000) of these activated CD8+ T cells are LCMV specific. *In vivo* depletion of CD8+ T cells prevents the bone marrow suppression, reverses the lymphopenia and protects the mice against a second pathogen. Thus, a population of activated CD8+ T cells, where the majority are not virus-specific, can mediate bone marrow damage during a chronic viral infection resulting in an impaired immune system. Such a polyclonally activated population of T cells may play a similar immunosuppressive role, perhaps through an autoimmune mechanism, in the course of other chronic viral infections.

N 218 CD28, HELPER T CELLS, AND ANERGY,
James G. McArthur, James P. Allison, and David H. Raulet, Department of Cell and Molecular Biology, Immunology Division, University of California at Berkeley, Berkeley, CA, 94720.
CD28 signalling is an essential costimulatory signal for IL-2 synthesis and the proliferation of cloned Th1 cells. Cloned Th2 cells secrete IL-4 with stimulation of the TCR, however, a second APC derived costimulatory signal is required for responsiveness to IL-4 and proliferation. We report here that soluble anti-CD28 mAb can provide this costimulatory signal and induce responsiveness to IL-4 in cloned Th2 cells. Furthermore, Th2 cell proliferation following stimulation with splenocytes and antigen is blocked with a combination of anti-CD28 F'Ab and IL-1 inhibitors.

Engagement of the TCR alone is not a neutral event, but induces a state of long term T cell unresponsiveness, termed anergy. We have shown that CD28 signalling can replace APC derived costimuli and prevent the induction of anergy in cloned Th1 cells (Nature 356(6370), p.607-609). We describe here that anergic cloned Th1 cells will proliferate when stimulated with soluble anti-CD28 mAb in addition to splenocytes and antigen indicating that the anergic state is reversible *in vitro* .

N 219 DOMINANT ROLE FOR Vβ17a$^+$ TH1 CELLS SPECIFIC FOR AN IMMUNODOMINANT VP2 EPITOPE IN THE PATHOGENESIS OF THEILER'S VIRUS-INDUCED DEMYELINATION IN SJL/J MICE,
Stephen D. Miller, William J. Karpus, Sheila J. Gerety and Jeffrey D. Peterson, Deptartment of Microbiology-Immunology, Northwestern University Medical School, Chicago, IL 60611.
Theiler's murine encephalomyelitis virus (TMEV) infection of the CNS induces a chronic T cell-mediated demyelinating disease in susceptible mouse strains which is characterized by an inflammatory mononuclear cell infiltrate. The present study identifies an 17 amino acid peptide on VP2 (VP2$_{70-86}$) as the immunodominant T cell epitope in TMEV-infected and immunized SJL/J mice, and demonstrates the ability of that sequence to prime for the majority of the SJL/J DTH T cell response to intact TMEV. The importance of VP2$_{70-86}$-specific T cell responses to the chronic demyelinating process was illustrated by experiments in which: a) SJL/J mice peripherally immunized with a fusion protein containing VP2$_{70-86}$ prior to intracerebral infection with a suboptimal dose of the BeAn strain of TMEV displayed an increased incidence and accelerated onset of clinical demyelination; b) i.v. adoptive transfer of a TMEV-specific, DTH-mediating, CD4$^+$, I-A^s-restricted Th1 line (sTV1) specific for the immunodominant VP2$_{70-86}$ epitope also led to increased incidence and accelerated onset of clinical disease only in TMEV infected recipients; and c) SJL/J mice rendered specifically tolerant of TMEV epitopes were protected from the development of clinical and histologic demyelination. Examination of a panel of VP2$_{70-86}$-specific T cell hybridomas derived from TMEV-infected mice indicated that they were predominantly Vβ17a. In contrast, examination of a similar set of hybridomas derived from mice immunized with either intact TMEV virions or with VP2$_{70-86}$ in CFA indicated a diverse pattern of Vβ usage, including Vβ 2, 3, 6, 7, 10, and 17a. Thus, the heterogeneity of the T cell repertoire used to recognize an immunodominant peptide may vary depending on the immunization conditions (peptide vs. viable virus) and/or the effects of tissue specific (peripheral vs. CNS) antigen processing/presentation. (Supported in part by USPHS NIH Grant NS-23349, NRSA Grant NS-09131 and NMSS Grant FG-934-A)

N 220 **EVASION OF THE CTL RESPONSE BY HTLV I IS FACILITATED BY QUASISPECIES NATURE OF TAX,**
Stefan Niewiesk, Claire Parker, Susan Daenke, Simon Nightingale, Jonathan Weber, Charles R.M.Bangham, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU

Human T-Cell Leukemia Virus (HTLV I) is the causative agent of Adult T cell Leukemia (ATL) and Tropical Spastic Paraparesis (TSP). In HTLV I infected individuals mRNA transcribed from the transactivator gene (TAX) and activated TAX-specific CTL can be found in freshly isolated PBL. We were interested to see whether CTL escape mutants are generated in HTLV I infected patients. The sequence analysis of 20 clones of the TAX gene of 2 healthy seropositives and 2 TSP patients confirmed previous reports that the individual consensus sequence differs very little (0.5-1.1%) from the reference sequence (Seiki). In contrast to this the variability within an individual is much higher (1.2-3.5%). 50-80% of the clones differ from the individual consensus sequence confirming the quasispecies nature of HTLV I. The finding that 70% of the mutations lead to coding changes indicates that strong selection forces are acting on the TAX protein. We assume that one of these might be pressure exerted by CTL, because we could demonstrate that an individual mounts a strong CTL response to multiple epitopes on the TAX protein simultaneously. We have started a longitudinal study concentrating on a previously described HLA-A2 octameric epitope. Two mutations in this epitope lead to failure of CTL recognition of the corresponding peptides.
It has been reported that there are heavy functional constraints on the amino acid sequence of the TAX protein. Whether these putative CTL escape mutations influence the transactivational activity of the TAX protein remains to tested.

N 221 A MURINE MODEL FOR EXAMINING IMMUNOLOGIC CONTROL OF MEASLES VIRUS GENE EXPRESSION.
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An in vitro system was developed for cultivation of human measles virus in murine cells that would permit future studies on viral immunity using homologous cell populations. A persistent infection with Edmonston strain measles virus was established in IC-21 cells, an SV-40 immortalized mouse macrophage cell line. Measles infected IC-21 cells fail to form syncitia and do not lyse. Infected IC-21 cells contain approximately ten-fold less virus intracellularly and in the media than similarly infected Vero cells. Infected IC-21 cells are morphologically indistinguishable from uninfected cells, have comparable rates of total protein synthesis and have a doubling time of 25-30 hrs compared to 20-25 hrs in uninfected cells. Immunofluorescence microscopy demonstrated a uniform distribution of measles virus throughout the cytoplasm of infected cells but variable quantities of measles antigen within individual cells. Flow cytometric analysis of the infected macrophages revealed a Gaussian distribution of viral proteins over an approximately 100-fold concentration range. Treatment of infected cells with a polyclonal rabbit anti-measles antibody downregulated synthesis of individual measles proteins and reduced the amount of live virus detectable in plaque assays. Antibody treatment led to cell lysis in the presence of complement but, in the absence of an intact complement system, no lysis was observed. With or without complement, measles virus protein synthesis and measles virus infective particles were reduced after antibody treatment. Therefore, anti-measles antibody induces a reduction in synthesis of measles proteins and live virus by mechanisms other than lytic destruction of cells.

N 222 MULTI-GENIC EVASION OF HOST-DEFENSES BY POXVIRUSES. [1]**Palumbo, G.J.**, [1]Buller, R.M.L., [2]Hu, F., [2]Pickup, D., and [3]Glasgow, W. [1]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892, [2]Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710, and [3]Laboratory of Molecular Biophysics, NIEHS, Research Triangle Park, NC 27709.
Poxviruses have recently been shown to encode genes with homologies to cytokine receptors and genes whose loss is associated with attenuation of the virus *in vivo*, although the functional significance and mode of action of these genes *in vivo* is poorly understood. Cowpox virus encodes a 38K gene that has previously been shown to inhibit the generation of an inflammatory response upon infection of the chorioallantoic membrane of the 12-day old chick embryo (*Virology.* 1989. 172:262). The wild-type CPV-BR-induced lesion is flat and red, while lacking the 38K gene the lesion is raised and white due to the massive inflammatory cell influx into the site of virus infection. Other poxviruses, however, often give intermediate pock morphologies though they possess a functional 38K gene, as detected by inhibition of 14(R),15(S)-diHETE formation. Together, these observations suggest that CPV-BR possesses a full complement of anti-inflammatory genes for the chick embryo model, while other orthopoxviruses lack a functional gene(s) involved in the inhibition of the generation of an inflammatory response at the site of virus infection. A detailed examination of the lesions induced by other orthopoxviruses indicated that there was an inflammatory response, although there were qualitative differences from the response generated by CPV-BR.D1 which lacks the 38K gene. Analysis of different CPV-BR mutants indicates that there are a minimum of at least four genes encoded by CPV-BR that are nonessential for virus replication in tissue culture, but are involved in inhibiting the generation of an inflammatory response at the site of virus infection.

N 223 VIRUS-INDUCED IMMUNE DEFICIENCY AS A CONSEQUENCE OF PROGRAMMED CELL DEATH (APOPTOSIS), Enal S. Razvi and Raymond M. Welsh, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655
Acute viral infections in mice and humans induce a transient immune deficiency reflected by loss in responsiveness of lymphocytes to mitogens. The acute infection of mice with lymphocytic choriomeningitis virus (LCMV) results in an antiviral cytotoxic T lymphocyte response peaking at day 7-9 post-infection, coincident with which is the non-responsiveness of lymphocytes to mitogen. We show here that crosslinking of the TcR/CD3 complex on T-cells from acutely LCMV-infected mice causes these cells to rapidly die by apoptosis (programmed cell death). This TcR-induced death was not inhibitable by exogenous interleukin-2 (IL-2), but was blocked by cyclosporin A. IL-2, in the absence of TcR-stimulation, enabled these cells to cycle without appreciable apoptosis above background. These results suggest that T-lymphocytes stimulated during LCMV infection become poised to undergo apoptosis upon TcR-crosslinking, and implicate apoptosis in the T-cell compartment as a possible mechanism for the *silencing* of the immune response to infection.

Blast T-cells, generated upon IL-2-mediated expansion from either acutely-infected (day 6 p.i) or immune (day 15 p.i) mice (IL-2 receptors being found on T-cells with activated or memory phenotypes) died a very rapid apoptotic death upon subsequent TcR/CD3-crosslinking. Thus, IL-2-dependent *cell cycling* predisposes T-cells for apoptosis upon TcR-signalling, and the exposure of memory T-cells *in vivo* to IL-2 induced during the antiviral immune response may *prime* them to subsequently die upon antigenic stimulation, thereby providing an explanation for the longstanding observation of antigen-specific immune suppression during acute virus infection.

N 224 POLYCLONAL ACTIVATION OF LYMPHOCYTES DURING LDV INFECTION. Raymond R. R. Rowland and Peter G. W. Plagemann, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455
Persistent viruses have developed a repertoire of strategies to evade the host immune response. The induction of peripheral tolerance appears to be the strategy employed by Lactate Dehydrogenase-elevating Virus (LDV), which infects a subpopulation of macrophages and causes a lifelong asymptomatic infection in mice. Even though anti-LDV antibodies are produced, the virus appears indifferent to the host immune response, since the virus is not cleared from the circulation, and the course of infection is unaltered in immunosuppressed mice. One central feature of infection is an early state of in vivo T cell suppression followed by the continuous polyclonal activatio of B cells and production of IgG2a and IgG2b antibodies. The purpose of these experiments was to study the polyclonal activation of B cells and the nature of T cell suppression by examining mitogen-stimulated proliferation, lymphokine production, and antibody synthesis in spleen cultures obtained from mice at different times p.i.. The most significant changes in T cell responses occurred during the first week p.i.. Decreases in proliferation, IL-2, and IL-2 receptor expression were observed in cultures stimulated with Con A, or the anti-CD3 antibody, 2C11. The Addition of IL-2 failed to recover the proliferative response to Con A. In contrast, Con-A stimulated cultures showed increased gamma-IFN when measured by either ELISA of gamma-IFN protein or by Northern analysis of gamma-IFN mRNA. IL-3 production was also increased, even in the absence of Con A. Decreased proliferation in response to LPS and increased production of IgG2a and -2b in spleen cultures were also observed. The magnitude and pattern of B and T cell responses suggest that both B and T cells are polyclonally activated during the acute stage of infection. B cells are stimulated to proliferate and produce antibody, whereas T cells, when stimulated, do not produce IL-2, fail to proliferate, and may become anergic.

N 225 THE ROLE OF THE LCMV ZINC-BINDING PROTEIN, P11 Z IN TRANSCRIPTION AND IN VIRUS-MEDIATED IMMUNOSUPPRESSION, Maria Salvato, Keli Shapiro, Eric Johnson, and Sharath K. Rai, Department of Pathology and Lab. Medicine, University of Wisconsin, Madison, WI, 53706
LCMV infection of laboratory mice results in a humoral immune response primarily to the structural protein p11 Z. During acute LCMV infection, humoral responses to several different viral proteins can be detected; however, during persistent infection, the early humoral response is predominantly to the Z protein. Our working hypothesis is that expression of the Z protein down-regulates expression of the envelope glycoprotein (GP) and thereby prevents sufficient viral expression for an acute infection. The Z protein is produced early in the virus life cycle from a subgenomic mRNA that is encapsidated within the virion. This is the first demonstration of a packaged early mRNA in the Negative-Strand RNA viruses. We have also been able to show that the Z protein is both a structural protein of the virus, and an essential component during transcription of viral RNA. Monoclonal antibodies to the Z protein inhibit transcription of LCMV-specific RNA in an *in vitro* transcription system.

N 226 CYTOMEGALOVIRUS INHIBITS MAJOR HISTOCOMPATIBILITY CLASS II EXPRESSION ON INFECTED HUMAN ENDOTHELIAL CELLS. Daniel D. Sedmak, Anna M. Guglielmo, Deborah A. Knight, Daniel J. Birmingham and W. James Waldman, Department of Pathology, The Ohio State University College of Medicine, Columbus, OH 43210

Persistent human cytomegalovirus (HCMV) infections are responsible for significant morbidity and mortality in immunocompromised individuals. One mechanism by which HCMV may develop persistence following primary infection is through inhibition of host cell human leukocyte antigen (HLA) class II expression with resultant escape from normal cell-mediated antiviral immune surveillance. Immunofluorescence flow cytometry of human umbilical vein endothelial cell (HUVEC) cultures infected with HCMV AD169 or with a clinical isolate propagated in HUVEC, (VHL/E), showed a marked reduction in interferon-γ (IFNγ)-induced surface expression of class II antigens. This inhibition did not occur when HUVEC were treated with UV-inactivated virus and IFNγ. HCMV, as determined by dual-labelling immunohistochemistry with monoclonal antibodies to HCMV early nuclear proteins and HLA class II antigens (anti-HLA DRα, anti-HLA class II beta chain, anti-HLA DP) inhibited induction of surface and cytoplasmic class II antigens in infected cytomegalic cells and not on adjacent non-infected cells within the cultures. Northern blot analysis of infected, IFNγ-treated HUVEC revealed an absence of class II mRNA in contrast to IFNγ-treated noninfected HUVEC. These results demonstrate that HCMV inhibits inducible HLA class II expression, a phenomenon which may contribute to the persistence of HCMV *in vivo*.

N 227 VACCINIA VIRUS ENCODES A NOVEL SECRETED IL-1β BINDING PROTEIN, Melanie K. Spriggs[1], Dennis E. Hruby[2], Charles R. Maliszewski[1], R. Mark L. Buller[3] and Judy Van Slyke[2], [1]Immunex Corporation, Department of Molecular Biology, 51 University Street, Seattle, WA 98101, [2]Department of Microbiology, Oregon State University, Corvallis, OR, and [3]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892

Poxviruses encode several proteins which function to assist the virus in evading the host immune response. These proteins include two soluble TNF receptor homologues, a complement binding protein, and an inhibitor of the interleukin-1 (IL-1) β convertase. Supernatants from vaccinia virus (VV)-infected CV-1 cells were examined and found to contain a 33 kd protein capable of binding murine interleukin-1β (mIL-1β). A VV open reading frame (ORF) that exhibits 30% amino acid identity to the type II IL-1 receptor was expressed in CV1/EBNA cells and shown specifically to bind mIL-1β. A recombinant VV was constructed in which this ORF was disrupted (vB15RKO). Supernatants from vB15RKO-infected cells did not contain an IL-1-binding protein. Supernatants from VV-infected CV-1 cells were capable of inhibiting IL-1-induced murine lymphocyte proliferation in vitro while supernatants from vB15RKO infected cells did not. Intracranial inoculation of mice with vB15RKO suggests that this ORF is involved in VV virulence. The possible role of this protein, as well as other candidate immunomodulators in vaccinia virus will be discussed.

N 228 TRANSFORMING GROWTH FACTOR-β PROTEIN EXPRESSION AND ACTIVATION DURING LCMV INFECTION OF EUTHYMIC, ATHYMIC, AND SCID MICE, Helen C. Su, Rika Ishikawa, Andrew T. Chan and Christine A. Biron, Division of Biology and Medicine, Brown University, Providence, RI 02912

We have previously shown that biologically active transforming growth factor-ß (TGF-ß) is produced during LCMV infection of euthymic mice and that NK cell proliferation is at least 100-fold more sensitive than T cell proliferation to TGF-ß-mediated inhibition. The experiments presented here were undertaken to assess the contribution of the adaptive immune response to production of biologically active factor. Immunohistochemical studies were carried out to examine *in vivo* production of TGF-ß protein. Detectable protein expression was induced in euthymic, athymic *nu/nu*, and SCID spleens during infection. The Mv 1 Lu biological assay was used to evaluate active TGF-ß production in media conditioned with splenic leukocytes isolated from infected mice. Cells from athymic or SCID mice produced less inhibitory activity than those isolated from euthymic mice. Furthermore, inhibitory factor production was of shorter duration. Administration of cyclosporin A (CsA) inhibited T cell activation and reduced inhibitory factor production in euthymic C3H/HeN and BALB/c *nu/+* mice at late times post-infection. These changes were accompanied by an enhanced NK cell response. Administration of CsA did not enhance NK cell activity in athymic *nu/nu* mice. Production of latent TGF-ß was evaluated by transient acid treatment to release the active form of TGF-ß. Euthymic, athymic, and SCID leukocytes all produced substantial amounts of latent factor in conditioned media. The presence of T and B cells was required for peak latent factor production at late times post-infection. The factor was shown to be TGF-ß1 by use of anti-TGF-ß1 neutralizing antibodies. Taken together, these data demonstrate that T and B cells can contribute to the decline in NK cell activity by facilitating production of latent and biologically active TGF-ß. Furthermore, the results help to explain observations of increased and prolonged NK cell activity in athymic and SCID as compared to euthymic mice.

N 229 ROLE OF ADENOVIRUS E3-Gp19K IN CLASS I EXPRESSION FOR CTL RECOGNITION OF ADENOVIRUS-INFECTED TARGET CELLS, Ralph A. Tripp[1], Terry Hermiston[2], Tim E. Sparer[1], Frances Rawle[1], William S. M. Wold[2] and Linda R. Gooding[1], Department Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322[1], Institute for Molecular Virology, St. Louis University, St. Louis, MO 63110[2]

We have examined the biology of the human adenovirus (Ad) early region 3 (E3) glycoprotein 19K (gp19K) which has been shown to inhibit cytotoxic T lymphocyte (CTL) recognition of Ad-infected target cells. The glycoprotein binds major histocompatibility complex (MHC) class I molecules and inhibits their transport to the cell surface. The gp19K-MHC class I binding is non-covalent, and does not require the MHC class I-associated β-2 microglobulin protein, or other Ad proteins. The lumenal domain of gp19K has been shown to bind the ∝1 and ∝2 domains of the mouse MHC class I, however the regions of gp19K which bind the class I molecule have not been investigated. Using a panel of gp19K deletion mutants, we have examined the amino acid region(s) involved in binding the MHC class I molecules, and have extended this relationship to CTL recognition of Ad E3-gp19K deletion mutant infected target cells. The data reveal that any deletion to the lumenal portion of the gp19K protein abolishes its ability to retain MHC class I in the endoplasmic reticulum of infected cells thus allowing for CTL recognition and lysis of infected target cells. Moreover, examination of newly synthesized gp19K-class I proteins and their relationship to CTL recognition and cytolysis revealed that maximal gp19K protein accumulation occurs 16-20 h. post-infection in mouse cells and that this accumulation directly correlates with maximal inhibition of CTL recognition and cytolysis. However, after 20-24 h. post-infection, intracellular levels of gp19K decrease, thereby allowing for increased CTL recognition. The addition of γ-IFN to Ad-infected target cells was suffcient to overcome the effect of gp19K, allowing for increased class I expression at the cell surface and resultant cytolysis.

N 230 CELL SOURCES AND REGULATION OF IL-12

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IL 12 is a heterodimeric cytokine composed of disulfide linked 40kDa (p40) and 35kDa (p35) subunits. It induces production of IFNγ by T and NK cells, NK cytotoxicity, and proliferation of activated T and NK cells. Coexpression of both subunits is necessary for production of high level activity in vitro. PCR analysis of mRNA from fractionated PBMC and cell lines reveals that transcripts for both subunits are detected in activated adherent cells, B cells and B cell lines. In contrast, non-activated T cells, PHA blasts, cultured NK cells and lines of a variety of cell types express p35 but little or no p40 mRNA. Stimulation with a variety of agents fails to induce p40 expression in a variety of cells other than monocytes. In cultures stimulated with Staph. aureus Cowan (SAC): 1) adherent cells express the highest levels of p40 and p35 transcripts; 2) CD14+ cells secrete high levels of the p40 subunit as well as IL12 activity; and 3) CD14+ adherent cells were sufficient for the response to SAC and for production of p40 and IL12 activity. Agents which induce p40 and IL12 expression in monocytes include SAC, LPS, and M. tuberculosis (H37RA). IL10 and IL4 prevent induction by SAC. The observation that monocytes are a major source of IL12 when induced by infectious agents suggests that IL12 expression may be a primary response to infection. The potent and pleiotropic activities of IL12 suggest that it may be central to subsequent immune and inflammatory responses.

Pathogenesis: Acute

N 300 COMPARTMENTALISATION OF T-LYMPHOCYTES TO THE SITE OF DISEASE: INTRAHEPATIC CD4+ T-CELLS SPECIFIC FOR HEPATITIS C VIRUS (HCV) IN PATIENTS WITH CHRONIC HEPATITIS C.

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Since the adult liver is an organ without constitutive lymphoid components, any intrahepatic T-cell found in viral hepatitis should have migrated to the liver after infection and inflammation. Therefore, information on frequency and function of liver T-cells may be relevant to the understanding of the pathogenesis of chronic hepatitis. We studied intrahepatic CD4+ T-cells from patients with chronic hepatitis C, and compared them to T-cells present in PBMC. We used six recombinant proteins (Core, E1, E2, NS3, NS4, NS5) of HCV to establish T-cell lines and clones from liver biopsies and PBMC. We found that only the protein NS4 was able to stimulate CD4+ T-cells isolated from biopsies, whereas with all the other HCV proteins we failed to establish liver-derived T-cell lines. To study compartmentalisation, NS4-specific T-cell clones from PBMC and liver of the same patient were compared for MHC restriction, fine specificity, lymphokine production, B-cell help ability and TCR V-region. The 22 PBMC-derived T-cell clones represent six distinct clonal populations that differ in the TCR V-region gene usage, whereas the 27 liver-derived T-cell clones appear all identical in the *N*-regions. Remarkably, none of the PBMC clones is identical to the liver-derived clone, demonstrating that this clone is compartmentalised to the liver. Altogether our results demonstrate that the protein NS4 is highly immunogenic for intrahepatic CD4+ T-cells primed by HCV *in vivo*, and that some CD4+ T-cells bearing a given TCR and with a peculiar ability to help IgA production by B-cells are compartmentalised to the liver. Studies are in progress to investigate the dynamics of intrahepatic T-cells in the course of disease and their appearance as memory cells in the PBMC.

N 301 PRIMING OF MEMORY T CELLS FOLLOWING RESPIRATORY INFECTION OF NEONATAL MICE WITH THE PARAMYXOVIRUS, HUMAN PARAINFLUENZA VIRUS TYPE 1.

Jane E. Allan and F. Suzette Wingo. Dept. of Immunology, St Jude Children's Research Hospital, 332 N Lauderdale, Memphis, TN, 38105.

Human parainfluenza virus type 1 (hPIV-1), an infection associated with respiratory tract disease in infants, has been targeted for the development of a childhood vaccine. Previously, we have described that adults, naturally immune to the virus, have high levels of circulating hPIV-1-specific memory cytotoxic T cells which recognize the viral nucleoprotein. Further studies on the immunogenic potential of this protein for inducing protective cellular immunity within the respiratory tract require a model system. We have therefore investigated the ability of mice to generate CD8+ T cells to hPIV-1.

Unlike many other viruses, intranasal infection of the neonatal (3 day old C57BL/6) mouse with hPIV-1 was found to result in a non-lethal, asymptomatic infection with low levels of viral replication in the respiratory tract. A strong hPIV-1 specific, CD8+ cytotoxic T cell response was recovered after in vitro restimulation of splenocytes obtained from 8 week old animals primed 5 to 10 days after birth by the intraperitoneal or intranasal routes. By using recombinant vaccinia viruses expressing the HN, P and NP proteins of hPIV-1 or the closely related Sendai virus, this T cell population was shown to recognize the viral nucleoprotein. The dominant T cell specificity was found to be encoded by the synthetic peptide NP 321-336 presented by Kb. Similar results were obtained for adult C57BL/6 mice.

We have shown that immunization of immunologically immature mice induces class 1 MHC-restricted memory CD8+ T cells of similar specificity to those primed in the mature animal. Furthermore, a strong memory CD8+ T cell response was found despite the increases in cell number associated with development from neonate to adult. This model will be useful for studying the immunogenic potential of viral proteins for cell-mediated immunity within the respiratory system of the immature host.

N 302 **SELECTION OF VIRAL VARIANTS IN SCID MICE**, Mary S. Asano, T. Somasundaram, Mehrdad Matloubian, Phu Pham, and Rafi Ahmed, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024-1747.
Viral variants with distinct phenotypic and genotypic differences are present in the central nervous system (CNS) and lymphoid tissues of mice persistently infected with lymphocytic choriomeningitis virus (LCMV). Previous studies have shown that this organ-specific selection correlates with a single amino-acid change at position 260 of the viral glycoprotein. This phenylalanine (F) to leucine (L) change is seen in the lymphoid tissue of persistently infected mice. Isolates with the parental sequence (F at residue 260) predominate in the CNS. In this study, we asked whether the organ-specific selection of the F to L change is immune-mediated. To address this issue, we examined the selection of viral variants in mice with severe-combined immunodeficiency (SCID). SCID mice were infected with the parental strain of LCMV. Viral isolates from the CNS and spleens of these persistently infected mice were analyzed for the presence of the F to L change. Fifteen days post-infection, both CNS and splenic isolates had the parental genotype. However, by ninety days post-infection, the majority of the splenic isolates displayed the F to L change, while the majority of the CNS isolates expressed the parental genotype. Since SCID mice lack functional T or B cells, these results show that the host immune system is not involved in the organ-specific selection of LCMV variants with the F to L change. While the selective pressures involved in the emergence of these organ-specific variants are not fully understood, this study demonstrates that such changes may occur independently of the host immune system.

N 303 THE SELECTIVE PROMOTION OF AG-SPECIFIC CD4+ TH1-LIKE LYMPHOCYTES IS DETERMINED BY MICROENVIRONMENTAL FACTORS DURING INFLAMMATORY RESPONSES, Vincenzo Barnaba°, Marino Paroli°, Alessandra Franco°, Guido De Petrillo°, Rosalba Bevenuto°, Manlio Ferrarini*, °Fondazione A. Cesalpino, I Clinica Medica, Università La Sapienza, Roma and *Istituto Nazionale Ricerca Cancro, Genova, Italy
We demonstrated that HBV-CAH patients have in the liver a conspicuous accumulation of CD4+ CD56+ T cells, which are not detected in periphery. These cells can be stimulated in vitro by HBenvAg to generate CD4+ T cell clones, that retain the same phenotype and have a Th1-like profile. By contrast, clones with these features represent a minority of the clones generated by HBenvAg from PBL of healthy vaccinated individuals: they comprised clones with an heterogeneous profile of cytokine secretion (either Th1 or Th2 or Th0). A group of them recognized HBenvAg peptide (193-207) in the context of the HLA allele DR2w15 by the same standard as liver-Th1-like clones. Together, these data suggest that the selective promotion of Th1-like or Th2-like response to HBenvAg seems not to be influenced by the nature of peptide or MHC haplotype but by liver microenvironment during inflammatory processes. These CD4+ CD56+ T cell clones displayed a strong cytotoxic activity and in an higher extent than their counterparts CD56-. The demonstration that freshly isolated CD4+ T cells infiltrating tissue with inflammatory processes, but not those derived from PBL, displayed cytotoxic activity and perforins strongly indicate that they may be cytotoxic in vivo and are generated in situ of severe immunopathologic responses. The priming by the whole virus presented by tissue-APC in the presence of inflammatory cytokines within the liver environment, which is the privileged site of infection, may facilitate the induction of cytotoxic CD4+ T lymphocyte with Th1-like profile. Indeed, the cells of vaccinated individuals that have an in vivo priming by soluble HBenvAg presented by different APC (perhaps in the presence of different cytokines) do not display an homogeneous functional profile.

N 304 CORRELATION BETWEEN THE BINDING OF DIFFERENT ARENAVIRUSES TO PUTATIVE CELLULAR RECEPTORS IN VITRO AND THEIR IN VIVO PATHOGENICITY, P. Borrow, V. Vedovato, J. Buesa-Gomez, J.C. de la Torre, and M.B.A. Oldstone, The Scripps Research Institute, La Jolla, CA 92037. Virus-receptor interaction, the first step in the infection process, is one of the key determinants of virus pathogenicity. We have been studying the cellular receptors used by members of the arenavirus family in order to elucidate whether receptor binding may underlie the differences in their in vivo biological properties. Using a virus overlay protein blot assay (VOPBA), we have identified 120-140kD cell membrane glycoprotein(s) as the putative receptor for lymphocytic choriomeningitis virus (LCMV). The expression of this putative receptor on tissue culture cell lines correlates with their susceptibility to LCMV infection. While binding of some LCMV isolates (Armstrong clone 13, Traub, Pasteur and WE clone 54) to the 120-140kD putative receptor is readily demonstrated, the interaction of other LCMV isolates (Armstrong parental strain, E350, and WE clone 2.2) with these or other proteins cannot be detected by VOPBA. There are correlations between binding to this putative cellular receptor and the in vivo biological properties of members of these two groups of viruses, e.g. the Armstrong parental strain and WE clone 2.2 produce a growth hormone deficiency syndrome on inoculation into neonatal C3H/St mice, whereas Armstrong clone 13 and WE clone 54 do not; and Armstrong clone 13 causes a generalized immune suppression on i.v. inoculation into adult mice, whereas the Armstrong parental strain does not. On the basis of this evidence, we hypothesize that receptor binding is likely a critical determinant of the in vivo pathogenicity of different arenavirus isolates.

N 305 **USING VIRUS AND MOUSE MUTANTS TO STUDY THE PATHOGENESIS OF THEILER'S VIRUS PERSISTENT INFECTION**, **Michel Brahic, Jean-François Bureau, Laurence Fiette, Nadine Jarousse, Andrés McAllister and Claudia Pena Rossi, Département des Rétrovirus, Unité des Virus Lents, Institut Pasteur, 75724 Paris Cedex 15, France**
Theiler's virus, a mouse picornavirus, is responsible for a persistent CNS infection accompanied by primary demyelination. Following intracranial inoculation, the virus infects grey matter cells first, mostly neurons, then migrates to glial cells of the white matter where it persists for the lifetime of the animal, if they are genetically susceptible to this late disease. In resistant mice, the virus never reaches the white matter and is cleared from the grey matter by the immune response.
The use of backcross mice, and the screening of the entire mouse genome with microsatellite markers allowed us to identify three loci responsible for susceptibility/resistance to persistent infection. One is H-2D and another one is Ifg. On the other hand, infecting ß2m$^{-/-}$ mice proved that CTL play a major role in clearing the virus from the CNS of genetically resistant mice. Furthermore, viral chimera obtained in the laboratory, and which are unable to migrate from the grey to the white matter, proved to be extremely useful in dissecting the role of CTL in the clearance of Theiler's virus from various grey matter cell types. From these studies, a coherent picture of the interaction of host and viral genes to determine the fate of Theiler's virus infection of CNS is emerging.

N 306 **ACUTE LCMV INFECTION IN MICE: VIRUS INDUCED T-CELL DEFECTS**

Eric A. Butz and Peter J. Southern, Department of Microbiology, University of Minnesota School of Medicine, Minneapolis, MN, 55455.

Acute infection of Balb/c mice by the Armstrong strain of lymphocytic choriomeningitis virus (LCMV) readily induces an MHC class I restricted, virus specific CTL response; paradoxically there is also a transient defect induced in the *general T-cell population*. Spleen and lymph node cells harvested from infected mice display a dramatic loss of *in vitro* proliferative response to the mitogen concanavalin A (ConA). Data is presented showing that this loss of function does not occur with *in vitro* infection of splenocytes, requires viral replication and is dose dependent. On day seven of acute infection, when the loss of function is most pronounced, the defect segregates with the T-cells of the infected animals. When stimulated, these cells produce much less IL-2 than normal cells. Splenocytes from infected mice are responsive to IL-2 alone, but much less so to the combination of IL-2 plus ConA, suggesting that T-cell receptor engagement is a negative signal in these cells. Cells from infected and uninfected mice respond equivalently to phorbol ester and ionomycin. These data are consistent with the nonresponsive T-cells having been rendered anergic. Anergy results from presentation of antigen to T-cells in the absence of a second signal, termed costimulation. *In situ* hybridization reveals that a significant percentage of macrophages and dendritic cells isolated on the third day of infection are positive for LCMV sequences. We hypothesize that infection of antigen presenting cells may compromise their ability to provide costimulation, resulting in the induction of anergy in the majority of T-cells in the infected animals.

N 307 DIFFERENTIAL REGULATION OF MHC CLASS I EXPRESSION BY CYTOMEGALOVIRUS INFECTION,

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Murine cytomegalovirus (MCMV) infection of H-2^b mice prevents priming of antigen-specific helper and cytotoxic T lymphocytes (CTL). In vitro, MCMV infection inhibits presentation of antigens to antigen-specific, MHC Class I-restricted CTL. This is accompanied by a significant decrease in surface expression of H-$2K^b$ and H-$2D^b$. We therefore examined the effects of MCMV infection on the intracellular expression of H-$2K^b$ and H-$2D^b$ using immunoprecipitation. Two major effects of virus infection were seen: 1) MCMV inhibited maturation of H-2K and H-2D heavy chains to the complex glycosylated forms. This suggests that MCMV infection interferes with transport of the molecules from the endoplasmic reticulum through the Golgi. 2) While there was a minimal reduction in the quantity of H-2D, H-2K expression was significantly reduced, suggesting that H-2K synthesis is selectively downregulated by virus infection. These two effects on MHC expression were mediated by an MCMV early gene product, as cells were infected in the presence of phosphonoacetic acid which inhibits DNA replication. Gamma interferon was unable to fully reverse the effects of MCMV on MHC transport and synthesis. The MCMV early gene product(s) did not act through beta-2 microglobulin (β_2m), as similar effects on H-2K and H-2D were seen in MCMV-infected, β_2m-deficient cells. Current studies aim to assess the molecular mechanisms of the differential regulation of these two Class I molecules by MCMV.

N 308 CYTOTOXIC T LYMPHOCYTES RESTRICTED BY HLA-A2 SPECIFIC FOR HEPATITIS C VIRUS (HCV) DERIVED PEPTIDES ARE PRESENT IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC HEPATITIS C,

Andreas Cerny, John G. McHutchison, Patricia Fowler and Francis V. Chisari. The Scripps Clinic and Research Institute La Jolla, CA 92037.

HLA class I restricted cytotoxic T lymphocytes (CTL) are a major defense mechanism in viral infections. CTL mediated lysis of virus infected host cells may lead to clearance of the virus or if incomplete to viral persistence and eventually chronic tissue injury. Viral persistence and immunologically mediated liver injury are thought to be important mechanisms leading to chronic hepatitis after infection with HCV. In order to assess the role of CTL in HCV infection we used the following experimental strategy: peripheral blood mononuclear cells (PBMC) of patients with chronic hepatitis C, having the HLA class I allele A2, were stimulated with HCV-derived peptides containing the HLA-A2 binding motif xLxxxxxxV. After two weeks of stimulation effector cells thus generated were tested for their capacity to lyse HLA-A2 matched peptide sensitized target cells in a 4-hour ^{51}Chromium release assay.

Our results so far indicate: 1). HLA A2 restricted CTL responses to HCV encoded peptides can be detected in the peripheral blood of patients chronically infected with HCV; 2). CTL responses in individual patients were found to be polyclonal and multispecific; 3). CTL responses are directed against peptides derived from both structural as well as nonstructural regions of HCV; 4). Some patients had no detectable CTL activity against the panel of HCV peptides used. Current efforts are directed to correlate the clinical status and the evolution of liver disease with the presence or absence of HCV specific CTL in the peripheral blood of patients during the natural course of disease and in response to antiviral therapy. Information derived from such an approach may be useful as a predictor for clinical evolution, as a means to monitor antiviral and immunomodulatory treatment and as a basis for a rational design of an HCV vaccine.

N 309 ROUTE OF NEUROINVASION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN THE MOUSE,

Peter C. Charles, Franziska B. Grieder, Nancy L. Davis, Kinuko I. Suzuki and Robert E. Johnston, Dept. of Microbiology and Immunology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27599-7290

Venezuelan Equine Encephalitis virus (VEE) is an enveloped, positive sense RNA virus that causes an epidemic encephalitis in horses and man. Experimental infection of the mouse with molecularly cloned VEE was characterized by a biphasic course of viral replication indistinguishable from infection with the biological virulent parent. Virus first replicated to high titer in the lymphoid organs and the reticuloendothelial system, producing a marked viremia. The lymphoid phase of the disease ended by 120 hours post inoculation (pi), with clearance from all organs and serum. A second central nervous system (CNS) phase of viral replication began in the brain approximately 48 hours pi, and continued until the death of the mouse. The route of entry of alphaviruses into the CNS is unknown. Several hypotheses exist, including transport of virus across endothelial cells in brain capillaries, direct neural spread from the peripheral nervous system, or macrophage/lymphocyte mediated spread. We have investigated the mechanism by which VEE gains access to the CNS using *in situ* hybridization and quantitative plaque assay. At 36 hours pi virus was detectable in the sensory neurons of the olfactory neuroepithelium and in the vomeronasal organ, as well as in the main and accessory olfactory bulbs. By 48 hours pi virus was demonstrable in the lateral olfactory tracts, pyriform areas, and the entorhinal cortex of the brain. At later time points virus was disseminated throughout the brain. These observations are consistent with the hypothesis that viral CNS invasion begins with hematogenous seeding of the olfactory neuroepithelium during the viremic lymphoid phase of the infection. Virus subsequently replicates locally in the neurons of the olfactory neuroepithelium, and is delivered to the olfactory bulbs via anterograde neuronal transport. The speed with which VEE can enter the CNS from this route may be important in the course of infection, as rapid clearance from the periphery may prevent the establishment of productive CNS infection at later times post infection.

N 310 PROTECTION OF MHC CLASS I-DEFICIENT MICE AGAINST INFLUENZA A INFECTION BY VACCINIA-INFLUENZA RECOMBINANTS EXPRESSING HEMAGGLUTININ AND NEURAMINIDASE,

Suzanne L. Epstein*, Julia A. Misplon*, Cassandra M. Lawson+, E. Kanta Subbarao+, Mark Connors+, and Brian R. Murphy+. *Molecular Immunology Laboratory, CBER, FDA, Bldg. 29, Rm. 522, Bethesda, MD 20892, and +Laboratory of Infectious Diseases, NIAID, NIH, Bldg. 7, Rm. 106, Bethesda, MD 20892.

Immunity to viral infections includes both antibody and T cell components. The contributions of humoral and cell-mediated immune responses vary depending on virus and host factors. We have used an *in vivo* challenge system to examine protective immunity to influenza A(H1N1) virus infection in immunocompetent B6 ($H-2^b$) mice, and in mice homozygous for disruption of the gene for $\beta 2$-microglobulin, termed $\beta 2\mu(-/-)$ mice. Such mice do not express conventional MHC class I complexes on the cell surface and lack CD8+ class I-restricted T cells. Ten vaccinia virus recombinants, each expressing one of the ten proteins of influenza virus, were used as vaccines. Normal mice were protected against challenge with influenza virus by vaccination with HA-VAC and NA-VAC, but not by any of the vaccinia vectors expressing one of the eight other influenza virus proteins nor by a mixture of all eight of the latter vectors. Similar results were observed in mice of $H-2^d$ or $H-2^k$ MHC haplotypes. The $\beta 2\mu(-/-)$ mice were also protected by HA-VAC and NA-VAC, demonstrating that vaccine efficacy did not require classical CD8+ cytotoxic T lymphocyte (CTL) responses. Depletion of CD4+ T cells in either normal or $\beta 2\mu(-/-)$ mice at the time of challenge had little or no effect on protection induced by H1-VAC or NA-VAC, suggesting that preformed antibody is the dominant mediator of protective immunity induced by these recombinants. Antibody responses to vaccinia virus antigens and the expressed influenza virus antigens were lower in titer in $\beta 2\mu(-/-)$ mice than in normal mice, suggesting an influence of CD8+ T cells or their products on antibody production.

N 311 THE ROLE OF T-CELL SUBSETS IN SEMLIKI FOREST VIRUS INDUCED CNS DEMYELINATION,

John K. Fazakerley, and Ian Subak-Sharpe, Division of Immunology, Department of Pathology, Cambridge University, Cambridge, UK.

Intraperitoneal inoculation of 3-4 week old BALB/c mice with the A7 strain of Semliki Forest virus (SFV) results 14 days later in a demyelinating encephalomyelitis. The virus can enter both neurones and glial cells. Viral replication is restricted in neurones and in athymic *nu/nu* mice, virus can persist in the CNS for the life of the animal. Adoptive transfer studies in *nu/nu* mice demonstrate that lesions of demyelination are dependent upon activated T-cells.

We have studied the *in vivo* depletion of $CD4^+$ and or $CD8^+$ T-cell populations with rat monoclonal antibodies. Depletion of $CD4^+$ cells, abrogated virus-specific IgG production resulting in elevated brain virus titres, delayed clearance of brain virus and increased demyelination. Depletion of $CD8^+$ cells affected neither antibody production nor brain virus titres, but greatly reduced the incidence of demyelination. Animals depleted of both T-cell subpopulations produced no virus specific IgG, had persistent CNS infections with minimal cytopathicity and no lesions of demyelination. In conclusion, clearance of Semliki Forest virus from the CNS requires $CD4^+$ T-cells. Lesions of demyelination are mediated by $CD8^+$ T-cells.

N 312 MOLECULAR GENETICS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS PATHOGENESIS IN MICE

Franziska B. Grieder, Nancy L. Davis, Debra C. Sellon, Judith F. Aronson and Robert E. Johnston, Dept. of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7290

Pathogenesis of Venezuelan equine encephalitis virus (VEE) in mice can be described as a sequence of steps which ends in death of the infected host. Distinct viral functions may be critical at each sequential step. Attenuated VEE mutants should be blocked at, and therefore define, different pathogenetic steps in the disease process. Single-site attenuated VEE mutants were generated by site-directed mutagenesis and differed from cloned virulent wild-type VEE at either E2 glycoprotein position 76 (lys for glu) or 209 (lys for glu). Wild-type VEE caused 100% mortality after footpad (fp) or intracerebral (ic) inoculation of mice. The E2 76 mutant was avirulent by both routes. Mutant E2 209 was avirulent by fp inoculation (0% mortality), but caused 89% mortality when inoculated ic. Both mutants induced a solid protective response to intraperitoneal challenge with wild-type VEE. The spread of wild-type and mutant virus was monitored by titration of tissue homogenates, immunocytochemistry, *in situ* hybridization, and histopathology. Following fp inoculation, wild-type VEE replicated at the injection site, spread to the draining lymph node within 4 hrs, to serum within 12 hrs, and to other lymphoid organs within 18 hrs post-inoculation. In contrast, the single-site mutant, E2 76, was blocked at the site of inoculation. Although sporadic spread beyond the footpad was observed, none of the 17 virus strains recovered from other organs was restricted to the footpad. All of the recovered viruses contained genotypic alterations. The spread of mutant E2 209 appeared similar to wild-type except that it was severely restricted in reaching the serum (in only 2 of 22 mice) and the brain (in 1 of 22 mice) following fp inoculation. Recovered viruses from these 3 animals are being analyzed for genotype and phenotype. The E2 209 mutation appeared to restrict the production of viremia and subsequent access of the virus to the brain. Consistent with this hypothesis, intravenous inoculation of E2 209 resulted in significantly increased virulence (33% mortality).

N 313 NEUROINVASIVENESS OF REASSORTANTS BETWEEN TWO ATTENUATED CALIFORNIA SEROGROUP BUNYAVIRUSES,

Christian Griot, Andy Pekosz, Dave Lukac, Steven Scherrer, Kara Stillmock, Dan Schmeidler, Michael Endres, Francisco Gonzalez-Scarano, Neal Nathanson, Departments of Microbiology and Neurology, University of Pennsylvania Medical Center, Philadelphia, PA 19104.

We have recently described two different attenuated reassortant clones of California serogroup bunyaviruses, both of which exhibit reduced neuroinvasiveness upon peripheral inoculation into suckling mice. Clone B1.1a. bears an attenuated middle RNA segment (neuroinvasiveness genotype vαv) and clone B.5 bears an attenuated large RNA segment (genotype αvv). A complete panel of reassortants was constructed between these two clones, and tested for their neuroinvasiveness (as measured by the $\log_{10}$ PFU/LD_{50} ratio) in suckling mice. It was found that clones with the neuroinvasive large and middle RNA segments (genotype vvv) were much more neuroinvasive (up to 10,000-fold) than either parent clone, whereas clones bearing both attenuated large and middle RNA segments (genotype ααv) were more attenuated (10 to 100-fold) than either parent clone. Furthermore, pathogenesis studies which followed replication of virus after inoculation by (i) tissue titration for virus, and (ii) immunofluorescence labeling for viral antigen showed that the highly neuroinvasive clones replicated in peripheral tissue (striated muscle) to high titers, produced an active plasma viremia and invaded the brain within 3 days. At a high input of 10^6 PFU/animal, clones with one or both attenuated segments failed to replicate to a significant amount in peripheral tissue, produced only a minimal passive plasma viremia during the first 24 hours, but nevertheless reached high titers in the brain and killed animals within 4 days. In view of these results, we also investigated the possibility that virus was reaching the central nervous system via neural pathways. Footpad inoculations of clone B1.1a at a moderate dose of 200 PFU/animal was preceded by sciatic nerve transsection or mock surgery. There were no differences in lethality, indicating that the sciatic nerve does not play a role in neuroinvasion following footpad inoculation. Thus the low level viremia is likely to be sufficient for penetration of the blood brain barrier.

N 314 IMMUNOGENETICS OF THE HUMAN T CELL RESPONSE TO HCMV. Huiling He, Charles R. Rinaldo, Jr., Penelope A. Morel. Departments of Medicine, School of Medicine and Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh Cancer Institute, Pittsburgh PA 15213

Human cytomegalovirus (HCMV) is associated with significant morbidity and mortality in immunocompromised hosts. The whole HCMV genome has been sequenced but the specific proteins which mediate the T cell response to HCMV are only partially delineated. We have studied the CD4 T cell response to selected HCMV proteins which have been shown to be immunologically relevant. The proteins that we selected include the two immediate early proteins (IE1 and IE2), gB, pp71 and MHC class I homolog (H301). In order to prepare pure preparations of these proteins, the maltose binding protein (MBP) fusion protein system was used. HCMV DNA fragments encoding these five proteins were each subcloned into the pMal-c vector and the fusion proteins were obtained by IPTG induction, and purified by passage over an amylose column. Using these purified proteins we have studied the T cell proliferative responses to HCMV proteins in 31 seropositive and 7 seronegative individuals. The whole HCMV was used as positive control and the MBP as negative control. Responses were considered to be positive when the stimulation index (test divided by negative control) exceeded 3.0. In an attempt to correlate these responses with the HLA type of each individual we have carried out DNA typing for HLA-DR on all individuals. Results showed that all of the fusion proteins were successfully made as determined by sequence analysis and could stimulate proliferative responses. None of the 7 seronegative individuals showed significant responses. For the 31 seropositive individuals, 10 of them responded to all five proteins, 11 responded to one to three proteins and 10 individuals responded to none of the proteins. The most commonly recognized proteins were the structural proteins: gB (16/31, 52%) and pp71 (16/31, 52%). The IE1 and IE2 were recognized by 11/31 (35%) and 13/31 (40%), respectively. Nine out of 23 (39%) responded to MHC class I protein. Lack of responsiveness to any of these proteins appears to be associated with DR2. In addition 3/4 of the individuals who responded to gB only were DR4 positive. These data suggest a role for HLA-DR in determining the immune recognition of specific HCMV proteins. The detailed correlation of HLA DR and DQ with response to these proteins will be discussed.

N 315 MOUSE HEPATITIS VIRUS INFECTION INFLUENCES THE ANTIGEN RECOGNITION BY CD8$^+$ CYTOTOXIC T CELLS. Mirjam H.M. Heemskerk, Henriette M. Schoemaker, [1]Willy J.M. Spaan and Claire J.P. Boog. Department of Infectious diseases and Immunology, Veterinary Faculty, University of Utrecht, The Netherlands. [1]Department of Virology, Faculty of Medicine, Leiden, The Netherlands.

Infection of mice with mouse hepatitis virus (MHV) causes a spectrum of diseases, ranging from acute hepatitis in the liver to acute encephalitis and chronic demyelination in the cental nervous system. A variety of mechanisms have been implicated in controlling MHV infections, which include the activity of macrophages, natural killer cells and lymphocytes. Because the role of cytotoxic T cells in this virus model is poorly understood, we are trying to generate MHV specific CTL's and determine their fine specificity and their biological activity in the protection against a lethal virus infection as well as their possible role in the establishment of chronic demyelination. Because the efficiency of MHV-A59 infection of syngeneic target cell lines was very low, we transfected EL4 cells with the receptor for MHV-A59. Transfectants show high expression of MHV-A59 and were used as targets in cytotoxicity assays, however no MHV-A59 specific CTL activity could be measured. Based on these results we measured the MHC class I expression on the infected target cells and observed a downregulation of the MHC class I molecules. Further studies on this finding are currently under investigation. Alternatively, we selected peptides from the structural proteins of MHV-A59 based on the recently reported K^b and D^b binding motifs of Rammensee at al. We analysed these peptides for binding to MHC class I molecules and tested them for CTL recognition, and we used them in vaccination studies.

N 316 SINDBIS VIRUS CONFORMATIONAL CHANGES AT THE CELL SURFACE DURING ENTRY: MAPPING OF TRANSITIONAL EPITOPES ON GLYCOPROTEINS E1 AND E2, Robert E. Johnston[1], William J. Meyer[1], Daniel C. Flynn[2], Randal J. Schoepp[1], Alan L. Schmaljohn[3], Judith A. Nicholas[4], Gary Greenwald[1] and Nancy L. Davis[1]. Dept. of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599[1], West Virginia University, Morgantown, WV 26506[2], USAMRIID, Ft. Detrick, Frederick, MD 21701[3], and Upjohn Co., Kalamazoo, MI 49001[4]

Conformational changes in the structure of the Sindbis virus (SB) glycoprotein spike were detected at the cell surface during entry. After attachment to baby hamster kidney cells at 4°C and during subsequent incubation of virus-cell complexes at 37°C, the constellation of glycoprotein epitopes accessible to their cognate monoclonal antibodies (MAbs) was altered. Epitopes accessible to MAb on virus-cell complexes, but not accessible on native virions, were termed transitional epitopes. Exposure of the transitional epitopes occurred normally in the presence of NH_4Cl and at both permissive and non-permissive temperatures in a Chinese hamster ovary cell line temperature-sensitive for endosomal acidification. When native virions in suspension were heated to 51°C for 5 min, many of the transitional epitopes became accessible, mimicking the cell surface conformational change. Because such virions remained infectious, the transitional epitopes were mapped by selecting and sequencing neutralization escape mutants. An E2 transitional epitope was mapped to E2 residues 200-202, a region between two of the major neutralizing antigenic sites of SB and near a putative site for receptor binding. A subset of the E1 transitional epitope MAbs were broadly cross-reactive among several alphaviruses. One of these mapped to E1 61 within a conserved domain. Using site-directed mutants, another such epitope was mapped to E1 81-95, the conserved, hydrophobic domain thought to be the alphavirus fusogenic peptide. These results suggest that the E1 81-95 domain may be exposed at the cell surface after rearrangement of the glycoprotein spike but prior to entry.

N 317 CD4+ HSV-SPECIFIC T CELL CLONES ISOLATED FROM GENITAL LESIONS OF PATIENTS WITH HSV-2 INFECTION, David M. Koelle, Hiyam Abbo, Annette Peck, Michael A. Tigges, Rae Lyn Burke, and Lawrence Corey, University of Washington, Seattle, WA 98195 and Chiron Corporation, Emeryville, CA 94608

Cells recovered from Ficoll-Hypaque treatment of superficial material from four recurrent human buttock HSV-2 lesions were plated at 10-200 cells/well with non-specific T lymphocyte growth stimuli. From 7.9% to 36.5% of wells had initial growth; between 29% and 100% of these displayed adequate growth for screening assays. T cell clones were screened for proliferative responses to HSV-2 antigen using autologous PBMC as APC. For the four lesions from three patients, 2/30 (6.7%), 3/29 (10.3%), 3/35 (8.6%), and 11/159 (6.9%) were repeatedly positive. All such clones were CD4+, CD8-; 11 of 16 were type-common, reacting with HSV-1 and HSV-2; 5 of 16 were type-specific for HSV-2, and 3 were not evaluated. Antigenic specificity data for the clones indicate: one clone each proliferates in response to CHO-cell derived gB2 and gD2 and one clone reacts with affinity purified gC2. The gD-specific clone lysed target LCL infected with recombinant vaccinia virus containing the gene for gD2 and displayed HLA-restricted cytotoxicity towards HSV-infected target LCL. The gC2-specific clone also displayed HSV-specific, HLA-restricted cytotoxic activity and reduced HSV-2 titer in an HLA-restricted fashion in a virus killing assay. One HSV-2 type-specific clone from each patient studied reacted with an unknown antigen(s) encoded by a HSV-1 X HSV-2 intertypic recombinant virus containing HSV-2 DNA from approximately 0.67-0.73 map units.

CD4+ T cell clones with specificity for HSV are enriched in recurrent buttock lesions, as LDA assays of PBMC gave values of $1/10^3$ to $1/10^4$ for these patients. T cell clones with reactivity for glycoproteins B, C, D and other antigens are present in lesions.

N 318 PROLIFERATIVE RESPONSES TO THE RECOMBINANT NUCLEOPROTEIN OF MEASLES VIRUS IN NATURALLY INFECTED AND VACCINATED INDIVIDUALS. **Alison C. Mawle, C.-G. Lu, K. Hummel, and W.J. Bellini, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA 30333**

Proliferative responses to measles virus as measured by the uptake of ^{3}H thymidine, have been hard to demonstrate in immune individuals who have been naturally infected. Previous attempts have used purified whole virus as the stimulus, and the levels of stimulation reported have been low and poorly reproducible. We have cloned the nucleoprotein gene from the Edmonston vaccine strain into a baculovirus expression vector and purified the recombinant nucleoprotein (rNP) using either a monoclonal antibody affinity column or ultracentrifugation. We have determined the kinetics of response using naturally infected individuals and obtained good responses, with stimulation indices (SI) ranging from 5-15. In a comparison between the cellular immune responses of naturally infected and immunized individuals, 13 individuals in each category, matched for sex and race were assayed for their ability to respond to RNP. Approximately 65% of each group had a positive response (natural=69%, vaccine=62%). The range of SI seen was 1-16 for naturally infected individuals and 1-20 for vaccinees. These differences are not statistically significant. Estimates of nucleoprotein-specific T cell frequency in the 2 groups determined by limiting dilution analysis will be presented. Elucidating the mechanisms of cell-mediated immunity to measles may help explain the current resurgence of the disease and aid in the design of future vaccines.

N 319 MOLECULAR ANALYSIS OF HUMAN ANTIBODY RESPONSES TO EPSTEIN BARR VIRUS (EBV):
Aberant responses in health and disease reflecting virus activity in vivo.

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A detailed immunoblot study was done with human sera collected world-wide from healthy seropositives (n=350) and patients (n > 200) with a variety of different EBV-associated disease syndromes.

IgA, IgM and IgG responses were analysed using natural and recombinant forms of EBV-proteins characteristic for different stages of the EBV replication cycle.

Comparing the number and nature of antigens identified by human serum antibodies allowed a clear differentiation of the humoral immune status in patients and healthy controls, as well as differentiation between patient groeps. Acute primary infection is characterized by IgM and IgG responses to viral replicative proteins (EA + VCA) whereas the latent infection in man is characterized by IgG response to latent phase (EBNA) proteins, few dominant structural virion proteins and the zebra-switch (IEA) protein. Nasopharyngeal carcinoma is characterized by IgG and IgA responses to replicative proteins (EA and VCA) that differ from those recognised in primary infection. Chronic severe EBV infection in children frequently is associated with a specific lack of responsiveness to the EBNA$_1$ protein, with fulminant antibody responses to replicative phase proteins.

Results will be discussed in view of site and activity of virus replication in vivo and the host immune surveillance.

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N 320 ROLE OF CYTKINES IN IMMUNOPATHOLOGY INDUCED BY VIRAL ENCEPHALITIS IN MICE F Mokhtarian and D Grob
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SJL/J mice are susceptible and B6 mice are resistant to the induction of experimental autoimmune encephalomyelitis (EAE). Prior infection of B6 mice with Semliki forest virus (SFV) makes them responsive to myelin basic protein (MBP) at 3-4 wks post infection(pi) and hence susceptible to EAE induction. In this study, the time course of production of pro-inflammatory cytokines (IFN-g, TNF) and anti-inflammatory cytokines (IL-4 and TGF ß-1) in response to MBP were measured in SFV-infected SJL and B6 mice. During the first week, the virus multiplication in the brains of infected mice peaked at day 3 and was cleared by day 10 pi. Lymphoproliferation and TNF and IFN-g production by splenocytes of SJL and B6 mice in response to autoantigen, MBP were measured on days 4, 7, 9, 15, 21 and 30 pi. Splenocytes from SJL mice proliferated significantly more to MBP (d4,p=0.02, d9, p=0.01,d21,p<0.03 , d30, p<0.05) than those from B6 mice (d7, p=0.03, d21 p<0.03) on all days pi (except for d7). IFN-g response to MBP by splenocytes from SJL mice was present on day 4 pi, reached a peak on d9 and decreased significantly on days 15, 21 and 30. IFN-g response and to MBP by splenocytes from B6 mice reached a peak on d7 and became nonexistent on the following days. On all days pi (except on d7) the IFN-g response to MBP was higher by splenocytes from SJL than by those from B6 mice. The TNF/LT response to MBP by splenocytes from SJL mice was present on d4, peaked on d9, decreased on d15 and became undetectable by d21. TNF/LT production by splenocytes from B6 mice in response to MBP was present on d4, was very high on d7 and became undetectable on d15 and 21 pi. On day 30 pi, following transient demyelination, both strains of mice responded to MBP by TNF/LT production. The production of TGF ß by splenocytes of SFV-infected B6 mice was significantly higher than by those of SJL mice, in the absence of MBP, on d7 (p<0.05), d15 (p=0.05) and d21 pi (p<0.01) and in the presensc of MBP, on d7 (p<0.003), d15 (p=0.01) and d21 pi (p<0.005), as determined by student's t test. Of particular interest was that detection of active TGF-ß did not require prior acidification. This indicated that the splenocytes of SFV-infected mice were producing the active form of TGF-ß. Supported by NIH grant NS24688 and a grant from Maimonides Res and Dev Fndn.

N 321 FUNCTIONAL CD4+ CLASS II MHC-RESTRICTED CYTOTOXIC T CELLS IN CLASS I MHC DEFICIENT MICE
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Mice deficient in ß2-microglobulin (ß2m -/-) lack functional class I MHC molecules and are deficient in mature CD8$^+$ T cells. Despite this apparent immune deficiency, these mice do not show an increased susceptibility to several viral pathogens. Lethal choriomeningitis after intracranial infection with lymphocytic choriomeningitis virus (LCMV) in normal mice is mediated by CD8$^+$ cytotoxic T lymphocytes. We previously showed that these ß2m -/- mice, despite the deficiency of CD8$^+$ cells, are susceptible to this immunopathologic process. However, the time from intracranial infection till death was increased in ß2m -/- mice, and all normal animals died while ß2m -/- mice showed only a 75% mortality. We also showed that these ß2m -/- mice show specific, anti-LCMV cytotoxic activity which is mediated by CD4$^+$, class II MHC-restricted T cells (Muller et al., Science 255:1576, 1992).

We now show that we can abolish mortality after intracranial infection using sublethal irradiation. We also show that *in vivo* treatment of these mice with anti-CD4 monoclonal antibody can reduce mortality after intracranial infection. Preliminary experiments show that we can transfer the observed mortality by the adoptive transfer of CD4+ T cells. We also show that overall cytotoxic activity toward LCMV is more variable in the ß2m -/- mice, and this variablity is reflected in the increased numbers of spleen cells required for transfer of mortality. Using in vitro studies we also establish the importance of the endosomal-lysosomal pathway in antigen processing of viral antigen for presentation to class II-restricted cytotoxic T cells, by showing that CD4$^+$ cytotoxic T cells are able to kill target cells incubated with killed virus.

In conclusion, these data suggest that CD4$^+$ T cells from ß2-microglobulin deficient mice can assume the cytotoxic functions of the CD8$^+$ cell population from normal mice, including immune-mediated pathology. However, differences in the antigen processing pathway for class II-MHC may influence the functioning of these cells in mediating immune injury.

N 322 T CELLS SPECIFIC TO SINGLE VIRAL PROTEINS CAUSE DISTINCT PATTERNS OF PROTECTION AND DISEASE

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Mice sensitized to individual respiratory syncytial (RS) virus proteins show distinct patterns of immunity and augmented pulmonary pathology during subsequent RS virus infection. In order to explore the immune mechanisms causing augmented disease, mice were primed by scarification with recombinant vaccinia viruses (rVV) expressing the major surface glycoprotein (G), fusion protein (F) or second matrix (22K) protein of RS virus. Virus stimulated spleen cell cultures from these mice gave rise to $CD3^+$, $\alpha\beta$ TCR^+ T cell lines. Those from 22K sensitised mice were mostly $CD8^+$; those from F sensitised mice, a mixture of $CD8^+$ and $CD4^+$; while those from G sensitised mice were mostly $CD4^+$. F and 22K-specific lines showed virus-specific CTL activity *in vitro*. The F specific lines released cytokines characteristic of "Th1" cells, while the G-specific line produced "Th2" cytokines. Virus-infected mice showed mild illness and recovered fully, but developed respiratory distress after injection of virus-specific cell lines. Dose-for-dose, the most severe (sometimes fatal) illness was seen in mice receiving G-specific cells. Injection of G-specific cells into RS virus-infected mice induced lung haemorrhage, pulmonary neutrophil recruitment ('shock lung') and intense pulmonary eosinophilia. Disease was further enhanced by co-injection of 22K-specific cells, which alone caused mild shock lung without eosinophilia. F-specific cells alone caused minimal enhancement of pathology, but did not affect disease caused by G-specific cells. Each of the cell lines reduced lung virus titre and combined injections eliminated infection completely.

Transfer of protein-specific T cells into naive RS virus-infected mice therefore reproduces the patterns of enhanced pathology seen in mice sensitised to individual RS virus proteins. T cells appear to be the main cause of pathology in this model of lung disease.

N 323 FURTHER CHARACTERIZATION OF THE IMMUNE RESPONSE TO LCMV IN MHC CLASS I DEFICIENT MICE.

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Mice lacking β_2-microglobulin (β_2m-) are deficient in MHC class I expression and, consequently, are deficient in mature $CD8^+$ T cells. In normal mice, death following intracranial infection with lymphocytic choriomeningitis virus (LCMV) results from $CD8^+$ T cell mediated attack on virally infected cells in the brain. We have previously shown that, despite their deficiency in $CD8^+$ T cells, β_2m- mice also die following intracranial infection with LCMV, albeit several days later than normal mice. We also described the induction of LCMV-specific, class II-restricted cytotoxic T cells (CTL) in β_2m- mice. We now show that, in accord with the delayed onset of meningitis, the kinetics of induction of CTL activity in these mice differs from that seen in normal mice. In control mice, maximum CTL activity is seen at 7 days post-infection, whereas in β_2m- mice maximum CTL activity is not reached until 9-10 days post-infection. In addition, the LCMV specific CTL in β_2m- mice are able to kill target cells that have been incubated with inactivated virus. Treatment of the target cells with chloroquine prevents killing, suggesting that the "exogenous" antigen presentation pathway is responsible for processing and presentation of LCMV to these class II-restricted CTL *in vitro*. This is consistent with the hypothesis that endocytosis and subsequent presentation of shed virus particles during LCMV infection *in vivo* is responsible for the induction of specific class II-restricted CTL in β_2m- mice. This mechanism may explain the delayed onset of CTL activity and meningitis following LCMV infection. We have also observed that viral induction of natural killer cell activity is similarly delayed. This might suggest a more fundamental defect in the elaboration of the cytotoxic response in β_2m- mice.

N 324 TARGETING SPECIFIC HOST AND VIRAL GENES TO CELLS OF THE CENTRAL NERVOUS SYSTEM: TRANSGENIC STUDIES,

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Viruses can cause neuronal injury by three distinct paths. Viruses such as herpes simplex and polio infect and replicate in neurons of the central nervous system (CNS), causing direct lytic injury. Other viruses, like human immunodeficiency virus (HIV), cause neuronal dysfunction indirectly; i.e., without the virus infecting neurons. Finally, viruses such as lymphocytic choriomeningitis virus (LCMV) can alter neuronal function without direct lysis by establishing persistence in neurons. In this instance, although LCMV actively replicates in neurons without causing cell death, significant alterations in neurotransmitter RNA levels are detected. For LCMV or any noncytolytic virus to establish persistence, it must also perfect strategies to evade host immune recognition. Neurons are deficient in the expression of the major histocompatibility molecules (MHC) necessary for immune recognition, while other CNS cells (astrocytes, oligodendrocytes) express little or no MHC, indicating that these cells can serve as a harbor for non-cytolytic viruses. In order to understand the principles by which viruses can persist in CNS cells, we have developed transgenic mice that express MHC genes in cells of the CNS using cell-specific promoters. Here we report successful in vivo expression of a MHC class I molecule (murine D^b) in neurons. It has been shown that transfection of a neuronal cell culture system with the D^b gene allows MHC class I heavy chain expression and permits CTL to lyse virally infected neurons. Using the neuron-specific enolase (NSE) promoter, the MHC class I molecule D^b was expressed in neurons of transgenic mice and the spliced transgene-derived D^b mRNA was detected by RNA PCR in brains of transgenic mice. Protein expression was shown by attachment of hippocampal neurons from transgenic mice on cover slips coated with a conformation-dependent monoclonal antibody directed against the D^b molecule. Neuronal expression of MHC class I glycoprotein was not detrimental since uninfected transgenic mice housed in microisolator cages for as long as 1 year have neither clinical disorders nor an enhanced death rate when compared to non-transgenic littermates. In preliminary studies, when such D^b-expressing neurons are persistently infected with LCMV in vivo, activated CTL given peripherally (intra-peritoneally) home to the CNS and cause disease. Other transgenic lines have been created in which the LCMV-NP is expressed in neurons using the NSE promoter. Studies with these mice as to neurotransmitter defects and of doubly transgenic mice that express both D^b and LCMV-NP in neurons are in progress.

N 325 **DIFFERENT *IN VIVO* EFFECTS OF VIRUS-SPECIFIC CD4+ T CELLS ON THE OUTCOME OF BORNA DISEASE DEPENDING ON THE TIME OF TRANSFER**,

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Borna disease virus (BDV), a noncytopathic single-stranded RNA virus, causes a persistent infection with meningoencephalitis in a wide range of naturally and experimentally infected animals. In our experimental model, the Lewis rat, BDV produces a biphasic disorder characterized by aggression and hyperactivity followed by listlessness and blindness. Both, meningoencephalitis and disease could be prevented by treatment of infected rats with immunosuppressive drugs, indicating that Borna disease (BD) is caused by an immunopathological mechanism. Drug-induced tolerance could be overcome by adoptive transfer of spleen- or lymph node cells from acutely infected, syngeneic adult rats or injection of syngeneic, BDV-antigen specific CD4+ T cells (NM1). In addition, transfer of the CD4+, BDV-specific NM1 cells before BDV-infection resulted in reduced virus titers in the central nervous system and protection against fatal BD. However, all protected animals developed a meningoencephalitis similar to the virus-infected control animals. Inoculation of control CD4+ T cells or inactivated NM 1 T cells failed to protect against clinical disease. These findings indicate that both protection against and induction of BD are mediated by BDV-specific CD4+ T cells, depending on time of transfer. Since only viable CD4+ T cells are able to induce both effects, the underlying mechanism is different from that of various CD4+ T cell-mediated autoimmune diseases.

N 326 CYTOTOXIC T CELL CROSSREACTIVITY BETWEEN VIRUSES DURING ACUTE INFECTION. Liisa K. Selin, Sharon R. Nahill, and Raymond M. Welsh. Univ. Massachusetts Medical Ctr., Worcester, MA.

During the examination of the mouse cytotoxic T cell (CTL) response to viral infections it was observed that challenge of lymphocytic choriomeningitis virus (LCMV)-immune mice with a second virus, either Pichinde (PV) or vaccinia virus, resulted in the generation of LCMV-specific memory CTL reactivation. Limiting dilution analyses (LDA) of these CTL generated in vivo revealed that some virus specific CTL clones were cross-reactive with the second virus. Mice receiving only one virus generated few if any cross-reactive clones during an acute infection. However, CTL from a LCMV-immune mouse when restimulated during an LDA with either LCMV or PV demonstrated a low but consistent precursor frequency for lysis of PV- infected targets. These results suggest that there exist remote cross-reactivities between non-related viruses and that the specificity of the CTL response to a virus is influenced by previous infections. Two experiments have demonstrated that prior immunity to LCMV enhanced the splenic precursor frequency to PV during a subsequent acute PV infection. In contrast, following the second viral infection (PV) there was an apparent decrease in splenic CTL memory precursors to the first viral infection (LCMV). It would appear that these remote viral cross-reactivities modulate future primary or secondary immune responses.

N 327 ROLE OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS IN THE PATHOGENESIS OF AN ACUTE VIRAL INFECTION, John Sheridan, Gerlinda Hermann, Cathleen Dobbs, and Ronald Glaser, Departments of Oral Biology, Medical Micro. and Immunol., Ohio State University, Columbus, OH 43210

One of the pathways by which physiological responses to stressors are mediated is the HPA axis which, ultimately, results in secretion of glucocorticoids (GC) by the adrenal gland. Anti-inflammatory and immunosuppressive properties of GC have been demonstrated. Experimental influenza viral infection has been shown to elevate plasma GC (Dunn et al., 1989), a response suggesting that infection may be perceived as a "stressor" and that GC is part of the neuroimmune communication that occurs during infection. This interaction may provide a means to limit the magnitude of the response and reduce immunopathology. The purpose of this study was to confirm activation of the HPA axis during infection of C57BL/6 mice with influenza virus A/PR8 and to investigate the role of the GC response in viral pathogenesis. Within 48h of i.n. infection with PR8, mice showed an elevation of plasma GC levels (166 ng/ml vs $<$50 ng/ml baseline). By 7d p.i., plasma levels of GC averaged 432 ng/ml and dropped to 100 ng/ml by 10d as the infection resolved. Behavioral paradigms, such as restraint (RST), elevate plasma GCs. When these behavioral treatments were imposed during an immune response to viral infection, the GC levels were amplified. Elevated GC levels were associated with reduced cell yields from the mediastinal lymph nodes (l.n.) compared with control/infected mice. Additionally, inflammation and pathosis in the lungs of PR8-infected mice were diminished in association with RST. To examine the role of GC more specifically, RU486 (GC receptor blocker), was admininstered on a daily basis prior to and post infection with PR8. The cell yields from l.n. were the same regardless of the behavioral treatment. Thus, blocking GC receptors via RU486 abolished the RST-associated decrease in cell number in the draining lymph node. Gross observations of infected lungs indicated severe congestion and consolidation. These studies indicate that GC may play a critical role in the regulation of the immune response during viral infection.

N 328 DISTRIBUTION, TROPISM AND PERSISTENCE OF THEILER'S VIRUS IN THE CNS OF MICE SUSCEPTIBLE AND RESISTANT TO DEMYELINATION, J. Pedro Simas and John K. Fazakerley, Division of Immunology, Department of Pathology, Cambridge University, Cambridge, UK.

Following intracerebral inoculation of 3-4 week old CBA or BALB/c mice, the GDVII strain of Theiler's murine encephalomyelitis virus (TMEV) produces an acute fatal encephalomyelopathy. The BeAn strain produces a chronic inflammatory demyelinating disease in CBA mice while BALB/c mice are resistant to demyelination.

The neuroanatomical distribution, spread and tropism of GDVII and BeAn have been determined in CBA and BALB/c mice. In both strains of mice, GDVII RNA was detected in specific areas including, cerebral cortex, hippocampus, substantia nigra, brain stem and spinal cord but not in corpus callosum or cerebellum. Large continuous areas were infected suggesting rapid cell to cell spread. In contrast, in both strains of mice CNS infection with BeAn was focal with scattered, single or small clusters of infected cells. This distribution sugests that BeAn replication is restricted in the CNS. Analysis of BeAn CNS virus titres demonstrated a difference in clearance in CBA and BALB/c mice. By double labelling, *in situ* hybridization and immunostaining GDVII and BeAn RNA could be observed in both neurons and astrocytes in CBA and BALB/c mice. *In situ* hybridization and slot blot analysis of RNA demonstrated BeAn virus persistence in the CNS of CBA mice several months after infection.

N 329 CORONAVIRUS-SPECIFIC CYTOTOXIC T CELLS DERIVED FROM THE CENTRAL NERVOUS SYSTEM, Steve Stohlman[1], Cornelia Bergmann[1], and Shigeru Kyuwa[2]. Department of Neurology and Microbiology, University of Southern California, School of Medicine, Los Angeles, CA 90033[1], and the Department of Animal Pathology, University of Tokyo[2]

The JHM strain of mouse hepatitis virus is a neurotropic variant that produces a panencephalomyelitis accompanied by primary central nervous system (CNS) demyelination. To determine the role of cytotoxic T cells (CTL) in the reduction of infectious virus and demyelination within the CNS, CTL lines were established from the CNS of Balb/c mice undergoing an acute fatal infection with JHMV. Twenty of twenty two lines were able to lyse JHMV-infected syngenic targets. The two remaining lines lysed both JHMV-infected and uninfected targets. Recombinant vaccinia viruses expressing the JHMV structural proteins (designated S, M, HE, and N) were used to determine the viral specificity of the remaining twenty cells lines. Fifteen of the T cell lines were able to specifically recognize only those targets expressing the viral nucleocapsid (N) protein. The remaining five lines recognize JHMV-infected syngenic targets but were unable to recognize any of the structural proteins, suggesting specificity for a nonstructural protein. Surprisingly, immunizations with recombinant vaccinia expressing the N protein or the N protein CTL epitope were unable to protect mice from a lethal infection or alter the progression of disease. Conversely the adoptive transfer of at least one of the T cell lines specific for the nonstructural protein was able to protect mice from lethal infection and prevent CNS demyelination. These preliminary data suggest that the vigorous CTL response to the N protein found in the CNS of infected mice does not contribute to either viral clearance or reduction of pathological changes.

N 330 ANALYSIS OF MHC CLASS I AND II EXPRESSION IN RELATION TO PRESENCE OF HPV GENOTYPES IN PREMALIGNANT AND MALIGNANT CERVICAL LESIONS, Marij J. Stukart[1], Frans V. Cromme[1], Chris J.L.M. Meijer[1], Peter J.F. Snijders[1], Anne Uyterlinde[1], Peter Kenemans[2] and Jan M.M. Walboomers[1]. Institute for Pathology[1], Section of Molecular Pathology, Dept of Gynaecology[2], Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

Cervical intraepithelial neoplasia (CIN) grades I to III lesions (n=94) and squamous cell carcinomas of the uterine cervix (n=27) were analysed for MHC class I and II expression and presence of HPV genotypes.
MHC class I and II expression was studied by immunohistochemistry and HPV typing was performed by general primer- and type-specific primer mediated PCR (GP/TS PCR). Both techniques were performed on paraffin embedded tissue sections.
Results show disturbed MHC class I heavy chain expression in CIN I to CIN III, as well as in cervical carcinomas. Upregulated MHC class II expression on dysplastic epithelial cells was also found in the different CIN groups and carcinomas. Prevalence of HPV genotypes increased with the severity of the lesion, mainly due to the contribution of the HPV types 16 and 18. No correlation could be established between the presence of specific HPV genotypes and any MHC expression pattern in the different CIN groups or cervical carcinomas. In some cases these data were confirmed by RNA in situ hybridisation showing HPV 16 E7 transcripts in the same dysplastic/neoplastic cells from which MHC status was determined. The results indicate that local differences may exist in the type of cellular immune response to HPV induced lesions.

N 331 PATHOGENESIS OF POLYOMAVIRUS INFECTION IN SCID MICE, Eva Szomolanyi-Tsuda, Leonard Shultz, Patricia Dundon, Isabelle Joris and Raymond Welsh, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA and Jackson Laboratory, Bar Harbor, ME.
Polyomavirus infection of newborn or adult nude mice results in the development of multiple tumors, but immuncompetent mice infected as adults are resistant to tumor induction by the virus. We investigated the role of natural killer (NK) cells in the resistance of mice to polyomavirus using BALB/C or C57BL/6 scid mice, which lack T and B cells. The pathogenesis of polyomavirus infection was examined in untreated mice and in mice depleted of NK cell activity by mAb to NK1.1 or by antiserum to asialo GM1. Infection of scid mice with $2x10^5$-$2x10^6$ PFU polyomavirus strain A2 intraperitoneally led to the death of 100%(62/62) of the animals between days 12 and 16 postinfection. Pathologic findings included splenomegaly, a large decrease in megakaryocytes and massive erythroblastosis in the spleen and bone marrow, thrombocytopenia, anemia and hemorrhagic lesions in skin. Multiple copies of unintegrated, episomal polyomavirus genome were found in the organs tested (spleen, kidney, liver,lung) at day 7 postinfection, as well as shortly before death. NK cell depletion did not influence the pathogenesis and the time of death, did not increase the polyomavirus titer in the spleen and kidney, and did not significantly change the amount of viral genome detected in DNA samples prepared from organs at day 7 postinfection or prior to death. FACS analysis of spleen cells from dying polyomavirus-infected animals showed high MHC class I and low class II antigen expression. Activated NK cells did not kill day 12 polyomavirus-infected spleen cells in vitro, suggesting that the spleen cells, which are mainly proliferating erythroblastoid cells in polyomavirus-infected scid mice are resistant to NK cells. Our results demonstrate that polyomavirus infection in scid mice leads to a very rapid, lethal hematologic disorder, characterized by massive erythroblastoid cell proliferation. This pathology is vastly different from the tumor induction "profiles" described in polyomavirus-infected newborn and adult nude mice.

N 332 MAPPING OF GENE SEGMENTS INVOLVED IN REOVIRUS PERSISTENCE AND VIRULENCE IN SCID MICE. Herbert W. Virgin IV and Barbara L. Haller, Departments of Medicine, Pathology, and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, 63110.
We evaluated genes involved in viral virulence in the absence of lymphocyte-dependent immunity using severe combined immunodeficient (SCID) mice and reovirus reassortants. Reovirus infection of adult SCID mice resulted in either early (12-20 days) or late (60-90 days) death depending on the reovirus used for infection. Reovirus type 1 Lang (T1L) was most virulent while type 3 Dearing (T3D) was least virulent. A panel of 12 T1L x T3D reassortant viruses were used to map the gene segments involved in this virulence phenotype. Four T1L gene segments (S1, L1, M1, and L2) were significantly associated with virulence in SCID mice. Three of the four gene segments (L1, L2, and M1) are viral core components possibly involved in viral RNA synthesis. The L1 and M1 gene products are estimated to be present in 1 copy per viral vertex while pentamers of the L2 gene product form the core spikes at each vertex. The S1 gene segment encodes the viral cell attachment protein as well as a non-structural protein of unknown function. Viral growth and capacity to generate hepatitis were also mapped using reassortants. In addition, reoviruses isolated from SCID mice after prolonged infection showed altered biochemical phenotypes in vitro and prolonged infection resulted in progressive increase in T3D titer in organs initially resistant to viral replication. These results demonstrate the importance of reoviral core proteins in virulence when lymphocyte based immunity is inoperative. Viruses isolated from chronically infected mice may provide interesting models for study of tissue tropism during chronic infection and molecular details of mutations occurring in the absence of lymphocyte-dependent selective pressures in vivo.

N 333 THE ROLE OF CD4 AND CD8 CELLS & THE THYMUS DURING DEVELOPMENT OF AUTOIMMUNE DISEASE IN A TRANSGENIC MOUSE MODEL, [1]Matthias von Herrath, [1]Janel Dockter, [2]Terri Laufer, [2]Laurie Glimcher and [1]Michael B. A. Oldstone, [1]The Scripps Research Institute, La Jolla, CA 92037; [2]Dept. of Cancer Biology, Harvard Medical School, Boston, MA 02115
Transgenic mice expressing nucleoprotein (NP) or glycoprotein(GP) of lymphocytic choriomeningitis virus (LCMV) under the control of the rat insulin promoter (RIP) in beta cells of the pancreas only develop insulin-dependent diabetes mellitus (IDDM) as an autoimmune disease when challenged with LCMV. The resulting pancreatic lymphocellular infiltrate is comprised of both $CD4^+$ and $CD8^+$ cells. Here we report that the presence of $CD4^+$ cells is not required for the development of infiltration or IDDM. Deletion of $CD4^+$ cells was accomplished by homologous recombination ("gene knockout") of the Ia (class II) molecule. Such mice were bred with the RIP-LCMV transgenics to yield progeny that were $CD4^-$ and $LCMV^+$. These mice developed IDDM after infection with LCMV with $CD8^+$ infiltrates but without infiltration by $CD4^+$ cells. As demonstrated by PCR technique, RIP-LCMV-NP mice express the viral NP transgene not only in the beta cells of the pancreas, but also in the thymus, whereas RIP-GP mice do express the transgene only in the pancreas. Interestingly, RIP-LCMV-NP mice require 1-3 months to develop IDDM after LCMV challenge. In contrast, RIP-LCMV-GP mice develop IDDM within 10 days after LCMV challenge. RIP-NP and Thy-NP mice (expressing LCMV-NP in the thymus) exhibit a partial unresponsiveness of LCMV-NP specific CTL after challenge with LCMV virus. This unresponsiveness can be transferred into SCID mice by grafting their renal capsule with thymi from newborn RIP-NP mice. Transplant of thymi from non-transgenic littermates does not cause unresponsiveness in SCID mice. Hence, the RIP-NP lines studies here negatively select out potential LCMV specific CTL, allowing limited numbers to reach peripheral organs. In contrast, RIP-GP lines positively select potential LCMV-specific CTL to reach peripheral organs of the immune system, thereby explaining the rapid onset of IDDM following viral challenge.

N 334 CD4+ T CELL ACTIVATION BY AUTOLOGOUS, HLA CLASS II-NEGATIVE, CYTOMEGALOVIRUS-INFECTED HUMAN ENDOTHELIAL CELLS.

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Cytomegalovirus (CMV), a source of serious complications among immunosuppressed individuals, infects endothelial cells *in vivo*. As the interface between the immune system and underlying tissues, the endothelium may be of primary importance in mediating protective or pathogenic immune responses to this virus; however, little is known of the role of the endothelium in the modulation of the human immune response to CMV. In an effort to model such immune interactions *in vitro*, we have investigated responses of spleen-derived CD4+ T lymphocytes (from cadaveric organ donors) to CMV-infected autologous human gonadal vein endothelial cells (GVEC). Microculture proliferation assays demonstrated dramatically enhanced responses by CMV-seropositive donor-derived T cells cocultured with CMV-infected GVEC, as compared to those elicited by non-infected cells. Similarly, as determined by limiting dilution analysis of IL2-producing cells, the frequency of CD4+ T cells responding to infected GVEC was generally found to exceed by an order of magnitude those responding to uninfected cells. Responses of CMV-seronegative donor-derived T cells to autologous GVEC, in contrast, showed no virus-related enhancement. The marked activation of CD4+ T cells is particularly intriguing since we have shown that CMV-infected GVEC do not express HLA Class II antigens. These studies demonstrate that CD4+ T cells can respond to CMV in the context of endothelial cells, potentially via a novel signalling mechanism, and thus, support an important role for the endothelium in the afferent recognition phase of the immune response to this virus.

Pathogenesis: Retroviruses

N 335 ***IDENTIFICATION OF A MEMBRANE ANTIGEN IMPORTANT FOR HTLV SYNCYTIA FORMATION.***

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HTLV-I is the causal agent of adult T-cell leukemia, but a disease association with HTLV II a related retrovirus, has not clearly been established. The use of different approaches has not been successful for the identification of the cellular receptor for the HTLV, but these experiments have shown that HTLV-I and II share the same receptor which is encoded by chromosome 17 ('Human Retroviruses" Ed: R.C.Gallo, G.Jay, 1991). Thus, characterization of the HTLV cellular binding and/or fusogenic receptor/s remains to be elucidated.

We have observed that cells infected with the HTLV-I isolate MT-2 and HTLV-II isolates MoT or FL-W are able to fuse with B cell lymphoma (BJAB) subclones designated WH (from Dr.William Hall), CC79, CC90, but not with another subclone CC84 (UPENN Cell Center). Based on these differences we generated antisera against structure(s) involved in syncytia formation of fusogenic subclones of target cells. BALB/C mice were immunized with BJAB-WH cells, antisera was obtained and adsorbed on BJAB-CC84 cells. Non-adsorbed sera killed both BJAB-WH and BJAB-CC84 target cells in complement dependent cell cytotoxicity. In contrast adsorbed sera killed only BJAB-WH cells. These sera inhibited 85-90% of control syncytia formation at a dilution of 1:120 in syncytia inhibition assays with a mixture of HTLV-I (MT-2) or HTLV-II (FL-W or MoT) infected cells and BJAB-WH or BJAB-CC79 target cells. Using adsorbed sera in immunoprecipitation studies we identified an 80 kD molecule from lysates of ^{35}S-cysteine labeled BJAB-WH and CC79, but not from lysate BJAB-CC84 cells. This structure presumably was involved in syncytium formation of target cells with two isolates of HTLV-II and one isolate of HTLV-I. In addition, it was shown that the BJAB-WH but not BJAB-CC84 cells are permissive for infection with cell free FL-W virus as measured by an HTLV-II p24 ELISA 20-30 days after infection and by syncytia formation 3-5 days after infection.

N 336 CARBOHYDRATE DETERMINANTS MODULATING THE ANTIGENIC ACTIVITY OF SURFACE GLYCOPROTEIN GP120 OF HIV-1

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In the present study we investigated to what extent the peripheral carbohydrate structure of N-linked glycans influenced the antigenic properties of gp120, specified by human immunodeficiency virus type 1 (HIV-1). Recombinant gp120 was purified from GMK cells, infected with a recombinant vaccinia virus, expressing gp120. The purified gp120 was coated onto ELISA 96 well microplates and subjected to sequential elimination of peripheral monosaccharide units.The modified or unmodified gp120 was then incubated with monoclonal antibodies defining specific epitopes of gp120 and with a reporter lectin to determine the extent of carbohydrate elimination. Antibody and lectin binding was quantified in an enzyme-linked system. We found that the carbohydrate structure, Sialic acid-Galactose (NeuAc-Galß(1-4)) of N-linked glycans, defined both by lectin reactivity and by specific glycosidases, was engaged in modulating the antibody binding of a number of epitopes of peptide nature. The antibody binding of one class of epitopes, situated in a region delimited by aminoacids 200-230, was strongly increased by removal of NeuAc-Galß(1-4), whereas the binding of epitopes in the V3 region was decreased and the binding of epitopes in the far N-terminal region was not altered by the treatment. These results suggested that peripheral structures of N-glycans are engaged in modulating the overall conformation of gp120.

N 337 DETECTIONOF HIV-SPECIFIC CELL MEDIATED CYTOTOXICITY IN THE PERIPHERAL BLOOD OF INFECTED CHILDREN.

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Cytotoxic T lymphocytes may play a significant role in containing the spread of HIV in infected individuals. Although HIV-infection is associated with immune suppression, a vigorous T lymphocyte response has been detected in infected adults. HIV can be transmitted from mother to child, either during pregnancy, when differentiation of the T lymphoid compartment is ongoing, or at birth when the neonate immune system is partially competent. The shorter asymptomatic period of pediatric infection could be related to differences in the host immune control of viral replication. HIV-specific cell mediated cytotoxicity (CMC) from fresh and in vitro stimulated PBMC of HIV-infected children was measured. CD8+CD3+ T lymphocytes were found to be the major effector population. The vast majority of children examined had detectable cytotoxic T cell responses. A cross-sectional analysis of CMC responses as a function of clinical status revealed that most children exhibiting positive primary assays belonged to the P2A CDC classification group (mild and non-specific symptoms), asymptomatic children (P1) recognized only the env glycoproteins, and most symptomatic children (P2B-F) had no detectable cytotoxic activities mediated by fresh PBMC. In contrast in vitro generated secondary CTL were consistently detected at all stages of disease, even in children with low CD4+ cells counts.

N 338 Cross-Reactive Lysis of Human Targets Infected with Prototypic and Clinical HIV-1 Strains by Murine Anti HIV-1 IIIB Env Specific CTL. Sunil Chada, Cataline E. DeJesus, Kay Townsend, William T. L. Lee, Lisa Laube, Douglas J. Jolly, Stephen M. W. Chang and John F. Warner. Departments of Molecular Virology and Immunobiology, Viagene Inc., 11075 Roselle St., San Diego CA 92121.

In order to evaluate the ability of murine anti-HIV-1 IIIB env CTL (CTLenv) to recognize and lyse HIV-1 infected cells, we have constructed a human cell line (Hu/D^d) expressing both the CD4 receptor and the murine H-$2D^d$ major histocompatibility complex (MHC) class I protein. This cell line can be productively infected with HIV-1 and can also function as a target for murine $CD8^+$, class I MHC restricted CTL directed against the envelope glycoprotein of HIV-1 IIIB. The ability of BALB/c anti-HIV-1 IIIB env CTL to specifically recognize and lyse Hu/D^d target cells infected with divergent HIV-1 strains was tested using both prototypic and clinical HIV-1 strains. CTL generated by immunization of mice with syngeneic cells expressing either the native or V3 loop-deleted (ΔV3) envelope glycoprotein from HIV-1 IIIB were able to recognize and specifically lyse Hu/D^d target cells infected with the HIV-1 prototypic isolates; IIIB, MN, WMJ II, SF2, CC as well as several HIV-1 clinical isolates. These results demonstrate that CTL determinants for HIV-1 env exist outside the hypervariable V3 region, anti-HIV-1 IIIB env CTL appear to recognize common determinants on diverse HIV-1 strains, and that classification of HIV-1 strains based on neutralizing antibody reactivities does not appear to correspond to CTL recognition and lysis. The results suggest that the cell-mediated components of the immune system may have a broader recognition of divergent HIV-1 strains than the humoral components.

N 339 THE CYCLOPHOSPHAMIDE SENSITIVITY OF THE SUPERANTIGEN MLS-1^a INDUCED ADULT TOLERANCE, Kai-ping N. Chow, Department of Microbiology and Immunology, Chang-Gung Medical College, *Kwei-shan, Taoyuan, Taiwan, Republic of China.*

The down-regulation of the development of cytotoxic T lymphocytes (CTLs) has been studied in C3H mice that were tolerized with CBA spleen cells(SC). These two strains of mice share the H-2^k haplotype, *but differ in the mls locus,* i.e., mls-1^b for C3H, and mls1^a for CBA. Seven days after 2000R irradiated CBA SC were i.v. injected into C3H, mice were immunized for the generation of CTL *in vivo*. We used Mls unrelated antigen trinitrophenol (TNP) to test whether these tolerized mice render CTL activity against the altered self targets. C3H SC-TNP along with CBA SC were injected into footpads in the immunization. Five days later, the percent of lysis against H-2^k-TNP in the tolerized mice were found suppressed up to 90%. This suppression was transferrable by a thy-1^+, CD5(Ly1)$^+$, CD4 T cells. However, the development of the CD4 T suppressor (Ts) during the 7-day period of tolerance induction was found to be cyclophosphamide (CY) sensitive. Administration of CY *in vivo* in day 3 to day 5 completely abolished the suppressor activity when transfer. Nevertheless, mature Ts was fully active in CY-treated recipients. Thus, the mechanism of the M1s-1^a induced adult tolerance is involved a CD4 Ts whose development *in vivo* is CY sensitive.

N 340 CYTOTOXIC T LYMPHOCYTE RESPONSES TO THE ENVELOPE PROTEINS OF ENDOGENOUS MURINE LEUKEMIA VIRUSES IN NORMAL AND INFECTED MICE. **Michael A. Coppola and William R. Green. Department of Microbiology, Dartmouth Medical School, Lebanon, NH 03756.**

C57Bl/6 (B6) mice generate vigorously lytic CTL in response to in vivo priming and subsequent in vitro stimulation with AKR/Gross virus-positive lymphomas. These CTL are H-$2K^b$-restricted and type-specific, in that they do not recognize H-2 matched Friend-Moloney-Rauscher virus-induced tumors. H-$2K^b$-transfected fibroblasts infected with either AKR623 MuLV or MCF247 MuLV, but not MCF13, were efficiently lysed by these anti-AKR/Gross virus CTL. We next constructed recombinant vaccinia viruses expressing the envelope genes of these MuLV. MC57 (H-2^b) cells infected with either 623EnvVac or 247EnvVac were also lysed by these CTL, suggesting that peptide(s) derived from the shared C-terminal half of the envelope protein may be recognized. These recombinant vaccinia viruses could also prime mice for anti-AKR/Gross CTL responses, and stimulate the in vitro generation of CTL from tumor-primed mice.

In other experiments, adult B6 mice infected with the SL3-3_{nb} MuLV progressively lost the ability to generate anti-AKR/Gross virus CTL. Mice became completely non-responsive within two to three weeks following intraperitoneal inoculation with as little as 5 x 10^4 X-C pfu of this retrovirus. This non-responsiveness appeared to be antigen-specific, in that anti-allo CTL responses of infected and uninfected mice were comparable. However, preliminary experiments suggested that infection with Friend MuLV, which is not recognized by anti-AKR/Gross virus CTL, may also abrogate the CTL response to AKR/Gross virus-positive tumors, indicating that the mechanism of non-responsiveness might involve anergy or suppression of T_h cells which recognize epitopes shared between these viruses, rather than a direct effect on the CTL or their undifferentiated precursors.

Taken together, these observations may allow the development of a model system for testing the efficacy of recombinant vaccines in preventing retroviral infection and/or modifying the outcome of infection in virus-positive hosts.

N 341 OCCURENCE OF VIRUS-SPECIFIC ANTIBODY-SECRETING CELLS IN THE INTESTINAL MUCOSA OF HIV-1 INFECTED INDIVIDUALS, Eriksson, K., Holmgren, J., Kilander, A., Hagberg, L., Norkrans, G., and Czerkinsky, C. Departmens of Medical Microbiology & Immunology and Infectious Diseases, University of Göteborg, Göteborg, Sweden.

We have examined the possible occurence, frequency and isotype distribution of HIV-1 specific antibody-secreting cells (ASC) in the intestinal mucosa of infected patients. For this purpose, punch biopsy specimens from the first duodenal segment were dispersed by a 2-step enzymatic procedure involving exposure of pre-sliced (100 x 150 mm) tissues to the cold acting enzyme thermolysine, followed by collagenase. Single cell suspensions obtained by this method comprised 40-60% highly viable (> 90%) mononuclear cells (MNC) as assessed by conventional histochemistry. Immediately following isolation, cell suspensions were assayed for numbers of total as well as HIV-1 specific (gp120 and gp160) IgA-, IgG-, and IgM-ASC by micromodified bichromatic ELISPOT assays. Peripheral blood MNC obtained from the same patients were assayed in parallel. Intestinal HIV-specific ASC were detected in all patients and in considerable numbers (120-1450 gp120- and 550-3500 gp160-specific ASC per million MNC), whereas circulating specific blood ASC were only occasionally detected and in low frequencies (< 50 specific ASC to either antigen per million MNC). Further, marked differences were noted with regard to isotype distribution of ASC to HIV-1 gp120 and gp160 (which comprises the transmembrane domaine gp41). These observations demonstrate that the enteromucosal immune system in HIV-1 infected individuals undergoes a state of pronounced and sustained B cell activation, and indicate that local expansion of the virus specific mucosal B cell repertoire operates independantly of systemic disease.

N 342 FUSION OF HUMAN B CELLS WITH HIV-1 ENVELOPE EXPRESSING T CELLS CAN BE INDUCED BY ANTIGEN SPECIFIC IMMUNOGLOBULIN (IG) RECEPTORS. Hana Golding, Dimiter Dimitrov, Robert Blumenthal, and Basil Golding. Divisions of Virology and Hematology, CBER, FDA, and LMMB, NCI, NIH.

The possible contribution of antigen specific immunoglobulin (Ig) receptors on B cells to syncytia formation was examined. Initially, in a model system, we used anti-TNP/TNP interactions between a panel of TNP-specific human EBV transformed lines, and HIV-1 envelope expressing human CD4$^+$ T cells, which were haptenated with TNBS. The B cell lines differed in their surface CD4 and IgM (αTNP) receptor expression. These cells were mixed with the CD4$^+$ 12E1-T cell line, which were infected with vaccinia-gp120/41 vector. The main findings were: (1) Syncytia formation was enhanced 10-fold if the gp120/41-expressing 12E1 cells were haptenated with TNP. (2) The enhanced syncytia were blocked by antibodies against CD4 and TNP, and by TNP-BSA. (3) One CD4$^-$ αTNP B-cell line produced syncytia with TNP-env-12E1 effectors which were only blocked by anti-TNP Ab. (4) Enhanced syncytia formation required TNP-haptenation of the gp120 at or close to the CD4 binding sites.
Based on the above findings, we screened a panel of hybrid B cell lines from HIV-1$^+$ individuals. Two of 6 lines produced anti-gp120 Ab, one of which, secreted IgG Ab directed to the CD4-binding site on gp120. Only this hybrid line formed syncytia with 12E1-gp120/41 effectors.
Our data suggest that in addition to CD4, certain anti-gp120 Ig on B cells (specific for the CD4-binding regions), may induce conformational changes in the gp120/41 complex leading to cell membrane fusion. This mechanism may lead to a selective elimination of such B cells in vivo.

N 343 ROLE OF THE SAG GENE IN THE MOUSE MAMMARY TUMOR VIRUS LIFE CYCLE. T. V. Golovkina[1], A. Chervonsky[2], J. Prescott[1] and S. R. Ross[1], [1]University of Illinois (m/c 536), Chicago, IL; [2]Yale University School of Medicine, New Haven, CT.

Endogenous mouse mammary tumor virus (MMTV) proviruses have recently been shown to co-segregate genetically with the minor lymphocyte stimulating (Mls) loci, also termed self-superantigens (sag). The antigenic activity has been localized to the open reading frame (ORF) protein encoded in the long terminal repeat (LTR) of MMTV. We generated previously transgenic mice carrying internally deleted version of the exogenous C3H MTV genome (pMTV.orf). Expression of the sag gene causes deletion of the Vβ14$^+$ T cells in transgenic mice and confers partial resistance to C3H exogenous virus-induced mammary tumors. In addition to the ORF transgenic mice we made a transgenic strain, Hybrid Provirus (HYB PRO), containing an entire copy of an MMTV provirus in which the 3' half (including 3' LTR, env and ORF genes) was derived from C3H exogenous virus and the 5' half (including 5' LTR, gag and pol genes) came from the Mtv-1 endogenous locus present in C3H mice. Like the ORF transgenic mice, the HYB PRO transgenic mice show specific deletion of their Vβ14$^+$ T cells and these mice are protected against exogenous C3H MMTV. None of the ORF transgenic mice could stimulate a proliferative response of either C3H/HeN MMTV(-) non-transgenic primary spleen cells or Vβ14-bearing hybridomas whereas HYB PRO spleen cells caused considerable proliferation of either type of responder cells. Newborn ORF and HYB PRO transgenic mice expressing high level of the transgenes have a high level of deletion of cognate thymocytes but in comparison with the HYB PRO transgenic mice this deletion in the ORF transgenic mice is not persistent. At the age of 10 days deleted thymocytes begin to reappear in the thymus of the ORF transgenic mice and this reappearance is complete at the age of 3 months. We have shown that this is not the level of the transgene expression that determines the difference we observed in the two types of transgenic mice. These discrepancies could be explain by the requirement an MMTV gene(s) in addition to sag. Genetic studies indicate that this maps to the env genes. Role of the sag and env genes in MMTV life cycle are discussed.

N 344 MODULATION OF HTLV-I REPLICATION THROUGH CD2 AND CD3 MEDIATED ACTIVATION OF INFECTED LYMPHOCYTES, Deborah J Guyot, William Harrington, Lee Ratner and Michael Lairmore, The Ohio State University, Comprehensive Cancer Center and Department of Veterinary Pathobiology, Columbus Ohio; University of Miami, Florida; Washington University, Missouri.

Human T Lymphotropic virus type 1 (HTLV-I) is considered the etiologic agent of both an adult T cell leukemia (ATL) and a chronic immune-mediated progressive myelopathy (HAM/TSP). The HTLV-I long terminal repeat (LTR) contains sequences responsive to the cellular second messengers cyclic AMP and protein kinase C, giving rise to the following hypothesis: Signal transduction initiated through normal cell signaling pathways, the TCR/CD3 complex and CD2 receptor promotes viral replication and leukemogenesis in HTLV-I-infected lymphocytes. CD2 and CD3 cross-linking protocols were optimized in peripheral blood lymphocytes using plate-bound OKT3 (1:200) and pairs of soluble anti-CD2 mAbs (T11.1/T11.2 and T11.2/T11.3, 1:200-1:400). Parameters of cell activation (calcium flux, ^{3}H-thymidine incorporation, viable cell counts) and HTLV-I replication (p24 antigen capture, RT-PCR) were assessed following receptor cross-linking of both persistently infected HTLV-I cell lines (FS-CSF, IR-CSF, HuT102, MT-2 and SP10.1) and PBLs from HAM/TSP patients. The cloned persistently infected cell lines constitutively express widely variable amounts of p24 antigen (from <250 to >600 pg/ml) and membrane CD2 and CD3 receptors (from 0-96% of cells positive). Cross-linking of these transformed cells had either minimal effects on cell proliferation and p24 antigen production or resulted in cell death. While the transformed cells responded to ionomycin stimulation with the expected calcium flux, these results may reflect uncoupling of signaling pathways, the down modulation of membrane receptor expression, or the activated state of the cells. CD2 cross-linking in 2 HAM/TSP patients resulted in a 2 fold increase in p24 production on a per cell basis as compared to unstimulated controls. Current studies are evaluating mRNA production in patient PBLs following CD2 and CD3 receptor-mediated cross-linking. These results suggest that stimulation of the CD2-mediated alternate pathway may enhance HTLV-I replication during physiologic lymphocyte activation.

N 345 ARE LY-1 B CELLS TARGET CELLS OF LP-BM5 MURINE LEUKEMIA VIRUS? Yasumichi Hitoshi, Yoshiaki Okada, Masahiko Makino, Toshiaki Mizuochi, and Kiyoshi Takatsu. Department of Immunology, Institute of Medical Science, University of Tokyo, Sirokanedai, Minato-ku, Tokyo, 108, Japan, and Department of Blood products, National Institute of Health, Japan, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162, Japan
The murine acquired immunodeficiency syndrome (MAIDS) caused by defective LP-BM5 MuLV is a disease that shows severe immunodeficiency with abnormal lymphoproliferation and hypergammaglobulinemia in susceptible C57BL/6 (B6) mice. Previous studies have proven that the complex cellular interactions between T and B cells are required for induction and development of MAIDS; B cell abnormalities are shown in the presence of T cells of CD4 phenotype, whereas B cells are required for induction of phenotypic and functional T-cell abnormalities in MAIDS. Interestingly, a recent report indicated that the majority of cells infected with the defective LP-BM5 MuLV belong to B cell lineage, suggesting that B cells trigger the induction and development of MAIDS. To examine the cellular mechanisms of development of MAIDS, we injected LP-BM5 MuLV intraperitoneally into B6 mice bearing the X chromosome-linked immunodeficiency (*xid*). *Xid* mice lack functionally mature B cells including Ly-1 B cells (also known as B-1 cells). All B6 mice died by 20 weeks after LP-BM5 MuLV inoculation. In marked contrast, *xid* mice have continued to survive without any sign of MAIDS-related symptoms till at least 20 weeks after the inoculation. The delayed progression of MAIDS in *xid* mice was appeared to depend on *xid* mutation, according to our experiments using both sexes of (B6.*xid* x B6) F1 and (B6 x B6.*xid*) F1 mice. Furthermore, Ly-1 B cells, enriched by a fluorescence activated cell sorter, was shown to integrate the defective genome and appeared to be a major virus-infected B-cell population. Our data corroborate that Ly-1 B cells play an important role in the induction and progression of MAIDS.

N 346 SEQUENCE VARIATION IN THE HUMAN IMMUNODEFICIENCY VIRUS AS A MECHANISM OF ESCAPE FROM CYTOTOXIC T LYMPHOCYTES

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Human immunodeficiency virus type 1 (HIV-1) isolates exhibit extensive sequence variation, and this variation has been proposed as a mechanism by which this retrovirus may evade the host immune response. Our goal is to examine the relationship between sequence variation occurring in HIV-1 CTL epitopes *in vivo* and recognition by HIV-1-specific CTL. CTL clones were obtained from HIV-1-infected subjects, including a laboratory worker infected with HIV-1 IIIB. Clones were screened for CTL activity against target cells expressing HIV-1 proteins and the fine specificity of these clones determined. HIV-1 DNA coding for CTL epitopes was amplified using a nested polymerase chain reaction technique with direct sequencing of PCR products. Sequence variation was observed within CTL epitopes and in an area immediately adjacent to a CTL epitope. Mutations were observed in one epitope which lead to a loss of recognition by a CTL clone isolated at an earlier time point. However, CTL clones isolated at a later time point were able to recognize the variant sequence and the index sequence, consistent with a broadening of the CTL specificity. In another instance, sequence variation occurred without any effect on CTL recognition. Preliminary examination of the effects of mutations in regions flanking CTL epitopes have not revealed any significant effect on CTL recognition. Sequence variation may result in escape from an existing immune response but may also be countered by new or altered specificity of host defenses.

N 347 BOTH NAIVE AND MEMORY CELLS BECOME ANERGIC DURING MAIDS, Susanne Koch, Girija Muralidhar, Linda M. Bradley and Susan L. Swain, Dept. of Biology and UCSD Cancer Center, University of California San Diego, La Jolla, CA 92093

Infection of C57BL/6 mice with the LP-BM5 mixture of retroviruses causes splenomegaly, lymphadenopathy and loss of T cell and B cell functions leading to severe immunodeficiency. CD4 T cells are required for the induction and progression of the disease, termed murine AIDS (MAIDS).The extent of lymphoproliferation and immunodeficiency was studied in adult thymectomized (ATX) animals and transgenic (Tg) mice, expressing aVβ3/Vα11 TCR specific for a fragment of pigeon cytochrome C (PCCF) and IE^k to evaluate the subsets of CD4 T cells required for disease progression. ATX animals are depleted of short-lived CD4 T cells of naive phenotype and retain only cells of memory phenotype and functions. Transgenic mice have a predominantly naive CD4 population. Both groups, ATX and Tg develop disease and the CD4 T cells, memory and naive respectively, are rendered unresponsive to mitogenic stimulation.

We further evaluated the extent of anergy in the memory population in an antigen specific system. KLH-primed mice were infected with BM-5 and the recall response of the CD4 population was analyzed. The naive population of transgenic mice was analyzed for PCCF-specific response following BM5 infection. CD4 enriched T cells from ATX and from Tg animals failed to proliferate or produce lymphokines in response to KLH or PCCF, respectively.

These results suggest that naive cells can be anergized, yet are not required for induction or progression of MAIDS and that memory cells retained after thymectomy are susceptible to induction of anergy by the virus.

N 348 FELINE IMMUNODEFICIENCY VIRUS MEDIATED NEUROPATHOLOGY IN THE CAT. Louis Lafrado, Michael Podell, Michael Oglesbee, Mary Hanlon and Lawrence Mathes, Center for Retrovirus Research, Ohio State University, Columbus, OH 43210.
The lentivirus, feline immunodeficiency virus (FIV), is associated with a syndrome similar to that seen in human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). Clinical signs of FIV infection include wasting, immunosuppression, gingivitis, and secondary infections. In addition, behavior modifications have been reported. This study was undertaken to analyze the effect of experimental FIV infection on the central nervous system. Six post-weanling cats were inoculated IM on Day -1 and Day 0 with methylprednisolone (4.0mg/kg of body weight) and IV on Day 0 with 1000 $TCID_{50}$ FIV_{MA} (Mt. Airy strain). Six age matched controls were handled as described for virus inoculated cats. Cats were evaluated at 3, 6 and 12 months post inoculation (PI) by electrodiagnostic techniques including visual and brain stem auditory evoked potential (VEP, BAEP), electroencephalography (EEG), and retinocortical times (RCT). All cats were assessed for FIV mediated immunosuppression and CD4/CD8 ratios. All FIV_{MA} inoculated cats were shown to have inverted CD4/CD8 ratios (0.9 FIV vs. 3.2 Control) 5 wks PI. Six to 8 wks PI 5/6 cats were shown to demonstrate behavior modifications characterized by "compulsive roaming". At 3 mon PI 3/6 FIV cats showed lower BAEP and VEP responses as compared to controls. VEP responses remained low (2/6 cats) for 12 mon PI while BAEP returned to normal values at 6 mon PI. Three of 6 FIV cats showed altered EEG patterns 3 mon PI; at 12 mon PI 2/6 FIV cats demonstrated altered EEGs characterized by increased activity as compared to age-matched controls. Spinal fluid analysis demonstrated the presence (3/6 cats) of FIV-specific antibody to p24 and gp130. All cats were necropsied (15 mon PI) and assayed by histopathologic techniques. Glial cell infiltrated were shown in the lumbar spinal cord. Glial cell nodules also were reported in the subcortical regions of the brain at 15 mon PI. Culture of brain and spleen cells with the feline lymphocyte cell line, 3201, demonstrated the reisolation of FIV from tissues of FIV_{MA} inoculated cats.

N 349 PREFERENTIAL OUTGROWTH OF TH2 CELLS AFTER HIV INFECTION,

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Before CD4+ T cells are depleted, T cells from asymptomatic HIV-infected individuals are non-responsive to activation via the CD3/T-cell receptor complex and release of TH1 cytokines is low. Functional abnormalities of T-cells might be secondary to the disturbance of antigen presenting cell function due to HIV infection. Incomplete activation by affected antigen presenting cells might lead to anergy of T cells. Indeed, T cells from HIV-infected individuals show characteristics of anergic cells as defined in murine models. Furthermore, these models showed that anergy is preferentially induced in T cells that have cytokine secretion patterns defined as TH1-like.

We investigated the type of T cells that could be cloned from HIV-infected individuals. T-cells randomly cloned from an HIV-infected individual showed a high proportion of IL-4 producing clones upon activation, whereas from an HIV-negative control, T-cell clones produced only low or not detectable levels of IL-4. T cells cloned from the HIV-negative control produced high levels of IL-2, whereas lower IL-2 production was found in clones derived from the HIV-infected individual. The finding that preferentially TH2-like clones can be isolated after HIV-infection was further substantiated by clones derived in parallel from cryopreserved material from the same individual before infection with HIV and three years after seroconversion. Again, predominantly clones producing IL-4 were found after HIV-1 infection in contrast to the clones derived from the time point before infection.

Thus, upon HIV-infection, predominantly TH2-like T-cell clones are generated which may be due to preferential anergy of TH1 cells. In addition, *in vivo*, TH2 cells could downregulate function of TH1 cells by secretion of cytokines as IL-10. This might contribute to the attentuation of the immune system and impaired responses to foreign antigens as observed in HIV-infected individuals.

N 350 MAINTENANCE OF HIGH LEVEL CYTOTOXIC T-CELL (CTL) RESPONSE IN LONG-TERM SURVIVORS OF HIV INFECTION, F. Miedema[1], M.R. Klein[1], C.A. van Baalen[2], R.J. Bende[1], K.C. Kuijpers[1], J.K.M. Eeftinck Schattenkerk[3], and I.P.M. Keet[4], [1]Dept of Clin. Viro-Immunol., Central Lab. of the Netherlands Red Cross Blood Transfusion Service and Lab. for Exp. and Clin. Immunology of the University of Amsterdam, 1066 CX Amsterdam; [2]RIVM, Bilthoven, [3]Academic Medical Centre, Amsterdam, [4]Municipal Health Centre, Amsterdam, The Netherlands

Long-term survival in HIV infection may be determined by effective host immune responses against HIV and/or variable HIV virulence. Among a large cohort of HIV-infected men, 21 HIV-1-infected long-term survivors (LTS) were identified based on normal and stable CD4 counts, normal polyclonal T-cell responses and the presence of only low-cytopathic, non-syncytium-inducing (NSI) HIV variants after 7 years of seropositivity. To investigate the role of HIV-specific CTL, one LTS was studied in detail, using autologous EBV-B cells infected with gag-recombinant vaccinia virus as stimulator and target cells. Unstimulated PBL did not show any detectable HIV gag-specific CTL activity, which in limiting dilution analyses (LDA) was shown to be due to suppression at high cell numbers. After Ag-specific stimulation, a high frequency of gag- specific class-I-restricted CD8+ CTL could be detected (267-348/10^6 PBL). Split-well analyses on a panel of HLA-typed EBV lines demonstrated that CTL activity was restricted by HLA-A3 and B18 and A25. Viral load in this patient was low: 3.6 infected cells per 10^6 PBL. Sustained anti-HIV cellular immunity may correlate with maintenance of the asymptomatic state in LTS by control of viral replication.

N 351 AGE ASSSOCIATED EXPRESSION OF ENDOGENOUS, INFECTIOUS ECOTROPIC C TYPE RETROVIRUS IN AD LIBITUM FED (AL) AND CALORIC RESTRICTED (CR) MICE. Murasko, D.M., Stewart, D., and Blank, K.J., Dept. Microbiology and Immunology, The Medical College of Pennsylvania and Dept. Pathology, Hahnemann University, Philedalphia, PA 19129

Virtually all strains of inbred mice have partial or complete DNA sequences of type C enodgenous retroviruses. Although the age associated expression of infectious, ecotropic C type virus has been extensively examined in high leukemic mouse strains, ie AKR, much less is known about age associated infectious ecotropic virus expression in other strains or the relationship of this expression to late onset lymphoma development. We examined the expression of infectious, ecotropic C type retrovirus in extracts of tails and spleens of C57BL/6, DBA/2, and (B6xDBA/2)F1 mice at three different ages: 2, 12-16 and 24-28 month old. Since CR has been shown to both extend lifespan and delay leukemia development in these strains of mice, the effect of CR on virus expression was examined. There was an age associated increase in endogenous virus expression in AL DBA/2 (17% to 67% to 100%) and C57BL/6 (0% to 60% to 50%) mice. CR delayed expression in both strains (33% and 0% at middle aged in DBA/2 and C57BL/6, respectively). There was little or no endogenous viral expression found in AL or CR (B6xDBA/2)F1 mice. The association of virus expression and late onset leukemia is currently being explored.

N 352 **DETERMINANTS OF HIV-1 MACROPHAGE TROPISM,** Marvin S. Reitz Jr., Franco Lori, Andrei Malykh, and Robert C. Gallo, Laboratory of Tumor Cell Biology, NCI/NIH, Bethesda, MD 20892

Attempts to define the genetic determinants responsible for HIV-1 macrophage tropism were made by constructing chimeras between two infectious clones of HIV-1 (HXB2 and LW/C) with only minor differences in their DNA sequence, but with striking differences in cell tropism. HXB2 replicates extensively in permanent T cell lines but not in primary macrophage/monocytes; the reverse is true for LW/C. The envelope proved to be the major determinant of macrophage tropism, but the V3 loop alone appeared to have little effect. Vpr and Nef both contributed to viral growth in macrophages, but had a similart effect in peripheral blood mononuclear cells, suggesting that their effect was not macrophage specific. We propose that HIV-1 tropism depends on several different determinants.

N 353 EFFICIENCY OF RETROVIRAL VECTOR ENCODING THE HIV_{LAI} NEF PROTEIN FOR ANTIGEN PRESENTATION TO AND RECOGNITION BY SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTL), Yves Rivière, Olivier Schwartz, Florence Buseyne, and Michael Robertson, Unité de Virologie et Immunologie Cellulaire and Laboratoire Rétrovirus et Transfert Génétique, Institut Pasteur, 28, rue du Dr. Roux, Paris, France 75724, Cedex 15

We have been able to detect Nef specific CTL in a number of HIV-1 infected donors, including patients in advanced stages of disease. These CTL activities were detectable in freshly isolated PBMC, as well as from CTL lines obtained by non-specific in vitro stimulation with an anti-CD3 monoclonal Ab and IL-2, using B-EBV targets infected with a recombinant vaccinia virus encoding the Nef protein (Vacc-Nef). For some patients, Nef specific CTL lines recognized an epitope located in a conserved region of Nef as defined by the lysis of target cells coated with Nef peptides. In one patient these CTL activities were detectable over a four year peirod and were directed against the same epitopes over this entire period. This patient's B-EBV cells were transduced with a Nef retroviral vector and were found to be efficiently lysed by Nef specific CTL lines generated by non-specific stimulation. In addition, these Nef transduced cells were very potent stimulators of Nef specific CTL lines from this patient's PBMC. Moreover, the CTL lines thus generated recognized the same epitopes as CTL lines generated by non-specific stimulation with anti-CD3 Ab and maintained lytic activity against autologous cells infected with Vacc-Nef. Taken together, these results demonstrate the ability of autologous target cells transduced with a Nef retroviral vector to stimulate antigen specific CTL activity as well as to serve as Nef specific target cells.

N 354 INDUCTION OF IN VIVO T CELL DYSFUNCTION IN MAIDS DEPENDS ON INFECTION BY DISEASE INDUCING DEFECTIVE GENOME, NOT HELPER VIRUS. A. S. Rosenberg, J.M.G. Sechler, J. W. Hartley and H.C. Morse III. Division of Hematologic Products, CBER, FDA and Laboratory of Immunopathology, NIAID, Bethesda, MD 20892. Mice inoculated with LP-BM5 murine leukemia viruses (MuLV) develop profound T cell dysfunction. Previously, we demonstrated that in vivo responses mediated by $CD4^+$ and $CD8^+$ T cells were markedly aberrant in LP-BM5 infected mice in that they lost the ability to reject MHC class II disparate bm12 skin grafts and exhibited delayed rejection of MHC class I disparate bml skin grafts, indicating loss of $CD4^+$ and $CD8^+$ T cell functions respectively. Because in vivo T cell deficits were observed in mice inoculated with BM5 mix, a mixture of replication competent (helper) and defective MuLVs, it was not clear if these deficits were induced solely by the replication defective virus (BM5d), known to be necessary and sufficient for induction of MAIDS. To clarify this point, groups of B6 mice were engrafted with bml and bml2 skin several weeks after inoculation with BM5 mix, helper ecotropic or MCF MuLVs, or an ecotropic MuLV pseudotype of molecularly cloned BM5d [BM5d(eco)], either alone or enhanced in replication by added MCF MuLV. Approximately equivalent $CD4^+$ T cell dysfunction was evident in mice inoculated with BM5 mix or BM5d(eco) but not in those given helper MuLVs alone, and there was no evidence of enhanced suppression in mice inoculated with BM5d(eco) plus MCF MuLV. No alterations in $CD8^+$ T cell function were discerned in mice inoculated with helper viruses, or with BM5d(eco), either alone, or with added MCF MuLVs. However, $CD8^+$ T cell dysfunction was evident in mice inoculated with BM5 mix suggesting that efficiency of expression of BM5d, mediated by helper virus activity, may affect some T cell populations more than others.

N 355 MARKEDLY REDUCED CD28 EXPRESSION IN CD8+ CELLS IN BRONCHOALVEOLAR LAVAGE (BAL) AND BLOOD FROM HIV SEROPOSITIVES. J.J. Saukkonen, H. Kornfeld, J.S. Berman, Pulmonary Center, Boston University School of Medicine, Boston, MA 02118.

HIV-infected patients have an intense accumulation of activated CD8+ T cells in the lung (CD8 alveolitis), when compared to the blood. CD8 alveolitis is largely accounted for by the presence of large numbers of CD8+ cytotoxic T cells (CTL) (CD8+D44+). However, CD8+ CTL are known to have defects in cytolytic ability, in proliferative potential, and memory cell number. The functionally significant surface receptor CD28, important in regulating the antigen-specific response of T cells, also identifies CTL in the CD8 subpopulation. To evaluate the expression of CTL markers in HIV infection, we performed BAL and venipuncture on five HIV-infected patients, all without respiratory symptoms, and on eight normal volunteers. We used monoclonal antibodies to CD8, D44 and to CD28 to identify the CTL population within the CD8+ T cell subset by flow cytomtery. We found no significant difference between normals and HIV seropositives in the percentage of CD8+D44+ cells in blood (29 ± 7.6% vs. 27.24 ± 5.6% respectively, p=0.88) or lung (54.9 ± 8.3% vs. 39.84 ± 12%, respectively ,p=0.31). The %CD8+CD28+ cells in blood of HIV patients was low compared to normals (50.34 ± 10% vs. 17.16 ± 3.0%, p=0.14); however, within the lung %CD8+CD28+ was significantly reduced in HIV BAL compared to normal BAL (5.35 ± 2.2% vs. 30.75 ± 5.7%, p=0.048). Comparison of the CTL marker ratios (CD8+D44+/CD8+CD28+) demonstrated marked relative reduction in CD28 expression in HIV BAL when compared to normal BAL (10.96 ± 3.0 vs. 2.11 ± 0.5 , p=0.005) and to a lesser extent in HIV blood (1.7 ± 0.4 vs. 0.66 ± 0.1, p=0.019). Thus, CD8+ CTL have dramatically down-modulated expression of CD28. Infection of CD28+ lymphoid cells (SUPT1) with HIV_{IIB} *in vitro* did not affect CD28 expression after 14 dys in culture. HIV+ individuals have CD8 lymphocytosis with reduced CD28 expression, which may have functional implications for the antigen-specific response of these cells. HIV infection alone does not appear to be responsible for CD28 down-modulation, which may be related instead to chronic activation.

N 356 EFFECT OF RESTRICTION ENDONUCLEASES ON HIV INFECTION, Joan M. G. Sechler, Kathleen A. Clouse, Klaus Strebel, Karis A. Weih, and Amy S. Rosenberg, Center for Biologics Evaluation and Research, FDA and Laboratory of Molecular Microbiology, NIAID, NIH, Bethesda, MD 20892
HIV RNA undergoes obligatory reverse transcription to a dsDNA form in the cytoplasm of infected cells. This step is critical for the establishment of infection. However, no treatment has yet been devised to target the dsDNA. If the dsDNA form of the virus could be destroyed before translocating to the nucleus and integrating into the host genome, then perhaps amplification of the virus within the organism might be prevented and disease progression halted.

Bacterial cells possess DNA site-specific restriction-modification systems that provide protection from viral infections. The type II restriction endonucleases recognize a particular sequence in DNA and cleave the DNA in the vicinity of that sequence. We investigated whether the type II restriction endonucleases could modify the course of HIV infection in human PBL. Studies performed to date indicate that enzymes known to cleave the dsDNA form of the virus inhibit the development of RT activity in cultures of infected human PBL whereas a control enzyme which lacks a restriction site on HIV dsDNA fails to inhibit virus replication. The replication kinetics were delayed and the absolute RT level achieved in the treated cultures was reduced. Additionally, morphologic changes associated with infection, such as syncytia formation, were significantly delayed. Of critical importance, the restriction endonucleases do not appear to damage host cell DNA as shown by their failure to inhibit proliferation of activated human PBL in vitro. Thus, type II restriction endonucleases may prove useful in the study and treatment of HIV infection.

N 357 CONTROL OF LEUKEMOGENIC RETROVIRUS INFECTION BY AUTOIMMUNE MECHANISMS.
V. Ter-Grigorov, E. Liubashevsky, R. Meirom, Z. Trainin, O. Jagujinskaya, V. Novicoff, Z. Mamiliaeva, Department of Immunology, Kimron Veterinary Institute, Bet-Dagan, 50250, Israel and Haematological Research Center, Moscow, Russia

It was found that progressive course of infection caused by Rauscher leukemia retrovirus (RV) in genetically susceptible mice is preceded and accompanied with formation of a series of autoimmune reactions against antigens involved in immune response regulation: MHC class II antigens (Ia proteins), T-cell surface markers CD4 and CD8, receptors for IL-2, etc. Passive transfer of these auto- antibodies or adequate monoclonal antibodies suppresses the genetically determined resistance of C57BL/6 mice to leukemogenic action of RV. On the other hand, both the maintenance of natural resistance to RV and naturally occurring regression of the disease were shown to be connected with active synthesis of antiidiotypic antibodies against autoantibodies of "progressor" type, mentioned above. We were able to induce such a synthesis in highly susceptible mice using autoantibodies or monoclonal antibodies conjugated to dialdehyde cellulose. Immunization with such insoluble complexes provide high therapeutic rather than prophylactic effect. Surprisingly the ability to produce autoantibodies to MHC class I gene products appeared to be another indispensable condition of resistance to RV infection. It turned out that immunization with monoclonal antibodies to $H\text{-}2D^b$ suppresses both anti-$H\text{-}2D^b$ autoantibody production and natural resistance to RV. Immunization of susceptible mice with fixed leukemic cells in addition to immunization with autoantibodies of "progressor" type provide both production of anti-MHC class I antibodies and high prophylactic effect to RV infection. These approaches might possibly be of use for therapy and prevention of retrovirus-induced diseases.

N 358 CD4+ T CELL PROLIFERATION INDUCED BY B CELL LINES FROM MICE WITH MURINE AIDS Bruce E. Torbett, Patricia A. Healy, and Donald E. Mosier. The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037

Susceptible strains of mice infected with the LP-BM5 murine leukemia virus (MuLV) mixture containing a nonpathogenic helper virus and a pathogenic 4.8 kB replication defective viral genome (LP-BM5D) develop immunodeficiency disease (MAIDS) that is characterized by extensive T and B cell proliferation and severe CD4 T cell unresponsiveness. The LP-BM5D genome codes for an altered *gag* precursor protein ($Pr60^{gag}$). Although the precise role of $Pr60^{gag}$ in disease is not known, it has been recently proposed that it is a viral "superantigen" based on the finding that one $Pr60^{gag}$ positive B cell lymphoma from a mouse with MAIDS stimulated proliferation of mouse CD4+ T cells expressing Vβ 5, 8, or 11 (Hügin *et al. Science* 252:424, 1991).

To determine if $Pr60^{gag}$ expression was always correlated with T cell stimulation, we generated a panel of transformed B cell lines by transfer of lymphocytes from LP-BM5 MuLV-infected mice to SCID mice. This protocol circumvented the difficulty of establishing cultured B cell lines expressing $Pr60^{gag}$ directly from infected mice. Enlarged spleen and lymph nodes were removed from SCID mice 3-5 weeks after injection of cells from infected mice, and the cells cultured for ~1 month. Four independent Ia+, B220+, H-2b donor-derived B cell lines were obtained. These transformed B cell lines stimulated proliferation of primary CD4+ T cells and selected hybridomas via CD3-dependent pathways. The three lines characterized expressed the 4.8 kB mRNA for LP-BM5D; however, only 2 of the 3 B cell lines expressed cell surface $p30^{gag}$ as determined by FACS analysis. In conclusion, it appears that expression of LP-BM5D defective genome correlates with the ability of these B cell lines to stimulate CD4+ T cells, but surface expression of mature *gag* proteins may not be required. We are continuing to generate additional B cell lines to detemine if in a larger sample size the ability to stimulate T cells coincides with the expression of $Pr60^{gag}$ protein.

N 359 KINETICS OF THYMIC, PERIPHERAL AND GUT-ASSOCIATED Vβ6+ T CELLS DELETION IN MMTV (SW) INFECTED BALB/c MICE

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Specific Vβ T cell deletion similar to that seen in Mls-1a mice, is observed in Mls-1b BALB/c mice which have been infected via maternal milk intake, with an exogenous MMTV (SW) whose 3'LTR ORF closely resembles that of endogenous Mtv-7 which is responsible for the Mls-1a phenotype. Here, we describe the kinetics of Vβ6+ T cell deletion in the thymus, spleen, lymph node and gut-associated lymphoid populations of these BALB/c mice from the neonatal period to six months of age. Deletion of CD4+Vβ6+ T cells was evident in the thymus but not in the spleen at 8 weeks of age. However, the earliest incidence of deletion was observed in the gut intraepithelial lymphocyte (IEL) population at 5 weeks of age. In addition, Mtv-7 (SW) RNA was only found in the gut in the first week of life, although could be detected after the second week in other lymphoid organs. Taken together, this report suggests that MMTV (SW) enters via the gut and is subsequently dissipated to the thymus and peripheral lymphoid organs, resulting in the initial deletion of gut CD4+Vβ6+ IEL, and thereafter CD4+Vβ6+ T cells in the thymus and peripheral lymphoid organs.

N 360 MURINE LEUKEMIA VIRUS INFECTION IN IMMUNOCOMPETENT BALB.K MICE Kathleen Tumas, Kenneth J. Blank[1], and Michael B. Prystowsky. Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104. [1]Department of Pathology, Hahnemann University, Philadelphia, PA 19102

The ecotropic murine leukemia virus, E-55+, will induce a thymic lymphoma/leukemia after a latency period of more than 4 months in all immunocompetent BALB.K mice that are injected as adults. The disease process involves an initial acute phase followed by a period when virus infected cells cannot be detected by PCR in any of the tissues of infected host. Sequence analysis of the env gene of the virus that is isolated from leukemic mice demonstrates that the E-55+ virus has undergone a double homologous recombination with the endogenous ecotropic virus, emv-1, carried within the genome of the BALB.K mouse. As a result, the new recombinant virus, designated E-55-, cannot be neutralized by antisera from BALB.K mice infected with the E-55+ virus. Preliminary data from adoptive transfer experiments indicate that bone marrow from BALB.K mice infected with the E-55+ virus can induce disease in both normal and thymectomized adult animals. When compared to normal adult animals receiving either virus or infected bone marrow, a decreased latency period is observed in the adoptively transferred animals with or without a thymus. Bone marrow colony assays using a variety of cytokines suggests that the target cell of the E-55+ virus is a hematopoietic progenitor cell.

N 361 DOES THE "Nef" GENE CONTRIBUTE TO THE DISEASE RESISTANCE OF SOOTY MANGABEY MONKEYS (SMM) NATURALLY INFECTED WITH SIVsmm? F. Villinger[1,3], S. Lauro[1], D. Clark[1], T.M. Folks[2], and A.A. Ansari[1,3]. Dept. of Pathology, Emory U. Sch. of Med.[1]; Retrovirus Disease Branch, CDC, Atlanta, GA[2]; and The Yerkes Reg. Primate Res. Center, Emory U. Atlanta, GA 30322.

The mechanisms by which certain species of nonhuman primates, such as the SMM, which are naturally infected with a lentivirus remain disease resistant have yet to be defined. A recent study demonstrated that disease susceptible rhesus macaques (RM) when experimentally infected with a pathogenic SIV clone with a deletion in the "Nef" gene remained resistant, whereas wild type SIVmac239 caused disease and death in recipients. These data prompted our laboratory to determine whether disease resistance in SMM was secondary to the selective in vivo propagation of SIV isolates with a defective "Nef" gene in this species. In efforts to avoid problems associated with propagation of in vitro selected isolates, the "Nef" gene was amplified and cloned directly from DNA samples of PBMC from the monkeys using oligoprimers in conserved sequences flanking the "Nef" gene.

Based on nucleotide sequence analysis of >12 "Nef" clones derived from individual SMM and RM, the clones appeared to define 2 "Nef" gene families for SIVsmm. There was >88% homology within each family and <80% homology between the 2 families. Of interest was the finding that "Nef" from in vitro derived SIVsmm isolates all belonged to one family, as well as "Nef" clones isolated from animals that had been experimentally infected with the in vitro derived SIVsmm9 isolate. Following experimental infection with SIVsmm9, however, a higher sequence divergence was recorded for "Nef" genes isolated from SMM than from RM after equivalent periods of time post infection.

More important, at the peptide level, whereas full length "Nef" clones were isolated from experimentally infected RM, all of the "Nef" genes found in SMM, whether naturally or experimentally infected, presented randomly distributed premature stop codons, resulting in truncated and most likely nonfunctional "Nef" proteins. These studies further document the importance of the "Nef" gene for SIV induced disease.
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N 362 CYTOKINE mRNA EXPRESSION IN HIV ASSOCIATED NEUROLOGICAL DISEASE, Steven L. Wesselingh, Christopher Power, Robert Fox, Steve Choi, Justin McArthur, Jonathan Glass, John Griffin and Diane Griffin, Department of Neurology, Meyer 6-181, 600 North Wolfe Street, Baltimore, MD 21287-7681

AIDS is associated with three major neurological syndromes: AIDS dementia (AD), vacuolar myelopathy (VM) and painful sensory neuropathy (PSN). The pathogenesis of these conditions remains unclear. They all demonstrate a marked increase in macrophage number and activation. The contribution of HIV replication in the tissue is unknown. It is therefore of interest to compare the levels of cytokine expression and viral transcription in these conditions.

Brains, spinal cords, and peripheral nerves were collected from AIDS patients with and without neurological disease and seronegative controls.

RNA was extracted and RT/PCR for cytokine and HIV mRNA was performed. *In situ* PCR was performed to determine the cell types expressing cytokine and viral transcripts.

We found increased TNF-α, decreased IL-1β, IL-4 and IFN-γ expression in AD, VM and PSN when compared to AIDS and seronegative controls. We found no consistent association between disease and viral transcription.

This profile of elevated TNF-α, decreased IL-1β expression associated with loss of TH2 cytokines such as IL-4 suggests mononkine/lymphokine dysregulation that may explain the observed macrophage activation and may play a role in the development of neuropathology and/or symptoms.

Vaccines

N 400 LYMPHOPROLIFERATIVE RESPONSES TO A CANDIDATE HIV-1 GP120 SUBUNIT VACCINE IN HUMAN VOLUNTEERS, Juerg Baenziger, Faruk Sinangil, Jeanette Reece*, Doreen Sakamoto, Stuart Rodda*, James Kahn#, David Chernoff, Cornelia Dekker, Kathelyn Steimer. The Biocine Company, Emeryville CA 94608, *Chiron Mimotopes Pty. Ltd. Clayton, Australia and #San Francisco General Hospital, San Francisco CA.

In a double blind, placebo controlled phase I clinical trial, HIV-1 seronegative human volunteers were immunized three times (0, 1 and 6 months) with 25 µg of native, glycosylated recombinant gp120 produced in genetically engineered mammmalian cells (rgp120$_{SF2}$). The antigen was administered in an oil in water adjuvant emulsion (MF59) with either 0, 1 or 10 µg doses of the immunomodulator muramyl tripeptide covalently linked with dipalmitoyl phosphatidylethanolamine (MTP-PE). There were six volunteers immunized with the antigen and two with a placebo. Two weeks after the third injection, the expected number of vaccinees at each adjuvant dose (six) showed evidence of circulating anti-gp120 antibodies. 17 out of these 18 gp120 seropositive volunteers showed a strong and specific lymphoproliferative response to the vaccine antigen, rgp120$_{SF2}$. Moreover, they also proliferated to HIV-SF2 gp120 produced in another expression system (yeast: env 2-3$_{SF2}$). This proliferative response was of the same order of magnitude as the mitogen induced proliferation in these same individuals. However, none of the gp120 seronegative volunteers, who presumably were vaccinated with the placebo formulations, demonstrated a lymphoproliferative response to either version of gp120$_{SF2}$. The frequencies of gp120 antigen-specific T cells of a subset of volunteers were measured two weeks after the third immunization and were found to range from approximately 100 to 200 precursors per million perpheral blood mononuclear cells. Short overlapping peptides spaning the entire sequence of gp120$_{SF2}$ have been synthesized and are currently being used to further analyze the vaccine induced helper T cell responses.

N 401 A HERPES SIMPLEX VIRUS (HSV)-ENCODED CYTOTOXIC T LYMPHOCYTE (CTL) RECOGNITION EPITOPE EXPRESSED IN A HETEROLOGOUS FUSION PROTEIN IS ABLE TO INDUCE CTL *IN VIVO*. Robert H. Bonneau, Tong-Ming Fu, and Satvir S. Tevethia. Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Based on previous studies by Hanke et al. (J. Virol. 65: 1177) we have identified a minimal CTL recognition epitope within herpes simplex virus (HSV) glycoprotein B (gB) that associates with the major histocompatibility complex (MHC) class I, H-2K^b molecule (gB498-505; SSIEFARL). Cells expressing this epitope in context of H-2K^b are recognized and lysed by both primary and memory cytotoxic T lymphocytes (CTL) obtained from mice previously immunized with HSV. The objective of this study was to determine the immunogenic potential of this individual octameric epitope (gB498-505) when expressed in context of a heterologous protein and to determine its contribution to inducing a protective immune response *in vivo*.

The simian virus 40 (SV40) large T antigen was used as an immunogenic vector in which to express this gB epitope to determine its ability to induce an epitope-specific CTL response *in vivo*. The coding region of the gB498-505 epitope was inserted into amino acid position 350 of SV40 T antigen by synthetic oligonucleotide fragment insertion and the resulting T antigen-gB epitope constructs were transfected into C57BL/6 mouse embyro fibroblasts from which a T antigen transformed stable cell line was derived. Immunization of naive, C57BL/6 mice with this cell line in the hind footpads was shown to induce the generation of primary gB498-505 specific CTL in the draining popliteal lymph nodes that were able to lyse both HSV-infected and gB498-505 peptide-pulsed syngeneic target cells. An intraperitoneal challenge with this cell line resulted in the generation of an epitope-specific memory CTL (CTLm) in the spleen. This cell line was also shown to stimulate the activation of gB498-505 specific CTLm from mice previously immunized with HSV. Studies are in progress to determine whether immunization with these cells is able to confer protection against subsequent HSV infection.

Overall, these findings support the use of heterologous proteins as vectors in determining the immunogenic potential of individual HSV-specific CTL epitopes and to assess their contribution in inducing a protective immune response *in vivo*.

N 402 CONDITIONS REQUIRED FOR THE GENERATION OF A CTL RESPONSE IN MICE WITH AN HSV gB2 SUBUNIT VACCINE, D. Cataldo, C. Walker and G. Van Nest Chiron Corporation, Emeryville, CA 94608

Several investigators have shown that MHC class I restricted cytotoxic T-cells can be primed by vaccinating mice with viral proteins delivered with various adjuvants/vehicles. We have investigated the parameters involved in priming a HSV-specific cytotoxic T-lymphocyte response in mice using recombinant, truncated glycoprotein B from HSV-2 (gB2). The parameters we have studied include adjuvant, immunization route and regimen. gB2 was delivered with PBS, complete Freund's adjuvant, DOTAP, a cationic lipid preparation and MF59, an oil-in-water emulsion. gB2 delivered with phosphate buffer does not prime a CTL response and gB2 with CFA generates a low CTL response. In contrast, gB2 delivered with DOTAP elicits a strong CTL response generating CTLs capable of lysing target cells infected with HSV or pulsed with the dominant CTL epitope from gB2 (aa495-509) in an *in vitro* ^{51}Cr release assay. gB2 delivered with MF59 elicits CTL activity equivalent to that seen with DOTAP. The addition of the muramyl peptide derivative MTP-PE to the vaccine neither enhances nor decreases the response. MF59 and DOTAP combined do not result in a CTL response higher than either alone. CTL activity was generated by subcutaneous immunization at the base of the tail with gB2/DOTAP but not by immunization IM or IV. Activity can be detected after a single immunization but higher levels of lysis *in vitro* are seen after three immunizations. The duration of the CTL response generated by HSV infection or gB2/DOTAP immunization was compared. The CTL response generated by gB2/DOTAP immunization is shorter than that generated by HSV infection but is still detectable at six months post immunization.

N 403 PERMISSIVE INFECTION OF CHANNEL CATFISH LYMPHOID CELL LINES BY CHANNEL CATFISH VIRUS, V.G. Chinchar and N.W. Miller, Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, 39216.

Channel catfish virus (CCV) undergoes productive and extensive replication in fingerling catfish and establishes latent infection in survivors. Although the site(s) of viral latency in carriers is unknown, a variety of tissues, including leukocytes, have been implicated. To explore the interaction of CCV and leukocytes, we examined infection of catfish leukocytes in vitro. To facilitate this study, continuous lines of cloned catfish B cells and non-cloned cell lines containing macrophages and/or putative T-cells were used. We have previously shown that, unlike their mammalian counterparts, catfish lymphoid cell lines are readily generated from catfish peripheral blood leukocytes without the need for exogenous growth factors or feeder cells. Our results indicate that all three leukocyte subpopulations were productively infected with CCV, but that the kinetics of infection and the final yields differed. B cells were as susceptible to infection as permissive brown bullhead cells and supported high levels of CCV replication even though viral gene expression terminated prematurely. In contrast, CCV yields in T cell and macrophage cultures were markedly lower, a result consistent with the delayed expression of viral genes. These findings indicate that catfish lymphoid cell lines support active CCV replication albeit with differing degrees of permissiveness. Furthermore, the ability to infect leukocyte cultures productively suggests that CCV-infected autologous macrophage or T-cell lines may be suitable targets for assays of catfish cytotoxic T cell activity.

N 404 A GENETICALLY ENGINEERED LIVE VIRUS VACCINE FOR VENEZUELAN EQUINE ENCEPHALITIS, Nancy L. Davis, Gary F. Greenwald, M. Loretta Valenski, Jonathan F. Smith[1] Curt Bartz[1], Will Laegreid[2], and Robert E. Johnston, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599, [1]U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701 and [2]Plum Island Animal Disease Center, Greenport, NY 11944

Live, attenuated virus vaccines have proved their effectiveness in controlling many virus diseases. Although they offer long-lived immunity and are relatively inexpensive to produce and administer, they also present the problem of reversion to a more virulent phenotype. The present investigational live virus vaccine for the alphavirus Venezuelan equine encephalitis (VEE), produces frequent adverse reactions that may be caused by such reversion events. We propose to overcome the disadvantage of reversion by using a molecular strategy to place multiple independently attenuating mutations into a single VEE vaccine strain. Attenuating mutations have been identified both in biologically selected attenuated virus strains and in attenuated mutants produced by targeted mutagenesis of conserved regions. These attenuating mutations have been introduced by site-directed mutagenesis into a full-length cDNA clone of VEE, which can be transcribed *in vitro* to give infectious RNA genomes. Loci affecting virulence have been identified in the E2 and E1 glycoprotein genes and in the 5'-untranslated region. Also, attenuated strains have been isolated that carry both a lethal mutation at the PE2 cleavage site and a resuscitating mutation in a glycoprotein gene. The feasibility of combining attenuating mutations to produce a stable vaccine strain has been demonstrated in rodents and in horses using three E2 glycoprotein mutations. Other multiple mutants are now being tested.

N 405 RECOMBINANT H1- AND H3-DERIVED HA2 INFLUENZA ANTIGENS ELICIT TYPE A CROSS-PROCTECTIVE T CELL RESPONSES IN MICE, S. Dillon[1], S. Demuth[1], M. Schneider[1], C. Weston[1], D. Horowitz[1], A. H. Nishikawa[2], C. Jones[2], C. Griffin[2], M. Scott[3] and A. Shatzman[3], Depts. of Molecular Virology and Host Defense[1], Protein Biochemistry[2] and Gene Expression Sciences[3], SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

Previous studies have shown that mice immunized with a chimeric recombinant protein ($NS1_{1-81}$-$HA2_{65-222}$; "D protein") derived from A/PR/8/34 (H1N1) are protected from lethal challenge with H1N1 and H2N2 viruses in the absence of a neutralizing antibody response. Studies with the H1 HA2 and NS1 components expressed separately showed that the $HA2_{65-222}$ protein retained the properties of the NS1-HA2 chimer in that $CD4^+$ and $CD8^+$ T cell responses and H1/H2 cross-protection were elicited. In contrast, the NS1 component elicited a $CD4^+$ response, but was not protective. To determine if HA2 subunits from other influenza subtypes would demonstrate similar properties, a chimer containing the $NS1_{1-81}$ region from A/PR/8/34 fused to the full length HA2 subunit from A/Udorn/72 (H3N2) virus (NS1-H3HA2) was expressed and partially purified using strategies similar to those developed for the D protein. Both inbred (CB6F1) and outbred (NIH:Swiss) mice immunized with NS1-H3HA2 were protected from lethal challenge with A/HK/68 (H3N2), but not with A/PR/8/34 providing evidence that the H3-HA2 is the active moiety. Antibodies specific for both the NS1 and H3HA2 regions were generated, but the antibodies had no neutralizing activity, and passive transfer of immune sera was not protective. Mice depleted of $CD4^+$ T cells by in vivo antibody treatment were not protected providing evidence that the mechanism of action is T cell mediated. Most importantly, mice immunized with a "cocktail" containing 10ug each of the NS1-H1HA2 and NS1-H3HA2 chimers were protected from lethal challenge with both H1 and H3-virus subtypes. These results indicate the potential utility of these proteins in the design of cross-reactive influenza vaccines. Studies are ongoing to further define the mechanism of action of the H3 construct.

N 406 GLYCOSYLATION OF A RECOMBINANT HIV ENVELOPE PROTEIN INTERFERES WITH PRIMING OF V3 LOOP-SPECIFIC, CLASS I MHC-RESTRICTED CTL RESPONSES IN INBRED MICE.

Barbara Doe and Christopher M. Walker. Chiron Corporation, Emeryville, CA, 94608

The purpose of this study was to compare recombinant HIV envelope proteins produced in yeast, mammalian, and insect cells for the ability to prime cytotoxic T lymphocyte (CTL) responses in inbred mice. Balb/c mice were immunized with denatured, non-glycosylated yeast-derived envelope proteins (env) from either the HIV-1 SF2 or LAI strains, and spleen cells were cultured for 5 days with a homologous V3 loop peptide of 20 amino acids that contain an H-$2D^d$ restricted CTL epitope. These cultured cells mediated class I MHC-restricted, HIV-strain specific CTL activity against ^{51}Cr labelled cells pulsed with the V3 loop peptides. Target cells were not sensitized for lysis when incubated overnight with the recombinant env proteins. CTL responses were also primed when mice were immunized with an HIV-1_{LAI} envelope protein produced in a baculovirus-insect cell expression system. Strong CTL responses were detected in mice receiving the yeast and insect cell-produced HIV envelope proteins in phosphate buffered saline (PBS), and thus specialized delivery vehicles were not required for class I MHC presentation of these antigens.

Significantly, immunization of mice with native, glycosylated envelope protein (gp120) produced in mammalian chinese hamster ovary (CHO) cells failed to elicit V3-loop specific CTL. Denaturation of the gp120 protein with 6M guanidine HCL followed by carboxymethylation did not facilitate priming of the CTL response. However, priming of mice with denatured gp120 that was treated with Endoglycosidase F/N-glycosidase F to remove N-linked carbohydrates did elicit CTL responses that were equivalent to those observed in mice receiving the yeast-derived env proteins. The V3 loop epitope does not contain a glycosylation site, and thus these results suggest that carbohydrate residues in flanking regions can influence processing and/or presentation of at least some CTL epitopes.

N 407 HCV SPECIFIC CYTOTOXIC T LYMPHOCYTE RESPONSES IN CHRONICALLY INFECTED CHIMPANZEES,

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$CD8^+$ T lymphocytes derived from the liver of chimpanzees chronically infected with hepatitis C virus (HCV) were studied for virus specific cytotoxic T lymphocyte (CTL) activity. $CD8^+$ T lymphocytes from liver homogenates were cloned by limiting dilution with anti-CD3 antibodies, IL2, and irradiated feeder cells. Specific lysis was assessed against autologous target cells infected with recombinant vaccinia viruses expressing various HCV proteins. HCV specific CTL clones were identified against both structural (core, E1 and E2) and non structural (NS2 and NS3) HCV proteins. $CD8^+$ cell lines with specificity for these HCV proteins ranged from 4-18% of the total number of clones screened. The HCV specific clones were class I ChLA restricted as shown by a dose dependent inhibition of CTL lysis when antibodies to class I MHC and CD8 proteins were added to the target cells. Epitopes recognized by NS3 specific clones from two animals were mapped using overlapping peptides spanning the entire NS3 coding region. Clones from these two subjects recognized a CTL epitope within the same 12 aa sequence of NS3. However, reciprocal lysis was not observed when clones from each animal were incubated with peptide pulsed heterologous target cells. This indicates that distinct ChLA restriction elements present the CTL epitope(s) contained within this peptide. Studies are currently under way, using truncated peptides spanning the 12 aa sequence, to map the CTL epitopes recognized by these NS3 specific clones.

N 408 PROTECTION AGAINST A HUMAN PAPILLOMA VIRUS TYPE 16-INDUCED TUMOR BY PEPTIDE VACCINATION WITH A CYTOTOXIC T CELL EPITOPE DERIVED FROM THE VIRAL ONCOGENE E7.

Mariet Feltkamp[1], Henk Smits[2], Michel Vierboom[1], René Minnaar[2], Barteld de Jongh[2], Jan Wouter Drijfhout[1], Jan ter Schegget[2], Kees Melief[1] and Martin Kast[1]. Dept. of Immunohematology, University Hospital Leiden, P.O. Box 9600, 2300 RC Leiden[1]. Dept. of Virology, A.M.C., Meibergdreef 15, 1105 AZ Amsterdam[2].

Human Papillomaviruses (HPV) are strongly associated with human cervical cancer. Over 90% of the cervical cancers contain HPV, especially HPV type 16. Peptides containing cytotoxic T lymphocyte (CTL) epitopes are able to induce a protective immune response in mice after *in vivo* administration against lethal virus infection (Kast et al., P.N.A.S. USA 88. 2283-2287). We investigated whether we could confer protection against an HPV16-induced murine tumor by peptide vaccination with a virus-derived cytotoxic T cell (CTL) epitope. This CTL epitope was identified after testing of a complete set of 240 overlapping synthetic peptides derived form the viral oncogenes HPV16-E6 and -E7 for binding to major histocompatibility complex (MHC) class I molecules. Peptides of 9 amino acids (aa) length and 8 aa overlap were analyzed for binding to "empty" MHC class I K^b and D^b molecules present on the antigen-processing defective cell line RMA-S. Over 30 peptides were identified as binders to K^b and/or D^b molecules, thereby meeting an important prerequisite for CTL recognition. The best H-$2D^b$-binding peptide, which partially overlaps with a helper T cell epitope, was chosen for the immunization of mice. This resulted in protection against a subsequent challenge with an HPV16-transformed tumorigenic cell line. Also a CTL response against this peptide was obtained which cross-reacted with the HPV16-induced tumor cell line, indicating that this peptide is naturally processed in HPV16-transformed cells of H-2^b origin.

N 409 BRUCELLA ABORTUS, A POTENTIAL CARRIER FOR HIV 1 VACCINE STIMULATES HUMAN TH-1 CELLS.

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HIV-1 infection is associated with impaired CD4+ cell function and decreased CD4+ T cell numbers. In order to stimulate antibody responses in HIV-1 infected individuals it would be necessary to use T-independent antigens. We have previously shown that *Brucella abortus* (BA) behaves as a T-independent antigen in human primary in vitro responses. HIV-1 proteins were conjugated to BA and shown to generate anti-HIV-1 neutralizing antibodies, even in mice that were depleted of CD4+ T cells. Lipopolysaccharide from BA was purified and shown to be considerably less toxic than LPS from *E. coli*. LPS from BA was found to behave as a T-independent type 1 carrier. In a separate set of experiments we conjugated V3 loop peptides to BA and used V3-BA to elicit antibody responses in normal and immune-deficient mice. Sera from these mice inhibited gp160-mediated syncytia.

BA antibody responses in mice are predominantly IgG2a. IgG2a is induced by IFNγ, a product of TH1 cells. It was therefore of interest to determine whether BA and LPS from BA could stimulate T cells to develop into cells of the TH1 phenotype. Purified human T cells, stimulated with Ba or Ba-LPS, secreted IFNγ and IL2, but not IL4 as determined by ELISA and sensitive cellular bio-assays. Both $CD4^+$ and $CD8^+$ T-cells produced IFNγ in response to Ba stimulation, but $CD4^+$ cells produced more. These results were confirmed by PCR which showed that BA and LPS from BA elicited IL2, IL2R and IFNγ mRNA but no IL4 mRNA was detected.

These data suggest that BA and LPS from BA would be useful as carriers for vaccines when T cell function is decreased and when TH1 cell expansion and/or IFNγ production would be beneficial.

N 410 **THE GENERATION OF CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR HIV I PEPTIDES**, Ethel J. Gordon[1,2], Joseph Dougherty[1], and Yacov Ron[1], [1]Department of Molecular Genetics and Microbiology University of Medicine and Dentistry of New Jersey and [2]Graduate Program in Microbiology, Rutgers University, Piscataway, NJ, 08854

HIV-1 infected individuals can mount a CTL response to various HIV proteins, however, these CTL are insufficient to eradicate all HIV-1 infected cells. *This may be a consequence of* reaction directed against a limited number of physiologically processed and presented viral peptides and/or a reaction against non-conserved regions of viral proteins. We have induced cytotoxic T lymphocytes (CTLs) specific for synthetic peptides which correspond to conserved regions of HIV-1 gag which were not described so far as natural targets for CTL in mice or in HIV-infected individuals. Predominantly, we have used a twenty residue form of p17 crosslinked to the surface of syngeneic T-cell-depleted mouse splenocytes which were used as stimulator cells both *in vitro* and *in vivo*. As target cells we used syngeneic tumor cells crosslinked to p17 or tumor cells infected with a vaccinia viral vector that expresses HIV-1 gag. As shown by a standard chromium release assay, our CTLs recognize both the synthetic p17 as well as the physiologically derived (processed) HIV-1 gag. The reproducibility of these experiments in mice of different haplotypes has broad implications for treatment of the disease in humans. By using synthetic peptides we hope to uncover other antigenic epitopes suitable for vaccine therapy.

N 411 ADJUVANT ACTIVITY OF IMIQUIMOD FOR A HERPES SIMPLEX (HSV) GLYCOPROTEIN (gly) VACCINE AS IMMUNOTHERAPY OF HSV-2 RECURRENT GENITAL DISEASE IN GUINEA PIGS, Christopher J. Harrison, Richard Miller, Eric Tepe, Ann Shahwan, and David I. Bernstein, Creighton Univ. and Univ. Nebraska M.C., Omaha NE, 3M Pharmaceuticals, St. Paul MN, J.N. Gamble Med. Res., Cincinnati, OH

Immunization of guinea pigs with HSV gly prior to HSV genital challenge reduces primary and recurrent genital HSV disease, but the degree of protection depends on the potency of the adjuvant. We have shown that gly + Complete Freund's adjuvant (CFA) is much more *effective than gly + alum or gly alone. We have also shown that* imiquimod, an immunomodulator that induces mostly interferon-α along with certain monokines (TNF and IL-1), provides an adjuvant effect when HSV naive animals are immunized with gly + imiquimod. These animals developed humoral and cell-mediated HSV-2 responses and exhibited reduced recurrent disease after HSV genital challenge. In addition, we have demonstrated that immunotherapy with gly + CFA given to latently infected animals during periods of frequent recurrences reduced the rate of recurrent disease, but was adjuvant dependent. We therefore investigated imiquimod's potential as an adjuvant to gly as immunotherapy for recurrent genital HSV disease.

At 14 and 35 days after HSV-2 vaginal infection, groups of latently infected guinea pigs remained unimmunized or were immunized with gly alone, gly + CFA, or gly + 1 or 5 days of subcutaneous imiquimod. Animals were observed for recurrent genital disease from day 15 to 90 after infection. In two separate esperiments of 93 guinea pigs, gly + imiquimod produced an overall 56-70% reduction in mean recurrence lesion days compared to no immunization. Peak protection with the imiquimod adjuvant occurred between days 42 and 63 (68-89% reduction). CFA + gly produced an overall reduction of 26-34% and peak protection of 39-60%. Both adjuvant regimens were superior to gly alone. HSV-2 antibody, proliferative responses and MHC-unrestricted cytolysis of HSV targets were assayed at selected intervals. Enhanced cell-mediated immune responses after gly + imiquimod immunotherapy were associated with the reduced recurrent HSV disease.

N 412 CELLULAR PROTEINS PHYSICALLY ASSOCIATED WITH IMMUNODEFICIENCY VIRUSES AND THEIR ROLE IN VACCINE DEVELOPMENT, Louis E. Henderson, J. L. Rossio, D. J. Waters, J. W. Bess, Jr., R. C. Sowder, II, D. G. Johnson, R. E. Benveniste, L. O. Arthur, National Cancer Institute-Frederick Cancer Research and Development Center, PRI/DynCorp, AIDS Vaccine Program, Frederick, MD 21702-1201

Macaques immunized with uninfected cells were protected against simian immunodeficiency virus (SIV) grown in the same cells (Stott et al. Nature 353:393, 1991), suggesting that immune responses to cellular antigens contributed to the protective response. To identify these antigens, we isolated cellular proteins from purified human immunodeficiency virus type 1 (HIV-1) and SIV and identified each protein by amino acid sequence analysis. Virus prepared from infected H9 cells (HIV-1) and from Hut78 cells (SIV) contained β-2 microglobulin (β_2m), HLA class II DR (α- and β-chains), ubiquitin, actin, histone H2b and histone H3, *and lesser amounts of other proteins, including* HLA class I (α-chain). In addition, thymosin β4 and cyclophylin A were identified in HIV-1. To determine if the cellular proteins were physically associated with the viral surface, antisera to many of these proteins were tested for the ability to precipitate intact virus. Whole viral particles were precipitated by antisera to β_2m, HLA class I or class II DR, but not by antisera to actin, thymosin β4, or ubiquitin. Preincubation of infectious virus with antisera to β_2m, HLA class I or class II DR blocked viral infections of Hut78 cells (SIV) or H9 cells (HIV-1), but did not block infection of CD4$^+$ HeLa cells. These data show that β_2m, HLA class I and class II DR are physically associated with viral particles and that antisera to these cellular proteins can exhibit a neutralizing response. The association appears to show some selectivity since H9 cells express class II DR, DP, and DQ, but only DR is found with the virus. The cellular proteins are not required for viral replication since HIV-1 grown in CEM$_{ss}$ cells does not contain β_2m, HLA class I or class II DR and can infect H9 cells. However, HIV-1 produced from human lymphocytes has associated β_2m, HLA class I and class II DR, and the immunological and pathological consequences of these viral associated cellular proteins must be considered.

N 413 **VIRUS INHIBITORY ACTIVITY OF PEPTIDES FROM THE ENVELOPE GLYCOPROTEINS OF HIV-1**

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The assembly of some enveloped RNA viruses has been shown to be inhibited by peptides from envelope glycoprotein sequences. This suggests a new approach to the development of antiviral drugs. Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins gp120 and gp41 encompass several domains associated with already defined viral functions, including the CD4 cell receptor binding site on gp120, and sites on gp120/gp41 involved in fusion of virus or virus-infected cells with uninfected target cells. Some of these functional domains bind synthetic peptides from other regions of gp120/gp41. The aim of the present study was: (a) to determine whether or not peptides from gp120/gp41 inhibit HIV-1 infection and (b) define the mechanism of action of inhibitory peptides. After screening synthetic peptides from HIV-1 IIIB envelope glycoproteins gp120/gp41, we found that only one of the peptides, IP1, derived from the extracellular portion of gp41, at concentrations ≥ 2 μM inhibited HIV-1 IIIB replication, virus-induced cell fusion and cytopathic effects to undetectable levels in both CD4$^+$ T and monocytic cell lines. This peptide also inhibited infection by several other HIV-1 isolates, including V32, a IIIB variant resistant to neutralization by V3 loop-specific antibodies; MN; RF; SF2; and AZT-sensitive and resistant clinical isolates. The peptide binds to the fusion domain at the N-terminus of gp41, and thereby inhibits HIV-1 induced fusion. Since this peptide has antiviral activity against both homologous and heterologous HIV-1 isolates and has no detectable cytotoxicity, it offers a novel approach to chemotherapy and prophylaxis of AIDS.

N 414 ENTEROTOXIN (LT) FROM E. COLI AS AN ADJUVANT FOR ORAL IMMUNIZATION WITH INFLUENZA VACCINE, Jacqueline M. Katz* and Sarah A. Young, Department of Virology/Molecular Biology, St Jude Children's Research Hospital, Memphis, TN 38101. (*Present address: Centers for Disease Control, Atlanta GA 30333)

Current parenteral administration of inactivated influenza virus vaccines provide only limited immunity at the site of viral infection in the respiratory tract. Adjuvants such as cholera toxin (CT) have been used to enhance mucosal immune responses induced by oral or intranasal vaccination. Like CT, the heat-labile toxin from E.coli (LT) has immunoregulatory potential but with less associated toxicity. This study has evaluated the efficacy of LT as an adjuvant for oral immunization of BALB/c mice with formalin-inactivated X-31 (H3N2) influenza virus. Levels of both humoral and cellular immunity as well as protection were compared to immune responses induced by parenteral vaccination. LT adjuvant substantially enhanced the production of influenza virus-specific IgG and IgA antibody in the serum and lungs of mice. Animals administered X-31 virus in the presence but not in the absence of LT were completely protected from infection for at least 3 months. LT adjuvant also enhanced T cell-mediated immunity since oral vaccination in the presence but not in the absence of LT induced a virus-specific proliferative and IL-2 secreting helper T cell response both in the spleen and regional lymph nodes. Taken together these results demonstrate that LT adjuvant when administered orally with inactivated influenza vaccine augments local and systemic B and T cell immune responses leading to potentiation of protection from infection.

N 415 THE NEUTRALIZATION ANTIBODY RESPONSES IN TAIWAN CHILDREN AFTER IMMUNIZATION WITH TWO STRAINS OF JAPANESE ENCEPHALITIS VIRUS VACCINE

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Immunization with Japanese encephalitis (JE) vaccine made with different strains (Nakayama, Beijing-1 or others) has shown the variations of immunogenicity in several studies. Taiwan children were immunized at a day 0,14 and 365 schedule. We collected children sera from 4 areas in Taiwan at 1-2 months after 2 doses immunization with either Nakayama(n=12) or Beijing (n=53) vaccines at 15-27 months of age as well as 1-2 months after 3 doses immunization with either Nakayama (n=47) or Beijing (n=32) vaccines at 27-39 months of age to measure their neutralization(nt) antibodies by using plaque reduction neutralization test (PRNT) against both 2 strains and 2 Taiwan local strains of wild type JEV. Our cross neutralization analysis showed that (1) children receiving 2 doses of Beijing vaccine had much higher seroconversion rates than those of Nakayama vaccine either against homologous virus (100%, n=53, vs 92%, 11/12) or against heterologous virus (72%, 38/53 vs 25%, 2/8); (2) all children immunized with 3 doses of Beijing-1 vaccine neutralized both Beijing-1 and Nakayama strains whereas children immunized with 3 doses of Nakayama vaccine had lower neutralization response against Beijing strain (87%,26/30) than that of Nakayama strain of JEV.(100%,32/32); (3) children sera with higher level of Nt titer against homologous strain had better capability to neutralize heterologous strains including currently circulating wild type JEV.In conclusion, since the natural infection rate of JEV is so high after 30 years of mass immunization health policy and since the ecology of this virus is so complicated, we advocate the reevaluation of immunogenicity(either humoral or cell mediated immune responses)of JE vaccine against changes of different strains of JEV in that country in order to prevent the severe epidemics of Japanese encephalitis which may result in 50% of case fatality rate .

N 416 EVALUATION OF IMMUNODOMINANT EPITOPES OF HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-I) BY MULTIVALENT SYNTHETIC PEPTIDE IMMUNIZATION

Michael Lairmore[*1,4], Alex Trevino[4], Renu Lal[5], Susan Kobs[2], Ann Marie DiGeorge[3] and Pravin Kaumaya[2,3]. Center For Retrovirus Research[1], Comprehensive Cancer Center[2], Department of Obstetrics & Gynecology[3], and Department of Veterinary Pathobiology[4], The Ohio State University, Columbus, OH; Retrovirus Diseases Branch[5], Centers For Disease Control, Atlanta, GA.

HTLV-I infection is etiologically linked with adult T-cell leukemia/lymphoma (ATLL) and a chronic, degenerative myelopathy. Several immunodominant structural motifs of the HTLV-I envelope have been defined by the use of either recombinant proteins or synthetic peptides. We sought to investigate the immunogenicity and immunoprotective capacity of selected regions of the HTLV-I envelope gp46 using synthetic peptides presented either as contiguous linear peptides coupled to BSA or by pairing with "promiscuous" T cell epitopes. A synthetic peptide derived from an immunodominant HTLV-I gp46 region (Env-5, aa242-257) linked to BSA elicited strong antibody responses in ELISA and immunofluorescent assays, but these antibodies failed to inhibit HTLV-I-mediated cell fusion or protect rabbits from HTLV-I infection. The peptide did not induce T-cell proliferative responses in rabbits; however, the peptide did elicit antibodies in T-cell deficient Balb c nu/nu mice suggesting that the immune response to this region of the viral envelope is predominantly a B-cell epitope. In an attempt to develop a universal HTLV-I vaccine able to elicit optimal B-cell, helper T-cell and cytotoxic responses, two regions of HTLV-I gp46 encoding a neutralizing epitope (SP2, aa 86-107) and an overlapping cytotoxic and B-cell epitope (SP4a, aa 190-206) were assembled on a multivalent template. Additionally, these constructs were designed to incorporate two "promiscuous" T-cell epitopes from measles virus and tetanus toxoid to overcome the genetic restriction associated with T- & B-cell chimera peptides. These multivalent constructs were highly immunogenic in rabbits and antibodies in 3 of 6 rabbits recognized whole virus preparations by ELISA. Among these rabbits 5 of 6 produced antibodies which inhibit HTLV-I-mediated cell fusion. Current studies include the testing of the immunoprotective capacity of various multivalent preparations linking T-cell and B-cell epitopes in rabbits and the evaluation of genetic restriction of these preparations in 3 strains of inbred mice. These data suggest that potential synthetic vaccines against HTLV-I infection may be required to contain multiple epitopes and elicit T-cell specific responses.

N 417 USE OF RETROVIRAL VECTOR MEDIATED EXPRESSION OF HBV PRECORE AND CORE ANTIGENS TO INDUCE IMMUNE RESPONSES IN MICE, William T.L. Lee, Joanne O'Dea*, Kay Townsend*, Douglas Jolly and Stephen Chang, Department of Viral Therapeutics, Viagene, Inc., 11075 Roselle Street, San Diego, CA 92121

Antigen-specific MHC Class I-restricted cytotoxic T lymphocyte (CTL) responses appear to have an important role in clearance of hepatitis B virus (HBV) during recovery from acute infection. Immunodominant CTL epitopes have been identified within the sequence common to HBV precore and core antigens (Penn et al., J. Exp. Med., 1991, 174:1565-1570). Viagene has previously shown that recombinant retroviral mediated expression of HIV-1 env and HSV-1 gB antigens induce potent and specific MHC Class I CD8+ CTL responses in mice. Therefore, we are investigating the use of recombinant retroviral vectors encoding HBV precore and core antigens to induce specific MHC Class I-restricted CTL responses in mice. The long-term goal is to use retroviral vector mediated expression of HBV antigens as a therapeutic for treatment of individuals chronically infected with hepatitis B virus.

Replication defective recombinant retroviral vectors encoding HBV precore antigen and core antigen were used to transduce mouse fibroblast and lymphoid cell lines of different haplotypes. Expression of precore and core antigens in the transduced cells was analyzed by EIA, Western blot, and immunofluorescent staining. These transduced mouse fibroblast cell lines, L mtk- and BC10ME, and mouse lymphoid cell line, EL4 were injected intraperitoneally into syngeneic C3H (H-2k), BALB/c (H-2d) and C57B1/6 (H-2b) mice, respectively. Splenocytes from immunized mice were harvested and restimulated in vitro with homologous cells stably expressing high levels of either precore or core antigen. The resulting primed effector cells are currently being tested on syngeneic target cells expressing epitopes specific to precore or core antigens.

* Participated equally in this work.

N 418 AN APPROACH TO A RETROVIRUS VACCINE: IMMUNIZATION OF MICE AGAINST FRIEND VIRUS DISEASE WITH A REPLICATION-DEFECTIVE FRIEND MURINE LEUKEMIA VIRUS (FMuLV), Frank Lilly and Ke-San Ruan, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

In an initial experiment to test the ability of replication-defective retroviruses to immunize against immunologically related pathogenic viruses, we have worked with the erythroleukemogenic Friend retrovirus complex (FV), which consists of a replication-competent helper component, Friend murine leukemia virus (FMuLV), and an immunologically related defective pathogenic component, spleen focus-forming virus (SFFV). We have introduced an in-frame, 81-base pair deletion into the p15E-encoding region of the *env* gene of a molecular clone of an otherwise replication-competent FMuLV provirus. Transfection of this clone into cells that package the viral RNA in MuLV coats led to release of infectious virus into the culture medium. Mouse fibroblasts infected with this virus, here called ΔFMuLV, expressed the truncated viral *env* gene product in their cytoplasm but not on cell surfaces, and culture fluids from these cells did not transmit the infection to fresh mouse fibroblasts. In preliminary experiments, immunization of mice of three *H-2*-congenic BALB/c strains with ΔFMuLV conferred levels of immunity to FV disease ranging from strong to relatively weak. Immunized mice developed anti-FV IgM and IgG antibodies and cytotoxic T-cells. Mice observed for 15 weeks after the first of two immunizations showed no detectable pathology, but ΔFMuLV DNA was detectable by PCR in livers of some immunized mice for at least 3-6 weeks. These results suggest that our approach to development of retrovirus vaccines may be a useful one.

N 419 HETEROTYPIC PROTECTION AGAINST INFLUENZA A BY INTRAMUSCULAR INJECTION OF A DNA EXPRESSION VECTOR ENCODING A CONSERVED VIRAL PROTEIN, M.A. Liu, J.J. Donnelly, 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.B. Ulmer; Department of Cancer Research, Merck Research Laboratories, West Point, PA 19486.

We describe a new and effective means of generating a cross-protective immune response against a heterologous strain of a virus by the intramuscular injection of a non-replicating DNA expression vector encoding a conserved viral protein. The injection of naked plasmid DNA results in the endogenous production of a foreign protein which results in the generation of peptide epitopes for association with MHC Class I molecules for subsequent recognition by cytotoxic T lymphocytes (CTL). CTL specific for conserved viral antigens are known to be capable of responding to different strains of virus, in contrast to antibodies which are usually strain-specific.

Plasmid DNA encoding influenza A nucleoprotein (designated NP DNA) was injected into the quadriceps of BALB/c mice. Nucleoprotein-specific CTL and primary CTL were generated that were able to kill epitope-pulsed or virally-infected targets. The primary CTL were exposed to Con A and IL2 but did not undergo specific *in vitro* restimulation prior to testing. NP DNA injection also resulted in the generation of high titer anti-nucleoprotein antibody which was shown by serum transfer *in vivo* to not protect against subsequent influenza challenge. Mice injected with NP DNA were challenged with an intranasal inoculation of live virus, using a strain of virus that was heterologous to the strain from which the NP gene was cloned. That is, the NP gene was cloned from A/PR8/34 (H1N1), and the mice were challenged with A/HK/68 (H3N2). The viral lung titers in NP DNA-immunized mice were decreased three logs in comparison to nonimmunized or control plasmid-immunized mice. The NP DNA-immunized mice also lost less weight (as an indicator of morbidity) and had significantly increased survival compared to control. This approach thus provided a simple yet powerful means of inducing an immune response capable of protecting against a heterologous viral infection.

N 420 IMMUNE RESPONSES OF RHESUS MONKEYS AFTER LOW DOSE INTRARECTAL INFECTION WITH SIMIAN IMMUNODEFICIENCY VIRUS, Debra MacKenzie[2,5], Peter Emau[1,3], Parul Trivedi[3], Kevin T. Schultz[1,4], Maria S. Salvato[1,3], Miroslav Malkovsky[1,2,5], and C. David Pauza[1,3], Immunology and Virology Research Group of the Wisconsin Regional Primate Research Center[1], and the Departments of Medical Microbiology and Immunology[2], Pathology and Laboratory Medicine[3], Pathobiological Sciences[4], and Human Oncology[5], University of Wisconsin, Madison, WI 53706

New attention is being focused on the role of the cell-mediated immune response in controlling HIV infection and disease progression. T-cells from seronegative individuals with known exposure to HIV, proliferate and produce IL-2 following *in vitro* stimulation with HIV antigens and peptides (Clerici et al, *J. of Infectious Dis.* 165:1012-1019, 1992). However, the long latent phase of HIV disease makes it difficult to determine whether these T-cell responses reflect exposure to antigen and immune clearance, or are indicative of an inapparent infection that has the potential to become fullblown disease. Rhesus monkeys infected intrarectally (i.r.) with low dose inoculums of SIVmac251 ($\leq$ 10 ID_{50}), remain seronegative and healthy more than one year after infection. However, transfusion of blood from one of these seronegative, asymptomatic animals to a naive recipient resulted in SIV infection. These animals provide an important and novel model for the study of long-term inapparent infection in humans. While infectious virus was consistently isolated by co-culture from the peripheral blood mononuclear cells (PBMC) of monkeys infected via the intravenous route, and from 3 of 4 monkeys infected i.r. with $\geq$ 100 ID_{50}, no infectious virus was isolated from the low dose i.r. infected animals. However, a transient low-level p27 antigenemia and an occasional weakly positive PCR was observed. The percentages of CD4 and CD8 T-cells and the CD4/CD8 T-cell ratio in the low-dose animals remained constant throughout the study. The ability of T-cells from the low-dose i.r. infected animals to proliferate to SIV antigens is under investigation. Preliminary data suggests that specific proliferative responses to gradient purified, heat killed SIV are present in these animals one year after infection. PBMC from these animals have been taken at regular intervals throughout the duration of the study and will be tested for patterns of immune responsiveness. These data will provide important information regarding the parameters of T-cell mediated immunity that develop following initial infection with low doses of virus which may be involved in the control of viral infection.

N 421 **INTRAMUSCULAR IMMUNIZATION OF GUINEA PIGS WITH A SUBUNIT VACCINE: ANTIBODY RESPONSES IN SERA, VAGINAL WASHES, AND SALIVA**
Owen B. Mason, Gary Van Nest, Gail L. Barchfeld Chiron Corporation, Emeryville, CA 94608
Mucosal surfaces act as a first line of defense against many infectious organisms. Intramuscular vaccination with subunit vaccines containing an advanced low oil emulsion adjuvant (MF59) has been shown to provide superior protection from mucosal herpes simplex virus 2 (HSV2) challenge in guinea pigs compared to alum. We were interested in determining if this approach results in measurable antibody at mucosal surfaces. Guinea pigs were vaccinated intramuscularly with recombinant HSV2 glycoprotein D (rgD2) without adjuvant, or combined with alum, MF59, or MF59 plus muramyl tripeptide-phosphatidyl ethanolamine (MTP-PE). Sera, vaginal washes and saliva were collected for the purpose of measuring rgD2 specific total immunoglobulin (Ig), immunoglobulin A (IgA), and HSV2 neutralizing antibodies. In sera and vaginal washes collected two weeks after the third monthly immunization total Ig was lowest with antigen alone, five fold higher in the alum and MF59 groups, and ten fold higher in the MF59 plus MTP-PE group. In saliva total Ig was detected only in the animal with the highest serum Ig. Serum Ig antibody titers above 31000 correspond to detectable Ig in vaginal washes, probably as a result of transudation. A titer is the reciprocal of the sample dilution that generates an ELISA value equal to 0.5 O.D. at A_{492}. Serum IgA ELISA titers in both the antigen alone and the alum groups were just above the detection limit of 10, while the MF59 group had a mean titer of 81 and the MF59 plus MTP-PE group had a mean titer of 140. No IgA was detected in either the vaginal washes or saliva. Vaccines with advanced adjuvants, which have been shown to produce a stronger antibody response than conventional vaccines, may therefore achieve a measure of their protective efficacy at the mucosa.

N 422 PROTECTION AGAINST HSV2-INDUCED DISEASE BY VACCINATION WITH A gH-DELETED HSV-1 VIRUS

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Deletion of the gene for glycoprotein H (gH) from the HSV-1 genome results in a virus which can be grown in a cell line expressing the gH protein (producing phenotypically normal particles), but which is incapable of multi-cycle replication in non-complementing cells. However since the gH protein is needed to confer infectivity on virus particles but not for virus assembly, the gH-deleted virus is capable of one complete round of replication in normal cells, producing virus particles which lack gH and which are therefore non-infectious.

Such a virus, which we have termed a DISC virus (Defective Infectious Single Cycle), therefore has potential as a vaccine, since it cannot spread within the host, yet should be capable of generating humoral and cellular immune responses against virtually all the virus antigens. This is borne out by experiments which show that in the mouse ear model of HSV-1 infection, vaccination with as little as 10^5 pfu of the gH-deleted HSV-1 virus is capable of providing effective protection against replication of wild type challenge virus and consequent disease, whereas an equivalent dose of inactivated wild type virus cannot.

These studies have been extended to the guinea pig vaginal model of recurrent HSV-2 infection. Vaccination of guinea pigs with the DISC HSV-1 virus, intra-vaginally or intra-epithelially, provided a high degree of protection against HSV-2-induced disease and substantially reduced the exent of virus replication at the site of challenge. The work supports the idea that a DISC HSV virus could represent an effective vaccine against HSV-induced disease.

N 423 **PROMISCUOUS T HELPER CELL EPITOPES OF THE MEASLES FUSION PROTEIN INCLUDE THE "FUSION PEPTIDE" DESPITE HOMOLOGIES WITH HUMAN PROTEINS: AN EXAMPLE OF SELF/NON-SELF DISCRIMINATION,**

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To elicit an efficient immune response against the measles virus, a synthetic vaccine must contain T cell epitopes, capable of inducing a T cell memory. To detect T cell epitopes of the measles fusion protein, we have studied the proliferative response of lymphocytes from adult convalescent donors to overlapping pentadecapeptides covering the whole protein sequence. Most donors reacted with 10 % of the peptides, but some reacted with up to 25%. Six regions were identified which reacted with more than half of the donors including the so-called "fusion peptide". This "fusion peptide" contains a promiscuous T_H cell epitope recognized by peripheral blood lymphocytes of DR1,DR2, DR5 and/or DR7-positive donors. The epitope contains the consensus motifs described for DR1 and DR2 binding peptides. In a human protein data bank 26 7/15-homologues of the epitope sequence were found. Among the two possible models, we chose the helical model to propose that four alanine residues are facing the T cell receptor and that a glutamine is important for interaction with the TCR. This glutamine was found in none of the 15 human homologues in which 3 or 4 of the above alanines were conserved. In contrast, 6 of the 11 human homologues with more than one substitution of an alanine contained the glutamine. Substitution analogues of the peptide F121 confirme a role of the glutamine in T cell stimulation. The T cell epitope described here is highly conserved in different paramyxoviruses. Competition between different T cell epitopes may, however, modulate its importance in other viruses. Besides the "fusion peptide", a number of peptides defined epitopes which are promiscuous with respect to MHC class II recognition. In particular such peptides are of interest for the development of synthetic subunit vaccines.

N 424 THE EFFECT OF ANTIGEN/ADJUVANT ASSOCIATION ON HUMORAL ANTIBODY RESPONSE: COMPARISON OF THE MF-59 EMULSION AND ISCOM ADJUVANTS IN A HERPES SIMPLEX GLYCOPROTEIN SUBUNIT VACCINE, Gary Ott*, Rae Lyn Burke*, Jina Kazzaz*, Karin Lövgren†, Bror Morein† and Gary Van Nest* Chiron Corporation*, Emeryville, Ca. and Department of Virology, The National Veterinary Institute, Uppsala Sweden†.

The substantially greater immunogenicity of particulate antigens relative to soluble subunit antigens has been attributed to both the presence of polyvalent antigen arrays on the particles and to enhanced transport of particulate antigens in the lymphatic drainage. Some part of the adjuvant effect of both oil in water emulsions and iscom structures has been attributed to association of antigen with the adjuvant particle (droplet). We have compared antibody titers generated with both iscom and MF-59 emulsion (squalene/H_2O) formulations in which antigen was either bound to the adjuvant particle (droplet) or free in solution. Under the standard dosage conditions used in these systems, antibody titers were independent of association. In addition antigen and adjuvant have been injected separately with up to 24 hours between injections. Preinjection of the MF-59 adjuvant results in equivalent titers to the standard coinjection. Our results suggest that these adjuvants provide stimulation by mechanisms other than antigen binding.

N 425 INJECTION OF EXPRESSION VECTORS CONTAINING ANTIGEN GENES INDUCE CELLULAR, HUMORAL AND PROTECTIVE IMMUNITY, Gary H Rhodes[1], VJ Dwarki[1], J Felgner[1], LH Hawe[2], JJ Donnelly[2], JB Ulmer[2], MA Liu[2], PL Felgner[1], AM Abai[1] SH Gromkowski[1] and SE Parker[1],; [1]Vical Inc., San Diego CA 92111 and [2]Merck Research Laboratory, West Point, PA 19486

Intramuscular injection of plasmid DNA results in expression of the genes encoded by the plasmid. We have investigated the immunological consequences of injection of an expression vector containing the genes for two well characterized antigens: the gp120 gene from HIV-1 and the nucleoprotein (NP) gene from influenza A virus. A single injection of DNA encoding for either gene induces IgG antibodies which appear 2 to 3 weeks after injection and persist for more than 12 months. The antibody response is dose dependent with 10 μg of DNA required for a consistent antibody response. Maximum antibody titers for both antigens were in the range of 1/10,000 to 1/30,000.

A single injection of DNA is also sufficient to produce cytotoxic T lymphocytes (CTL) which recognize target cells coated with synthetic peptides representing the major T cell epitopes. Priming of CTL may occur independently of antibody since some animals injected with low amounts of DNA develop CTL activity without measurable IgG antibodies. Once induced, the CTL activity persists for more that 6 months.

The induced immunity appears to be functional in vivo as animals injected with the gene for influenza NP from strain A/PR/8/34 (H1N1) are protected from a subsequent lethal viral challenge of a homologous (H1N1) and heterologous (H3N2) strains of influenza virus.

The ability to directly prime CTL may allow the development of subunit vaccines which are more efficacious than killed virus or recombinant protein derived vaccines. Similarly, the induction of CTL could be useful for a therapeutic vaccine against chronic viral infections.

N 426 USE OF DIRECT DNA INOCULATIONS TO ELICIT PROTECTIVE IMMUNE RESPONSES

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Avian and murine influenza virus models have been used to demonstrate that direct DNA inoculations can raise protective immune responses. Plasmid DNAs that express the influenza virus hemagglutinin protein have been tested for their ability to protect against lethal influenza virus challenges. Vaccination and a single boost have consisted of the inoculation of 100 ug of DNA by each of 3 routes (iv, ip, and sc; or iv, ip, and im). Challenge has been via the nares at one to two weeks post boost. In four trials, 28/56 chickens vaccinated with p188 DNA (a plasmid DNA in which a defective retrovirus expresses an H7 hemagglutinin) survived the lethal H7N7 influenza virus challenge. By contrast, only 1/55 control-DNA-inoculated chickens survived. In five trials, 19/30 chickens inoculated with PCMV-H7 DNA (a plasmid DNA in which the CMV immediate early promoter expresses H7) survived. In these trials, 1/30 of the control chickens survived. In two trials in the murine model, 12/12 PCMV-H1-DNA-inoculated mice and only 1/12 control-DNA-inoculated mice survived a lethal H1N1 influenza challenge. The DNA inoculations appeared to prime antibody responses. This was suggested by the rapid appearance of high titers of anti-hemagglutinin antibody post challenge.

N 427 **IMMUNIZATION WITH SOLUBLE VIRAL PROTEINS ELICITS MHC CLASS I-RESTRICTED CD8+ CYTOTOXIC T LYMPHOCYTE RESPONSES *IN VIVO*.**

Reinhold Schirmbeck and Jörg Reimann; Institute of Microbiology, University of Ulm, Albert-Einstein-Allee 11, D-7900 ULM, FRG.

Immunization with soluble protein antigens usually stimulates CD4+ but not CD8+ T cells. 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 efficiently lysed syngeneic transfectants of different histotypes expressing HbsAg. $H\text{-}2^d/L^{d+}$ mice (BALB/c) were responders, $H\text{-}2^d/L^{d-}$ (dm2) and $H\text{-}2^b$ (B6) mice were non-responders. Target cells pulsed *in vitro* with HBsAg particles were specifically lysed by anti-HBsAg CD8+ CTL. In contrast to the single immunization with "naked" S-antigen particles that efficiently primed $CD8^+$ CTL, immunization with S-antigen adjuvanted with either aluminium hydroxide, or incomplete Freund's adjuvants did not induce a CTL respons.

(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. 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, 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 1-restricted antigen presentation to CD8+ CTL.

N 428 NAIVE AND MEMORY T-LYMPHOCYTES IN THE CONTEXT OF A PHASE I gp160 VACCINE THERAPY TRIAL.

Sitz, Karl V.; VanCott, Thomas C.; Darden, Janice M.; Redfield, Robert R.; Birx, Deborah L.; Department of Retroviral Research, Walter Reed Army Institute of Research, Washington, D.C.

Objectives: T-lymphocytes can be classified into naive or memory subsets based on their CD45 isotype expression. Schnittman et al. have demonstrated that memory CD4 T-lymphocytes are preferentially infected *in vitro* by HIV-1 and serve as the primary reservoir of HIV-1 *in vivo*. In HIV vaccine trials in infected individuals, there has been speculation that repeated antigenic stimulation might convert naive cells into memory cells thus enhancing HIV infection and subsequent destruction of these cells. Therefore we compared the naive and memory phenotypes of patients who were participating in a gp160 vaccine protocol at three time periods over approximately a one year time period.

Methods: Twelve (five presented in this abstract) HIV-infected patients from the phase I gp160 trial were selected based on the following criteria: initial CD4 > 500, new antibody response to HIV epitopes, and new peak LSI > 15 in a proliferation assay to the gp160 vaccine. Fresh heparinized blood specimens were stained with panels of monoclonal antibodies using a three color approach (FITC, PE, PerCP) and processed using a whole blood lysis protocol. Comparison of results was performed with a Wilcoxin signed-rank test.

Results: There were no statistically significant differences (p<.05) in any parameter between the three time points. All values in the table are averages.

Phenotype	681 Days	917 Days	1068 Days
CD4+RA-RO+	53%	55%	55%
CD4+RAdROd	7%	11%	9%
CD4+RA+RO-	39%	34%	36%
CD8+RA-RO+	35%	40%	43%
CD8+RAdROd	15%	21%	16%
CD8+RA+RO-	49%	39%	41%

Conclusion: Comparison of naive and memory phenotypes in a group of early stage HIV-infected patients receiving the gp160 vaccine at three time points over approximately one year did not reveal statistically significant differences in any of the phenotypes listed. Specifically, there did not appear to be a depletion of memory CD4 or CD8 cells. Analysis of the larger group of 12 patients will be completed shortly. A large blinded, prospective trial is underway with the gp160 vaccine and will better answer whether there are any changes in the naive and memory phenotypes with repeated immunization.

N 429 HBcAg INDUCED T-CELL INDEPENDENT ANTI-HBc PRODUCTION IN CHRONIC HBsAG CARRIERS.

Staffan P.E.Sylvan and Ulla B.Hellström, Department of Environmental Health and Infectious Diseases Control, Karolinska Hospital, Stockholm, Sweden.

The hepatitis B core antigen(HBcAg) can function as a T-cell independent antigen in mice. This capacity of the HBcAg has been suggested to be responsible for the enhanced immunogenicity exhibited by HBc-particle based chimeric vaccines in non-human species like rabbits and guineapigs, where HB is not a natural infection. In man, however, little is known regarding the contribution of a T-cell independent B-cell reactivity induced by HBcAg for the development of protective immunity to HB. Therefore, we analyzed the HBcAg induced IgG anti-HBc production in vitro in supernatants from T-cell depleted B-cell cultures obtained from 10 chronic HBsAg carriers, 8 individuals with natural acquired immunity to HB and 7 HB-susceptible controls by an ELISA developed at our laboratory. Spontaneous production of IgG anti-HBc was only observed in some B-cell cultures from chronic HBs-Ag carriers, but not from HB-immune donors or HB-susceptible controls. In HBcAg stimulated B-cell cultures IgG anti-HBc production increased significantly in the majority of chronic HBsAg carriers, whereas HB-immune donors were non-responders in vitro. The results indicate that, in man, the capacity for T-cell independent B-cell activation exhibited by HBcAg is related to the chronic HBs-Ag carrierstate and is not a feature of established immunity to HB.

(Sylvan et al., Archives of Virology, 1992, Suppl 4:29-35.

N 430 LIFESPAN OF VIRGIN AND MEMORY T CELLS, David F. Tough and Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Using the thymidine analogue 5-bromo-2-deoxyuridine (BrdU) in the drinking water, we have examined the turnover of virgin and memory T cells; these cells were defined on the basis of CD45RB, Pgp-1 (CD44) and MEL-14 (LECAM-1) expression. For peripheral CD4 and CD8 T cells from normal mice, BrdU incorporation was apparent in both memory (CD45RB^{lo}, Pgp-1^{hi}, MEL-14^{lo}) cells and virgin (CD45RB^{hi}, Pgp-1^{lo}, MEL-14^{hi}) cells. The density of BrdU labelling in these two types of cells, however, was quite different: memory cells labelled strongly (BrdU^{hi}) whereas virgin cells labelled weakly (BrdU^{lo}). A comparison of normal and adult thymectomized (ATx) mice revealed that, unlike the BrdU^{hi} cells, the subset of BrdU^{lo} virgin cells was missing in the ATx mice, implying that these cells represent recent thymic emigrants. After exit from the thymus, most of these cells remained in interphase for prolonged periods. By contrast, in both normal and ATx mice the BrdU^{hi} memory-phenotype cells showed a high turnover. However, 10-20% of the memory-phenotype cells ceased dividing and appeared to enter interphase. Some of these non-dividing cells retained expression of memory markers for a month or more. However, others downregulated these markers and reacquired a virgin (resting) phenotype. D.F.T. is the recipient of an MRC of Canada Fellowhsip.

N 431 A NEUTRALIZING B CELL EPITOPE WITHIN HCMV GB INDUCES CLASS I AND 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 antibodies in patients are directed against gB. Recently a major 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 (gB 618-628) was found to be the optimal peptide for recognition by HLA-A2-restricted CD8+ cytotoxic T cells. We now report that the very same peptide is also presented by several HLA-DR4 subtypes. GB-specific CD4+ T cell clones generated from one HCMV-positive individual secrete IL-2, IFN-γ and TNF-α, but not IL-4. A 30 amino acid long region of gB is therefor not only inducing neutralizing antibodies, but also capable of inducing class I-restricted cytotoxic T cells and class II-restricted, potential helper T cells. This epitope is conserved in HCMV labstrains as well as patient strains, which suggests an important function of it for the virus. Taken together this epitope appears to be a good candidate for a subunit vaccine.

[1]Utz et al. (1989), J. Virol. 63:1995.
[2]Utz et al. (1992), J. Immunol. 149:214.

N 432 SYSTEMIC CYTOKINE RESPONSE IN MICE VACCINATED WITH AN INFLUENZA VACCINE AND OIL EMULSION ADJUVANTS, Jean-Paul Valensi and Gary Van Nest, Chiron Corporation, Emeryville, CA 94608

A potent adjuvant formulation, MF59, has been developed for subunit vaccine use in humans. MF59 is an oil-in-water emulsion designed to produce an immune response similar to that of Freund's-like adjuvants without the deleterious side effects. It contains metabolizable squalene (replacing mineral oil), Tween 80 and Span 85 surfactants (replacing Arlacel A), and may include the synthetic muramyl peptide MTP-PE (N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanine-2- [1,2-di-palmitoyl-sn-glycero-3-(hydroxyphosphoryl-oxy)] ethylamide) replacing the killed mycobacteria cell component of Freund's. MF59 adjuvants have been tested in a wide variety of animals and humans, and have been shown to elicit high antibody and T_h cell responses. To better understand the mechanism of action of MF59 adjuvants, we have undertaken a study of the systemic cytokine profile in Balb/c mice. Our goal is to elicit desirable cytokine responses by optimization of the MF59 adjuvant formulation. A commercially available trivalent influenza vaccine was injected three times at weekly intervals without adjuvant, with MF59 adjuvant, and with MF59 adjuvant and MTP-PE. Antibody titres generated using MF59 and MF59/MTP-PE adjuvants were seven and five-fold higher respectively, than with vaccine alone. Serum cytokine levels were measured by ELISA assay at various time points after the third immunization for the following cytokines: IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, GM-CSF, γ-IFN and TNF-α. Immunization with vaccine alone increased levels of IL-3 and IL-5. Vaccine with MF59 increased levels of IL-3, IL-5 and IL-6. The only additional cytokine to increase with the MF59/MTP-PE formulation was γ-IFN. There were no measured increases for IL-1α, IL-2, IL-4, IL-10, GM-CSF, and TNF-α with any of the vaccine/adjuvant combinations.

N 433 EFFECTS OF COSTIMULATION OF CD28 BY ITS LIGAND B7 ON HUMAN T-CELL SUBSETS, René van Lier, Heleen Kuiper, Miranda Brouwer, Paul Parren, Mark de Boer*, Centr. Lab. Blood Transfusion Service and Lab. Exp. and Clin. Immunology of the University of Amsterdam, 1066 CX AMSTERDAM, The Netherlands; *Cetus Corporation, Emmerville, CA, USA

Clonal expansion of $CD4^+$ T cells not only depends on ligation of the T cell receptor (TCR) by MHC/peptide complexes but also requires costimulatory signals that can be generated by accessory molecules present on specialized antigen presenting cells (APC). The T-cell membrane molecule CD28 appears to be a transducer for such signal. We tested induction of proliferation by CD3 mAb presented by transfected mouse fibroblasts, expressing both CD32 and B7, in highly purified T-cell subsets, i.e. (naive) $CD45RO^-$ and $CD45RA^-$ (memory cells). Optimal stimulation of the TCR with large amounts of fibroblasts presenting high dose of CD3 mAb (5 μg/ml), resulted in a more efficient proliferation of naive cells compared to memory cells. In marked contrast when low dose of CD3 mAb were applied (5 ng/ml) memory cells are still efficiently induced whereas respons in naive cells was almost completely lost. Analysis of cytokine production showed similar results. These data show that naive cells can effectively respond to the CD28-ligand B7. Moreover it is suggested that for their optimal respons a relatively high degree of TCR/CD3 cross linking is required.

N 434 TYPE-SPECIFIC AND COMMON CTL RESPONSES AGAINST THE SIMIAN IMMUNODEFICIENCY VIRUS ENVELOPE PROTEIN IN INFECTED RHESUS MACAQUES.
Christopher M. Walker, Vanessa Hirsch[*], Philip Johnson[#], Tiluhan Yilma[+], Luis Giavedoni[+], Marta Marthas[+] and Ann Erikson. Chiron Corp., Emeryville, CA 94608, [*]NIH/Twinbrook, Rockville, MD, [#]Ohio State University, Columbus, OH, and [+]University of California, Davis, CA 95616.

Cytotoxic T lymphocyte (CTL) responses against the envelope protein of the simian immunodeficiency virus (SIV) were studied in three rhesus macaques infected with SIV MAC239. All animals had serum antibodies to SIV proteins, and virus was detected in the plasma and peripheral blood mononuclear cells (PBMC) of two of the three animals using PCR techniques. PBMC from the three animals were isolated on Ficoll Hypaque gradients and cultured for 5 days in medium containing Con A. CTL responses were measured against autologous ^{51}Cr lymphoblastoid cell lines (LCL) that were infected with recombinant vaccinia viruses expressing the SIV envelope protein. High levels of lysis were observed when the Con A stimulated PBMC were incubated with LCL infected with VV expressing the gp130 protein of the SIV from the homologous MAC239 isolate. Target cells infected with the SIV SmH4 isolate, which is approximately 80% homologous in amino acid sequence to SIV MAC239, were also lysed. CTL activity appeared to be mediated by class I MHC restricted CTL, as enriched CD8+ cells contained the lytic activity. In addition, antibodies to human class I MHC (W632) or CD8 antigens blocked most of the lytic activity.

To further define the specificity of the response, CD8+ cells from these cultures were cloned by limiting dilution in the presence of IL 2 and irradiated human feeder cells. Six clonal cell lines were derived that lysed target cells infected with SIV MAC239, but not the SIV SmH4 isolate. These results demonstrate that a heterogenous population of envelope-specific CTL recognizing both type-common and specific epitopes are present in the peripheral blood of SIV infected animals.

N 435 **EFFECTIVE VACCINATION AGAINST PAPILLOMA DEVELOPMENT BY IMMUNIZATION WITH L1 OR L2 STRUCTURAL PROTEIN OF COTTONTAIL RABBIT PAPILLOMAVIRUS,** Y.L. Liu, L.A. Borenstein, R. Selvakumar, R. Ahmed, and F.O. Wettstein, Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles, CA 90024.
Immunization of rabbits with either L1, the major structural protein, or L2, a minor structural protein of cottontail rabbit papillomavirus (CRPV), protected against challenge with the virus. Neutralizing antibodies were elicited by both the L1 and L2 *trp*E fusion proteins. Neutralization with anti-L1 serum, however, was more efficient than with anti-L2 serum. In contrast, when tested on Western blots the immune response to L2 was stronger than to L1. Rabbits were also protected against CRPV infection by immunization with L1 expressing recombinant vaccinia virus. Sera from two of three rabbits immunized with recombinant vaccinia virus were negative on Western blots but all three were positive in ELISA's with nondenatured fusion protein or in immunoprecipitations. Two approaches are being used now to identify neutralizing epitopes of L1. In one approach, L1 subfragments are tested for their ability to protect rabbits from papilloma development; in the other, L1 subfragments are assayed for their potential to induce a neutralizing response. Preliminary results indicate that subfragments of the L1 protein result in seroconversion of the immunized rabbits but in contrast to the results seen with the whole L1 protein, no neutralizing antibody is made and the rabbits remain susceptible to CRPV infection. Taken together, these results suggest that both the viral structural proteins, L1 and L2, merit consideration in the development of a vaccine against papillomavirus and retaining conformational epitopes in the vaccine preparation is particularly important.

Late Abstracts

STIMULATION OF MUCOSAL, CELL MEDIATED AND PROTECTIVE IMMUNITY USING LIPID MATRIX BASED VACCINES.
Susan Gould-Fogerite and Raphael J. Mannino,
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Oral vaccination against pathogen challenge provides advantages over other routes of immunization including cost, convenience and safety. The goal of oral vaccination is to prime both circulatory and mucosal immunity.

Over the past several years we have been developing lipid matrix based immunogens which are highly immunogenic in the absence of additional adjuvants while being non-toxic and non-inflammatory. These formulations can be designed to stimulate antigen specific B cells, T helper cells and/or cytotoxic T cells. Previously we demonstrated that intranasal immunization of a protein-lipid formulation could induce circulating and mucosal immunity and protection from intranasal pathogen challenge.

Recently we have formulated an insoluble, second generation antigen lipid complex designed to survive the stomach and dissociate in the small intestine. Oral administration of this formulation induces circulating neutralizing antibodies and protects from pathogen challenge on a mucosal surface in a influenza virus model system. Studies to further characterize this oral vaccine formulation in terms of murosal antibody response and induction of cellular immunity are in progress.

Cytotoxic T cell responses have been shown to be important for clearance of infected or tumorigenic cells. We have shown that in vivo CD+8 CTL responses can be generated using fusogenic lipid matrix formulations containing peptides, proteins or whole killed pathogens. Current studies are investigating the structure-function relationships between subunit vaccines and the induction of cytolytic immune responses, using antigens derived from SIV, HIV, influenza and parainfluenza viruses. Variables being investigated involve the role of T cell help and the route of immunization.

NON-RESPONDERS TO HEPATITIS B VACCINE CAN PRESENT HBsAg TO T LYMPHOCYTES. Geert Leroux-Roels, Isabelle Desombere, Pierre Voet, Pierre Hauser. Department of Clinical Chemistry, University of Gent and SmithKline Beecham Biologicals, Rixensart, Belgium.
Objective : To unravel the cellular mechanisms causing non-responsiveness to HBsAg vaccines in man.
Methods : To examine whether hepatitis B vaccine non-responders can adequately take-up, process and present HBsAg, PBMC from 8 HLA-DR2$^+$ non-responders were used to present HBsAg or preS2-S particles to HBsAg-specific T cells from a haploidentical high-responder vaccinee. The proliferative response of these lines was used to evaluate the efficacy of antigen presentation. Concomitantly the proliferative response of PBMC from these non-responders upon stimulation with HBsAg and tetanus toxoid (TT) was measured. HLA typing was performed with serological and molecular biological techniques.
Results : PBMC from 8 non-responders did not react to HBsAg in vitro, whereas they vigourously proliferated upon stimulation with TT. PBMC from 5 DR2(w15)$^+$ non-responders [3 subjects with DRB1*1501 and 2 with DRB1*1502] were able to present HBsAg to DRB1*1501-restricted T cell lines. Three DR2(w16)$^+$ non responders were unable to present HBsAg in a DR2(w15)-restricted fashion. PBMC from 3 DPw4$^+$ non-responders [2 subjects with DR2(w15) and one with DR2(w16)] were able to present HBsAg in a DPw4-restricted fashion.
Discussion : Non-responsiveness to HBsAg is a selective immune defect since all subjects examined displayed an in vitro response to TT. All DR2(w15)$^+$ non-responders were able to present HBV *envelope* antigens to HBsAg-specific, DRB1*1501-restricted T cells irrespective of their being DRB1*1501 or DRB1*1502. One DR2(w16), DPw4$^+$ non-responder was able to present HBsAg via DPw4. Whether the inability of the other 2 DR2(w16)$^+$ non-responders to present HBsAg to the T cell lines was due to histoincompatibility or to an antigen-uptake, -processing and/or -presentation defect remains unclear.
Conclusions : The non-responsiveness to HBsAg of 6 out of 8 HLA-DR2$^+$ vaccinees is not due to a defect in antigen-uptake, -processing or -presentation, but to another mechanism that has yet to be defined.

CELL-MEDIATED IMMUNITY TO SIMIAN IMMUNO-DEFICIENCY VIRUS (SIV) AND VIRAL BURDEN DURING INFECTIONS WITH VIRULENCE-ATTENUATED STRAINS, Barbara Lohman, Marta Marthas, Ellen McGowan, Niels Pedersen and Michael McChesney, California Regional Primate Research Center, University of California - Davis, Davis, CA 95616-8542
Cell-mediated immunity is an important component of the host response to viral infections and in the case of SIV infection, this response may control the virus load. The moleculary cloned SIVmac1A11 is a model for a live virus vaccine because monkeys infected with this virus are transiently viremic, do not develop disease and withstand low-dose challenge with a pathogenic strain of SIVmac. The mechanism of protection is unknown. Anti-viral cytotoxic T lymphocyte activity (CTL) is a classical measure of cellular immunity. Previous attempts to measure CTL activity in monkeys infected for over a year with SIVmac1A11 were negative, while monkeys persistantly infected with the pathogenic virus, SIVmac239, had detectable secondary CTL responses. We have followed CTL responses during acute infection with SIVmac1A11 by a chromium-release assay measuring specific lysis of targets expressing $p55^{gag}$ and $gp160^{env}$. Both primary and secondary CTL were detectable in some monkeys at 2 and 4 weeks post inoculation, and an anti-gp160 response in secondary cultures was detected at 4 and 6 weeks from monkeys that were still viremic. We will monitor the CTL response through the viremia transition period, together with end-point dilution quantitation of viremia. We hypothesize that the level of CTL activity will decline when the monkeys become aviremic and will increase following a second inoculation with 1A11. In addition, recombinant viruses constructed from the genomes of SIVmac1A11 and SIVmac239 produce low-level persistant viremia (unlike the transient viremia with SIVmac1A11) and no disease after two years. CTL responses and viremia will be measured in animals infected with these viruses, before and after challenge with pathogenic SIV.

RECOMBINANT VIRUS VACCINES WHICH ENCODE THEIR OWN CYTOKINE GENES, Ian Ramshaw, Janet Ruby and Alistair Ramsay, Viral Engineering Group, John Curtin School of Medical Research, PO Box 334, Canberra 2601, Australia.
The type of immune response induced is determined, in part, by the pattern of cytokines secreted by different subsets of T lymphocytes. One subset of T lymphocyte (Th1) secretes IL-2 and IFN-γ and promotes cell-mediated immunity, whilst another population (Th2) secretes IL-4, IL-5, IL-6 and IL-10 and preferentially induces antibody responses. Given this information, it is not surprising that attempts have been made to modify the immune response to vaccines by the administration of cytokines. However, cytokines generally have a very short half-life in vivo and there are major difficulties associated with toxicity and targetting. We have attempted to overcome these problems by constructing live recombinant virus vaccines which encode their own cytokine genes. Thus, during replication, the virus produces its own factor which is secreted from the infected cells with profound immunological consequences. Based on their effect on the growth and immunogenicity of the virus, the cytokines studied to date fall into 2 categories; those that alter the pathogenesis of the virus and those that selectively stimulate specific immune responses. Both of these properties are relevant to the development of safe and effective vaccines. The expression of IL-2, IFN-γ or TNF dramatically alter the virulence of the virus vector. On the other hand, IL-5 expression selectively stimulates an IgA response whilst IL-6 stimulates an enhanced IgG response to coexpressed antigens.

THE ROLE OF IFN-γ IN THE CLEARANCE OF VACCINIA VIRUS.
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In different experimental models, it has been found that vaccinia virus infection can be cleared in the absence of classic anti-viral T cells. For instance, recombinant vaccinia viruses (rVV) which encode IL-2, IFN-γ or TNF-α did not produce a lethal infection in athymic, nude mice, and the mice were able to resolve infections with any of these rVV (Ramshaw *et al.*, Immunol Rev 127, 1993). In addition, β_2-microglobulin⁻ mice, which lack CD8 T cells were able to clear VV (Spriggs *et al.*, PNAS 89, 1992). In the case of the cytokine-producing rVV, we have shown that the local secretion of IFN-γ is crucial to the resolution of vv-IL-2 (Karupiah *et al.*, J exp Med 172, 1991). In normal mice, we have also demonstrated that the anti-viral activity of effector CD8 T cells is dependent on IFN-γ (Ruby and Ramshaw, Lymphokine Cytokine Res 10, 1990).

In order to try to distinguish between the alternate hypotheses that IFN-γ is required for the upregulation of class I MHC on the infected cell or is directly anti-viral, we have infected mice with rVV which encode $H\text{-}2K^d$. We will present data which shows that the clearance of this virus by CD8 T cells is still dependent on IFN-γ, arguing that the secretion of anti-viral cytokines by effector T cells may be their predominant function *in vivo.*