MOLECULAR ASPECTS OF VIRAL IMMUNITY

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Plenary Sessions

Poster Sessions

Molecular Aspects of Viral Immunity

Keynote Address

J2-001 GAME OF CHESS PLAYED BETWEEN THE VIRUS AND THE HOST, Michael B.A. Oldstone, The Scripps Research Institute, La Jolla, California.

The moves viruses make against the host and its immune system that allow them to persist and the countermoves made by the host is the topic of this presentation. The focus is on the ability of non-lytic viruses to initially avoid immunologic recognition, infect and persist in cells and subsequently interfere subtly with the cell's ability to produce specific differentiated products as hormones, neurotransmitters, cytokines and immunoglobulins, etc., in the absence of virus induced cytolysis or inflammation. Despite replication, the infected cell maintains its normal anatomic architecture and yet the virus can disorder the cells' function leading to disturbances in homeostasis and disease. Thus viruses can underlie a wide variety of diseases, currently of unknown etiology, that affect the endocrine, immune, nervous and other differentiated systems. However, in several instances, the persistent infection can be cured followed by reappearance of the cell's normal homeostatic function.

New Strategies for Molecular Understanding of Viral Pathogenesis

J2-002 THE ROLE OF T CELL MEDIATED CYTOTOXIC ACTIVITIES TO CONTROL INFECTIONS, TUMOR GROWTH AND ALLOGRAFT SURVIVAL

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$CD8^+$ cytotoxic T cells and natural killer cells are crucially involved in the elimination of some viruses, in graft rejection, antitumor activities, in immunopathology and some autoimmune diseases. The granule exocytosis model of cytolysis proposes that cytolytic lymphocytes lyse target cells dependent on Ca^{2+} by directed exocytosis of perforin; however residual cytolytic activity in the absence of Ca^{2+} could still be observed.

With our perforin deficient mice generated by homologous recombination, we demonstrated the existence of mainly two types of cytolytic activities, one perforin - the other Fas-mediated.

Lysis of virus infected or allogeneic target cells by antigen specific perforin deficient effector T cells were abolished against fibroblasts and more or less diminished against Fas-expressing target cells. Perforin deficient mice failed to clear LCMV and the elimination of certain tumor cells (fibrosarcoma, lymphoid tumor and melanoma cells) proceeded in a markedly reduced fashion. Elimination of Listeria monocytogenes is not significantly affected during primary but substantially decreased when analysed with primed $CD8^+$ T cells in adoptive transfer experiments. Survival times of allografts with allogeneic differences on MHC class I antigens were prolonged in perforin deficient animals.

Fas-based cytotoxicity was established by in vitro assays using a large panel of targets of normal and tumor cell types with various levels of Fas-expression. However the in vivo significance (e.g. the possible role in immunoregulation) has still to be established.

J2-003 T LYMPHOCYTE FUNCTION AND ONTOGENY IN GENE-TARGETTED MUTANT MICE, Tak W. Mak, Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Canada M4X 1K9.

T lymphocytes recognize their antigen peptides and Major Histocompatibility Complex products with the use of their T cell antigen receptors (TcR). In addition to the α and β chains of TcR, the interaction between T cells and their target cells or antigen presenting cells is also assisted by a series of other cell surface polypeptides. Most notable of these are CD4 and CD8, which are selectively expressed on mature helper/inducer and killer/suppressor T cells, respectively. Upon engagement of their ligands, a series signals are being transduced intracytoplasmically via some of these molecules and their associated proteins. Perhaps the most important enzyme in this signal transduction process is the lymphocytes specific tyrosine kinase lck. Another important component is the cell surface tyrosine phosphatase CD45. This molecule is alternatively spliced and the different isoforms are expressed on the various hemopoietic and lymphopoietic cells. Signalling thru the TcR-CD4/CD8-lck-CD45 complex is thought to be insufficient to activate T lymphocytes. A co-stimulatory signal is believed to be essential. Many investigators have suggested that CD28, a ligand for B7/BB1 is an essential co-stimulatory signal. In an attempt to gain better understanding on the roles of these molecules in T lymphocyte functions and ontogeny, we generated a series of mutant mice with disruptions in these genes. These mutant mice are being analysed in order that we can evaluate the importances of these genes in T cell development.

J2-004 COSTIMULATORY SIGNALS AND VIRAL IMMUNITY, Frank Borriello[1], Elizabeth A. Tivol[1], Gordon J. Freeman[2], Mehrdad Matloubian[3], Rafi Ahmed[3] and Arlene H. Sharpe[1], [1]Brigham and Women's Hospital, [2]Dana-Farber Cancer Institute, [2,3]Harvard Medical School, Boston, MA, USA, and [3]UCLA School of Medicine.

Costimulatory signals appear pivotal in determining the functional outcome of T cell antigen recognition, since their absence results in functional inactivation of mature T cells, leading to a state of hyporesponsiveness. Signaling via the B7:CD28/CTLA4 costimulatory pathway can provide a potent costimulatory signal. Learning how to positively control this pathway may lead to new immunization strategies for infectious agents. The discovery of a second costimulatory molecule, B7-2, as the major, early activating costimulator in this pathway indicates that signaling through this pathway is more complex than previously thought. It is unclear whether B7-1 (CD80) and B7-2 (CD86) mediate distinct or overlapping costimulatory functions. B7-1 and B7-2 interact with two receptors, CD28 and CTLA4, on T cells. Although they demonstrate only modest amino acid conservation, B7-1 and B7-2 have been shown to equally costimulate T cell proliferation and IL-2 production through CD28 in vitro. The early expression of B7-2 has led to the hypothesis that B7-2 may participate in the initiation of the immune response, thereby playing a pivotal role in the decision between T cell activation and anergy, whereas mB7-1, being expressed later, may serve to amplify or regulate the immune response. To analyze the in vivo function of the costimulatory molecules B7-1 and B7-2, we have generated mice lacking or overexpressing B7-1 or B7-2. These mice provide a definitive means for determining whether B7-1 and B7-2 have complementary or overlapping functions in vivo. The importance of B7-1 for regulating in vivo T cell responses previously had been inferred from studies with CTLA4Ig fusion protein. The B7-1 deficient (B7 -/-) mouse strain provided the first in vivo evidence for the existence of functional alternative CTLA4 counter-receptors. Despite lacking B7-1 expression, activated B cells from B7-1 -/- mice still bound CTLA4, demonstrating that alternative CTLA4 ligand(s) exist. Initial evaluation of the B7-1 -/- mice revealed only a partial defect in the immune response. The lack of a more dramatic phenotype can be explained by the existence of additional CTLA4 counter-receptors. Unstimulated B7-1 -/- mice have normal numbers of B and T cells, normal levels of serum immunoglobulins, and respond normally to mitogens. B7-1 -/- mice exhibit normal primary CTL responses to lymphocytic choriomeningitis virus and clear the virus similarly to wild type mice. However, activated B7-1 -/- B cells have a 70% reduction in costimulation of the response to alloantigen, with a corresponding reduction in IL-2 production. The residual allogeneic mixed lymphocyte response is inhibited by CTLA4-Ig, demonstrating that alternative CTLA4 counter receptors are functionally important in vivo. In addition, B7-1 deficient mice appear to have enhanced antibody responses to T dependent hapten-protein conjugates, consistent with a negative regulatory role of B7-1 in the generation of immune responses. The finding of functional alternative CTLA4 counter receptors in the B7-1 -/- mouse strain provided impetus to search for additional CTLA4 counter receptors and resulted in the cloning of murine mB7-2. Our initial characterization of the B7-2 deficient mouse strain is in progress.

J2-005 RECOGNITION OF ENDOGENOUSLY PROCESSED VIRAL AND TUMOR ANTIGENS IN HLA TRANSGENIC MICE, Linda A. Sherman , Matthias Theobald , Drake La Face , Per Peterson , and Judith Biggs , Dept. of Immunology , The Scripps Research Institute , 10666 N.Torrey Pines Rd. La Jolla CA 92037.

The major barrier to recognition of human MHC molecules (HLA) by mouse T cells is poor recognition of HLA by the murine co-receptor molecules, CD4 and CD8. In the case of CD8 T cells, this deficiency can be overcome by constructing a chimeric molecule composed of the $\alpha 1$, $\alpha 2$ domains of HLA ligated to the $\alpha 3$ domain of a murine class I molecule. This provides the antigen presentation capacity of HLA along with the ability of the $\alpha 3$ domain of H-2 to interact with mouse CD8. An alternative strategy is to express human CD8 as a transgene product. Both models have provided CTL responses to viral and tumor cell antigens presented in association with HLA-A2.1. Results will be presented describing and contrasting the use of such transgenic mice in identifying endogenously processed and presented antigens.

Molecular Aspects of Viral Induced Inflammatory Disease

J2-006 CYTOKINE REGULATION OF NK AND T CELL RESPONSES TO VIRAL INFECTION. C.A. Biron, L.P. Cousens, J.S. Orange, T.P. Salazar-Mather, and H.C. Su. Division of Biology and Medicine, Brown University, Providence, RI 02912.

Cytokine expression and function are poorly understood during viral infections.[1] Our laboratory has been characterizing these for regulation of NK and T cell responses during infections of mice with lymphocytic choriomeningitis virus (LCMV). In immunocompetent mice, NK cells are activated to undergo blastogenesis and acquire elevated killing at early times post-infection. Virus-specific CTLs and $CD8^+$ T cell expansion are activated at later times. We have shown the following. The early response is accompanied by a redistribution of T and B cell populations into splenic white pulp areas and NK cell trafficking and cytokine delivery to splenic marginal zones. Virus-induced interferon-α/β expression is pivotal for induction of these changes. At later times during infection, when T cells are expanding, interleukin (IL)-2 and transforming growth factor-β expression are observed. IL-4 and IL-10 are first detected with CTL activation but peak at later times. IL-2 does not appear to be a major mediator of normal virus-induced early NK cell responses as peak IL-2 production is later and as NK cell activation and proliferation are cyclosporin A (CsA) resistant. It has not been possible to demonstrate induction of IL-12 factor during this infection, and administration of IL-12 induces toxic levels of tumor necrosis factor-α in LCMV-infected mice. These studies indicate that cytokines act to coordinate different phases of immune responses to viral infections, and that particular cytokines are beneficial whereas others are detrimental for promoting protection against viruses.

The requirement for IL-2 in T cell proliferative responses was examined in IL-2-deficient mice. These mice have been shown to have reduced but activated CTL function in response to LCMV infection.[2] Although LCMV-infected IL-2-/- mice had CTL responses, $CD8^+$ T cells were not induced to express the high affinity IL-2 receptor, and, in comparison to cells from infected IL-2+/+ or +/- littermates, the $CD8^+$ T cell proliferation was reduced by >90%. Studies carried out in the $CD8^+$ T cell-deficient $\beta 2$-microglobulin ($\beta 2$-M)-/- mice demonstrated that these mice were induced to express IL-2 during LCMV infection. In contrast to normal mice, however, a profound CsA-sensitive NK cell response was observed at late times post-infection. $\beta 2$M-/- IL-2-/- mice revealed a requirement for IL-2 in this NK cell response. Thus, although IL-2-independent pathways for both NK cell and CTL activation exist, the experiments show that there are specific *in vivo* conditions where IL-2 is required for proliferation of these cell subsets. The studies demonstrate both the balance of influences mediated by cells and cytokines and the plasticity of immune responses to viral infections.

1. Biron, C.A. *Curr Opin Immunol* 1994, 6:530-538.
2. Kündig, T.M., H. Schorle, M.F. Bachmann, H. Hengartner, R.M. Zinkernagel, and I. Horak. *Science* 1993, 262:1059-1061.

J2-007 T-CELL MEDIATED AND CYTOKINE INDUCTION DURING NEUROTROPIC MOUSE HEPATITIS VIRUS INFECTION. Michael J. Buchmeier, Monte Hobbs, and Bradley D. Pearce, The Scripps Research Institute, La Jolla, CA 92037.

Mechanisms of T-cell mediated clearance of viruses from the central nervous system are poorly understood, but likely to differ from those employed in the periphery because the CNS lacks lymphatic drainage and constitutive expression of MHC class I antigen, and the unique structure of the CNS vasculature imposes constraints on access by leukocytes and soluble immune mediators. To study the mechanism by which viruses are cleared from neurons in the central nervous system, we have developed a mouse model involving infection with a neurotropic variant of mouse hepatitis virus (OBLV60). After intranasal inoculation, OBLV60 grew preferentially in the olfactory bulbs of Balb/c mice. Using *in situ* hybridization, we found viral RNA localized primarily in the outer layers of the olfactory bulb, including neurons of the mitral cell layer. Virus was cleared rapidly from the olfactory bulb between 5 and 11 days. Athymic nude mice failed to eliminate the virus demonstrating a requirement for T lymphocytes. Immunosuppression of normal mice with cyclophosphamide also prevented clearance. Both $CD4^+$ and $CD8^+$ T-cell subsets were important as depletion of either of these subsets delayed viral clearance. Gliosis and infiltrates of $CD4^+$ and $CD8^+$ cells were detected by immunohistochemistry at 6 days. The role of cytokines in clearance was investigated using an RNase protection assay for IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, TNFα, TNFβ and IFNγ. In immunocompetent mice there was upregulation of RNA for IL-1α, IL-1β, IL-6, TNFα and IFNγ at the time of clearance. Nude mice had comparable increases in these cytokine messages with the exception of IFNγ. Induction of MHC-I molecules on cells in infected brains was demonstrated by immunohistochemistry in normal and nude mice, suggesting that IFNγ may not be necessary for induction of MHC-I on neural cells *in vivo*.

J2-008 CYTOTOXIC T LYMPHOCYTES CAN CLEAR HEPATITIS B VIRUS FROM THE HEPATOCYTE WITHOUT KILLING THE CELL. Luca G. Guidotti, Kazuki Ando, Tetsuya Ishikawa, Lisa Tsui and Francis V. Chisari. The Scripps Research Institute, La Jolla, CA 92037

Although cytotoxic T lymphocytes (CTL) are known to clear viral infections by killing infected cells, recent studies suggest that they can also suppress the replication of certain viruses by noncytolytic mechanisms. We have examined this area by monitoring the immunopathological and antiviral consequences of antigen recognition by hepatitis B virus (HBV) specific CTL in HBV transgenic mice that express the viral gene products in their hepatocytes. We have shown that intravenously injected CTL rapidly trigger their target hepatocytes to undergo apoptosis, but that the direct cytopathic effect of the CTL is minimal in comparison with the cytopathic effects of the antigen-nonspecific intrahepatic inflammatory response that they activate. In addition to killing the hepatocyte, the same CTL also downregulate HBV gene expression and completely abolish HBV replication in the hepatocytes that they don't destroy. This noncytolytic antiviral CTL effect is mediated by at least two distinct processes in these animals. First, the CTL cause a quantitative reduction in the steady state content of all HBV mRNA species in the hepatocyte, and this is followed by disappearance of all of the corresponding viral proteins in the liver and serum. The CTL initiate this process by secreting IFNγ and TNFα when they are activated by antigen recognition, since the regulatory effect of the CTL can be prevented completely by prior administration of the corresponding antibodies. Nuclear run-on experiments reveal that viral mRNA transcription is unaffected despite the profound reduction in HBV mRNA content in the liver, suggesting that the CTL-derived cytokines accelerate viral mRNA degradation in the hepatocyte. A second noncytolytic antiviral pathway is also activated by the CTL. We have recently shown that HBV nucleocapsid particles, and the replicative HBV DNA intermediates that they contain, disappear from the transgenic mouse liver following either CTL administration or partial hepatectomy, the latter of which triggers hepatocellular regeneration without any change in hepatocellular HBV mRNA content. These results suggest that preformed HBV nucleocapsid particles may be actively degraded during hepatocyte turnover, and they raise the possibility that similar events might also occur in nondividing hepatocytes that are activated by noncytolytic signals delivered by the CTL. We propose that, in addition to their pathogenetic effect, the combined effects of the CTL response at the HBV mRNA, nucleocapsid and replicative DNA levels may represent a curative antiviral stimulus during HBV infection. Since the virus must contain molecular elements that respond to these CTL-induced antiviral signals, inactivating mutations at these loci could be very efficiently selected by immune pressure, because a single mutation could abrogate the antiviral effect of a wide spectrum of T cell responses, irrespective of epitope specificity. Identification of these viral response elements and the intracellular pathways that interact with them may lead to the development of new strategies for antiviral drug design.

Molecular Mechanisms of Immune Suppression in Viral Infection

J2-009 **Inhibition of antigen presentation to CD8+ T lymphocytes by herpes simplex virus (HSV) immediate early (IE) protein ICP47,** **David C. Johnson[1], Ian A. York[1], Frank L. Graham[2], David W. Andrews[3], Roman Tomazin[1] and Stanley R. Riddell[4],** Departments of Pathology[1], Biology[2] and Biochemistry[3], McMaster University, Hamilton, Ont. Canada L8N 3Z5, [4]Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Human fibroblasts infected with HSV are resistant to lysis by CD8+ cytotoxic T lymphocytes (CTL), yet human B cell lines can be efficiently lysed by these CTL. The effect on human fibroblasts is rapid (within 2 hr of infection of cells), occurring before synthesis of MHC class I is altered by virus infection. A recombinant HSV, F-US5MHC, which expresses mouse MHC class I proteins does not render human fibroblasts sensitive to lysis by mouse CTL. MHC class I molecules are retained in the ER of HSV-infected fibroblasts in a misfolded, unstable form and stability of the MHC complex can be restored by addition of exogenous peptides. Using a panel of HSV mutants and Ad expression vectors we demonstrated that the HSV IE protein ICP47 was both necessary and sufficient to cause retention of class I and ICP47 expression in fibroblasts caused the cells to resist lysis by CD8+ T lymphocytes. ICP47 is a soluble, cytosolic protein and we have found no evidence of membrane association. Therefore, it appears that ICP47 inhibits cytosolic stages of the antigen presentation pathway so that antigenic peptides do not reach the ER. To date, polyclonal and monoclonal antibodies directed to ICP47 have not specifically precipitated any of the previously described components of the antigen presentation pathway and we have not found ICP47 associated with TAP transporter proteins or proteosomes in these experiments. The effects of ICP47 are being assessed in proteosome and TAP transporter assays. GST-ICP47 fusion proteins tightly bind a 8.5 kDa cytosolic cellular protein which is found in a number of adherent human cell lines but not lymphocytes. The protein has been purified and sequencing is in progress. In addition, radiolabelled ICP47 binds to a single cellular protein of ≈55 kDa on ligand blots. These proteins are good candidates as cellular targets of ICP47 and as novel components of the antigen presentation pathway. Preliminary experiments support the hypothesis that ICP47 is very effective in blocking CD8+ T lymphocyte responses in vivo, perhaps explaining the predominance of CD4+ vs. CD8+ anti-HSV CTL in vivo. We expect that ICP47 may be very useful, not only to elucidate antigen presentation pathways, but also to prevent immune recognition of gene transfer vectors and as a immunosuppressive agent.

J2-010 SUSCEPTIBILITY TO POLYOMA VIRUS-INDUCED TUMORS IS CONFERRED BY AN ENDOGENOUS MMTV SUPERANTIGEN.

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Susceptibility to tumors induced by mouse polyoma virus varies among inbred mouse strains. We have previously shown that polyoma tumor susceptibility is controlled by products of MHC as well as non-MHC genes. In crosses between MHC-nonidentical strains differing in tumor susceptibility, resistance correlates with dominant/codominant inheritance of the resistant H-2 haplotype. We have observed the opposite pattern of inheritance of susceptibility in crosses between MHC-identical strains. In crosses between the highly susceptible C3H/BiDa mouse and the highly resistant but MHC-identical ($H\text{-}2^k$) C57BR/cdJ mouse, polyoma tumor susceptibility is conferred by a single autosomal dominant gene, which we have designated *Pyv*^{S}. *Pyv*^{S} does not encode cell receptors for the virus, affect viral dissemination or anti-viral antibody responses, or affect intracellular events essential for productive infection or cell transformation by the virus. Whole-body irradiation renders C57BR/cdJ mice fully susceptible to polyoma-induced tumors, indicating an immunological basis for this strain's resistance. We hypothesized that *Pyv*^{S} encodes an Mtv superantigen (SAG) that confers susceptibility to C3H/BiDa mice by deleting precursors of polyoma-specific T cells. We found that tumor susceptibility in (C3H/BiDa x C57BR/cdJ) x C57BR/cdJ backcross mice cosegregated with *Mtv-7*. Inheritance of *Mtv-7* showed perfect concordance with absence of peripheral Vβ6$^+$ T cells. Genotyping of backcross mice using markers of simple sequence repeat polymorphisms flanking *Mtv-7* showed no evidence of recombination between *Pyv*^{S} and *Mtv-7*. Strongly biased usage of Vβ6 by (a) polyoma-specific CD8$^+$ CTL from virus-infected C57BR/cdJ mice and by (b) CD8$^+$ T cells infiltrating a polyoma tumor in a virus-immune C57BR/cdJ host provide further evidence that T cells bearing this Mtv-7 SAG-reactive Vβ domain are critical anti-polyoma tumor effector cells. These results indicate identity between *Pyv*^{S} and *Mtv-7 sag*, and demonstrate a novel mechanism of inherited susceptibility to virus-induced tumors based on effects of an endogenous superantigen on the host's T cell repertoire.

J2-011 **IMMUNOSUPPRESSION BY IMMUNOPATHOLOGY?**

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Infection of mice with lymphocytic choriomeningitis virus (LCMV) causes a transient to longlasting immunosuppression dependent upon virus-isolate dose of virus and age, H-2, non H-2, level of CD4$^+$ T cells, of CD8$^+$ T cells and kinetics of neutralizing antibodies of the host. The immunohistological analysis suggests that CD8$^+$ T cell dependent disappearence of marginal zone macrophages of follicular dendritic cells and of virus infected cells in general correlates with immunosuppression. The details of mechanisms responsible for these findings are now being analysed. A role of this CD8+ T cell dependent immunosuppression in the establishment of a LCMV carrier state in immunocompetent mice is suggested by the following experiments: The otherwise slow and low neutralizing antibody response against LCMV is accelerated and enhanced by CD8$^+$ T cell depletion at the time of infection, suggesting virus-specific immunopathology being responsible at least partially. The ELISA antibody response is not significantly altered under the same conditions but is abrogated if LCMV-specific T cell receptor transgenic mice are infected with high doses of LCMV, indicating, that suppression of the specific antibody response depends upon the relative kinetics of CTL versus antibody responses. Whether exhaustion of specific CTL responses is enhanced by similar mechanisms remains to be tested. The role of interleukins of the relative distribution of virus in the mouse and in the various aspects of immunosuppression are now being studied. Immunosuppression, caused by CD8$^+$ T cell-dependent immunopathology, may also be operational in HIV infection in humans. Such a pathogenesis of HIV-triggered AIDS could explain several aspects of the disease process not readily fitting the (unproven) conventional idea that HIV is causing immunodeficiency via direct viral pathogenicity.

Antigen Processing and Presentation in Viral Infections

J2-012 CELLULAR IMMUNITY AGAINST DNA TUMORVIRUSES: POSSIBILITIES FOR PEPTIDE BASED VACCINES AND IMMUNE ESCAPES, Rene E.M. Toes[1], Mariet C.W. Feltkamp[1], Maaike E. Ressing[1], Rienk Offringa[1], Howard M. Grey[2], Alessandro Sette[2], Cornelis J.M. Melief[1], and W. Martin Kast[1], [1]Department of Immunohematology and Blood Bank, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands and [2]Cytel Corporation, San Diego, CA 92121.

The cellular immunity against two DNA tumor viruses (i.e. human adenovirus type 5 (Ad 5) and human papillomavirus type 16 (HPV16)) was studied with respect to possible immune escape mechanisms and to the development of CTL epitope based peptide vaccines. After identifying an immunorelevant CTL epitope in the Ad 5E1A protein to which CTL clones were directed that could eradicate Ad 5E1 induced tumors in nude mice, an amino acid replacement study of this epitope revealed a point mutation that totally eliminated the possibility to recognize the mutant peptide by the CTL clones directed against the wild-type peptide sequence. New viral constructs were made that contained this point mutation and used to transform mouse embryo cells. However, these mutant tumor cells were still immunogenic and CTL clones specific for these mutant tumor cells were shown to react with a peptide derived from the Ad 5E1B protein. These Ad 5EIB specific CTL clones, however, were as effective as the Ad 5E1A specific CTL clones in the eradication of Ad 5E1 induced tumors in nude animals, indicating that a choice can be made of immunorelevant epitopes to which an immunization strategy could be developed. In addition, we discovered that by supertransfection of Ad 5E1 induced tumor cells with the activated ras oncogen the possibility of Ad 5E1B specific CTL to recognize the Ad 5E1 induced tumors was eliminated whereas the Ad 5E1A specific CTL could still kill these tumor cells. This might indicate a new mechanism of tumors to escape CTL.
In an HPV16 induced mouse tumor model an immunosubdominant CTL epitope was identified in the E7 protein that could, upon immunization with that peptide, protect mice against a subsequent challenge with HPV16 induced tumor cells. By changing the anchor residues in that peptide an even more immunoprotective peptide could be generated. Combined, these data indicated a successful use of a CTL epitope based peptide vaccine in the prevention of HPV16 induced tumors in mice. Subsequently this led us to identify relevant CTL epitopes of HPV16, that is highly associated with cervical carcinoma in humans, for the major HLA-A alleles (i.e. HLA-A *0101, A *0201, A*0301, A*1101 and A *2401). Together these alleles cover a majority of all humans. CTL epitopes were identified through peptide-MHC binding assays followed by *in vitro* peptide immunizations with high affinity binding peptides to induce primary CTL responses and immunogenicity studies in HLA-A transgenic mice. Thereafter, memory CTL responses were measured in cervical cancer patients against selected peptides. Combined, these data led us to develop a CTL epitope based peptide vaccine that could be of use in HPV16 induced cervical cancer patients. A clinical trial for this disease is scheduled to start in the fall of 1994.

J2-013 CLASS II PRESENTATION OF AN ENDOGENOUSLY SYNTHESIZED GLYCOPROTEIN. Carol S. Reiss[1,2,3], Shirley M. Bartido[1], Miriam Stein[1], and Stephanie Diment[3,4], Biology Department[1], and Center for Neural Science[2], New York University, New York NY 10003, Kaplan Comprehensive Cancer Center[3] and Pathology Department[4], New York University Medical Center, New York NY 10016.

In contrast to Class I presentation which is well characterized to use peptide fragements of proteins sythesized in the cytoplasm, exogenously administered experimental antigens enter the class II MHC pathway through endocytosis. We have been studying the recognition of the glycoprotein of vesicular stomatitis virus (VSV) which can enter either the exogenous or endogenous pathways for presentation to CD4+ T cells. Investigations of the intracellular sites involved, the proteolytic processes involved in the epitope generation, will be discussed.

The glycoprotein studied in detail is a truncated form of the wt type 1 glycoprotein, termed *poison tail* (Gpt). Expressed with a vaccinia virus vector, the Gpt remains Endo H sensitive and never becomes Endo D sensitive, indicating that it is restricted to the endoplasmic reticulum. Gpt is degraded in the ER, and we believe the degradation products include the immunogenic epitopes recognized by a panel of I-A^d and I-E^d T cell clones and hybridomas. Immunofluorescence studies have confirmed the ER localization. Flow cytometric evaluations show that the Gpt never appears on the cell surface, in contrast to the wt G.

The peptides generated are not secreted; using an innocent by-stander assay, Gpt-infected cells are incapable of sensitizing ^{51}Cr-labeled uninfected APC. This contrasts with the rapid ability of supernatants from wt G-vaccinia virus-infected cells to sensitize APC for T cell recognition.

Investigations of the characteristics of the enzymes contributing to the degradation of the Gpt have shown that a reducing environment is essential, as diamide treatment of cells prevents degradation. Lysosomotropic drugs (eg. NH_4Cl and leupeptin) do not alter the half-life of the protein, but do prevent presentation of the peptides; this is inconsistent with an autophagic component to the proteolysis. pH optima are physiological, as pH8 environment inhibits the enzyme activity. Inhibitors of enzyme classes are consistent with a trypsin-like, and not cystein-, cathepsin B-, or chymotrypsin-like class. Supported by NIH grant AI 18083 to CSR.

Humoral Immunity in Viral Recovery and Protection

J2-014 **IMMUNE RESPONSE TO CARDIOVIRUS VACCINE VECTORS,** Ann C. Palmenberg[1], Lee Martin[1], Jorge Osorio[1], Ralf Altmeyer[2], Nicolas Escriou[2], Marc Girard[2], and Sylvie van der Werf[2]; [1]Institute for Molecular Virology and Department of Animal Health & Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706; [2]Unité de Virologie Molécular, Institut Pasteur, Paris France.

Encephalomyocarditis virus (EMCV) and Mengovirus are related members of the cardiovirus genus of picornaviruses. Their RNA genomes encode a large polyprotein which is cleaved proteolytically in co- and post-translational reactions to yield all mature viral proteins necessary to establish an infection. Although originally thought to be exclusively murine in host range, both viruses actually infect a wide range of mammals. EMCV has caused devastating epizootics in captive primates (eg: macaques, chimps and baboons), domestic pigs and exotic zoo collections (elephants, lions and tigers). Death, following ingestion of virus-contaminated material, is rapid, and caused by extensive meningoencephalitis and virus-induced damage to the CNS. Myocarditic lesions are common in older animals. When administered intracerebrally, the LD_{50} for EMCV strain R is about 1 pfu. We are studying the pathogenesis of EMCV and Mengo with engineered cDNA plasmids containing infectious viral sequences. Many plasmids contain truncated versions of the unusual 5' noncoding homopolymeric poly(C) tract that is a hallmark of these cardioviruses. Short poly(C) Mengoviruses grow very well in tissue culture but are 10^6-10^{12} fold less pathogenic to mice than the wild-type strains. Animals receiving sublethal doses of short-tract Mengo strains develop high titers of neutralizing antibodies, exhibit potent CTL responses and acquire lifelong protective immunity against challenge with wild-type virus. The genetic stability of the short-tract strains, even upon serial brain passage, mark them as safe, efficacious live vaccines. Currently, we believe the poly(C) phenomenon is due to interference by the wild-type virus sequences (long poly(C) tract) with normal cellular cytokine induction mechanisms (ie: IFα and IFβ) during the initial stages of animal infection. The targeted cells are probably macrophages, and their singular ability to correctly respond or not respond to poly(C) tract length during the first few hours of infection determines whether an inoculated animal will live (protectively vaccinated) or die. The short-tract viruses probably induce IF in the macrophages, and are consequently killed then rapidly cleared from the host. In related experiments we've found that attenuated Mengo strains can easily carry large heterologous insertions within their genomes, and express these sequences into protein them during replication in animals. The resulting immune response (B cell and CTL) to the chimeras is directed towards the foreign sequences (epitopes) as well as towards the Mengo proteins. A chimeric HIV vaccine, a rabies vaccine and an LCMV vaccine have been developed and tested. The LCMV chimera seems especially effective, as a single pfu of this engineered Mengo strain, administered orally to a mouse, is sufficient for complete immunogenic protection against intracerebral challenge with wild-type LCMV virus.

Local Immunity and Virus Infection of Privileged Sites

J2-015 PROGRESS TOWARD THE DEVELOPMENT OF A LIVE ATTENUATED RESPIRATORY SYNCYTIAL VIRUS (RSV) VACCINE, Brian R. Murphy[1], James E. Crowe, Jr.[1], Phuong T. Bui[1], William R. Elkins[1], Cai-Yen Firestone[1], Robert M. Chanock[1], Michael D. Lubeck[2], Ruth Karron[3], Mary Lou Clements[3], and Peter F. Wright[4], George R. Siber[5],[1]National Institutes of Health, NIAID, Bethesda, MD 20892-0702, [2]Wyeth Ayerst Research, Radnor PA 19101-8299, [3]Johns Hopkins University, Baltimore, MD 21205-1901, [4]Vanderbilt University School of Medicine, Nashville, TN 37232-2581,[5]Massachusetts Public Health Biologic Laboratories, Jamaica Plain, MA 02130

RSV is the most common cause of serious viral lower respiratory tract disease in infants and children. We have recently renewed our efforts to generate a safe and effective live attenuated RSV vaccine for topical administration that will overcome the deficiencies of previously studied live and non-living RSV vaccines. This vaccine will be a bivalent vaccine consisting of subgroup A and B live attenuated virus components. Since the peak incidence of severe disease caused by RSV is in the 2-month old infant, an RSV vaccine will need to be effective when given to 1-month old infants. Based on the success of live poliovirus vaccines given early in infancy, it is anticipated that the intranasally administered live virus vaccine will infect and induce a protective local and systemic immune response even in infants with passively acquired maternal antibodies. The main approach that we have taken in this effort to develop the live RSV vaccine is to introduce one or more *ts* mutations by chemical mutagenesis into a cold-passaged virus (*cp*RSV) that had been partially attenuated by the acquisition of host-range mutations selected by passage in cells of a heterologous host species. We have developed a large set of *cp*RSV subgroup A *ts* mutants (termed *cpts* mutants) that contain the host-range mutations selected during cold passage and two or more *ts* mutations introduced by chemical mutagenesis. These mutants have been evaluated *in vitro* for their level of temperature sensitivity and *in vivo* in rodents, chimpanzees, and humans. A large set of RSV subgroup B *cpts* mutants has been similarly produced and evaluated.

The immunogenicity and protective efficacy of three candidate live attenuated RSV vaccine strains that represent a spectrum of attenuation were evaluated for protective efficacy in chimpanzees. Prior to infection some of these animals were given RSV immune globulin by the IV route to simulate the condition of the very young infant who possesses passively-acquired maternal RSV antibodies. The three candidate vaccine strains were immunogenic and induced significant resistance to RSV challenge in both groups of chimpanzees. Interestingly, the chimpanzees infused with RSV antibodies prior to immunization were primed more effectively for an unusually high serum neutralizing antibody response to infection with challenge virus than chimpanzees which did not receive such antibodies. This high booster response occurred despite marked restriction of replication of the challenge virus. The evaluation of two candidate vaccines in seronegative human infants will also be described.

J2-016 DISEASE ENHANCEMENT BY IMMUNITY TO RESPIRATORY SYNCYTIAL (RS) VIRUS, Peter J.M. Openshaw, Respiratory Medicine, St. Mary's Hospital Medical School, London W2 1PG, United Kingdom.

RS virus is immunologically interesting for at least two reasons: 1) Upper respiratory reinfection occurs despite previous exposure and demonstrable immunological memory: 2) Humans or rodents previously immunised against virus infection can show enhanced disease during reinfection. Others have shown that passive transfer of antiviral antibody either protects against virus infection or has no effect, and there is no evidence of antibody enhancement of disease *in vivo*. By contrast, T cell immunity appears closely associated with disease augmentation. We have focused on examining the immunological mechanisms of disease enhancement in mice.

Initial studies showed that transfer of $CD8^+$ cytotoxic T lymphocytes (CTL) causes rapid virus clearance from the lungs of RS virus-infected mice, but also increased disease severity with alveolar haemorrhage and polymorphonuclear (PMN) cell recruitment to the lung. This disease (reminiscent of shock lung) could sometimes be fatal, whereas normal mice recover well from similar doses of RS virus. Next, we compared the effects of $CD4^+$ and $CD8^+$ T cells, using polyclonal T cells separated immunomagnetically from mixed lines grown *in vitro* with viral antigen. $CD4^+$ T cells were more pathogenic than $CD8^+$ T cells in a dose-for-dose comparison, but that the type of pathology varied depending on the type of cell injected.

While testing recombinant vaccinia viruses expressing single RS viral proteins for their ability to protect mice against infection, we observed that animals sensitised to the major surface glycoprotein G (attachment protein) developed lung eosinophilia after challenge with RS virus intranasally. T cell lines from the spleens of mice sensitised with various recombinant vaccinia viruses were established. Those form mice primed with the M2 (22K) protein were predominantly $CD8^+$ CTL, and that produced few cytokines. Those from mice primed with fusion protein (F) generated mixed T cell lines with both Th1 $CD4^+$ T cells, and CTL. Mice primed to G protein gave rise to predominantly $CD4^+$ T cells producing Th2 cytokines. *In vivo* transfer of these cell lines into naïve RSV infected mice reproduces the patterns of disease seen in mice sensitised *in vivo* with the respective antigens. The mouse model of RS virus disease therefore has excellent potential for illustrating mechanisms of lung immunopathology.

J2-017 INFLAMMATORY RESPONSES TO HERPES SIMPLEX VIRUS INFECTIONS IN THE EYE. Barry T. Rouse[1], John S. Babu[1], Donna M. Bouley[1], Johnson Thomas[1], and Sally M. Atherton[2]. [1]Department of Microbiology, University of Tennessee, Knoxville, TN 37996-0845 and [2]Department of Anatomy, University of Texas, San Antonio, TX.

The eye is a complex organ whose function is to transmit light images through different cell and tissue layers and liquid media to a neurosensory retina. Its design permits little tolerance for aberration or distortion of its conducting elements as could occur when invading pathogens arrive and an inflammatory response with its swelling, plasma protein extravasation, leukocyte infiltration and tissue damage results. Thus it is in the eye's functional interest to limit inflammatory responses when possible and rely on immune defenses which do not involve tissue distortion and damage. Restricting tissue damaging responses is not always effective and the process is best developed in response to agents delivered to locations such as the anterior chamber. Infections of the cornea with herpes simplex virus is an example where an inflammatory response is initiated which may result in ocular impairment. Such herpetic stromal keratitis (HSK) is a common cause of blindness in man. Animal model studies indicate that HSK is a multi-step process initiated by virus in an avascular structure. HSK fails to occur in the absence of T cells or replicating virus. However, replicating virus disappears several days before a visible inflammatory response becomes evident. Evidence will be presented that the secondary agonists which drive the inflammatory response may not be viral antigen(s) per se. Multiple cell types are involved in HSK, with the respective role of functional sets of lymphocytes changing according to the clinical phase of the disease. In addition, nonspecific inflammatory cells such as neutrophils and NK cells also influence the severity of lesions. Basically the reaction begins with T cells that produce type one cytokines, particularly IFN-γ, dominating the scene, but during remission type 2 cytokines, notably IL-10, appear as mechanistically involved. From the use of knockout mice for various immunological parameters, evidence will be presented that numerous mechanisms of pathogenesis may be at play during HSK. Damage to corneal tissues in all systems appear to involve TNFα.

A second ocular damaging event in which immunopathology is at least partially involved is herpetic retinal necrosis. Evidence that this disease may involve the immunopathological role of $CD4^+$ T cells and protective effects by $CD8^+$ T cells will be presented, as will be suggestions by which the pathological events are mediated at the molecular level.

Memory and the Immune Response to Virus

J2-018 IMMUNOLOGICAL MEMORY TO VIRUSES, Rafi Ahmed, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles.

Acute viral infections and live vaccines often confer long-term immunity. The nature of T and B cell memory is different. B cell memory is manifested not only by the presence of memory B cells but also by continuous antibody production. In contrast, the effector phase of the T cell response is short-lived and long-term T cell memory is due to the presence of 'quiescent' antigen specific memory T cells that are present at higher frequencies and are able to respond faster upon re-exposure to virus due to increased levels of adhesion molecules. In this talk I will present our results on: (i) the bone marrow as a major site of long-term antibody production after acute viral infection; (ii) the role of $CD4^+$ T cells and B cells (immune complexes) in maintaining $CD8^+$ T cell memory, (iii) the role of *fas* antigen in regulating T cell responses, and (iv) the efficacy of various antigen delivery systems in inducing long-term T cell memory.

J2-019 COMPARTMENTALIZED B CELL RESPONSE TO SENDAI VIRUS INFECTION OF MICE, C.Coleclough, L,Hyland, R.Sealy and M.Sangster, Department of Immunology, St.Jude Children's Research Hospital, Memphis, Tennessee.

Sendai virus is a natural respiratory viral pathogen of mice. Intranasal infection of mice with the virus provokes a virus specific antibody-forming-cell reaction that exhibits a distinct kinetic pattern in the lymph nodes that drain the respiratory tract, in the spleen, and in the bone marrow. The bone marrow AFC population is extremely long-sustained, and supports an active humoral response that essentially persists for the lifetime of the infected animal. Thus the conventional categories of "primary" and "secondary" response may not apply to the humoral response of mice naturally exposed to respiratory viruses. Paradoxically, the population of B cells that reacts most rapidly to Sendai virus infection does not itself secrete antibody, but can be demonstrated by the recovery of hybridomas that secrete "polyspecific" antibodies. The activation of this polyspecific B cell population is, like the humoral response, extremely persistant. Viral infection thus sets in train multiple B cell "memory" processes. Variation in the rules of development and turnover of different B cell populations constrains the mechanisms that may operate to generate these different forms of memory.

J2-020 ESTABLISHMENT AND MAINTENANCE OF T CELL MEMORY TO RESPIRATORY VIRUSES, Peter C. Doherty, Sam Hou, Christine Ewing, David Topham, Anthony McMickle, James Houston, and Ralph Tripp, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105.

The analysis of the development and memory phases of the $CD4^+$ "helper" (Th) and $CD8^+$ cytotoxic T lymphocyte (CTL) responses to the respiratory pathogens, influenza virus and Sendai virus (parainfluenza type 1) have been characterized by a combination of limiting dilution analysis (LDA) for determining Th and CTL precursor (p) frequency and FACS separation of lymphocytes with different activation phenotypes. The interpretation at this stage, largely based on the analysis of the CTL response, is that the development phase of T cell memory and the primary response are synonymous. Virus-specific CTLp are produced in considerable excess of the numbers required to provide the effector CTL that terminate the primary infection, with only a fairly small proportion localizing to the target organ (the lung) that supports virus growth. Even when many of the proliferating CTLp are killed by administration of a small dose (20 mg/Kg) of the DNA-targeted drug cyclophosphamide (Cy), there is no indication of immune exhaustion. The $CD4^+$ Th response has, at this stage, not been analyzed through the course of the primary infection. Use of the LDA approach to determine Thp frequencies is inherently more difficult, as the "read-out" is lymphokine production and there is considerable "bystander" activation in these primary responses to respiratory viruses. Memory Thp and CTLp are characterized initially by the expression of an "activated" phenotype: CD44-high, L-selectin-low, CD49d (VLA-4) high. After some months, an increasing proportion of the memory T cells revert to the L-selectin-high CD49d-low form typical of naive CTLp. The change, which is never absolute, seems to occur first with CD49d and the rate varies for different viruses. Current experiments are addressing the possibility that intercurrent infection, particularly with the mouse γ-herpesvirus 68 which causes persistent infection of lymphoid tissue, may be inducing a switch back to the activated pattern, as a consequence of "bystander" effects, or "low affinity" stimulation via the clonotypic TCR in responding lymphoid tissue. The question of such cross-reactivity and/or exposure to "high lymphokine" environments for the long-term maintenance of memory is also being addressed.

J2-021 **GENERATION AND PERSISTENCE OF T CELL MEMORY.** Susan L. Swain, Richard W., Dutton, Linda Bradley, Elizabeth Broome, Laura Carter, Michael Croft, Caroline Dubey and Laura Haynes. University of California San Diego, La Jolla, CA 92093-0063.

To study the factors which regulate the generation and persistence of specific T cell memory we have used model systems utilizing T cell receptor transgenic mice as a source of enriched naive cells which can be either cultured in vitro to generate effector populations or restimulated in adoptive hosts. In either case one can visualize the development of an expanded effector population. We have documented that the proliferation and IL-2 production of the naive T cells depends on their activation by APC expressing high levels of co-stimulatory molecules. We find that B7.1 and ICAM-1 as costimulators strongly synergize and that increased T cell receptor triggering can both increase the magnitude of the response and decrease its dependence on costimulation.

When cytokines IL-4 Vs IL-12/IFNγ are present at the initiation of the response of either CD8 or CD4 cells they dictate that the effectors generated will be polarized either towards IL-4 and IL-5 secretion or IL-2 and IFNγ secretion, respectively. The fate of the effector population generated and followed in vitro, also is tightly regulated by Ag, cytokines and probably by costimulation. CD4 effector cells not re-exposed to Ag, produce no cytokines and they die within 3-4 days. Effectors restimulated with Ag make massive amounts of cytokines, regardless of the presence of cytokines, at low densities of Ag and with little dependence on costimulation. When there is little IL-2 produced and no cytokines added, effectors die rapidly by apoptosis. However the combination of IL-2 and TGFβ block apoptosis and support expansion of the effector population which is greatly enhanced by periodic Ag stimulation. Some conditions favor the reversion of effector-like cells to a more resting memory phenotype and these are being further explored.

We have also examined the development and maintenance of memory after transfer of effector cells to adoptive hosts. Long-lived polarized memory populations are generated from the polarized effectors and these persist for prolonged period in the absence of apparent Ag stimulation. This supports the idea that factors other than antigenic stimulation, present in situ can support the expansion and maintenance of memory cells.

Molecular Immunology of Neurotropic Virus Infections

J2-022 REVERSIBLE CONFORMATIONAL CHANGES OF THE RABIES GLYCOPROTEIN THAT MASK OR EXPOSE EPITOPES INVOLVED IN VIRULENCE, Anne Flamand, Patrice Coulon, Yves Gaudin, Florence Lafay, Hélène Raux and Christine Tuffereau. Laboratoire de Génétique des Virus, CNRS, 91198 Gif Cedex, France.

The rabies glycoprotein (G) is the only external protein of the virion and is therefore responsible for any interaction that rabies makes with the host cell during the first steps of the virus cycle. The G protein is also the target of neutralizing antibodies. There are around 450 trimers of G at the virion surface which constitute the spikes visible by electron microscopy. Upon exposure to slightly acidic pH, the glycoprotein undergoes a conformational change which results in longer and less regular spikes. Strikingly and quite differently from influenza hemagglutinin, this conformational change is reversible: if the pH is risen back to 7.0, the spikes regain their neutral configuration (1). Probably as a consequence, the viral infectivity is totally preserved after an exposure of 2 hours at pH 6.4 and 37°C, which induces the conformational change, followed by an incubation at neutral pH. Since the conformational change is reversible, there is a pH-dependant equilibrium between the native and the low-pH conformation: the higher the pH, the more spikes are in their native configuration.

Two main antigenic sites and several minor sites have been identified on the native rabies glycoprotein (2). Specific amino acids belonging to each of the two major antigenic sites are important or essential for viral virulence. For instance a lysine in position 198, which is part of antigenic site II, is important, although not essential, for the viral virulence. Similarly, the arginine 333, which belongs to antigenic site III, is essential for pathogenicity while dispensable for multiplication in cell culture (reviewed in 3). Viral strains mutated at arginine 333 have lost the capability to penetrate certain categories of neurons, suggesting that this mutation affected the recognition of specific receptors or subsequent interactions necessary for the penetration of the virus at nerve terminals. Therefore the two main antigenic sites are regions of the glycoprotein which also interact specifically with neurons in the animals.

We have found that neutralization requires the fixation of at least one or two IgG for every three spikes, irrelevant of the antigenic site recognized by the antibody (4). Most neutralizing antibodies recognize conformational epitopes which are accessible on the native configuration of the protein. Some epitopes remain accessible also on the acidic configuration while others are not. In addition, a minority of antibodies recognize epitopes which are only accessible on the acidic conformation. This is not unlikely in view that each spike has a certain probability to undergo a conformational change, even at neutral pH. In consequence the surface of the virus probably fluctuates and G epitopes which are not accessible on the native glycoprotein could be transiently exposed. Conformational flexibility at neutral pH and physiological temperatures has also been observed for poliovirus (5). Structural flexibility of external proteins could have important implications in virus-host interactions.

(1) Gaudin et **al.**, J. Virol. **67**, 1365; (2) Benmansour et **al.**, J. Virol. **65**, 4198; (3) Coulon et **al.**, Current Topics in Microbiology and Immunology, Lyssaviruses, vol.**187**, 69; (4) Flamand et **al.**, Virology **194**, 302; (5) Li et **al.**, J. Virol. **68**, 3965.

J2-023 THEILER'S VIRUS-INDUCED DEMYELINATING DISEASE - ROLE OF VIRUS-SPECIFIC TH1 CELLS IN CNS IMMUNOPATHOLOGY AND THEIR REGULATION BY ANTIGEN-SPECIFIC TOLERANCE, Stephen D. Miller, Jeffrey D. Peterson, Jonathan G. Pope, and William J. Karpus, Northwestern University Medical School, Chicago, IL 60611

Theiler's murine encephalomyelitis viruses (TMEV) are endemic enteric pathogens of wild and colony-reared mice. Intracerebral inoculation of susceptible mouse strains leads to a chronic, progressive inflammatory demyelinating disease of the central nervous system (CNS) characterized clinically by an abnormal gait, progressive spastic hind limb paralysis and urinary incontinence, and histologically by parenchymal and perivascular mononuclear cell infiltration and demyelination of CNS white matter tracts. Demyelination is related to persistent CNS viral infection. Due to the similarity in clinical and histological presentation, TMEV-induced demyelination is considered to be a highly relevant model of multiple sclerosis (MS). Our current interests are in determining the phenotype, fine specificity, lymphokine profile and TCR usage of CNS-infiltrating cells involved in the effector stages of TMEV-induced demyelination. Based on a variety of experimental evidence, it is clear that demyelination induced in SJL/J mice by infection with the BeAn strain of TMEV is a Th1-mediated event: (a) disease induction is suppressed in T cell-deprived mice and by *in vivo* treatment with anti-I-A and anti-CD4 antibodies; (b) disease susceptibility correlates temporally with the development of TMEV-specific, MHC-class II-restricted DTH responses and with a predominance of anti-viral IgG2a antibody; (c) activated (*i.e.*, IL-2R$^+$) T cells infiltrating the CNS are exclusively of the CD4$^+$ phenotype, and (d) pro-inflammatory cytokines (IFN-γ and TNF-β) are predominantly produced in the CNS. We have mapped the predominant Th1 epitope on the virion to amino acids 74-86 of the VP2 capsid protein. A Th1 line specific for VP2$_{74\text{-}86}$ exacerbates the onset of demyelination in recipient mice infected with a suboptimal dose of TMEV. TMEV-infected SJL/J mice fail to exhibit peripheral DTH and T cell proliferative responses to the major myelin proteins, MBP and PLP, and pre-tolerization with neuroantigens has no affect on the incidence or severity of TMEV-induced demyelinating disease, whereas neuroantigen-specific tolerance prevents the induction of relapsing experimental autoimmune encephalomyelitis (EAE). In contrast, tolerance induced with intact TMEV virions specifically anergizes virus-specific Th1 responses and results in a dramatic reduction of the incidence and severity of clinical disease and CNS demyelination in SJL/J mice subsequently infected with TMEV. These results have important implications for a possible viral trigger in MS as they indicate that chronic demyelination in TMEV-infected mice is initiated in the absence of demonstrable neuroantigen-specific autoimmune responses and are consistent with a model wherein early myelin damage is mediated via primarily by mononuclear phagocytes recruited to the CNS and activated by pro-inflammatory cytokines produced by TMEV-specific Th1 cells.

J2-024 MOLECULAR PATHOGENESIS OF PRION DISEASES Stanley B. Prusiner, University of California, San Francisco, CA 94143-0518

The concept that prions are novel pathogens which are different from both viroids and viruses has received increasing support from many avenues of investigation over the past decade. Enriching fractions from Syrian hamster (SHa) brain for scrapie prion infectivity led to the discovery of the prion protein (PrP). Prion diseases of animals include scrapie and mad cow disease; those of humans present as inherited, sporadic and infectious neurodegenerative disorders. The inherited human prion diseases are genetically linked to mutations in the PrP gene that result in non-conservative amino acid substitutions. Transgenic (Tg) mice expressing both SHa and mouse (Mo) PrP genes were used to demonstrate that the "species barrier" for scrapie prions resides in the primary structure of PrP. This concept was strengthened by the results of studies with mice expressing chimeric Mo/SHa transgenes from which "artificial" prions have been synthesized. Similar chimeric Mo/human (Hu) PrP transgenes were constructed which differ from MoPrP by 9 amino acids between residues 96 and 167. All of the Tg(MHu2M) mice developed neurologic disease ~200 days after inoculation with brain homogenates from three patients who died of Creutzfeldt-Jakob disease (CJD). Inoculation of Tg(MHu2M) mice with CJD prions produced MHu2MPrP^{Sc}; inoculation with Mo prions produced MoPrP^{Sc}. The patterns of MHu2MPrP^{Sc} and MoPrP^{Sc} accumulation in the brains of Tg(MHu2M) mice were different. About 10% of Tg(HuPrP) mice expressing HuPrP and non-Tg mice developed neurologic disease >500 days after inoculation with CJD prions. The different susceptibilities of Tg(HuPrP) and Tg(MHu2M) mice to human prions indicate that additional species specific factors such as chaperone proteins are involved in prion replication. Diagnosis, prevention and treatment of human prion diseases should be facilitated by Tg(MHu2M) mice. In other studies, Tg mice were compared expressing wt and mutant MoPrP. Overexpression of the wtMoPrP-A transgene ~8-fold was not deleterious to the mice but it did shorten scrapie incubation times from ~145 d to ~45 d after inoculation with murine scrapie prions. In contrast, overexpression at the same level of a MoPrP-A transgene mutated at codon 101 (corresponding to codon 102 in HuPrP) produced spontaneous, fatal neurodegeneration between 150 and 300 d of age in two lines of Tg(MoPrP-P101L) mice designated 2866 and 2247. Genetic crosses of Tg(MoPrP-P101L)2866 mice with gene targeted mice lacking both PrP alleles (Prn-p$^{0/0}$) produced animals with a highly synchronous onset of illness between 150 and 160 days of age. The Tg(MoPrP-P101L)2866/Prn-p$^{0/0}$ mice had numerous PrP plaques and widespread spongiform degeneration in contrast to the Tg2866 and 2247 mice that exhibited spongiform degeneration but only a few PrP amyloid plaques. Another line of mice designated Tg2862 overexpress the mutant transgene ~32-fold and develop fatal neurodegeneration between 200 and 400 d of age. Tg2862 mice exhibited the most severe spongiform degeneration and had numerous, large PrP amyloid plaques. While mutant MoPrP^{C}(P101L) clearly produces neurodegeneration, wtMoPrP^{C} profoundly modifies both the age of onset of illness and the neuropathology for a given level of transgene expression. Our findings and those from other studies suggest that mutant and wtPrP interact, perhaps through a chaperone-like protein as noted above in studies of Tg(MHu2M) mice, to modify the pathogenesis of the dominantly inherited prion diseases.

Vaccine Design and Strategies

J2-025 **GENERATION OF VIRAL PEPTIDES RECOGNIZED BY MHC CLASS I-RESTRICTED T CELLS,** *Jack R. Bennink, Luis Anton, Heidi T. Link, and Jonathan W. Yewdell,* Laboratory of Viral Diseases, NIAID, Bethesda, MD 20892-0440.

$CD8^+$ lymphocytes (T_{CD8+}) play an important role in host immunity to viruses and other intracellular parasites. Virus-specific T_{CD8+} recognize MHC class I molecules in association with peptides of 8 to 10 residues derived from viral proteins. This presentation will focus on how and where antigenic peptides are generated by cells. To begin to characterize the nature of proteases involved in the generation of antigenic peptides from cytosolic proteins, we used a panel of recombinant vaccinia viruses expressing different forms of influenza virus nucleoprotein (NP). We found that the efficiency of generation of two NP peptides is related to the metabolic stability of the source gene product. There has been considerable speculation that such short lived proteins are degraded by proteasomes in a ubiquitin-targeted process. Our observations, however, call into question the importance of ubiquitin targeted-proteolysis in generating antigenic peptides from exogenously provided or endogenously synthesized viral proteins. We also examined the extent to which antigenic peptides can be generated in the endoplasmic reticulum (ER). We found that antigenic peptides could be produced from short precursors (17 residues) but not from a number of full length proteins (influenza virus hemagglutinin, NP, ovalbumin) that are targeted to the ER by a NH_2-terminal signal sequence. Peptides were generated much more efficiently from the COOH-terminus of the 17 residue precursor than from the NH_2-terminus. These findings indicate that the ER has a much more limited capacity than the cytosol to generate antigenic peptides, but that ER proteases (particularly aminopeptidases) could perform the final proteolytic steps in the generation of class I binding peptides from precursors imported from the cytosol by TAP, the MHC encoded peptide transporter.

J2-026 DEVELOPMENT OF SYNTHETIC PEPTIDE VACCINES, Jay A. Berzofsky, Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892

Potential advantages of synthetic peptide or engineered recombinant vaccines are that they can be limited to contain only the specific antigenic determinants for desired responses without other determinants that elicit unwanted responses, and that the sequences of the determinants themselves can be modified to enhance potency or breadth of crossreactivity. However, they can have the disadvantage that any single determinant may be presented by only a limited selection of major histocompatibility complex (MHC) molecules of the species. To overcome the problem of MHC polymorphism, we have identified determinants presented by multiple MHC molecules, and have also located multideterminant regions of the HIV-1 envelope protein that contain overlapping determinants each presented by different class II MHC molecules, so that the whole multideterminant region is presented by multiple MHC molecules of both mouse and human. We have made use of "cluster peptides" spanning these multideterminant regions of the HIV-1 envelope to provide help for neutralizing antibody (Ab) and $CD8^+$ cytotoxic T lymphocyte (CTL) responses to peptides attached to these helper regions. These synthetic peptide vaccine constructs containing the P18 peptide from the V3 loop of HIV-1 IIIB or MN, elicited both neutralizing Ab and CTL in multiple strains of mice. The cluster peptides inducing helper T cells were essential for elicitation of Ab and CTL to the P18 segment of both IIIB and MN strains of HIV-1 in mice of several MHC haplotypes. Several adjuvants were compared for their ability to elicit both CTL and Ab simultaneously, without one response inhibiting the other. A single formulation in incomplete Freund's adjuvant (IFA) could elicit all 3 responses, neutralizing Ab, CTL, and TH1 helper cells. The CTL specific for the MN strain P18 peptide crossreacted with strains SC, SF2, Z321, and CDC4. The peptides in IFA also elicit high titers of antibodies in rabbits. Boosting was found to enhance CTL responses as well as Ab responses. These constructs are being prepared for a human immunotherapy trial. These vaccine constructs are potent and also avoid sites on gp160 that are known to elicit enhancing antibodies or autoimmune responses that might contribute to disease pathogenesis. However, we can potentially improve on these by tinkering with the internal structure of the individual epitopes. We have found that replacing a negatively charged glutamic acid residue with an uncharged amino acid in one of the helper determinants makes it 10 to 100-fold more potent in binding to the class II MHC molecule and in eliciting murine helper T cells that still recognize the natural HIV-1 sequence. Thus, such a modified peptide should be more potent as a vaccine, while retaining the ability to elicit T cells that will respond to HIV proteins that of course do not have the altered sequence. We are currently mapping the critical residues for presentation of one of these peptides by human HLA-A2, with the intent of developing modified peptides that will be more potent as components of a human vaccine. Thus, by learning how these peptides bind to MHC molecules and T-cell receptors, we can design internally modified determinants to construct more potent or more crossreactive second generation vaccines. We are testing these vaccine approaches in a mouse model in which mice can be protected against tumor cells expressing HIV proteins as would an HIV-infected cell.

J2-027 EFFICACY AND IMMUNOGENICITY OF DNA VACCINES FOR VIRAL DISEASES, Margaret A. Liu, Jeffrey Ulmer, John Shiver, Arthur Friedman, William McClements, and John Donnelly, Merck Research Laboratories, West Point PA 19486

DNA vaccines, comprised of non-replicating plasmids encoding viral proteins, are capable of generating protective immunity in animal models of several viral diseases. In preclinical models of influenza infection, reduced viral shedding was observed in DNA-vaccinated ferrets after challenge with the human clinical virus strain, A/Georgia/93. Cross-strain protection was conferred by DNA encoding the major internal proteins (nucleoprotein, NP, and matrix, M1) and the surface protein haemagglutinin (HA) from the antigenically-distinct previous virus strains, A/Beijing/89 and A/Hawaii/91. This protective efficacy was greater than that seen by immunization with the widely-used clinical vaccine composed of killed A/Beijing/89 virus. Thus, compared to a killed virus vaccine, protection seen with the DNA vaccine against a drifted virus strain was greater. We previously demonstrated that immunization of mice with NP DNA generated MHC Class I-restricted cytotoxic T lymphocytes. Mice likewise were protected from death and morbidity following cross-strain challenge[1]. HA DNA vaccines generated neutralizing antibodies in mice, ferrets and primates, and provided protection in mice[2] and ferret models of influenza.

In animal models of other viral diseases, immune responses and protection against viral challenge have been seen after immunization with DNA encoding viral proteins. DNA encoding HIV gp120 generated CTL and neutralizing antibodies in monkeys. Antigen-specific proliferative responses and, in mice, secretion of high levels of γ-IFN relative to levels of IL-4, months after immunization were also observed . Immunization of rabbits with DNA encoding L1, the major viral capsid protein of Cotton tail Rabbit Papilloma Virus (CRPV), resulted in neutralizing antibodies and protected against the development of warts after inoculation with CRPV. Mice immunized with DNA encoding the glycoprotein gD from Herpes Simplex Virus type 2 (HSV-2), developed neutralizing antibodies and were protected from death when subsequently challenged with HSV-2.

DNA vaccines were protective in animal models of various viral diseases. Neutralizing antibodies, helper T cells (T_h1) and cytotoxic T cells were generated. Cross-strain protection due to cellular immunity was demonstrated.

[1]Science, 1993 259:1745-1749, [2]DNA Cell Biol, 1993, 12:777-783.

HIV and SIV Infections and the Immune Response

J2-028 FACTORS DETERMINING THE NEUROPATHOGENESIS OF SIV INFECTION IN MACAQUES, Opendra Narayan, Edward B. Stephens, Sanjay V. Joag, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, Kansas 66160-7424.

The profile of a neurovirulent virus is determined by its mechanism of entry into the CNS (neuroinvasion), the type of CNS cell in which it replicates (neurotropism) and its ability to cause pathologic effects in the brain (neurovirulence). Whereas neuroectodermal cells, especially neurons, are the target cells of most neurovirulent viruses, the main target cell in the brain for SIV and other lentiviruses is the macrophage. Infection in, expression of viral antigens by and products of SIV replication exported from these cells result in inflammation and degenerative changes in the brain and concomitant loss of neurons. SIV strains that are mainly T-cell tropic cause transient activation of T-cells and during this period, infected T-cells cross the blood brain barrier and localize in the brain causing persistent but minimally productive infection and minimal neuro pathologic effects. Viral proteins but not virions are produced continuously. By virtue of the tropism of the virus for CD4 T cells, many infected animals eventually become immunosuppressed and develop AIDS, but not classical neurological disease. Viruses which are macrophage tropic invade the brain presumably also in T lymphocytes and the viruses infect macrophages in the brain. However, productive virus replication is minimized by antiviral CD8 T cells which suppress (kill?) all virus producing cells throughout the body, including the CNS. Productive virus replication in brain macrophages and accompanying inflammatory changes develop only when CD8 cells fail i.e. after profound immunosuppression sets in. The neurological disease that results from productive virus replication in macrophages in the brain therefore depends on presence of an appropriate macrophage-tropic viral phenotype invading the neuropil and development of immunosuppression in the host. The neurological disease could therefore be defined as one of the AIDS syndromes.

Molecular Pathogenesis of Viral Infections

J2-029 MULTIPLE FUNCTIONS OF ADENOVIRUS E3 IMMUNOREGULATORY GENES: EFFECTS ON ACUTE VIRAL PATHOGENESIS AND PROLONGATION OF SURVIVAL OF PANCREATIC ISLET ALLOGRAFTS, Marshall S. Horwitz[1,2], Gyorgy Fejer[1], Ildiko Gyory[1], JoAnn Tufariello[1] and Shimon Efrat[3]. [1]Departments of Microbiology and Immunology, [2]Pediatrics and [3]Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

The adenovirus (Ad) early transcription region (E3) codes for more than 7 polypeptides, four of which have already been shown to alter the immune response to Ad infection. The amount of the class I major histocompatibility complex (MHC) on the plasma membrane can be reduced by the binding of the Ad E3 gp19K protein to the MHC heavy chain, which prevents transport of the complex out of the endoplasmic reticulum. This process interferes with presentation of viral peptides to cytotoxic T lymphocytes. Cytolysis by tumor necrosis factor-α (TNF) is inhibited by 4 distinct viral polypeptides, 3 of which (the Ad E3 14.7K or the complex of the 10.4K and 14.5K proteins) are coded in the E3 region. The E3 polypeptides are translated from a family of viral mRNAs, that are synthesized from a single viral promoter and processed by alternative splicing. We have studied the functions of the E3 polypeptides in several murine models. The goals of these experiments were to determine the effects of the Ad E3 polypeptides in acute and persistent viral infections as well as in a transplantation model designed to measure whether these viral immunoregulatory proteins would abrogate allogeneic graft rejection. In a vaccinia virus (V.V.) pneumonia model, in which the isolated Ad E3 14.7K or Ad E3 gp19K genes were inserted into the V.V. pathogen, the Ad anti TNF polypeptide increased viral virulence but the Ad anti MHC had no effects.

In addition to manipulating the Ad E3 genes in viral constructs, several transgenic mouse lines containing the Ad E3 genes have been constructed for these experiments. The E3 genomic DNA behind the rat insulin promoter (RIP) has been used to generate transgenic animals. Islets from RIP-E3 transgenic animals (H-$2^{b/d}$) have been transplanted allogeneically to H-2^{d} recipients and remained viable, secreting insulin until the end of the experiment at 94 days; in contrast, control nontransgenic islets of the same genotype were rejected by 21-28 days. The E3 genes behind the native E3 promoter have been inserted into mouse embryos to generate transgenic animals, and the expression of the transgene monitored in multiple organs. The E3 promoter of the transgene is responsive to stimulation by the Ad E1A following infection with an E3 minus Ad 7001 and can also be upregulated by administration of bacterial lipopolysaccharide. The effects of this transgene on Ad pathogenesis are currently being studied. Thus, these viral immunoregulatory genes have been shown to alter viral pathogenicity during acute infection and to downregulate the host immune response sufficiently to permit islet cell transplantation. These results on manipulating the Ad E3 genes for the control of the host immune response also have implications for designing adenovirus vectors for gene therapy.

J2-030 IMMUNOLOGICAL FEATURES OF A MURINE GAMMA-HERPESVIRUS INFECTION. Anthony A Nash, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH

Murine herpesvirus (MHV) 68, a gamma-herpesvirus, is a natural pathogen of small rodents. When MHV-68 is administered intranasally to inbred mice, a productive infection is established in the lung, involving alveolar epithelium and mononuclear cells. Infection resolves in the lung by day 10, but the virus is maintained in B lymphocytes in a latent form. The number of latently infected B lymphocytes in the spleen rises dramatically between the 2nd and 3rd week post infection. Thereafter, the numbers decline, with approximately 1 in 10 5 virus positive lymphocytes detected 1 year later. Recovery from the primary infection is dependent upon CD8+ T cells, since their depletion result in an overwhelming infection of lung with spread of virus to other tissues. In contrast, CD4 T cells do not appear to regulate the infection in the lung, but are the major effector cell in the induction of splenomegaly, seen in MHV-68 infected mice. Another pathological feature of the infection is the induction of lymphomas, characterised by monoclonal B lymphocytes. The possibility that this disease arises from infected lymphocytes is strengthened by the ability of B cells taken from infected animals to spontaneously transform *in vitro* to generate cell lines. Many of the biological properties displayed by MHV-68 are similar to Epstein-Barr virus infection. The ability to genetically manipulate the virus and the host in order to define gene function, makes this a powerful model to study the interaction of a gamma-herpesvirus with its natural host.

Emerging Viral Infections: Immunology and Molecular Biology

J2-031 EMERGING VIRAL INFECTIONS: COMMON THEMES, Stephen S. Morse, The Rockefeller University, New York, NY 10021-6399

"Emerging" infections can be defined as infectious diseases that either have newly appeared in the population, or that are rapidly increasing their incidence or expanding their geographic range. Recent viral examples include AIDS, Ebola, and Hantavirus Pulmonary Syndrome (first identified in a 1993 outbreak in the southwestern U.S.). Emerging viral infections show a number of common features. Most "new" viruses derive from existing viruses that move into new areas or acquire new hosts ("viral traffic"). Many are zoonotic (originating from animal sources) (even pandemic influenza appears usually to be a reassortant originating in wildfowl). Ecological or environmental changes (either natural, or, often, man-made) may precipitate emergence of new diseases by placing people in contact with a previously unfamiliar zoonotic reservoir or by increasing the density of a natural host or vector of a pathogen, increasing the chances of human exposure. Upon introduction into a human population from a zoonotic reservoir, the newly introduced virus may cause localized outbreaks of disease. Some may show rapid variation and evolution upon introduction, and some evidence suggests a role for immune selection in this process. A few viruses (such as HIV) may succeed in establishing themselves and disseminating in the human population, becoming truly "human" infections. Human activities can also play an important role in establishment and dissemination. Migrations from rural areas to cities, now an accelerating worldwide phenomenon, or other displacements, can introduce remote viruses to a larger population; the virus may then spread along highways and (globally) by air travel. The development of an effective system of surveillance and rapid response is essential, but resources for this are presently inadequate. Vaccine development, production, and deployment problems also need to be addressed. Immunopathology may be a key feature of many of these infections, a number of which manifest as hemorrhagic fevers. Many of the life threatening complications are due to increased vascular permeability. The resemblances to septic shock suggest that cytokines (such as TNF) are likely to be important in the pathogenesis of these infections. The response of cells, such as the macrophage, that induce or synthesize key cytokines, may be an important element, and the ability to infect these cells may be one common denominator. Why some viruses elicit this response, while other closely related viruses do not, cannot yet be predicted from molecular data. Better understanding of these aspects of the immune response should lead to additional therapeutic strategies. (Supported by NIH grant R01 RR03121.)

J2-032 NEWLY RECOGNIZED HANTAVIRUSES, S.T. Nichol[1], S. Morzunov[1], V. Chizhikov[1], C.F. Spiropoulou[2], H. Feldmann[1], E. Ravkov[1], M. Bowen[1], A. Sanchez[1], W. Thayer[1,2], P.E. Rollin[1], T.G. Ksiazek[1], J.E. Childs[1], M. Monroe[1], S. Trappier[1], E. Tkachenko[3], J. Pilaski[4], L.T. de Souza[5], L.B. Iversson[6], J.E. Rowe[7], S. St. Jeor[7], C.J. Peters[1], [1]Special Pathogens Branch, Division of Viral and Rickettsial Disease, Centers for Disease Control and Prevention, Atlanta; [2]Department of Microbiology and Immunology, Emory University, Atlanta; [3]Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia; [4]Medical Institute of Environmental Hygiene, Heinrich-Heine Universitat Dusseldorf, Germany; [5]Instituto Adolfo Luiz, Sao Paulo, SP, Brazil; [6]FSP, University of Sao Paulo, Sao Paulo, SP, Brazil; [7]Department of Microbiology, University of Nevada, Reno.

Genetic approaches have been used to detect and characterize numerous previously unidentified hantaviruses. Puumala/Prospect Hill/Sin Nombre-like viruses or virus variants are present throughout North and South America, Europe and Russia. Several of the American viruses identified are associated with the newly recognized Hantavirus Pulmonary Syndrome (HPS), a severe respiratory illness with high mortality. The genetic relationships of these and previously characterized hantaviruses have been studied by phylogenetic analysis of the nucleotide sequence differences located in PCR fragments amplified from the G2 encoding region of the virus M segments. The relationships observed are consistent with a long-term association of viruses with their primary rodent reservoirs and suggestive of coevolution of host and virus. A Sin Nombre virus isolate is now available and its genetic characterization has been completed. Various virus antigens have been expressed and are being used to probe the interaction of the virus with the host immune system.

J2-033 PROSPECTS FOR IMMUNIZATION TO HANTAVIRUSES. Connie S. Schmaljohn and David J. McClain. Virology Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland, U.S.A. 21702-5011

Hantaviruses cause significant morbidity and mortality throughout the world. More than 200,000 cases of hemorrhagic fever with renal syndrome (HFRS) are reported annually in Asia, Europe and Scandinavia. The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses, with Hantaan virus causing the most severe form of the disease. In 1993, a new hantavirus was discovered in the United States (initially termed Four Corners virus), and was identified as the etiologic agent of hantavirus pulmonary syndrome (HPS). Vaccines for hantaviruses are not readily available, although a number of inactivated viral preparations have been made and tested in Asia. Recurrent problems with inactivated hantaviral vaccines have been lot to lot variability, the need for repeated immunizations, and their inability to elicit long-lasting neutralizing antibody responses in immunized volunteers. Because of such limitations on traditional vaccine development for these viruses, as well as the viruses' hazardous nature and slow, low-titer replication in cell culture, we used a recombinant DNA approach to develop a vaccine for HFRS. Our vaccine is a recombinant vaccinia virus expressing the M segment of Hantaan virus under control of the vaccinia virus 7.5 K promoter and the S segment under control of the 11 K promoter. The M segment, which encodes the G1 and G2 envelope proteins, was included because of our findings that: (1) immunization with vaccinia or baculovirus-expressed G1 and G2 induced a neutralizing and protective immune response in hamsters; and, (2) neutralizing antibodies to G1 or G2 could passively protect hamsters from challenge with virulent virus. The S segment, which encodes the nucleocapsid protein (N), was included because of our finding that hamsters immunized with baculovirus-expressed N also were protected from subsequent infection. Although the protective immune response to N is probably cell-mediated, the importance of such a response is presently not well defined. Assessment of our vaccine in preclinical studies, indicated that immunized hamsters developed neutralizing antibodies and were protected from displaying viral antigen in their lungs after challenge. In a Phase I, dose escalation, clinical study, the vaccine induced neutralizing antibodies in individuals immunized subcutaneously with approximately 10^7 PFU of the recombinant virus. In addition to humoral responses, immunized volunteers developed a cell-mediated immune response as indicated in lymphocyte proliferation assays. Larger clinical studies, including alternate routes or booster immunizations, are planned. Based on these studies, we anticipate that the vaccine will be efficacious for preventing HFRS caused by Hantaan and the antigenically closely related Seoul virus. We are studying the cross-protective properties of this vaccine with more distantly related hantaviruses such as Puumala virus. Although we expect this vaccine to be safe as well as effective, we also are investigating the use of more attenuated pox-viruses as vaccine vectors.

Self-Non-Self and Viruses

J2-034 CROSSREACTIVE SPECIFICITIES AND APOPTOTIC EDITING OF THE VIRUS-INDUCED CYTOTOXIC T LYMPHOCYTE (CTL) RESPONSE, Raymond M. Welsh, Enal S. Razvi, and Liisa K. Selin, Univ. Massachusetts Medical Ctr., Worcester, MA 01655.

Infection of mice with lymphocytic choriomenigitis virus (LCMV) results in a profound expansion in the number of spleen CD8 T cells and in the induction of virus-specific CTL activity. Thereafter, the CD8 T cell number declines, and the CTL activity diminishes, though the frequency of LCMV-specific precursor CTL per CD8 cell, as assessed by limiting dilution assays (LDA), is remarkably stable throughout long-term immunity. The decline in T cell and total spleen leukocyte number at the late stages of acute infection is associated with high levels of apoptosis, as detected by the in situ nucleotidyl transferase assay. Apoptosis occurred in both the T cell and B cell populations, with the B cells dying in clusters. This apoptosis was also seen in transgenic mice ectopically expressing Bcl-2 in the T and B cells and in C57BL/6 *lpr/lpr* mice, which have a mutation in the *fas* gene. T cells from the infected animal underwent apoptosis in vitro when stimulated through the TcR with anti-CD3, thereby explaining some of the immunosuppression seen during acute viral infections. Memory cells persisted for over a year and could be found in blast-size cell populations. Challenge of LCMV-immune mice with either Pichinde virus, vaccinia virus, or murine cytomegalovirus led to the reactivation of the LCMV-specific CTL response. LDA analyses showed unexpectedly that these heterologous viruses crossreacted with subpopulations of LCMV-specific memory T cells. This memory T cell response to virus from an earlier infection was associated with enhanced immunopathology and enhanced clearance of virus during a heterologous virus challenge. Over the course of the acute infection, CTL specific for the second virus were preferentially expanded over the crossreactive CTL, and after the acute infection, when the T cell response had subsided, CTL memory to the first infection had decreased. There is therefore a network of memory T cells which contribute to and are modulated by infections with putatively unrelated viruses, and apoptosis plays a homeostatic role in the course of these T cell responses.

Late Abstracts

IMMUNE RESPONSES TO LIVE ATTENUATED RETROVIRAL VACCINES, R. Paul Johnson*†, Cara Wilson†, Kelledy Manson§, Michael Wyand§, Bruce Walker†, Ronald C. Desrosiers* *New England Regional Primate Research Center, Southborough, MA 01772 †Infectious Disease Unit, Massachusetts General Hospital, Boston, MA 02114 §TSI/Mason, Worcester, MA

Immunization of rhesus macaques with live attenuated retroviruses deleted in *nef* can induce protective immunity against challenge with pathogenic SIV. Development of protective immunity in these vaccinated animals occurs only after several months of infection, with maximal protection observed after one year. The specific immune responses responsible for mediating protection have not been defined, and little is known about the cellular immune responses in animals vaccinated with these live attenuated retroviruses. We have analyzed cellular and humoral immune responses in rhesus macaques and chimpanzees infected with live attenuated retroviruses. SIV-specific neutralizing antibodies were present in vaccinated animals, but did not clearly correlate with protection against challenge. CTL specific for envelope and gag were identified in vaccinated macaques studied 12 or more months after vaccination. Quantitation of SIV-specific CTL activity in one of these animals using limiting dilution analysis revealed a relatively high precursor frequency of cytotoxic T lymphocytes, up to 1/5000 for gag and 1/8500 for envelope. CD8+ lymphocytes obtained from vaccinated macaques were also able to suppress SIV replication in autologous CD4+ cells. Suppression mediated by unstimulated CD8 autologous cells was maximal when cells were in direct contact with SIV-infected lymphocytes, but CD8+ cells activated by an anti-CD3-specific monoclonal antibody were able to release a potent soluble inhibitor of SIV replication. In contrast to the relatively vigorous CTL response present in vaccinated macaques, we were not able to detect consistent CTL activity in chimpanzees infected with a HIV-1 molecular clone (NL43) or attenuated viruses at periods up to one year after infection, despite the use of a variety of stimulation techniques. Proliferative responses to HIV p24 and gp160 were observed in chimpanzees infected with NL43 and attenuated variants. Although the relative contribution of these immune responses to protective immunity is not known, the relative vigor of the cellular immune responses observed in vaccinated macaques suggest they may play a role in mediating resistance to challenge.

HIV-1-specific CTL response and TCR usage in HIV-1 infection

Spyros A. Kalams*, Thomas Harrer*, Ellen Harrer*, R. Paul Johnson, Peter Barbosa¶, Mark Feinberg¶, Susan Buchbinder†, Bruce D. Walker*

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Objectives: To analyze the magnitude and specificity of the CTL response to HIV-1, and to determine the TCR usage by clonal CTL responses in infected persons, including persons with documented infection of up to 15 years with CD4 cells > 500/mm^3.

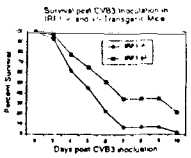
Methods: HIV-1-specific CTL activity was evaluated in PBMC as well as in PBMC stimulated in vitro with HIV-1 infected autologous CD4 cells, using target cells infected with recombinant vaccinia viruses expressing HIV-1 proteins. CTL epitopes recognized by these individuals were determined using cloned effector cells. Quantitative cultures were performed by endpoint dilution, and viral quantitation was determined by QC-PCR. TCR analysis was performed by PCR, using both family-specific primers and anchored PCR, followed by sequencing. Sequence analysis of CTL epitopes in autologous viruses was determined by PCR amplification and sequencing. Clonal frequency was analyzed in PBMC by oligonucleotide probe to the CDR3 region of the TCR.

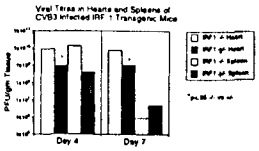
Results: Studies performed in long-term non-progressing persons indicate the presence of a vigorous and broadly directed CTL response. Detailed epitope mapping in a person infected for 15 years, who by QC-PCR had <167 viral RNA molecules per ml and who tested negative by b-DNA assay, revealed CTL clones directed at six different epitopes, with detectable recognition of three of these epitopes using freshly isolated PBMC as effector cells. The CTL clones were broadly reactive against many known HIV-1 variants, and both the p17 and p24 epitopes were cross reactive with SIV. Sequence analysis of autologous virus within the three dominant CTL epitopes revealed the lack of immune escape variants in PBMC, plasma, and virus obtained from coculture supernatant. Analysis of TCR usage by clones to defined epitopes in persons at different disease stages revealed heterogeneity in TCR usage and heterogeneity in recognition of virus variants within these epitopes. Analysis of clonal frequency using an oligonucleotide probe to the CDR3 region of the TCR in an individual with a vigorous response to gp41 suggested that approximately 6% of circulating T cells were a single HIV-1-specific clone.

Conclusions: Our results indicate that cytotoxic T lymphocytes are part of the immune response in persistent non-progressive HIV-1 infection, and that prolonged infection can occur without the development of viral immune escape variation within defined CTL epitopes. In addition, vigorous circulating CTL responses can occur in the setting of an extremely low viral load. Heterogeneity in the ability of clones from different individuals to recognize sequence variation within CTL epitopes may be important in the pathogenesis of infection. CTL to conserved epitopes or CTL which are broadly reactive with multiple viral strains may be important in contributing to maintenance of the asymptomatic state.

New Strategies for Molecular Understanding of Viral Pathogenesis

J2-100 IRF-1 TRANSGENIC MICE SHOW INCREASED SUSCEPTIBILITY TO COXSACKIEVIRAL MYOCARDITIS. Karen Aitken, Joseph Penninger, Tak Mak, Fayez Dawood, Wen-Hu Wen, Tamara Martino, Michael Sole and Peter Liu, The Toronto and Princess Margaret Hospitals, University of Toronto, Canada. The Interferon regulatory factor-1 (IRF-1) gene is essential for the transcriptional activation of inducible nitric oxide synthase (iNOS) in murine macrophages. It also plays an important role in the activation of Interferon and IL-6 and IL-7 receptor genes. To investigate the role of IRF-1 related systems of defense against viral myocarditis, the transgenic mice were innoculated with 10^5 pfu of coxsackie virus B3 (CVB3). Homozygous IRF-1 (-/-) and heterozygous IRF-1 (+/-) mice were randomized into groups to determine heart and spleen viral titres, cardiac pathology and mortality rates at early and late stages of the disease. The mortality was dramatic in the IRF-1 -/- mice. The hearts of the homozygous animals also had increased mononuclear cell infiltration, necrosis and viral replication. *We conclude* that IRF-1 regulated gene products such as iNOS and IFN are essential for the early defense against CVB3-induced myocarditis by attenuating viral replication and inflammatory responses.





J2-101 COXSACKIEVIRUS B3 INFECTION OF IMMUNO-COMPROMISED MICE. Andreas Henke[1,2], Axel Stelzner[2], J. Lindsay Whitton[1]. The Scripps Research Institute, Dept. of Neuropharmacology[1], La Jolla, CA 92037; Institute of Virology, Friedrich Schiller University, 07745 Jena, Germany[2].

Coxsackievirus B3 (CVB3), a member of the Picornavirus family, is a common cause of acute or chronic human myocarditis. However, whether cell damage results primarily from direct virus-mediated effects or from immune-mediated destruction of the heart tissue during the infection remains unclear. We used a cardiovirulent variant of CVB3 which is able to infect several strains of mice. The infection results in a disease which strongly implicates immunopathogenetic mechanisms in myofiber necrosis. To characterize the role of the immune system in this disease we used various transgenic knockout (ko) mice with different immune defects. CVB3 is able to infect normal C57BL/6 mice and immunocompromised (CD4 ko and β2M ko) mice and causes a lethal disease after i.p. injection in a viral dose dependent manner. We found elevated LD50s in immunocompromised mice, which also die slightly later than normal mice. This virus replicates in the hearts of all mice used with maximal titers 3-5 days p.i.. Large pathological changes were detectable only in heart tissue of CD4 ko mice, and were maximal at 1-2 weeks after infection. These results suggest the possibility that $CD4^+$ T cells may limit tissue damage by an as yet undefined mechanism; the roles of $CD4^+$ and $CD8^+$ T cells in this disease are under investigation.

Research supported by a grant of the DAAD, Germany.

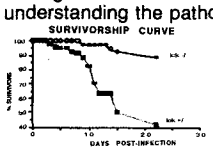
J2-102 EFFECT OF THE ADJUVANT MF59 ON INFLUENZA VACCINATION IN YOUNG AND OLD MICE, Deborah A. Higgins, Karlene Lee-Tung, Julia R. Carlson and Gary Van Nest, Chiron Corporation, Emeryville, CA. 94608

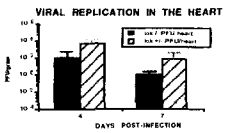
The flu season is a yearly problem, especially in the elderly population. Annual vaccination lacks broad protection and antibody titers induced in the elderly are frequently lower than those acheived by younger vaccinees. Using the mouse model, we are investigating the differences in immune response between old and young mice to influenza vaccine and at the same time testing the ability of a potent adjuvant to boost the response in the the elderly.

Our studies show defects in the immune response of the elderly. Antibody titers after immunization are lower in the elderly for both seronegative and seropositive mice. CMI studies show that helper T-cell response (proliferation) to in-vitro antigen stimulation is also decreased in the elderly. FACS analysis of WBCs show the elderly mice have lower leukocyte and lymphocyte populations, lower CD4 and B-cell populations and a higher proportion of macrophages and CD8 cells than young mice. It was also noted that the young mice had very consistent WBC profiles while the elderly profiles had greater variability. Serum cytokine studies show lower levels of IL2, IL4 and IL5, but higher levels of IL6 and IFNg in the elderly as compared to the young mice.

The addition of MF59 to the immunization increased the antibody titers of both naive and seropositive young and old mice. The helper T-cell response of the young mice was unaffected, while the response of the elderly group was increased. Cytokine profiles show an increase in IL2, IL4 and IL5 levels for both young and old, while IL6 and IFNg levels went up for young animals but remained constant for the elderly mice.

J2-103 MICE LACKING $p56^{lck}$ ARE RESISTANT TO COXSACKIEVIRUS B3 INDUCED HEART DISEASE. Tamara Martino, Karen Aitken, Joseph Penninger, Jano Nikhilanandhan, Tak Mak, Fayez Dawood, Wen-Hu Wen, Lily Wee, Martin Petric, and Peter Liu, The Toronto and Princess Margaret Hospitals, University of Toronto, Canada. Coxsackievirus B3 (CVB3) causes severe heart disease in adult mice, and is a useful model for the human cardiac conditions of myocarditis and dilated cardiomyopathy. In this study, we have demonstrated that transgenic mice lacking the T-cell signalling molecule $p56^{lck}$ are resistant to CVB3 induced heart disease. The virus replicates in the heart, and is cleared from the myocardium at a rate comparable to "normal" littermates, but there is only minimal mortality, or damage to the cardiac tissue. However, in lck-deficient mice depleted of natural killer (NK) cells prior to viral infection, there is increased CVB3 replication, and some infiltration by mononuclear cells in the myocardium. To further elucidate the role of the immune system in CVB3-induced heart disease, cytokine expression in the hearts of CVB3 infected normal, lck-deficient, and NK-cell depleted mice is under investigation. *We conclude* that T-cell activation is critical to produce substantive myofiber necrosis after CVB3 infection, that NK-cells play an fundamental role in protecting the mice from severe virus infection, and that lck transgenic mice serve as an important model for understanding the pathogenic mechanisms involved.





J2-104 CD4+ CTL FROM ß2M-/- MICE FOLLOW SIMILAR KINETICS TO CD8+ CTL FROM NORMAL MICE

Daniel Muller[1,2], Arnor Vikingsson[2], David Hildeman[2], Bjorn Ludviksson[1], and Katrina Pederson[1]. Departments of [1]Medicine and [2]Medical Microbiology and Immunology, Medical School, University of Wisconsin-Madison, Madison, WI 53792

Mice genetically engineered to be deficient in ß2-microglobulin (ß2m-/-) are deficient in functional class I MHC molecules and, in consequence, are deficient in mature CD8+ T cells. Despite this apparent immune deficiency, these mice do not show an increased susceptibility to several viral pathogens. Immunopathologic meningitis after intracranial infection with lymphocytic choriomeningitis virus (LCMV) is mediated by CD8+ cytotoxic T lymphocytes (CTL) in wild-type C57BL/6 mice, and CD4+ CTL in ß2m-/- mice.

We now show that CD4+ T cells from ß2m-/- mice exhibit antigen specific release of serine esterase and interferon-gamma. Further, as quantitated by flow cytometry, the early decrease in CD4+ T cells seen in normal mice 3 days after LCMV infection, previously presumed to be due to the activity of CD8+ CTL, still occurs in ß2m-/- mice. However, ß2m-/- mice show an earlier rise in percentage and absolute numbers of CD4+ T cells by 7 days after infection. CD4+ T cells from ß2m-/- mice rise to above baseline levels with kinetics paralleling the early rise in CD8+ T cells in normal mice after LCMV infection. CD4+ CTL activity is lower in ß2m-/- mice and have a later peak than CD8+ CTL from normal mice. These differences may explain the lower mortality and longer time course of immunopathologic meningitis in ß2m-/- mice.

In conclusion, these data suggest that CD4+ T cells from ß2m-/- mice can assume some of the cytotoxic functions of the CD8+ cell population from normal mice, including immune-mediated pathology. Further, CD4+ T cells from ß2m-/- mice have the capacity to release serine esterase, and show kinetics similar to CD8+ T cells from normal mice.

J2-105 **NORMAL PATTERNS OF LUNG DISEASE AND VIRUS CLEARANCE IN RESPIRATORY SYNCYTIAL VIRUS INFECTED MICE DEFICIENT OF IL-3.**

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Respiratory Syncytial (RS) virus is the most important respiratory pathogen of infants, causing the majority of cases of bronchiolitis, it can be life threatening in very young infants and those with underlying cardiopulmonary disease or immunodeficiencies. T cell immunodeficiency can lead to prolonged infection in children and T cell transfers clear virus in the murine disease model. Mice can be readily infected with RS virus and have proved a valuable model of its pulmonary pathology and the T cell response to it.

IL-3 has a broad spectrum of activities on proliferation and terminal differentiation of early blast cells and committed progenitors of many lineages. Less is known about the effects of IL-3 on T cell development and proliferation and its effect on the host response to infection by common pathogens. The IL-3 gene was disrupted in 129/Sv embryonic stem (ES) cells by homologous recombination after transfection with omega-type constructs flanked with a herpes simplex thymidine kinase gene. These cells were used to produce a mouse strain deficient of IL-3. Mice were infected intranasally with A2 strain RS virus; pathological changes in the lung were monitored by cytology of bronchial lavage, and virus persistence by nested RT-PCR of lavage fluid and lung homogenate. No differences were found in pathology or virus persistence between knockouts and normal mice. IL-3 deficiency is therefore compatible with apparently normal responses to viral infection.

J2-106 CD40 LIGAND HAS POTENT ANTIVIRAL ACTIVITY

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Signalling of B cells via the constitutively expressed CD40 molecule is an essential component of the T cell help required for an antibody response. The interaction between CD40 and its ligand, in the presence of B cell acting cytokines, such as interleukin 4 (IL-4), is sufficient to trigger B cells to proliferate and differentiate and to induce immunoglobulin class switching. A recombinant vaccinia virus encoding both factors, CD40L and IL-4, did not, however, stimulate an enhanced antibody response in mice infected with the construct. Instead, the antibody levels were lower than those of mice infected with a control virus. The reduced antibody response reflected the diminished growth of CD40L-expressing viruses, to the extent that immunocompromised mice were able to resolve these infections. The kinetics of virus clearance indicate that the anti-viral mechanism of CD40L is rapidly activated and has generalized tissue distribution. The CD40L-mediated clearance of virus is independent of the anti-viral cytokines, TNF and IFN-γ. The anti-viral activity of CD40L may represent a surprising and potent effector mechanism of T cells activated during a virus infection.

J2-107 CELLULAR AND HUMORAL IMMUNE RESPONSES TO VIRAL ANTIGENS CREATE BARRIERS TO LUNG-DIRECTED GENE THERAPY WITH RECOMBINANT ADENOVIRUSES.

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Recombinant adenoviruses are an attractive vehicle for gene therapy to lung in the treatment of cystic fibrosis (CF). First generation viruses deleted of E1a and E1b transduce genes into airway epithelial cells *in vivo*, however, expression of the transgene is transient and associated with substantial inflammatory responses, and gene transfer is significantly reduced following a second administration of the virus. In this study, we have used mice deficient in immunological effector functions in combination with adoptive and passive transfer techniques to define antigen-specific cellular and humoral immune responses that underlie these important limitations. Our studies indicate that MHC class I restricted $CD8^+$ cytotoxic T lymphocytes (CTLs) are activated in response to viral antigens leading to destruction of virus infected cells and loss of transgene expression. MHC class II associated presentation of viral antigens activates $CD4^+$ T helper (T_H) cells of the T_{H1} subset and, to a lesser extent, of the T_{H2} subset. $CD4^+$ cell mediated responses are insufficient in the absence of CTLs to completely eliminate transgene containing cells, however, they contribute to the formation of neutralizing antibodies in the airway which block subsequent adenovirus mediated gene transfer. Destructive CTLs are activated to newly synthesized viral proteins while blocking humoral responses are to input viral proteins. Definition of immunological barriers to gene therapy of CF should facilitate the design of rationale strategies to overcome them.

Molecular Aspects of Viral Induced Inflammatory Disease

J2-108 PERSISTENT ASYMPTOMATIC INFECTION OF MICE WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV) AND ESCAPE FROM HOST IMMUNE RESPONSES.

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LDV establishes a life long viremic infection in mice which is not controlled by anti-LDV immune responses. Anti-LDV antibodies are rapidly generated but they neutralize LDV infectivity only poorly. Here we show that LDV-specific cytotoxic T cells (CTLs)are generated within 7 days p.i. CTLs were detected by H-2 specific lysis of LDV-specific target cells. These target cells were generated by transfection of 3T3-NIH cells with a retrovirus vector carrying the gene (ORF 7) for the LDV nucleocapsid (N) protein. Spleen cells from LDV-infected mice were incubated in vitro with LDV-infected macrophages or transfected 3T3-NIH cells and cultured for 5 days in the presence of IL-2. The CTLs had largely disappeared in mice by 30 days p.i. The following experiments were conducted to further explore the mechanism of immune escape. In situ hybridization was used to localize LDV - infected cells in tissues of infected mice. These were found in persistently infected mice only in the liver, testis, and lymphoidal tissues. In addition large amounts of virion or virion debris accumulated in the germinal centers of the spleen and lymph nodes beginning about 3 days p.i. The accumulation of large amounts of LDV antigen in the germinal centers may be causally related to the suppression of the CTL response as well as to the polyclonal activation of B cells associated with LDV infection. To determine whether immune selection plays a role in LDV immune escape, LDV was reisolated from nude and immunocompetent BALB/c mice at various times p.i. The ORFs for the N and the two envelope glycoproteins were RT/PCR amplified and sequenced.

J2-109 ADULT NUDE MICE ARE RELATIVELY RESISTANT TO INFECTION WITH MURINE ROTAVIRUS BUT DO NOT DEVELOP IMMUNITY AFTER A PRIMARY INFECTION,

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The immune mechanisms involved in clearance of and protection from rotavirus infection are poorly understood. Although SCID mice become chronically infected, nude mice clear infection with murine rotavirus.

To confirm the importance of the immune system in clearing rotavirus infection we infected Rag 2 knockout mice with murine rotaviruses. Both knockout pups and adults developed chronic infections.

To determine if a T cell independent antibody could be mediating the clearance of rotavirus infection in nude mice we measured IgG, IgM and IgA in faeces and serum of rotavirus infected mice. A low and transient IgM response was found in serum but not faeces of infected nude mice but not in age matched naive nude mice. The protein specificity of these antibodies is being determined.

To determine if a primary infection in nude mice would induce protection (memory) against a secondary infection, we challenged six week old mice that had been infected as pups with the murine EW strain of rotavirus with the murine Cambridge strain of rotavirus. The previously infected mice, as well as naive nude mice, both shed very low but equal levels of rotavirus in the stools. Interestingly adult naive heterozygous littermates shed more virus than the nudes. The factors responsible for this relative resistance of adult nude mice to rotavirus infection as well as the mechanism for viral clearence in nude mice will be discussed.

J2-110 A SPECIFIC Th1/Th2 CYTOKINE PATTERN IS RAPIDLY ELICITED FOLLOWING INFECTION WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS,

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The role of cytokines in initiating the immune response to Venezuelan Equine Encephalitis (VEE) was investigated. Mice were infected in the left rear foot pad with either cloned virulent VEE (V3000) or an isogenic single-site attenuated VEE mutant (V3032) (single amino acid change at E2 glycoprotein position 209 from glu→lys) and at 1, 6, 24, 48, 72, and 96 hours post-infection, the popliteal lymph nodes and spleen were harvested and examined for TNF-α, IL-12, IFN-γ, IL-2, IL-4, IL-6, and IL-10 gene expression using a quantitative RT-PCR. In both strains elevations of TNF-α, IL-12, IFN-γ, IL-10, and IL-6 were detected in the lymph node, with no changes in either IL-2 or IL-4; however, whereas cytokine elevations were detected by 6 hours after injection of V3000, elevations were delayed until 24 hours following infection with V3032. Cytokine mRNA elevations in the spleen were less substantial, but elevated levels of IFN-γ, IL-10, and IL-12 were also observed. These findings suggest that VEE infection induces an early highly specific cytokine pattern associated with simultaneous elevations in both IFN-γ and IL-10 and that a single amino acid mutation can induce temporal changes in this pattern without alterring either the actual cytokines induced or the degree of their elevation. We are currently performing intervention experiments to investigate the effect of intravenous anti-IL-12 and/or anti-IFN-α/β antibody administration on this early in vivo cytokine response to examine in particular whether these cytokines may be required for the observed elevations in either IFN-γ or IL-10.

J2-111 SEVERE AND PROLONGED INFLAMMATORY RESPONSE RESULTS IN LOCALIZED POXVIRUS INFECTION OF C5-DEFICIENT MICE,

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In addition to intracellular proteins required for viral replication, it is now evident that viruses encode proteins which mediate an active evasion of the host defense (Kotwal and Moss, Nature 1988, 355:176-179; Kotwal et al., UCLA Symp. 1990, 121:149-160). The great majority of the poxvirus encoded proteins actively mediating the evasion of host defense are secretory proteins termed "virokines". Viruses produce proteins which can either compete with or antagonize molecules critically involved in generating immune responses. The vaccinia virus complement control protein (VCP) was the first virokine to be reported that has structural similarity to human/mouse complement control proteins, with the greatest similarity to the human complement 4b binding protein (hC4-BP) and the first protein to have a postulated role in the viral evasion of host defense (Kotwal and Moss, Nature 1988, 355:176-179). Bioactive VCP, purified from serum-free medium has been shown to be more potent than the hC4b-BP (Kotwal, Am. Biotech. Lab., 1994) and has also been shown to bind to two important complement components, C3 and C4, and block the complement cascade at multiple sites *in vitro* (Kotwal et al., Science 1990, 250:827-830; Mckenzie, Kotwal et al., J. Inf. Dis. 1992, 166:1245-1250). The importance of this protein was demonstrated *in vivo* using recombinant virus lacking an intact DNA coding for VCP (Isaacs, Kotwal and Moss, PNAS 1992, 89:628-672) and further underscored by the Smallpox virus genomic sequence analysis, indicating the presence of a highly conserved homolog of VCP (Massung et al., Nature 1993, 366:748-751). In order to determine the precise effects of VCP at the site of infection, a mouse model has been developed. Measurement of the specific swelling response and microscopic examination of the histological changes in BALB/c and DBA/2 mice has revealed that VCP is capable of regulating inflammatory response *in vivo* (Jayaraman and Kotwal, 1993 Mol. Immunol. 30:20). In order to ensure that the possiblity of the unrelated mouse strains sharing identical H-2 haplotypes but differing at numerous other loci was not overlooked, we performed similar experiments in congenic strains. Well characterized C5-deficient mice injected with poxviruses in comparison to normal mice, produce a significant inflammatory response, accompanied with severe ulceration that persists for several days. The data suggests that the host complement response is a critical factor in regulating poxvirus pathogenesis.

J2-112 CYTOKINE PROFILES IN SENDAI VIRUS INFECTION OF GENETICALLY RESISTANT AND SUSCEPTIBLE MICE, X.Y. Mo, Sally Sarawar, and Peter C. Doherty. Department of Immunology, St. Jude Children Research Hospital, 332 N. Lauderdale, P.O.Box 318, Memphis, TN 38101-0318

Previous studies have suggested that immune response was one of the major factors that contributed to the host resistant/susceptible mechanism to Sendai virus infection (Brownstein, 1981). Various immune regulatory molecules were investigated in inbred susceptible 129/J (129) mice and resistant C57BL/6J (B6) mice which share the same MHC haplotype. When they were given 200 EID_{50} of Sendai virus intranasally, 129 mice showed histologically diffuse interstitial pneumonia compared to the local moderate inflammation in B6 mice. Virus was eliminated from the infected lung 3 days earlier in B6 mice than in 129 mice. The cytokine levels and cytokine-producing cells were investigated in the acute pneumonic lung. Also the recall cytokine production in the draining mediastinal lymph node (MLN) were studied. The maximal numbers of IL-2, IFN-γ, IL-10, and IL-4 producing cells were comparable for each strains of mice, while more IL-6 producing cells were present in the lung of 129 mice. However, the numbers of these cytokine producing cells peaked in 129 mice 3 days later than in B6 mice. Analysis of soluble cytokines in bronchoalveolar lavage fluid showed that at least two-fold higher levels of IFN-γ, IL-10, and IL-6 were present in 129 mice than in B6 mice. Recall cytokine production in the draining MLN showed only the presence of IL-2, IFN-γ, IL-10, and IL-6, while no IL-4 was detected. Generally, higher levels of these cytokines were detected throughout the whole infection process in 129 mice. Therefore, the susceptibility of 129 mice to Sendai virus is not due to insufficient cytokine production in these two anatomical sites, yet heavier and prolonged virus burden in susceptible mice may drive the more rigorous and protracted induction of immune regulatory molecules.

J2-113 ROLE OF TNF IN IL-12-INDUCED TOXICITIES DURING VIRAL INFECTION. J.S. Orange, T.P. Salazar-Mather, S.M. Opal, R.L. Spencer*, A.H. Miller#, B.S. McEwen§, and C.A. Biron. Brown University, Providence, RI 02912; *University of Colorado, Boulder, CO 80309; #Emory University, Atlanta, GA 30322; and §Rockefeller University, New York, NY 10021.

In mice infected with lymphocytic choriomeningitis virus (LCMV), interleukin-12 (IL-12) doses of >100 ng/d induce profound immunotoxicities characterized as almost complete inhibition of virus-induced $CD8^+$ T cell expansion and CTL activation, and up to 2 log increases in viral replication [Orange, Wolf, and Biron, *J. Immunol.* 152:1253, 1994]. Serum tumor necrosis factor (TNF) is also observed under these conditions. The studies reported here further characterize the expression and function of TNF in this context. Northern blot and *in situ* hybridization analyses demonstrated that IL-12 induced TNF-α expression and that LCMV infection synergized with IL-12 for this induction. Administration of antibodies neutralizing TNF reversed the IL-12-induced immunotoxicities in LCMV-infected mice and restored anti-viral defenses. The TNF-mediated immunotoxicities appeared to result from an induced cellular sensitivity to the factor, as splenic leukocytes and $CD8^+$ T cells isolated from LCMV-infected mice were more sensitive to TNF-mediated cytotoxicity in culture than were equivalent populations prepared from uninfected mice. Additional physiological changes were observed in IL-12-treated uninfected mice and were dramatically elevated in IL-12-treated virus-infected mice, including: 1) decreases in body weights; 2) elevation of circulating glucocorticoid levels; and 3) decreases in thymic mass. These changes were also reversed by anti-TNF. The results delineate a unique TNF-mediated immunotoxicity and have significant implications concerning detrimental consequences of *in vivo* TNF and/or IL-12 for protective anti-viral responses.

J2-114 ROLE OF CYTOKINES IN VIRAL PATHOGENESIS. I.A. Ramshaw, J. Ruby, D.P. Sharma, A. Ramsay. KH Leong and M Rolph John Curtin School of Medical Research, Australian National University, Canberra Australia

Cytokines play a decisive role in determining the type of immune response elicited by an antigen, both in driving Th cell differentiation along Th1 or Th2 pathway and, ultimately, the effector and regulatory activity of these subsets. We have studied the role of cytokines in virus infections by constructing recombinant virus vectors encoding cytokine genes. During replication in mice, these vectors produce the encoded factor which is secreted from the infected cells with profound immunological consequences. Cytokines studied todate fall into two categories; either altering viral pathogenesis or selectively stimulating specific immune responses. The expression of IL-2, IFN-γ, TNF-α or IL-7 in recombinant vaccinia virus (rVV) dramatically alters pathogenicity, such that immunodeficient mice resolve the normally lethal infection. On the other hand, expression of IL-4 by rVV suppresses the induction of cytotoxic T cells (CTL) inhibiting the clearance of virus which leads to an increase in viral pathogenicity. The expression of IL-4 by rVV was shown to inhibit the host IL-2 response required for the development of CTL's. NO production was also significantly suppressed by IL-4. rVV's expressing IL-5 or IL-6, although not affecting pathogenicity, preferentially stimulates IgA reactivity to coexpressed antigens. Encoding cytokines in recombinant viruses has clearly demonstrated the important role of cytokines as anti-viral effector molecules and in regulating appropriate immune responses.

J2-115 LOSS OF MEMORY B CELL RESPONSE TO CYTOCHROME DURING INFECTION WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS CORRELATES WITH THE PRESENCE OF VIRUS WITHIN THE SPLEEN FOLLICLES, Raymond R.R. Rowland*, Peter G. W. Plagemann, and Ronald Jemmerson. Department of Microbiology University of Minnesota, Minneapolis, 55455. *Department of Biology-Microbiology, South Dakota State University, Brookings, SD, 57007.

Lactate dehydrogenase-elevating virus (LDV), a naturally occurring virus, causes a persistent infection in mice and presents an ideal model for the study of immune modulation during acute and persistent virus infections. Within a few days following infection with LDV there is a pronounced polyclonal activation of B cells followed by the suppression of primary B cell responses to T-dependent Ag. We investigated the effect of acute and persistent LDV infection on the development of a memory B cell response to the model protein antigen, horse cytochrome c (cyt), by employing a modification of the splenic fragment assay. About a 50% decrease in the frequency of responding Ag-specific memory B cells was observed in BALB/c mice infected with LDV, whether the mice were immunized with cyt at the time of LDV infection or three weeks later. This may be due in part to a defect in T cell help, since in cultures of normal memory B cells and T cells derived from LDV acutely-infected mice the frequency of responding B cells was also decreased two-fold. In situ hybridization using a cDNA probe specific for LDV revealed two patterns of LDV RNA within the spleen. Twenty-four hr p.i. LDV RNA was located within the marginal zone, surrounding each follicle. This pattern is consistent with on-going virus replication within permissive macrophages. During persistence viral RNA could no longer be detected in the marginal zone, but was located within the follicles. The absence of LDV-permissive cells within the follicular region suggests that the source of LDV RNA is not due to ongoing viral replication. One possibility is that circulating virus is trapped by a specific cell population within the follicle. The effect of virus trapping within the spleen provides a mechanism by which LDV and other viruses can modulate immune cell function during persistent infections.

J2-116 VIRUS-INDUCED NK CELL TRAFFICKING AND DELIVERY OF CYTOKINES TO SPLENIC MARGINAL ZONES. T.P. Salazar-Mather, R. Ishikawa, and C.A. Biron. Brown University, Providence, RI 02912.

IFN-γ can be produced by activated NK cells. This cytokine enhances immune responses by augmenting macrophage antigen presentation. Viral infection induces IFN-α/β and NK cell activation. Changes in splenic architecture, cell trafficking, and cytokine expression were examined during viral infections of C57BL/6 mice. At times coinciding with IFN-α/β production and NK cell activation, there was a redistribution of nucleated cells from red pulp to white pulp regions in spleens isolated from mice infected with either lymphocytic choriomeningitis virus (LCMV) or murine cytomegalovirus (MCMV). Cell transfer experiments with dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate- or PKH26-GL-labeled bone marrow cells isolated from normal mice demonstrated an infection-induced accumulation of non-T/non-B cell populations along recipient splenic marginal zones. Flow cytometric analyses demonstrated that approximately 10% of the transferred bone marrow cells accumulated in spleens after 20 hrs and 30% of these expressed the NK cell marker, NK1.1+. *In vivo* antibody treatment procedures, to eliminate cell subsets in donor mice, demonstrated that the cells localizing at the marginal zone were derived from AGM1+ and NK1.1+ populations. A small subpopulation of marginal zone cells in infected mice were shown to be expressing high levels of IFN-γ mRNA by *in situ* hybridization. Treatment with anti-AGM1 or anti-NK1.1 antibodies eliminated both endogenous NK cells and the IFN-γ mRNA positive cells. These data demonstrate that newly derived NK cells accumulate along marginal zones. The results also suggest that this trafficking pattern may act to enhance immune responses by facilitating delivery of cytokines to specialized antigen presenting cells.

J2-117 ACTIVATION DEPENDENT RECALL OF A PRODUCTIVE VSV INFECTION IN PERSISTENTLY INFECTED PRIMARY B CELLS, Madelyn R. Schmidt and Robert T. Woodland, Dept. of Molecular Genetics and Microbiology, Univ. of Massachusetts Medical School, Worcester, MA 01655

We have previously shown that primary resting murine B lymphocytes are non-permissive for vesicular stomatitis virus (VSV), however, a productive infection can be induced when infected B cells are activated with anti-immunoglobulin (a-Ig) plus IL-4 or lipopolysaccharide (LPS). We posit VSV in unactivated primary B cells provides a paradigm of persistently infected lymphocytes and activation dependent recall of an active infection. Analysis of the behavior of virus in unstimulated B cells during long term culture and the requirements for subsequent induction of productive infection has been limited by the poor survival of primary cells in culture. We circumvented this limitation by using highly purified small B cells from mice transgenic for the bcl-2 proto-oncogene, expression markedly extends in vitro survival of unstimulated primary B cells. Overexpression of bcl-2 does not alter B cell infection or induction of a productive infection by activators during acute infection. Infection does not effect B cell survival in culture. Unstimulated virus infected B cells produce primary viral mRNAs but not viral proteins or infectious particles (PFU) during culture. Persistently infected B cells stimulated with a-Ig plus IL-4 produced a fully productive VSV infection at all times analyzed, up to 3 weeks post infection. In contrast, VSV production in persistently infected B cells activated with LPS markedly declined relative to acutely infected activated cells (50-100 fold by week 1 and 1,000 fold by week 2). Cells were not completely refractory to LPS activation as VSV protein was produced. The selective LPS deficiency is unique to persistently infected cells as uninfected cultured B cells proliferate and differentiate to produce antibody upon LPS activation. These data show that a persistent infection may selectively alter the host cell response to previously productive activators which may as a consequence interfere with immune regulation.

J2-118 THE ROLE OF CYTOKINES IN MURINE RETROVIRUS INFECTION

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Cytokine expression has been shown to correlate with protective or ineffective immune responses in a number of disease models. Recently there has been the suggestion that immunity to some retroviruses is associated with the production of certain patterns of cytokines. To explore this further we have have used Rauscher murine leukemia virus (R-MuLV) infection of C57BL/6 (resistant) and BALB/c (susceptible) mice to elucidate the role of cytokines immunity to retroviruses.
Initially the *in vitro* proliferation of spleen and lymph node cells from infected mice was examined. In response to stimulation with immobilised anti-CD3 antibodies the proliferation of spleen but not lymph node cells from infected mice was found to be rapidly suppressed. Suceptible BALB/c mice exhibited a much greater suppression than resistant C57BL/6 mice. The cause of this suppression is under investigation however, the immunosuppressive molecules nitric oxide and prostaglandins are not involved.
In vitro cytokine production by spleen and lymph node cells from R-MuLV infected mice was determined. In response to stimulation with immobilised anti-CD3 antibodies, spleen cells from infected BALB/c mice produced diminishing amounts of IFN-γ and IL-2. In contrast spleen cells from infected C57BL/6 mice produced IFN-γ and IL-2 to levels that were only slightly less than uninfected controls. IL-6 production by spleen cells from infected mice of both strains was at levels higher uninfected controls. Anti-CD3 stimulated lymph node cells from infected mice produced elevated IFN-γ suggesting that suppressed cytokine production is spleen specific. Expression of cytokine genes *in vivo* is currently being investigated using RT-PCR to detect cytokine mRNA in the spleens of infected mice.

J2-119 RSV-G GLYCOPROTEIN SPECIFIC T CELLS PREFERENTIALLY SECRETE IL-5 AND PREDISPOSE TO PULMONARY EOSINOPHILLIA., Anon Srikiatkhachorn, and Thomas J. Braciale, The Beirne B. Carter Center for Immunology Research and the Departments of Microbiology, Pathology, and Pediatrics, University of Virginia Health Sciences Center, Charlottesville, VA 22908

We studied the immune responses to two different glycoproteins of respiratory syncytial virus (RSV) in a murine model. BALB/C mice were immunized with recombinant vaccinia virus expressing either RSV-fusion glycoprotein (VAC-F), attachment glycoprotein (VAC-G) or β-galactosidase (as a control). These mice were given RSV intranasally three weeks after priming and then sacrificed 5 or 14 days later. Spleens and bronchial lymph nodes were harvested for *in vitro* culture and lungs were harvested for histologic studies . We found that bulk cultures obtained from both VAC-F and VAC-G immunized animals secreted both Th1 and Th2 type cytokines when stimulated with RSV infected spleen cells . However, the levels of IL-5 and IFN-γ were higher in bulk cultures derived from VAC-G primed animals while the levels of IL-2 were higher in the bulk culture from VAC-F primed animals. The IL-4 and IL-5 production was relatively short lived since spleen cells and bronchial lymph node cells obtaind form mice sacrificed 14 days after intranasal inoculation produced much lower levels of IL-4 and IL-5 while the levels of IL-2 and IFN-γ production were comparable to bulk cultures obtained from mice at the peak of infection. There was little inflammatory response in the lungs obtained from mice immunized with the control vaccinia. In contrast , lungs from mice immunized with VAC-F or VAC-G showed significant infiltration of inflammatory cells. There was a striking infiltration of eosinophils in the lungs from mice primed with VAC-G. These eosinophils could be detected aroud major bronchi and blood vessels, as well as, in some cases, in lung parenchyma. This study suggests that the immune responses to different viral glycoproteins may be distinct and may play important roles in viral pathogenesis.

J2-120 IL-4 and TGF-ß DURING VIRAL INFECTION OF NORMAL AND ß2-MICROGLOBULIN-DEFICIENT (ß2M-/-) MICE. H.C. Su, M.T. Kasaian, L.D. Fast, and C.A. Biron. Brown University, Providence, RI 02912.

During infection of normal mice with lymphocytic choriomeningitis virus (LCMV), NK cell responses peak on day 3 and subside as CD8+ T cell responses are activated at day 7 post-infection. In contrast, ß2M-/- mice, lacking CD8+ T cells, have dramatically elevated NK cell responses on day 7 post-infection. The ß2M-/- response is evidenced by increased NK cell activity, as well as up to 5-fold increases in blast and total NK1.1+CD3- cell numbers. NK cell responses in normal mice are cyclosporin A (CsA)-resistant and interleukin (IL)-2-independent, whereas day 7 NK cell responses in ß2M-/- mice are CsA-sensitive and IL-2-dependent. To investigate the role of additional cytokines in regulating cellular responses during acute viral infections, production and function of IL-4 and transforming growth factor-ß (TGF-ß) were examined. Induction of IL-4 mRNA, at late times post-infection of normal mice, was shown by *in situ* hybridization of T cell-enriched splenic leukocytes and polymerase chain reaction (PCR) amplification of cDNA from RNA. ELISAs of media conditioned with cells isolated on days 0, 3, 5, 7, 9, and 14 post-infection demonstrated delayed induction of IL-4 protein as compared to CTL activation. TGF-ß, evaluated in biological and ELISA assays, was induced maximally at days 7 to 9 post-infection. The kinetics of TGF-ß production by cells from infected ß2M-/- mice was similar to that of normal mice. However, cells from ß2M-/- mice produced IL-4 at early but not at late times post-infection. Together, these results suggest that either IL-4 is a critical cytokine for shutting off NK cells during normal responses to viral infection, or that the ß2M-/- context modulates responsiveness of NK cell subsets to other late cytokines. Studies are in progress to distinguish between these two possible mechanisms.

Molecular Mechanisms of Immune Suppression in Viral Infection

J2-121 CONTROL OF FEVER BY THE VACCINIA VIRUS IL-1β RECEPTOR. Antonio Alcamí, Niall C. Higbee and Geoffrey L. Smith. Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK.

The induction of fever in response to infection is an important host defense mechanism that enhances aspects of the immune response and restricts the replication of some microorganisms. Vaccinia virus, a member of the poxvirus family, is a complex cytoplasmic DNA virus that encodes a variety of proteins that interfere with host immune functions, such as complement regulatory factors and soluble receptors for IL-1β, TNF and IFNγ.

Here we show that expression of the vaccinia virus IL-1β receptor (vIL-1βR) in the WR strain prevents the febrile response and reduces the severity of infection in intranasally inoculated mice. Fever was recorded on days 1-6 after infection of mice with a vIL-1βR deletion mutant, but not in animals infected with wild type WR or a virus revertant. These studies were extended to other virus strains that were used as smallpox vaccines, and expression of the vIL-1βR was consistently found to prevent the onset of fever. Vaccinia virus induced a severe hypothermia after 6 days in infected mice that was independent on vIL-1βR expression and correlated with virus replication in the brain, the organ that controls body temperature.

These results represent the first example of a virus mechanism to inhibit the host febrile response and suggest a central role for soluble IL-1β in the induction of fever in poxvirus infections.

J2-122 MEASLES VIRUS INFECTION IN THE SCID-hu MOUSE: DISRUPTION OF THE HUMAN THYMIC MICROENVIRONMENT, Paul G. Auwaerter*, Hideto Kaneshima+, J. Michael McCune+, Gordon Wiegand* and Diane E. Griffin*, +Systemix, Palo Alto, CA 94304 and *Neurovirology Laboratories, Johns Hopkins University School of Medicine, Baltimore, MD 21093

Measles virus (MV) infection can depress cell-mediated immune responses for months following clinical disease. MV is known to infect the thymus during human illness and this may contribute to immune suppression. We have used the SCID-hu mouse with co-implants of human fetal thymus and liver to determine the effect of virulent and avirulent strains of MV on the thymus. SCID-hu mice were infected by direct inoculation of the graft with 10^3 PFU of either a wild type strain of MV(Chicago-1,Chi-1) or an attenu-ated strain (Moraten, Mor) and sacrificed at intervals over 28 days. Peak viral titers, as judged by plaque assay on Vero cells, were reached by Chi-1 on d4 ($10^{5.7}$ PFU/ third of implant), and Mor on d21 ($10^{3.2}$ PFU/ third of implant). Hematoxylin/eosin stained sections of Chi-1-infected thymuses showed marked distortion of the cortex and medulla by d4 with thymocyte poiknosis and decreased cellularity. By d14, these implants were mostly devoid of normal thymocytes. Mor-infected thymuses showed relatively preserved architecture and cellularity. Suspensions of the cells from implants stained with mAbs to CD3,CD4 and CD8 were analyzed by flow cytometry. There were significant decreases in the CD4+CD8+ cell pop-ulation by d10 with complete loss of all such cells by d28 with Chi-1, and only modest reductions with Mor. Immune fluorescence staining of sections with a MV mAb to hemagluttinin(HA) and Abs for either human cytokera-tins(AE1/AE3) or CD15 co-localized MV predominantly to epithelial and monocytic cells. Additionally, MV antigen was present diffusely by d4 in both cortex and medulla in Chi-1 infection whereas Mor-infected implants had only patchy distribution by d21. Only rare cells stained both with MV HA and CD2 or CD4. MV HA was not expressed over background on any CD4+ cells judged by FACS. We conclude that MV replicates in the SCID-hu thymic implant primarily in epithelial and monocytic cells, and that the attenuated virus reproduces more slowly and with less cellular disruption. Little MV HA could be demonstrated in thymocytes, therefore the data suggest that significant infection of the thymic epithelial stroma disrupts the thymic microenvironment which normally supports and aids in selection of immature T cells. Part of the long-term immune suppression seen in MV infection may be due to infection of the thymic epithelial stroma with subsequent loss of thymocytes.

J2-123 CD4 SURFACE EXPRESSION IS DOWNREGULATED IN A RABBIT T CELL LINE INFECTED WITH MYXOMA VIRUS, Michele Barry[1], Siow Fong Lee[2], Judy Hannon[2], Lynn Boshkov[2] and Grant McFadden[1]. [1]Dept. Biochemistry, U. of Alberta, Edmonton, Alberta, Canada, T6G 2H7, and [2]Dept. of Laboratory Medicine and Pathology, U. of Alberta Hospital, Edmonton, Alberta, Canada.

It is becoming increasingly evident that many poxviruses contain genes that enable the virus to evade the host's immune system. Myxoma virus is a *leporipoxvirus* and is the causative agent of myxomatosis, a rapidly lethal disease in the European rabbit (*Oryctolagus cuniculus*). One possible mechanism of immune evasion is virus-induced downregulation of cell-surface receptors important for an immune response. Cell-surface levels of several receptors on a rabbit T cell lymphoma cell line (RL-5) were monitored by flow cytometry. Following infection with Myxoma virus, cell-surface levels of CD4 were found to drop dramatically. Other cell surface antigens such as CD18, CD43, and CD45 were unaffected during infection with myxoma virus. Further more, the downregulation of CD4 by myxoma virus could be inhibited by treating cells for an extended period of time with PMA, suggesting that the downregulation was not simply a masking of the epitope via viral antigens. Analysis of CD4 levels in the presence of cytosine arabinoside indicates that late gene expression is not necessary for the modulation. Since the tyrosine specific protein kinase $p56^{lck}$ associates with the cytoplasmic domain of CD4 we have also examined the association of $p56^{lck}$ with CD4 as well as steady state levels of $p56^{lck}$ during viral infection. The modulation of surface CD4 has also been described in HIV infected T cells suggesting that the loss of cell-surface CD4 may be a common viral immune evasion tactic by lymphotrophic viruses.

J2-124 DETECTION OF HIV-SPECIFIC CELL MEDIATED CYTOTOXICITY IN THE PERIPHERAL BLOOD OF INFECTED CHILDREN. Florence Buseyne[1], Stéphane Blanche[2], Claude Griscelli[2] and Yves Rivière[1]
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Cytotoxic T lymphocytes (CTL) may play a significant role in containing the spread of HIV in infected individuals. Although HIV-infection is associated with immune suppression, a vigorous CTL response has been detected in infected adults. HIV can be transmitted from mother to child. One third of vertically infected children has a rapid evolution toward disease, with onset of AIDS before 18 months. The other two thirds remain asymptomatic for years. The bimodal course of disease evolution in HIV-infected children could be related to differences in the host immune control of viral replication.

HIV-specific CTL response from fresh and in vitro activated PBMC of HIV-infected children was measured. The vast majority of infected chidren had detectable HIV-specific CTL, which where CD3+CD8+. We previously showed that among children with a slow disease progression, fresh CTL were more frequent in the P2A(paucisymptomatic) group than in the P1(asymptomatic) and the P2B-F groups (symptomatic group).

The cohort of children has now been followed during 4 years, and 46 children have been tested at least once. We found that CTL responses were less frequent in the children with a rapid disease progression than in the children with a slow disease progression at the same age. Our data suggest that CTL response is an important factor in delaying disease evolution.

J2-125 TWO INDEPENDENT LOCI WITHIN THE HUMAN CYTOMEGALOVIRUS US COMPONENT DOWN-REGULATE MHC CLASS I HEAVY CHAIN EXPRESSION, Ann E. Campbell[1], Laura K. Hanson[1], Lei Sun[2], Jacquelyn S. Slater[1], Richard M. Stenberg[1], and Thomas R, Jones[2], [1]Department of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk, VA, 23507 and [2]Molecular Biology Section, Medical Research Division, American Cyanamid Company, Pearl River, NY 10965

Human cytomegalovirus (HCMV) infection results in a significant reduction in MHC class I heavy chain expression both within the cell and at the cell surface. We hypothesized that an HCMV gene which is nonessential for viral growth in tissue culture may be responsible for this phenotype. HCMV recombinant deletion mutants, representing at least 18 nonessential open reading frames, were tested for their ability to down-regulate MHC class I expression by flow cytometry and immunoprecipitation analyses. One of these mutants, RV670, which is deleted from IRS1-US11, lost the ability to down-regulate MHC class I expression. Additional HCMV mutants were constructed to further define the gene(s) involved. Results using these mutants identified two regions within the US component which can independently down-regulate MHC class I expression: US11 and a gene within US2-6. In addition, stably-transfected cell lines expressing either US11 or US2-6 gene products significantly reduced levels of MHC class I heavy chain. Studies are in progress to further define the mechanism by which these viral gene products alter immune recognition.

J2-126 CTL EPITOPES WITHIN THE HUMAN PAPILLOMA VIRUS E6 PROTEIN. Benjamin M. Chain, Liquan Gao , Paul Travers and Hans Stauss. Dept. Immunology, University College Medical School, London. The E6 protein of Human Papilloma Virus type 16 (HPV16) is expressed in a considerable proportion of carcinomas of the cervix, and is a potential target antigen for immunotherapy. To test the immunogenicity of this protein, we used a recombinant vaccinia construct expressing the E6 protein to immunise mice , and then analysed the CTL response. Using a panel of overlapping peptides we identified a single major epitope , contained within the region 131-140, and presented by the H-2K molecule, against which all the CTL activity in B10 ($H\text{-}2^b$) mice was directed. This region of the E6 molecule did not contain the predicted amino acid motif previously identified for this restriction element. Further analysis using sets of mutant peptides identified two key "anchor" residues at positions 131 (Arg) and 140 (Met). Unexpectedly, CTL analysis of lysates of cells expressing E6 which were fractionated using reverse phase HPLC suggested that these cells contained a heterogeneous mixture of peptides which all contained the basic epitope identified by the synthetic peptide studies. These results, in conjunction with molecular modelling studies, suggest that the E6 epitope may bind within the groove of H-2K molecules in a non-conventional way, allowing some variation in peptide length. The E6 molecule contains another region (43-50) which does contain a predicted H-2K binding epitope. A synthetic peptide spanning this region binds strongly to the class I molecule, and can be used to generate CTL in vivo or in vitro. However, these peptide specific CTL fail to recognise target cells expressing E6 protein. The failure to present E6 may lie in the selectivity of peptide transport of class I epitopes into the ER.

J2-127 THE MMTV *SAG* GENE IS REQUIRED FOR VIRAL INFECTION, Tatyana V. Golovkina[1], Jaquelin P. Dudley[2], Aron Jaffe[1] and Susan R. Ross[1], [1]Department of Microbiology/Cancer Center, University of Pennsylvania, Philadelphia, PA 19104; [2]Department of Microbiology, University of Texas at Austin, Austin, TX 78712

MMTV encodes a superantigen (*sag*) gene, whose gene product causes the stimulation of cognate T cells bearing specific β chains of the T cell receptor. We, as well as others, have proposed that Sag function is critical to the ability of milk-borne MMTV to infect mice. To determine whether this is the case, we created transgenic mice (HYB PRO/Cla) with a frameshift mutation int the *sag* gene. Young HYB PRO/Cla mice (< 10 weeks of age) showed no deletion of their cognate Vβ14$^+$ T cells, unlike transgenic mice carrying a functional *sag* gene. However, a slow, progressive loss was seen in the HYB PRO/Cla mice as they aged, indicating that it was due to expression of wild type Sag protein. Thus, as the HYB PRO/Cla mice aged, there was production of virus that appeared to lose the Cla mutation.

The HYB PRO/Cla mice produced transgene RNA in their lactating mammary gland and shed virus in their milk. Their nontransgenic offspring of showed infection with transgene-encoded MMTV because they had the typical slow deletion of Vβ14+ T cells characteristic of C3H MMTV infection and because we detected transgene-derived MMTV RNA in their mammary glands. Cloning and sequencing of the viral RNA produced by the nontransgenic offspring of the HYB PRO/Cla mice showed that recombination between the *Mtv*-1 endogenous viral RNA and the transgene-encoded RNA occurred, such that the frameshift introduced by the Cla mutation was repaired.

These results show that there is selection of infectious virus that contains a functional *sag* gene. Thus, it appears that the only virus that is capable of being transmitted by the milk borne infection pathway is that which encodes a functional Sag protein.

J2-128 PREVENTION OF CLASS I MHC RESTRICTED CTL RECOGNITION BY HEPATITIS C VIRUS, Young S. Hahn[1] and Charles M. Rice[2], [1] Department of Pathology, Beirne Carter Center for Immunology, University of Virginia, Charlottesville, VA 22901, [2]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

Hepatitis C virus (HCV) is an emerging human pathogen. HCV infection in human can result in varied clinical outcomes including the establishment of persistent infection which may lead to the development of hepatocellular carcinoma. Persistent viral infections are increasingly being recognized as a significant cause of human morbidity and mortality. An important aspect of viral persistence involves the evasion of immunological surveillance. To elucidate a potential mechanism involved in immune modulation by the HCV, we have studied whether HCV gene product(s) has the inhibition effect on influenza HA antigen presentation to class I MHC restricted cytotoxic T lymphocytes (CTL). CTL is activated early in viral infection and plays a critical role in the clearance of virus infection by eliminating infected cells.

Two vaccinia recombinant viruses, vHCV1 and vHCV2, were tested for their ability to inhibit the recognition of HA antigen by HA-specific CTL. vHCV1 expresses the structural proteins C, E1, E2 as well as the nonstructural proteins NS2 and a portion of NS3. vHCV2 expresses the nonstructural proteins, a portion of NS2, NS3, NS4A, NS4B, NS5A, NS5B. When HA transfected B cell lymphoma line (YG4) was infected with vHCV1 or vHCV2 or vSC11 as a negative control, cells infected with vHCV1 showed a dramatic reduction of HA recognition by HA-specific CTL in contrast to vHCV2 or vSC11 infected YG4 cells. The level of inhibition was reproducibly in a range of 40% - 60% relative to uninfected YG4 cells. However, vHCV1 did not affect the charging of peptides at the cell surface of antigen presenting cells. It suggests that HCV gene product(s) may interact with proteins involved in the antigen presentation machinery to inhibit the presentation of endogenous antigen to CTL. It implicates that the protein involved in the presentation of CTL recognition may regulate the immune system leading to the establishment of persistent viral infection.

J2-129 MURINE CYTOMEGALOVIRUS MEDIATED INHIBITION OF MACROPHAGE MHC CLASS II EXPRESSION IN SCID MICE, Mark T. Heise, Jessica L. Pollock, and Herbert W. Virgin IV, Center for Immunology and Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110.

The macrophage (Mϕ) is thought to play an important role in human and murine cytomegalovirus (MCMV) infection. We therefore examined Mϕ activation during MCMV infection. Intraperitoneal challenge with 10^3 pfu of MCMV or 10^6 pfu of herpes simplex virus type-1 (HSV) resulted in an inflammatory infiltrate consisting largely of MHC class II positive Mϕ in CB17 SCID mice on day 7 post infection. Increasing doses of MCMV resulted in decreased Mϕ class II expression, with class II expression absent 7 days after infection with 10^6 pfu of MCMV. Limiting dilution analysis demonstrated that fewer than 1:250 Mϕ was productively infected. However, preliminary in situ PCR results suggested that approximately 40% of the peritoneal exudate cells contained MCMV genome. Infection of normal CB17 mice with 10^6 pfu of MCMV induced Mϕ class II expression, suggesting that the specific immune response overcomes the lack of class II. Consistent with this, Mϕ from SCID mice reconstituted with MCMV immune spleen cells and challenged with 10^6 pfu of MCMV, express high levels of class II. The deficiency in class II expression was MCMV specific, since several HSV-1 strains induced Mϕ class II expression. MHC class I and ICAM-1 levels were increased on the class II negative Mϕ, making general inhibition of Mϕ cell surface protein expression unlikely. *In vivo* administration of recombinant mouse IFNγ induced high levels of class II on Mϕ from SCID mice challenged with either HSV or uv inactivated MCMV, but not live MCMV. This suggests that MCMV infection inhibits IFNγ induced Mϕ class II expression in the absence of specific immunity. This inhibition of one component of IFNγ dependent Mϕ activation has implications for the progression of acute MCMV infection and viral latency.

J2-130 DOWNREGULATION OF MHC CLASS I MOLECULES BY HUMAN CYTOMEGALOVIRUS (HCMV): CYTOKINES CONTROL THE ANTIGEN PRESENTATION FUNCTION. Hartmut Hengel[1], Christine Eßlinger[1], Jos Pool[2], Els Goulmy[2] and Ulrich H. Koszinowski[1], [1]Department of Virology, University of Heidelberg, Germany, and [2]Department of Immunohematology and Bloodbank, University Hospital, Leiden, The Netherlands.

Infection with human Cytomegalovirus (HCMV) results in a loss of surface expressed MHC class I molecules. The reduction of surface expressed MHC class I molecules is selective since other surface molecules like CD54 (ICAM-1), CD71 (transferrin receptor) or CD44 (pgp-1) are not affected. Steady state levels of MHC class I complexes and unassembled MHC class I heavy chains are found largely decreased, whereas free $ß_2$-microglobulin remains abundant. To determine the functional consequences of this viral effect, CD8+ cytotoxic T cell (CTL) clones with specificity for defined minor and major histocompatibility antigens were used to monitor antigen presentation in HCMV-infected cells. At the immediate early stage of virus replication the antigen presentation function was intact, but at the early and late phase of the viral replication cycle the presentation of all antigens tested was abolished. Remarkably, interferonγ (IFNγ) and tumor necrosis factorα (TNFα) counteracted the impaired MHC class I complex formation by a strong increase in MHC class I synthesis and assembly. This resulted in a rescue of the peptide and allo-MHC class I presentation. The effect of the cytokines required the exposure of cells to the factors before HCMV infection. Although the cytokines compensated the negative viral effect on the antigen presentation function, the viral interference with MHC class I complex formation was still active. Thus the cytokine effect upon antigen presentation did not act through antiviral activities but was associated with the induction of MHC class I genes of the host.

J2-131 HEPATITIS B VIRUS (HBV)-SPECIFIC T CELL RESPONSE IN CHRONIC HBV INFECTION, Cheng-po Hu, Pei-yun Shu, Ching-hung Shen, Meei-yun Lin, Jaw-ching Wu, and Chungming Chang, Department of Medical Research and Division of Gastroenterology, Veterans General Hospital, and Graduate Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan

Hepatitis B virus (HBV) causes acute and chronic liver diseases and is closely associated with hepatocellular carcinoma. In order to understand the cellular immune response against HBV in chronic HBV infection, T cell proliferation, cytotoxicity and cytokine production were studied. We found that although the majority of asymptomatic HBsAg carriers and patients of chronic hepatitis B (CHB) had no proliferative response to HBsAg, some individuals in both groups showed significant T cell proliferation against HBsAg. In contrast, the proliferative T cell response to HBcAg in asympatomatic HBsAg carriers was significantly stronger than that in patients of CHB with acute exacerbation. In addition, the frequency of HBcAg-reactive T cell precursors measured by limiting dilution assay was much higher in asymptomatic HBsAg carriers than in patients of CHB. Therefore, T cell responses against HBsAg and HBcAg are regulated differently in chronic HBV infection. Furthermore, we demonstrated HBsAg- and HBcAg-specific cytotoxic T lymphocyte (CTL) activity in asymptomatic HBsAg carriers, using autologous HBsAg- and HBcAg-expressing lymphoblastoid cell lines (LCL) as target cells, respectively. The cloned CTL were able to produce IFN-γ, TNF-α or GM-CSF after stimulation. These findings demonstrate that T cell response to HBV is not completely suppressed in asymptomatic HBsAg carriers. Most of them have strong HBcAg-specific response and some of them have HBsAg-specific response.

J2-132 ALTERATION IN LANGERHANS CELL PHENOTYPE FOLLOWING IN VIVO INFECTION WITH THE FLAVIVIRUS WEST NILE. L.J. Johnston, G.M. Halliday* and N.J.C. King, Department of Pathology, Department of Dermatology*, University of Sydney, N.S.W. 2006, Australia.

Flaviviruses are arthropod-borne viruses whose route of infection is via the skin. They are mostly neurotropic and responsible for significant human morbidity and mortality. The classic cell-mediated immune response to a viral infection may be influenced by the ability of these viruses to modify expression of cell-surface molecules involved in the presentation of antigen to, and activation of, T cells. The skin Langerhans cell is the prototypic nonlymphoid dendritic cell and as such is uniquely placed to participate in a response against epidermally-acquired viral infections. The migratory properties of these cells contribute to their role as initiators of T cell-mediated immune responses within the draining lymph node. We have previously shown infection of epidermal cells *in vitro* by the flavivirus West Nile (WNV) results in an increase in MHC class I and II expression on the majority of epidermal cells and Langerhans cells respectively. In this study a technique for infecting the epidermis with WNV *in vivo* was developed. Time-dependent increases in the surface expression of a number of antigens which are involved either directly or in a co-stimulatory capacity in initiating a cell-mediated immune response, were detected on both the majority of epidermal cells and the Langerhans cell population using flow cytometry. These increases were detectable as early as 16 hours after infection. A significant decrease in the percentage of Langerhans cells remaining in the epidermis was observed within 48 hours of infection. The phenotypic changes observed *in vivo* are analogous to those described following *in vitro* culture of Langerhans cells. These results, together with the reduction in Langerhans cell numbers, may represent the *in situ* maturation and concomitant migration of these cells as a consequence of virus-induced cytokines within the skin microenvironment.

J2-133 Transcription and Tax_1 Transactivation of the Human T-Cell Lymphotropic Virus Type I Promoter by an α-Amanitin Resistant Polymerase, Fatah Kashanchi, Graziella Piras, Janet F. Duvall, Michael F. Radonovich, Ales Cvekl* and John N. Brady, Laboratory of Molecular Virology, *Laboratory of Molecular and Developmental Biology, National Institutes of Health, Bethesda, MD 20892

The human T-lymphotropic virus type I (HTLV-I) promoter contains the structural features of a typical RNA polymerase II (pol II) template. The promoter contains a TATA box 30 bp upstream of the transcription initiation site, binding sites for several pol II transcription factors, and long poly A+ RNA is synthesized from the integrated HTLV-I proviral DNA *in vivo*. Consistent with these characteristics, HTLV-I transcription activity was reconstituted *in vitro* using TBP, TFIIA, rTFIIB, rTFIIE, rTFIIF, TFIIH and pol II. In HeLa whole cell extracts, however, the HTLV-I LTR also contains an overlapping transcription unit (OTU). HTLV-I OTU transcription is initiated at the same nucleotide site as the RNA isolated from the HTLV-I-infected cell line, MT-2, but was not inhibited by the presence of α-amanitin at concentrations which inhibited the adenovirus major late pol II promoter (6 μg/ml). HTLV-I transcription was inhibited when higher concentrations of α-amanitin were used (60 μg/ml), in the range of a typical polymerase III (pol III) promoter (VA-I). Purified Tax_1 transactivates this promoter 5- to 10-fold *in vitro*. Interestingly, basal and Tax_1-transactivated transcriptional activity of the HTLV-I LTR could be reconstituted with the 0.5 M phosphocellulose fraction. These observations suggest that the HTLV-I LTR contains overlapping Tax_1-responsive promoters, a typical pol II promoter and a unique pol III promoter which requires a distinct set of transcription factors. Tax_1 further *in vitro* transactivates a polymerase II template containing the 21 base pair repeats cloned upstream of the ovalbumin promoter and G-free cassette. Tax_1-transactivated transcription was concentration dependent and inhibited by low concentrations of α-amanitin.

J2-134 EARLY INTERFERON-INDEPENDENT INDUCTION OF CD54 BY FLAVIVIRUS INFECTION OCCURS IN QUIESCENT BUT NOT PROLIFERATING FIBROBLASTS, Nicholas J. C. King, Jie Shen and *Jannine M. Devery, Department of Pathology, University of Sydney, N.S.W. 2006, Australia and *The Heart Research Institute, Missenden Road, Camperdown, N.S.W., Australia.

Flaviviruses are arthropod-borne, positive, single-stranded RNA viruses which cause a wide variety of illnesses with high morbidity and mortality in humans throughout the world. Their high genomic stability argues for a survival strategy related more to interaction with the vertebrate host immune response, than a dependence on viral genetic mutation. Our previous work has shown that West Nile Virus (WNV) infection of many cell types directly induces functional increases in class I and II MHC expression. We report here that WNV infection of human embryonic fibroblasts (HEF) results in the increased expression of CD54 by two distinct mechanisms. An early, direct cytokine-independent mechanism operates within 2 h of virus infection, while an indirect mechanism, regulated by type 1 interferon (IFN), operates within 24 h of virus infection. CD54 expression increased by 4-5 fold within 2h of WNV infection on HEF, and by 6-7-fold within 24h. WNV-inactivated, conditioned supernatants removed from infected HEF cultures after 4 h incubation did not alter CD54 expression on unstimulated HEF, whereas conditioned supernatants from 24 h-infected cultures increased CD54 expression by about 1.5-2-fold after incubation for 24 h, but not after 4 h, similar to CD54 induction by 200U/ml of IFN-β. Increased CD54 expression on HEF by WNV was also cell-cycle dependent. CD54 increased only in quiescent, contact-inhibited infected HEF in G_0 phase. In contrast, induction of CD54 by types 1 and 2 IFN was not cell-cycle dependent. Other viruses, including double-stranded DNA viruses, vaccinia, and adenovirus 2 and 5, and the single, positive-stranded RNA alphavirus, Semiliki Forest virus, did not induce CD54 expression on HEF after 24 h. Another alphavirus, Ross river, was able to induce CD54 but only by the indirect mechanism of type 1 IFN-dependent release. Poly I.C, also, increased CD54 expression to the same extent as IFN-β after 24 h, making it unlikely that the early increase was due to a nonspecific viral effect. The closely related flavivirus, Kunjin, induced increased CD54 expression in a manner similar to WNV. The ability of flaviviruses to induce increased CD54 expression directly within a few hours of infection may be an important virus-host survival strategy promoting cell-cell adhesion and hence possible further viral infection/replication.

J2-135 IMMUNE ESCAPE IN HIV INFECTION

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Recognition of viral peptides presented on the cell surface in association with class I MHC molecules leads to lysis by cytotoxic T cells (CTL) and forms an important part of the immune response to HIV infection. HIV virus has a high mutation rate and variation in the region of the viral epitope may allow evasion of this immune response. Variation could theoretically affect processing of the antigen, binding of the epitope to the HLA molecule or recognition of the presented epitope on the cell surface. We have studied proviral sequence variation in gag and CTL responses in a number of HLA B8 patients infected with HIV. Amino acid substitutions, such as a lysine to arginine change at position 3 of the P17 gag nonamer GGKKKYKLK, lead to loss of recognition of the peptide by CTL from the patient whose provirus contained this sequence. These variant peptides bind to HLA B8 with comparable affinity to the index peptide suggesting that this loss of recognition is likely to be caused by changes in the interaction between the HLA-peptide complex and the T cell receptor. Other changes, such as lysine to arginine or glutamine at position 7, not only cause loss of recognition, but also lead to inhibition of lysis of targets bearing the index peptide. Thus it appears that in addition to loss of recognition by cytotoxic T cells, naturally occurring epitope variants may act as "antagonists", as has been demonstrated in MHC class II systems. Antagonism may be an important mechanism allowing immune escape by the HIV virus.

J2-136 THE YB-1 DNA-BINDING PROTEIN REPRESSES INTERFERON-γ INDUCED MHC EXPRESSION BY INDUCING SINGLE-STRANDEDNESS IN THE PROMOTER, Gene H. MacDonald, Yoshie Itoh-Lindstrom and Jenny Panyun-Ting, Lineberger Comprehensive Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599

Yb-1 is a member of a newly defined DNA and RNA binding proteins, the Y box factors. These proteins have been shown to affect gene expression at both the transcriptional and translational levels. Recently, we showed that YB-1 represses interferon-γ induced transcription of human major histocompatibility (MHC) genes (Ting et al., 1994, J. Exp. Med., 179;1605-1611). Expression of MHC genes is essential for the development of antigen specific immune responses. In addition to our observations, others have shown that YB-1 can activate expression of reporter genes through HIV and HTLV-I promoters (Kashanchi et. al., 1994, J. Virol., 68; 561-565). YB-1 is a host protein that potentially can be used by viruses to activate viral expression, while repressing IFN-γ induced MHC expression.

We have characterized the DNA binding properties of purified, recombinant YB-1 on the MHC class II DRA promoter. We demonstrate that YB-1 specifically binds single-stranded templates of the DRA promoter with a much greater affinity than the double-stranded template. Most importantly we also demonstrate by Mung Bean Nuclease (MBN) and $KMnO_4$ analyses that YB-1 can induce or stabilize single-stranded regions in the X and Y elements of the DRA promoter. We propose a model of transcriptional repression in which YB-1 binding results in single-stranded regions within the promoter preventing, loading and/or function of other DRA-specific transactivating factors. Currently the mechanism by which YB-1 regulates the HIV and HTLV-I promoters are being investigated, in addition to affects of viral infection on YB-1 expression.

J2-137 EXPRESSION OF HPV-16 E5 PROTEIN IN KERATINOCYTES LEADS TO POST-TRANSCRIPTIONAL LOSS OF MHC-1 AND TAP-1. Dennis J. McCance[1]., Frans V. Cromme[2]., Samuel W. Straight[1]., Grace Tsao[1]., Hidde L. Pleogh[3]., Chris J.L.M. Meijer[2]., Jan M.M. Walboomers[2]. Dept. of Microbiology & Immunology[1], University of Rochester, Rochester, NY 14662, USA, Institute of Pathology[2], Section of Molecular Pathology, Free University Hospital, Amsterdam, Netherlands, Center for Cancer Research[3]. M.I.T., Cambridge, MA, USA.

Viral proteins can be recognized by cytotoxic T-cells when presented at the surface as small peptides bound by major histocompatibility complex class I molecules. These peptides are translocated into the lumen of the endoplasmic reticulum (ER) by the peptide transporter, encoded by the TAP-1 and -2 genes. Subsequent complex formation between peptide, class I and β2-microglobulin in the ER results in stable cell surface expression of the trimeric MHC-1 molecule. In previous studies we showed that in HPV-16 positive cervical carcinomas there was a loss of MHC-1 protein expression, which correlated at the single cell level with loss of TAP protein.

In this study we investigated whether loss of TAP and MHC-1 is mediated by an HPV-16 encoded protein. Human keratinocytes were transfected with various HPV-16 constructs including pAT16, the full length genome, pAT16E5X, the full length genome with a premature stop codon in E5, pUC.ET16, the E6 and E7 oncogenes only, and pKVE5, expressing E5 from mouse Moloney LTR. The different constructs were transfected into primary keratinocytes, cloned cells grown in medium supplemented with and without γ-interferon (γ-IFN) for 48 hours. Cells were harvested and total RNA and protein harvested for Northern and Western blots respectively. Western blots showed very low steady state levels of TAP-1 and MHC-1 heavy chains in the cells with pAT16 as well as those containing E5 alone, which was marginally increased by γ-IFN. In contrast, primary keratinocytes, pAT16E5X and pUC.ET16 lines showed comparable TAP-1 and MHC-1 protein levels, which increased after γ-IFN treatment. Northern blots showed no differences in the amounts of TAP-1 and MHC-1 mRNA between the different cell lines. The data indicate that expression of HPV-16 E5 leads to post-transcriptional loss of MHC-1, presumably by interfering with TAP.

J2-138 **IDENTIFICATION OF ADENOVIRUS E1A REGIONS WHICH AFFECT MHC CLASS I EXPRESSION AND SUSCEPTIBILITY TO CTLs,**
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To map and characterize functional differences between E1A of Ad5 and Ad12, we previously constructed a series of hybrid Ad5/12 E1A genes and used them with Ad12 E1B to transform primary Hooded Lister rat kidney cells. At least two regions within the first exon of Ad12 E1A were identified which influenced tumorigenicity. This study further examines the role of these regions in tumorigenicity by analyzing their affect on cell surface MHC class I expression and sensitivity to class I-restricted CD8+ CTLs as well as to non-class I-restricted NKs. Expression of either of the Ad12 E1A regions implicated in tumorigenicity coincided with down-regulation of cell surface class I expression. However, neither class I down-regulation nor sensitivity to syngeneic NKs or allogeneic CTLs strictly correlated with the tumorigenic capacities of rat cells transformed by hybrid Ad5/12 E1A genes plus Ad12 E1B. We also provide evidence suggesting that CTL epitopes may be encoded in the first exon of Ad5 E1A. These epitopes were encoded by Ad5 E1A sequences which were replaced by Ad12 E1A sequences required for tumorigenicity in the strongly tumorigenic hybrid Ad5/12 E1A plus Ad12 E1B transformants. Expression of Ad5 E1A epitopes was shown to confer susceptibility to syngeneic Ad5 E1-specific CTLs *in vitro*, a finding which may influence the tumorigenicity of Ad5 E1- and Ad12 E1-transformed cells *in vivo*.

J2-139 VACCINIA AND COWPOX VIRUSES ENCODE SOLUBLE IFN-γ RECEPTORS WITH NOVEL BROAD SPECIES SPECIFICITY.

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Vaccinia and cowpox viruses, members of the poxvirus family, are complex cytoplasmic DNA viruses that encode a variety of proteins that interfere with host immune functions, such as complement regulatory factors and soluble cytokine receptors.

Soluble receptors for IFN-γ were found to be secreted from cells infected by 17 orthopoxviruses including vaccinia, cowpox, rabbitpox, buffalopox, elephantpox and camelpox viruses. The B8R open reading frame of vaccinia virus strain WR, which has similarity to the extracellular binding domain of the cellular IFN-γ receptor, is shown to encode the IFN-γ binding activity by expression in the baculovirus system. The virus soluble IFN-γ receptors bind IFN-γ and, by preventing its interaction with the cellular receptor, interfere with the antiviral effects induced by this cytokine.

In contrast to cellular IFN-γ receptors, which are highly species specific, the virus IFN-γ receptors possess a broad species specificity. The vaccinia and cowpox virus IFN-γ receptors bind human, bovine and rat IFN-γ, but not mouse IFN-γ. This novel broad species specificity would aid virus replication in different species and suggests that vaccinia and cowpox viruses may have evolved in several species, possibly including humans but excluding mice. Lastly, the conservation of an IFN-γ receptor in all orthopoxviruses tested emphasises the importance of IFN-γ in defence against poxvirus infections.

J2-140 THE ROLE OF ADENOVIRUS EARLY REGION PROTEINS IN THE PATHOGENESIS OF ADENOVIRUS INFECTIONS.

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Human adenoviruses (Ad) establish asymptomatic, persistent infections in normal individuals. Our laboratory is investigating the different mechanisms by which Ads avoid triggering an immune/inflammatory response. The E3 region has been shown to alter both innate (TNF) and the specific responses (CTL). *In vitro* the E3 proteins 10.4K/14.5K and 14.7K provide protection against TNF induced activation of phospholipase A_2 thus blocking apoptosis. To examine the role of these proteins *in vivo*, B10 mice were inoculated intranasally with deletion mutants that lacked either 10.4K or 14.7K or both and the pulmonary pathogenesis was scored. When both 10.4K and 14.7K are deleted there is a marked increase in alveolar pathogenesis that parallels the *in vitro* protection of virally infected cells against TNF cytolysis. The specific immune response is altered by another E3 protein, gp19K. Gp19K prevents CTL recognition *in vitro* and was expected to alter the cellular infiltration caused by CTLs and perhaps even prevent *in vivo* CTL priming. Surprisingly, when B10 mice where immunized with a mutant lacking gp19K, there was no change in the pathology or in CTL generation. To further test the role of CTL in Ad pathogenesis, viruses lacking the CTL epitopes were tested. When mutants that lack the immunodominate CTL epitope in E1A where used, a second immunorecessive epitope in E1B becomes the predominate target of CTLs. These findings are important since human Ad is currently being tested as a vector for gene therapy of cystic fibrosis. Our data suggest that when constructing Ad vectors to be used for gene therapy, one must retain either the 10.4K or 14.7K genes to decrease pathology and that deleting the genes that encode the antigens that are recognized by CTLs does not prevent the generation of Ad specific CTLs.

J2-141 STRUCTURE-FUNCTION ANALYSIS OF THE EPSTEIN-BARR VIRUS INTERLEUKIN-10 HOMOLOGUE.

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The BCRF1 open reading frame of Epstein-Barr virus exhibits remarkable sequence homology with the coding sequences of interleukin-10 from a variety of organisms. Many of the numerous immunological properties ascribed to interleukin-10 are shared by the product of BCRF1 and this has led to it being termed viral interleukin-10.

In order to investigate the activity of viral interleukin-10 (vIL-10) and its interactions with the human interleukin-10 receptor we have expressed the protein in a bacterial and the eukaryotic COS-7 expression systems. The bacterially expressed vIL-10 was partially purified and used to set up two assays to measure IL-10 activity: i)the increase in IgM secretion from an EBV transformed B cell line - MT4.L and ii)the downregulation of class II HLA expression on the human monocytic cell line THP-1.

A series of deletion mutants (both N- and C- terminal as well as an internal deletion to remove a putative heparin binding domain) were constructed to identify possible domains within the vIL-10 protein that interact with the hIL-10 receptor and confer its biological activity. A number of these mutants have been expressed in the COS-7 expression system and their structure and biological activity are currently being assessed.

The identification of the domains within vIL-10 that interact with the receptor or accessory proteins may aid in the understanding of the possible role of vIL-10 within the EBV life cycle and in the pathogenesis of the numerous diseases associated with the virus.

J2-142 **VACCINIA VIRUS ENCODES A NOVEL SOLUBLE TYPE I INTERFERON (IFN) RECEPTOR UNRELATED TO EXISTING IFN CELL SURFACE RECEPTORS**

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The interferons (IFNs) are a family of cytokines whose functions include the protection of cells against viral infection. Type I IFNs include the 15 IFNα subtypes and IFNβ that compete for binding to the same cell surface receptor, while type II IFN (IFNγ) binds to a different receptor. The orthopoxviruses, of which vaccinia virus (VV) is the prototypic member, have developed a number of anti-IFN strategies. The VV E3L protein competitively binds dsRNA and prevents the activation of IFN-induced and dsRNA-activated protein kinase (PKR), while the VV K3L protein shows sequence similarity to the eukaryotic initiation factor 2α (eIF2α) that is phosphorylated and inactivated by PKR. The K3L protein competitively binds the kinase and blocks host eIF2α phosphorylation and hence IFN-induced inhibition of host protein synthesis. Orthopoxviruses also suppress cytokine action by expressing soluble cytokine receptors that bind and sequester the ligand; to date soluble receptors for interleukin-1β, tumour necrosis factor and IFNγ have been described. Supernatants from VV-infected cells were found to contain a soluble inhibitor of type I IFN that was conserved in most of the orthopoxviruses tested. The inhibitor was produced early in infection and did not inhibit IFNγ. The IFNα/β inhibitor was mapped and the gene expressed from recombinant baculovirus. The inhibitor blocked the binding of ^{125}I-IFNα to U937 cells and binding of ^{125}I-IFNα to supernatants from baculovirus and VV-infected cells demonstrated that the inhibitor functioned as a soluble receptor for IFNα/β. Direct binding of ^{125}I-IFNα to VV WR supernatants revealed that the soluble IFNα/β receptor had a high affinity for type I IFN. Deletion of the gene from the VV genome and ligand blotting of the soluble receptor demonstrated that IFN binding was encoded by a single protein. Competitive binding curves using IFNα from other species revealed that the poxvirus soluble IFNα/β receptor bound human and bovine IFN with high affinity but murine IFN with relatively low affinity. Interestingly, the soluble IFNα/β receptor is highly conserved in variola virus. Given the importance of IFN in antiviral defense it is likely that the soluble IFNα/β receptor plays an important role in the virulence of the orthopoxviruses.

Antigen Processing and Presentation in Viral Infections

J2-200 CELLULAR LOCALIZATION OF THE COMPARTMENTS INVOLVED IN ENDOGENOUS PROCESSING OF A VIRAL GLYCOPROTEIN FOR CLASS II MHC PRESENTATION, Shirley M. Bartido[1], Miriam Stein[1], Stephanie Diment[3,4], and Carol S. Reiss[1,2,3], Dept of Biology[1], Center for Neural Science[2], New York University, New York NY 10003, Kaplan Comprehensive Cancer Center[3] and Pathology Dept[4], New York University Medical Center, New York NY 10016

Endogenous processing of a viral glycoprotein for presentation to $CD4^+$ T cells has defined a previously under-investigated pathway in antigen processing and presentation. It may be important not only for pathogens, but also for self-proteins, and thus may be involved in self-tolerance.

We have been characterizing the processing of the ER-restricted Gpt glycoprotein of vesicular stomatitis virus (VSV) biochemically and enzymatically, by cellular localization using confocal immunofluorescence, cellular fractionation, and by T cell recognition assays.

By flow cytometry, Gpt is undetected on the plasma membrane; in contrast, the wild type protein (G) is readily found following infection of A20 cells with a vaccinia virus vector, leading to endogenous synthesis.

The Gpt can be found exclusively in the ER compartment using co-localization with markers for ER (signal peptide binding protein, calnexin), and not in the Golgi compartment (α-mannosidase II, wheat germ agglutinin), endosome, lysosome, or surface plasma membrane. This is consistent with the characteristics of the localization of the proteases which appear to be responsible for its degradation.

Work is in progress to localize the site of peptide binding to MHC heterodimers.

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J2-201 IDENTIFICATION OT T CELL RECEPTOR RECOGNITION RESIDUES FOR AN INFLUENZA VIRUS NONAMER PEPTIDE EPITOPE PRESENTED BY HLA B27, Paul Bowness and Andrew J. McMichael, Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, U.K.

The fine specificity of T cell recognition of peptide analogues of the influenza nucleoprotein epitope NP 383-391 SRYWAIRTR was studied using HLA B27-restricted influenza-specific cytotoxic T cell (CTL) clones, of defined T cell receptor (TcR) usage, derived from unrelated individuals following natural infection.

Synthetic analogue peptides were synthesized containing single amino acid substitutions, and tested both for binding to HLA B*2705 *in vitro*, and for presentation to CTL clones by HLA B27-positive targets. Even conservative amino acid substitutions of the peptide residues P 4, 7, and 8 profoundly influenced CTL recognition, without affecting binding to HLA B*2705. These amino acid side chains are thus probably directly contacted by the TcR. CTL clones which used the TcR Vα14 gene segment (but not those using TcR Vα12) were also sensitive to P1 substitutions, suggesting that the TcR alpha chain of these clones lies over the N terminus of bound peptide, and that the "footprint" of certain TcRs can span all exposed residues of a peptide bound to MHC class 1.

These results, taken together with previous structural and functional data, suggest that, for nonamer peptides bound to HLA B27, P1, P4 and P8 are "flag" residues with TcR accessible side chains.

J2-202 PRESENTATION OF AN OUT-OF-FRAME CLASS I RESTRICTED EPITOPE. T.N.J.Bullock and L.C.Eisenlohr, Department of Immunology, Thomas Jefferson University, Philadelphia, PA 19107.

Antigen presentation by class I MHC molecules is thought to require the degradation of fully formed proteins in the cytosol. This degradative process supplies oligopeptide epitopes for transport into the endoplasmic reticulum (ER) where they can interact with and stabilize class I molecules. Stable class I molecules, associated with β2-microglobulin, can then proceed to the cell surface where they present the epitopes to T cell receptors.

The generally accepted model for protein translation, the scanning hypothesis proposed by Kozak, is thought to describe the traditional method of translation for the majority of proteins. We wished to test the hypothesis that any internal methionine that is in good translation initiation context can be a source of short peptides, which may then be processed into class I epitopes.

We have created a frameshift mutation in the influenza PR8 nucleoprotein gene (NP), the target of the CTL response of several inbred mouse strains. NP contains three class I restricted epitopes at amino acids 50-57 (H2-K^k), 147-155 (H2-K^d) and 366-374 (H2-D^b). The frameshift was introduced 26 amino acids upstream of the H2-K^d epitope. The mutated genes were then recombined with vaccinia virus and tested for presentation using CTL restricted to each of the epitopes described above. We found that, whilst presentation of the H2-K^k epitope was unaffected by the frame shift, the epitope proximal to the frameshift (H2-K^d) was no longer presented to appropriately restricted CTL. However, presentation of the distal H2-D^b epitope was retained. Therefore we have shown, using a viral protein and a viral expression system, that out-of-frame epitopes can be processed and presented to CTL. Work is ongoing to confirm that internal methionines are capable of providing a platform for the initiation of translation for in-frame and out-of-frame epitopes.

J2-203 CONSERVED CYSTEINE RESIDUES WITHIN THE E3/19K PROTEIN OF ADENOVIRUS TYPE 2 ARE ESSENTIAL FOR BINDING TO MHC ANTIGENS, Hans-Gerhard Burgert and Martina Sester, Spemann Laboratorium, Max-Planck-Institut für Immunbiologie, D-79108 Freiburg, Germany

The E3/19K protein of human adenovirus type 2 (Ad2) is a resident transmembrane glycoprotein of the endoplasmic reticulum. Its capacity to associate with class I histocompatibility (MHC) antigens abrogates cell surface expression and the antigen presentation function of MHC antigens. At present, it is unclear exactly which structure of the E3/19K protein mediates binding to MHC molecules. Apart from a stretch of approximately 20 conserved amino acids in front of the transmembrane segment, E3/19K molecules from different adenovirus subgroups (B and C) share little homology. Remarkably, the majority of cysteines is conserved. In this report, we examined the importance of cysteine residues (Cys) for structure and function of the Ad2 E3/19K protein. We show that E3/19K contains intramolecular disulfide bonds. By using site-directed mutagenesis, individual cysteines were substituted by serines and alanines, and mutant proteins were stably expressed in 293 cells. Based on the differential binding of monoclonal antibody Tw1.3 and cyanogen bromide cleavage experiments, a structural model of E3/19K is proposed, in which Cys 11 and Cys 28 as well as Cys 22 and Cys 83 are linked by disulfide bonds. Both disulfide bonds (all four cysteines) are absolutely critical for the interaction with human MHC antigens. This was demonstrated by three criteria: loss of E3/19K coprecipitation, lack of transport inhibition and normal cell surface expression of MHC molecules in cells expressing mutant E3/19K molecules. Mutation of the three other cysteines at position 101, 109 and 122 had no effect. This indicates that a conformational determinant based on two disulfide bonds is crucial for the function of the E3/19K molecule, namely, to bind and to inhibit transport of MHC antigens.

J2-204 THE EFFECT OF ACUTE LCMV INFECTION ON THE IMMUNE RESPONSE OF MICE TRANSGENIC FOR A K^b/OVALBUMIN 257-264-REACTIVE T-CELL RECEPTOR, Eric A. Butz, Francis R. Carbone, and Michael J. Bevan, Howard Hughes Medical Research Institute, University of Washington, Seattle, WA, 98103.

Acute infection of adult mice by lymphocytic choriomeningitis virus (LCMV) is known to cause a profound transient suppression of immune responses to coinfecting agents and of in vitro responses to T- and B-cell mitogens. OVA-tcr-I mice are transgenic for a T-cell receptor specific for a chicken ovalbumin peptide (SIINFEKL) in the context of H-2 K^b. Most of the T-cells in these mice are $CD4^-CD8^+$, and of these, greater than 90% express the transgenic receptor. We have examined the immune responses of OVA-tcr-I mice to acute LCMV infection. T-cells baring the transgenic receptor do not recognize the antigens presented by LCMV-infected target cells, but these mice mount weak anti-LCMV CTL responses. Following LCMV infection there is an augmentation of the anti-SIINFEKL lytic response by spleen cells from OVA-naive OVA-tcr-I mice, but the proliferative response of these cells to the same peptide is reduced.

J2-205 THE $CD8^+$ T-CELL RESPONSE TO THE IMMUNODOMINANT SENDAI VIRUS $NP_{324-332}/K^b$ EPITOPE IS EXTREMELY DIVERSE IN TERMS OF TCR REPERTOIRE AND FINE SPECIFICITY, Geoffrey A.Cole, Twala L. Hogg, and David L. Woodland, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105

The CTL response to Sendai virus in C57BL/6 mice is directed almost exclusively to a single H-$2K^b$-restricted epitope derived from the virus nucleoprotein, $NP_{324-332}$ (SEV-9). Analysis of 18 independent T cell hybridomas generated from C57BL/6 mice following primary Sendai virus infection has shown that a very diverse repertoire of TCR is selected in response to this epitope. Crystallographic analysis of SEV-9 bound to K^b has shown that the side chains of peptide residues Phe^{P1}, Gly^{P4}, Asn^{P5}, and Ala^{P8} protrude towards the solvent and are potentially available for recognition by the TCR. Notably, residues Gly^{P4} and Asn^{P5} protrude prominently from the peptide binding site due to their localization on a bulge in the center of SEV-9. To determine the importance of each of these residues for T cell recognition, we analyzed hybridoma responses to SEV-9 analogs substituted at each of these four positions. Preliminary data showed there generally appeared to be dominant recognition of Gly^{P4} and Asn^{P5}. However, individual hybridomas exhibited distinct patterns of fine specificity for residues Phe^{P1} and Ala^{P8}. Thus, individual hybridomas were dependent on one, both, or neither of these residues for recognition of SEV-9. These data are consistent with a critical role for the Gly^{P4} and Asn^{P5} in governing TCR-SEV-9/K^b recognition and suggest a structural basis for the diversity of the TCR repertoire selected by this epitope.

J2-206 VACCINE STRATEGIES FOR PREVENTION OF CMV INFECTIONS IN IMMUNOCOMPROMISED ADULTS, Don J. Diamond[a], Takuya Tsunoda, Joanne York, Christine Wright, John A. Zaia[b], and Stephen J. Forman, Departments of Hematology and BMT, and Pediatrics[b], and the Division of Immunology[a], City of Hope National Medical Center and the [a]Beckman Research Institute, Duarte, CA 91010

Previous studies have suggested that several abundant CMV proteins are major immunogenic targets in seropositive adults. We are interested in defining the major viral protein targets of a $CD8^+$ CTL response, in order to derive a vaccine strategy for individuals who are unable to mount immune responses which are lymphokine-dependent because of immunosuppression. HLA-typed and CMV-positive normal volunteers who have HLA-A alleles that represent ~75% of the U.S. population are being tested to determine which of 5 abundant CMV proteins they recognize by a $CD8^+$ CTL response: p28, p65, p150, IE, and gB. T cell lines will be derived in order to unambiguously determine the HLA restriction of the $CD8^+$ CTL response to each of these proteins. Proteins which are recognized by the most HLA diverse population will be further characterized in terms of mapping of Class I epitopes through the use of T cell clones derived from the polyclonal cell lines by limiting dilution. The defined epitopes will form the basis of a vaccine strategy to augment the memory responses of seropositive volunteers against CMV. These epitopes will be used to boost the CTL precursor frequency of bone marrow transplant donors as a means to transfer cellular immunity to immunosuppressed hematologic transplant recipients.

An alternative strategy is to immunize seropositive individuals with recombinant viral proteins as a means to boost immunologic memory. We are pursuing that strategy in a transgenic murine model of HLA-A2.1 developed by Dr. L. Sherman (Scripps Institute, La Jolla). We are vaccinating the transgenic mice with two well defined CMV proteins, p65 and gB together with either of two lipid-based adjuvants, commercially available DOTAP™ (Boehringer-Mannheim) or MF59™ (Chiron, Emeryville, CA). Our preliminary studies with HSV-2 gB demonstrate that both adjuvants are effective at eliciting murine Class I restricted responses against the protein. Current studies are evaluating the recognition properties of the adjuvant- CMV protein complexes by HLA-A2 as a restriction element in the transgenic model.

J2-207 DEFINITION OF A HUMAN T CELL EPITOPE FROM INFLUENZA A NON-STRUCTURAL PROTEIN 1 USING HLA-A2.1 TRANSGENIC MICE, Victor H. Engelhard, Stephen Man, Michael H. Newberg, Victoria L. Crotzer, C. John Luckey, Noelle S. Williams, Ye Chen, Eric L. Huczko, and John P. Ridge. Beirne Carter Center for Immunology Research and Department of Microbiology, University of Virginia, Charlottesville VA 22908

Previous results from this laboratory demonstrated that the dominant influenza A epitope recognized by HLA-A2.1 restricted CTL from HLA-A2.1 transgenic mice was the M1 peptide epitope that is immunodominant in human CTL responses. However, analysis of a large number of CTL lines revealed a subset of influenza A/PR/8/34-specific murine CTL that recognized an HLA-A2.1 restricted epitope distinct from M1. Using recombinant vaccinia viruses encoding different influenza gene segments, the epitope recognized by these CTL was shown to be derived from the A/PR/8 NS1 protein. Because these CTL did not recognize targets infected with the A/Alaska/6/77 strain of influenza, candidate peptide epitopes were synthesized based on sequences that included an HLA-A2.1 specific binding motif and that differed between A/PR/8 and A/Alaska. All of these CTL recognized a nonamer and a decamer peptide which contained a common 8 amino acid sequence and two distinct sets of binding motif residues. However, the nonamer peptide was able to sensitize CTL for half maximal lysis at 80-2500 fold lower doses than either the octamer or decamer. The homologous peptide derived from A/Alaska NS1 contained conservative amino acid changes at positions 4 and 8 and was not recognized at any tested concentration, although it bound with higher affinity to HLA-A2.1 than the peptide from A/PR/8. The A/PR/8 NS1 nonamer epitope was also recognized by human influenza A specific CTL derived from two individuals. These results substantiate the general utility of HLA class I transgenic mice for the identification of human CTL epitopes for other pathogens.

J2-208 PROCESSING, PRESENTATION AND RECOGNITION OF A VIRAL CTL EPITOPE EXPRESSED IN A CELLULAR PROTEIN, [1]T-M. Fu, [2]S. Alam, [1]M. J. Tevethia, [2]K. Verner, and [1]S. S. Tevethia, [1]Dept. of Microbiology and Immunology and [2]Dept. of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

CD8+ cytotoxic T-lymphocytes recognize small epitope peptides in complex with MHC class I molecules on the cell surface. In this study, we have attempted to determine whether a viral CTL epitope, when expressed in a cellular protein, can be appropriately processed and presented and recognized by the CTL. An *H-2K^b*-restricted CTL epitope from herpes simplex virus type 1 (HSV-1) glycoprotein B (gB epitope, residues 498-505, SSIEFARL) was cloned into the mouse dihydrofolate reductase protein (DHFR) at amino acid position 87. The recombinant DHFR was expressed through a vaccinia virus recombinant. To distinguish the recombinant DHFR proteins from the endogenous DHFR, an antibody epitope (recognized by monoclonal antibody PAb901), derived from SV40 T antigen residues 684-698, was tagged to the C-termini of recombinant DHFR proteins. The in vivo expression of recombinant DHFR was assessed by immunoprecipitation with the monoclonal antibody PAb 901. The *H-2^b* cells infected with recombinant vaccinia virus expressing the recombinant DHFR were specifically lysed by gB epitope-specific CTLs. Furthermore, the recombinant DHFR was functional in the induction of gB epitope-specific CTL response upon immunization of C57Bl/6 mice. These results indicate that an viral epitope expressed in a cellular protein can be efficiently processed, presented and recognized by epitope-specific CTL, and suggest that the cellular proteins can be used to express CTL epitopes for induction of CD8+ immune responses.

J2-209 PROMINENT ROLE OF AUXILLIARY RESIDUES SIDE CHAIN IN CONTROLING PRESENTATION OF LCMV H-2D^b VIRAL PEPTIDES: IMPLICATION FOR THE PREDICTION OF MHC-RESTRICTED CTL EPITOPES. Jean Edouard Gairin[1,2], Denis Hudrisier[1], Honoré Mazarguil[1] and Michael B. A. Oldstone[2]. [1]Laboratoire de Pharmacologie et Toxicologie Fondamentales, CNRS, 31400 Toulouse, France and [2]Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, CA92037, USA.

Virus infected cells present only a very limited number of peptides intracellularly processed from a viral protein to CTL even when many peptides bearing the MHC class I-restricted binding motif are present in the protein. Infection of H-2^b mice with lymphocytic choriomeningitis virus (LCMV) induces a CD8$^+$ CTL response directed against three well-characterized epitopes presented by H-2D^b molecules: NP396-404 (FQPQ-NGQFI), GP33-43 (KAVYNFATCGI) and GP276-286 (SGVEN-PGGYCL). The H-2D^b motif is characterized by a sequence of 9 to 11 a.a. with two anchor residues: Asn at position 5 and hydrophobic (Met, Ile, Leu) at the C-terminus. The LCMV NP and GP proteins contain thirty-one other peptides exhibiting the D^b motif. However, no CTL response against one (or more) of these peptides has been characterized. Peptide binding to MHC is a critical step in antigen presentation. The aim of this study was therefore to analyze the binding properties of the potential D^b LCMV peptides. The 34 LCMV peptides and 11 known D^b-selective peptides were synthesized and their MHC binding affinities measured in two D^b-specific binding assays. Most of the LCMV peptides (28/34) did not bind to D^b. The other 6 (including the 3 epitopes) and all the known D^b peptides showed good affinity. Comparison of the sequences (good vs. non binders) allowed the identification of auxilliary anchors required for high binding affinity or of negative elements hampering MHC binding. In addition to the main anchors, the positive and negative factors at secondary residues play a crucial role in governing peptide-MHC interactions. Knowledge of such factors might be of importance for the prediction of MHC-restricted CTL epitopes.

J2-210 CHARACTERIZATION OF ANTIGEN PRESENTING CELLS FROM MEDIASTINAL LYMPH NODES AND LUNGS OF INFLUENZA-INFECTED MICE, Ann Marie Hamilton-Easton and Maryna Eichelberger, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105

To understand the requirements necessary to initiate a protective immune response to viral infection, it is important to identify the principal antigen presenting cell (APC) in a primary influenza infection. As with other mucosal antigens, it is believed that priming of naive T cells occurs in the draining lymph nodes. Kinetics experiments demonstrated the presence of influenza virus in the mediastinal lymph nodes (MLN) of C57BL/6 mice at 2 days post-intranasal infection with X31, an influenza A virus. Virus-specific cytotoxic T lymphocytes (CTL) were generated a day later at this site. To determine which APC was capable of stimulating virus-specific CTL precursors in the MLN, B, T and dendritic cells from the MLN of influenza-infected mice were separated and examined for the presence of virus. The predominant cell type which contained infectious virus was the dendritic cell. B and T cells from the MLN contained little, if any, virus. The APC capacity of these populations was tested by their ability to stimulate virus-specific T cell hybridomas. Only dendritic cells from the MLN of influenza-infected mice were able to stimulate virus-specific T cell hybridomas, although all APC populations from both naive and influenza-infected mice were effective stimulators after in vitro pulsing with the appropriate influenza peptide. Potential APC populations were also separated from the lung. Virus was detected in bronchioalveolar macrophages and dendritic cells but not B or T cells. Both macrophages and dendritic cells isolated from influenza-infected lungs could stimulate virus-specific T cell hybridomas. The ability of the MLN and lung APC populations to stimulate naive CD8$^+$ T cells and generate virus-specific CTL is currently being examined.

J2-211 RECOGNITION OF CARBOHYDRATE BY MHC CLASS I RESTRICTED, GLYCOPEPTIDE SPECIFIC CYTOTOXIC T LYMPHOCYTES, John S. Haurum, Gemma Arsequell*, Annemarie Lellouch*, Raymond Dwek* and Tim Elliott, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU and the ‡Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

Cytotoxic T lymphocytes (CTL) recognise peptide antigens presented by class I MHC. However, it remains to be determined whether post-translationally modified peptides also are presented for recognition by T cells. In order to address this issue, we studied the immunogenicity and MHC restricted recognition of a synthetic glycopeptide carrying an O-linked N-acetylglucosamine (GlcNAc) monosaccharide substituted serine. This glycosylation of cellular and viral proteins is catalysed by a recently described cytosolic glycosyltransferase.

We have demonstrated that glycosylation did not reduce peptide binding to MHC class I and that glycopeptides elicited a strong CTL response which is MHC restricted and glycopeptide specific. Furthermore, glycopeptide recognition by CTL is dependent on the structure of the glycan as well as its position within the peptide.

Our data show that post-translational modifications of peptides may constitute part of the natural T cell repertoire. Furthermore, acquisition or loss of glycosylation might be a novel viral mechanism for masking of epitopes or the creation of neo-epitopes.

Ongoing work in our laboratories is now aimed at determining whether glycosylated peptides may be presented by MHC in vivo.

J2-212 **PEPTIDE-BINDING SPECIFICITY OF RAT MHC CLASS I RT1.A MOLECULES CORRELATE WITH THE TRANSPORT CAPACITY OF THE NATURALLY ASSOCIATED TAP ALLELES.**

Etienne Joly, Andrea Gonzalez, Carol Clarkson, Jonathan C. Howard and Geoffrey W. Butcher. Laboratory of Immunogenetics, Department of Immunology, The Babraham Institute, Cambs CB2 4AT, UK.

Tap transporters from rats can be divided into two allelic groups, depending on their capacity to provide the RT1.A^a molecule with an appropriate level of suitable peptides[1]. Recent results suggest that this might correlate with the RT1.A^a molecule requiring arginine-ended peptides (Powis et al., manuscript submitted), which the Tap^b allele of the transporter is unable to translocate across the ER membrane efficiently[2,3]. RT1.A alleles are naturally linked with the Tap^a or the Tap^b allelic group[4]. We have set out to characterise various alleles for the RT1.A molecule, and find that, for the majority of TAP^a-associated RT1.A molecules, 3 acidic residues line the C/E pocket, dictating Arginine as C-terminal anchor residue for the bound peptides. On the other hand, in Tap^b-associated RT1.A molecules, one acidic residue at the most is found in the C/E pocket, which certainly results in a different anchor residue for the bound peptides. The selective pressure of viral infections must have driven this co-evolution which affects dramatically the array of peptides presented to cytotoxic T lymphocytes.

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2) Heemels M.T.,*et al.* 1993 *Science*, **262**: 2059-2063.
3) Momburg F. *et al.* 1994 *Nature*, **367**: 648-651.
4) Joly E. *et al.* 1994 *Immunogenetics*, **40**: 45-53

J2-213 ROLE OF STRUCTURAL-MOLECULAR CONTEXT ON ANTIGENICITY OF VIRAL T HELPER EPITOPES. Fabrizio Manca, Daniela Fenoglio, Giuseppina Li Pira, Anna Ferraris, Daniele Saverino, Peifang Sun and Annalisa Kunkl; Dept. Immunology, San Martino Hosp. Univ. of Genoa, 16132 Genoa, Italy.

Th epitopes present on viral proteins can be recognized by specific Th cells if appropriately expressed by antigen presenting cells (APC) as a result of uptake and processing. Since viral epitopes are not simply present in the context of viral proteins, but also in the context of whole viral particles, it is important to determine the role of the molecular and/or structural context on antigen uptake-processing-presentation.

Therefore we have generated panels of CD4+ human T cell lines and clones specific for different HIV antigens (gp120, p66, p24), in order to test their ability to respond to the same epitopes present within synthetic peptides, recombinant proteins or inactivated virions (provided by G. Lewis, Dept. Microbiology, Univ. Maryland, Baltimore).

We could identify T cell lines and clones that were able to discriminate the molecular and structural context of the epitopes. Certain T cells, in fact, responded to peptides and proteins, but not to viral particles, whereas other T cells were also able to proliferate when challanged in vitro with autologous APC and viral particles.

The data suggest that in the human Th cell repertoire specific for viral antigens T cells exist that can discriminate the molecular-structural context of Th epitopes. It will be interesting to ascertain whether T cells specific for epitopes that can only be recognized when provided in the context of a soluble molecule, but not of a viral particle, have any relevance in in vivo protection, or are a simple by-product of the cellular immune response.

J2-214 IMMUNOGENIC HIV VARIANT PEPTIDES THAT BIND TO HLA-B8 BUT FAIL TO STIMULATE CYTOTOXIC T LYMPHOCYTE RESPONSES, Stephen N. McAdam, Paul Klenerman, Lynda G. Tussey, Sarah Rowland-Jones, David Lalloo, Andrew Leigh Brown* Frances M. Gotch and Andrew J. McMichael, Molecular Immunology, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DU England and *Centre for HIV Research, Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, EH9 3JN, Scotland

Cytotoxic T lymphocyte responses in HIV infection can be impaired due to variation in the epitope regions of viral proteins such as gag. We show here an analysis of variant epitope peptides in three gag epitopes presented by HLA B8. Seventeen variant peptides were examined for their binding to HLA B8; all but one bind at concentrations comparable to known epitopes. All except two could be seen by CTL clones grown from HLA B8 positive HIV-1 infected patients and were therefore immunogenic. However, in one haemophiliac patient studied in detail, there was a failure to respond to some of the peptides that represented virus present as provirus in his peripheral blood. In one case his CTL had previously responded to the peptide. Thus there was a selective failure of the CTL response to variant epitopes. This impaired reaction to new variants and failure to maintain responses to some epitopes late in HIV infection could contribute to the loss of immune control of the infection.

J2-215 QUANTITATIVE ANALYSIS OF MHC CLASS I ANTIGEN PROCESSING EFFICIENCY,

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Listeria monocytogenes is a Gram positive bacterium that infects macrophages and secretes proteins into host cell cytosol. The murein hydrolase p60 is secreted by *L. monocytogenes* and is required for complete bacterial septation. In the infected macrophage secreted p60 is processed by the host cell into the nonamer peptide p60 217-225 and is presented to cytotoxic T lymphocytes by the H-2K^d MHC class I molecule. We have used strains of *L. monocytogenes* that secrete different amounts of p60 to show that the rate of p60 217-225 production is proportional to the amount of antigen secreted into the host cell cytosol. p60 is degraded in the host cell cytosol with a half life of 90 minutes. The appearance of p60 217-225 is coupled to the degradation of newly synthesized p60. We have determined the rate of intracellular p60 secretion and by accounting for the rate of p60 degradation we estimate that approximately 35 p60 molecules are degraded to produce one p60 217-225 epitope. This ratio is maintained over a range of intracellular antigen concentrations. Our findings provide an estimate of the efficiency of antigen processing and demonstrate the remarkable capacity of the MHC class I antigen processing pathway to accommodate new epitopes.

J2-216 CHARACTERIZATION OF AUTOLOGOUS VIRAL SEQUENCE RECOGNITION BY THREE HLA-B7-RESTRICTED CYTOTOXIC T LYMPHOCYTE CLONES SPECIFIC FOR THE THIRD VARIABLE REGION OF HIV-1 GP120. Jeffrey T. Safrit, Charla Andrews, Alexander Lee, and Richard A. Koup, The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

We have isolated and characterized three cytotoxic T lymphocyte (CTL) clones from the peripheral blood of two acute seroconversion patients and one patient in the first trimester of pregnancy. These clones were CD8+ and class I HLA-restricted by the B7 molecule. All three clones recognized IIIB and RF but not MN strains of HIV-1. Using vaccinia vectors expressing truncated versions of the HIV-1_{IIIB} envelope, the clones were found to recognize an epitope within amino acids 287-364, but not including 312-328 of gp120. Further mapping of the epitope with synthetic 20-mer peptides overlapping by 10, or 25-mers overlapping by 8, was unsuccessful. The sequence of the region of gp120 recognized by these clones was compared to the predicted HLA-B7 peptide binding motif and a possible matching region was found. Using shorter peptides corresponding to this potential epitope recognition site, the minimum epitope recognized by the clones was determined to be the 10 aa sequence RPNNNTRKSI spanning amino acids 298-307 (BRU sequence). With the exception of the MN strain, this region of the V3 loop is well conserved among clade B isolates of HIV-1. Using peptides based upon virus sequences present within each patient at or shortly after presentation, it was determined the clones are capable of recognizing autologous virus. A serine to arginine change at P9 of the epitope (associated with a switch from NSI to SI phenotype viruses) abrogated clone recognition. The variation of the V3 loop sequences from these patients will be analyzed longitudinally to determine if viruses emerge which can escape this particular CTL response.

J2-217 LMP2 PROTEASOME SUBUNIT REQUIRED FOR EFFICIENT CLASS I ANTIGEN-PROCESSING IN A T-CELL LYMPHOMA, Catherine Sibille, Keith Gould, Karen Willard-Gallo, Stuart Thomson, Jennifer A. Rivett, Simon Powis, Geoffrey W. Butcher and P. De Baetselier, Department of cell. Immunology, Free University Brussels, 1640 St Genesius Rode, Belgium

A variant of the mouse BW5147 T-lymphoma, termed SP3, displays a selective defect in MHC class I-restricted presentation of influenza antigens when expressed as long polypeptide. sequences. Of the MHC-encoded genes implicated in the class I pathway, TAP1, TAP2 and LMP7 are expressed at normal levels in SP3 cells, werheas LMP2 is underexpressed. IFN-γ-transfected SP3 cells simultaneously restore LMP2 expression and antigen presentation to CTL. In contrast, expression of antisense mRNA for LMP2 in IFN-γ transfected SP3 cells represses specific antigen recognition and surface class I MHC induction in these cells. These results are the first evidence that LMP2 subunit can influence directly both class I antigen presentation and class I surface expression.

J2-218 SEROLOGICALLY IDENTICAL HLA B35 ALLELES WHICH DO NOT CROSS-PRESENT MINIMAL CYTOTOXIC EPITOPES TO CD8+ CTL, Takuya Tsunoda, Hsinyi Chang, Phyllis Hao, Stephen J. Forman, and Don J. Diamond[a], Department of Hematology and BMT and the [a]Division of Immunology, City of Hope National Medical Center and the [a]Beckman Research Institute, Duarte, CA 91010

Work done in the last two years has shown that a viral matrix protein referred to as pp65 is most often recognized by CD8+ CTL as part of the immune response of seropositive humans to CMV. To replicate that immune response *in vitro*, it is mandatory to cleave the protein and prime autologous target cells for CTL mediated killing (McLaughlin-Taylor *et al* (1994) J. Med. Virol. 43;103). Alternatively, it is possible to use immortalized autologous B cells (EBV-LCL) infected with a vaccinia virus expressing pp65 (kind gift of Dr. William Britt, University of Alabama Medical Center) to achieve Class I restricted cytotoxicity by memory T cells from seropositive individuals [Tsunoda *et al*, submitted].

We have further pursued a strategy to define a minimal cytotoxic epitope for a vaccine against CMV infection using T cell clones derived from individuals who have the MHC B35 gene (kind gifts of Drs. Riddell and Greenberg, Fred Hutchinson Cancer Research Center and Dr. Robert Siliciano, Johns Hopkins University Medical Center). We tested by chromium release assay (CRA) the recognition of a series of B35 allelic variants of EBV-LCL, by B35 restricted and CMV or HIV-specific T cell clones. Several conclusions quickly became apparent. The previously described B*3501 peptide epitope from pp65 was not able to prime the autologous B35 EBV-LCL for killing by the pp65-specific CTL, whereas a recombinant vaccinia virus expressing whole pp65 could cause the same cell line to be recognized and killed in the same experiment. In addition, an HIV gp41-specific CD8+ CTL which has a defined minimal cytotoxic epitope will only recognize and kill a subset of B35 EBV-LCL. The two T cell clones will not recognize each other's autologous EBV-LCL. The resolution of this interesting phenomena comes from sequence analysis of the HLA Class I B genes from both EBV-LCL. EBV-LCL which contain the B*3502 allele are recognized and killed by the pp65-specific T cell clone, and cell lines carrying B*3501 alleles are recognized by the HIV gp41-T cell clone. We conclude that the reported CMV pp65 B*3501 restricted epitope is not correct, since the CTL in question will only recognize B*3502 alleles in combination with the correct pp65 epitope.

J2-219 THE HEMAGGLUTININ GLYCOPROTEIN OF INFLUENZA VIRUS IS PROCESSED FOR CLASS I PRESENTATION BY A CYTOPLASMIC MECHANISM THAT IS MAINLY TAP DEPENDENT, Scott S. Tykodi,*§† Young S. Hahn,*§‡ and Thomas J. Braciale,*§‡ *Beirne B. Carter Center for Immunology Research, Departments of §Microbiology and ‡Pathology, University of Virginia Health Science Center, Charlottesville, VA 22908, †Division of Biology and Biomedical Sciences, Immunology Program, Washington University School of Medicine, St. Louis, MO 63110.

CD8+ cytotoxic T lymphocytes detect virally infected cells by recognizing a processed viral peptide associated with class I MHC molecules. The generation of antigenic peptides from cytoplasmic or nuclear antigens for class I presentation is thought to require the cytoplasmic degradation of antigen, possibly by a proteasome complex, and peptide transport into the endoplasmic reticulum (ER) by a heterodimeric complex of the MHC-encoded Tap-1 and Tap-2 genes thereby allowing for peptide-MHC complex assembly within the ER. It remains unclear for viral glycoproteins if cytoplasmic degradation and Tap transport are required processing events. The cotranslational translocation of viral glycoproteins into the ER and subsequent antigen degradation within the ER would result in a Tap-independent antigen processing pathway. The RMA-S cell line has a mutation in the Tap-2 gene resulting in the functional disruption of peptide transport from the cytoplasm into the ER. Using the RMA-S cell line transfected with the MHC class I K^d gene, we show that processing of the influenza virus hemagglutinin (HA) glycoprotein for presentation to a panel of K^d restricted CTL is dramatically inhibited by the disruption of Tap function. Residual and highly inefficient K^d charging in the RMA-S/K^d cell can be elicited both with influenza virus as well as recombinant Sindbis virus expression vectors carrying influenza HA gene fragments encompassing known K^d epitopes. Sindbis vectors carrying HA gene fragments with or without a signal sequence sensitize RMA-S/K^d to a similar limited extent. This data is consistent with an inefficient movement of peptides from the cytoplasm into the ER by a Tap independent mechanism and does not reveal a processing competent compartment within the secretory pathway.

J2-220 THE FLANKING REGION OF EPITOPE INFLUENCES THE TRANSPORT OF EPITOPE BY TAP

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Peptide transport by the transporter associated with antigen processing (TAP) was studied using a microsome system as previously reported by Heemels et. al.. In this system, a radiolabeled synthetic peptide which can be N-link glycosylated is used as the indicator peptide for the transport studies. The transport efficiency of synthetic peptides corresponding to antigenic peptides restricted to the murine Kd molecule was measured by inhibition of labeled peptide transported into the microsomes. The transport efficiency of three Kd epitopes in the type A influenza virus NP147-155, HA204-212 and HA210-219 was found to be similar. An 11 amino acid peptide corresponding to HA204-214 which contains the 204-212 epitope was transported at a similar efficiency as the 9 amino acid minimum epitope. However, when the peptide sequence is further extended by one amino acid to residue 215, this peptide is poorly transported. These results suggest that the flanking region of an epitope can dramatically influence the transport of the epitope. When the transport kinetics of TAP was studied using the microsome system, the Vmax for transporting the indicator peptide (a variant of NP epitope that has the sequence TYNRTRALI) was found at 260.8 fmole/minute (+/- 30.5). The Km for this peptide was found to be 231.9 nM(+/- 31.8).

J2-221 BYPASSING A BLOCK IN ANTIGEN PROCESSING FOR CLASS I-RESTRICTED CYTOTOXIC T CELL RECOGNITION. Amy J. Yellen-Shaw and Laurence C. Eisenlohr, Thomas Jeferson University, hiladelphia, PA., 19107.

Previous work from our laboratory showed that processing of an influenza nucleoprotein (NP) epitope (amino acids 147-155) expressed endogenously from a recombinant vaccinia virus "minigene" is severely impaired when a flanking sequence (the dipeptide threonine-glycine) is appended to the C-terminus of the construct (147-158/R-). The inhibition of processing is overcome by placing the unprocessed peptide in the context of the full-length NP molecule, demonstrating that regions of a protein outside the epitope itself critically affect the ability of the proteolytic machinery to fragment the protein appropriately. To determine the requirements for bypassing the block in antigen processing, we have constructed an array of "minigene"-expressing vaccinia recombinants in which the unprocessed epitope is extended by varying lengths toward either the C-terminus or the N-terminus of the NP molecule. Our results show that while an extension of the C-terminus by only one amino acid restores processability, a much longer extension of the N-terminus ($75 < n < 100$ amino acids) will also allow the substrate to be processed. It is therefore clear that a full-length, properly folded molecule is not required for liberation of the blocked epitope, and that probably more than one mechanism can contribute to enhancement of substrate proteolysis. We hypothesize that the C-terminal extension allows recruitment of an endopeptidase versus exopeptidase ("trimming") activity which is capable of cleaving the difficult bond. We considered the possibility that the N-terminal extension rescues processing by recruitment of the ubiquitin-dependent degradation system. To address this possibility we replaced all available ubiquitination sites (lysine residues) in one of the rescued constructs (50-158/R-) to see if the construct would still be processed and presented. The six available lysine residues were changed to arginine using PCR-based mutagenesis. The resulting construct (termed 6R) was recombined into vaccinia virus and tested for presentation to NP-specific CTL. The 6R construct was presented at a level equivalent to that seen with the wild-type 50-158/R-construct. This result provides clear evidence that entry into the ubiquitin-dependent degradation pathway is not responsible for rescue of presentation in this system and more importantly, that ubiquitination is not required for processing of all large substrates.

Humoral Immunity in Viral Recovery and Protection; Local Immunity and Viral Infection of Privileged Sites

J2-222 DENGUE VIRUS VIRULENCE AND ITS INTERACTIONS WITH MACROPHAGES AND LYMPHOCYTES, Yun-Chi Chen, Chia-Chi Ku, Li-Jung Chien ,and Chwan-Chuen King, Institute of Epidemiology, National Taiwan University, Taipei, Taiwan, R.O.C.

Dengue virus (DEN) can cause dengue fever (DF) and dengue hemorrhagic fever (DHF) / dengue shock syndrome (DSS) and DEN-2 was the most common serotype found in DHF outbreaks globally. Current hypotheses suggested that DHF may be associated either with antibody-dependent enhancement (ADE) or with viral virulence. DEN can replicate predominantly in monocytes/macrophages (M/M), but whether peripheral blood lymhocytes (PBLs) are the target cells of DEN still remain controversial. In order to compare whether various clinically derived DEN-2 will interact with M/M and lymphocytes in different manners, we used two isolates -- PL046 strain (obtained from a DF patient during Taiwan 1981 outbreaks) and 16681 strain (isolated from a DHF patient in Thailand by CDC, USA) to infect primary M/M and lymphocytes as well as several types of cell lines. Primary lymphocyte culture was nonadherent cells obtained after 24 hr adherence of PBMCs, whereas the primary M/M culture was collected by depletion of lymphocytes using anti-CD3/CD19 mAb and complement prior to adherence procedure and the purity of M/M culture was checked by CD14 surface marker staining. Supernatants (SN) of virus were harvested at various time points post infection after with several or without treatments. Our prelimanary data showed that DHF-associated DEN-2 strain had higher viral yield in certain age of M/M and a promonocytic cell line (HL-CZ) than Taiwan DF-associated DEN2 strain. In addition, this DHF-DEN2 strain was more likely to infect the promonocytic (HL-CZ) than well differentiated monocytic (CTV-1) and lymphocytic (H9) cell lines and also had higher peak yields than DEN-1 virus in HL-CZ cells. Interestingly, DHF-DEN2 strain replicated much more efficiently in primary lymphocytes no matter these cells were activated with PHA or not, whereas Taiwan DF-DEN2 strain virus was hardly detectable in SN of both activated and non-activated lymphocyte cultures. Therefore we conclude that (1) different strains of dengue virus could orchestrate quite differently with immune cells, (2) different stage of M/M differentiation might be an important permissive determinants for dengue virus infection and replication, and (3) DEN virus strain virulence -- a more important factor than lymphocyte activation status -- seemed to determine whether this strain would infect human PBLs. Further studies should be focused on searching for detaied mechanisms of virus and immune cell interactions.

J2-223 **IMMUNE ENHANCEMENT OF CLINICALLY DIFFERENT DENGUE VIRUS STRAINS**, Li-Jung Chien and Chwan-Chuen King, Inst. of Epidemiology, National Taiwan University, Taipei, Taiwan . The pathogenesis of dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) involves how could virus efficiently infect target cells and spread into blood stream. Two major hypotheses have been proposed:(1) antibody depedent enhancement (ADE) hypothesis focused on history of dengue viral infection in hosts and (2) viral virulence hypothesis emphasized the importance of viral strain. To understand the role of antibody and viral virulence, we compared the ADE ability of different strain DEN-2 virus in a human monocytic cell line (HL-CZ) by infecting HL-CZ cells with DEN-2 prototype (New Guinea Strain C) and PL046 (DEN-2 Taiwan local strain isolated from a DF patient) at different m.o.i. (ranging from 0.01 to 0.0001) with or without the presence of monoclonal antibody 4G2 (100ug/ml). Experimental results demonstrated that (1)DEN-2 viral yields can only be detected by addition of 4G2 and (2) DEN-2 prototype viral yields appeared earlier and higher than PL046 ($2.5*10^3$pfu/ml on day3 vs $2*10^2$pfu/ml on day4 for m.o.i.=0.01). This phenomena paralled well with the severity of clinical syndrom. In conclusion, (1) ADE did occur but virus strain determined the enhancement folds and (2) when viral yields were enhanced early than day5 post infection, it provided tremendous opportunity to attack the immune system and finally may lead to severe disease.

J2-224 SURFACE-EXPOSED CRYPTIC LINEAR EPITOPES OF HUMAN PAPILLOMAVIRUS (HPV) TYPE 16, J. Dillner and P. Heino, Microbiology & Tumor Biology Center, Karolinska Institute, Stockholm, Sweden

HPV 16 the major cause of anogenital precancers in man. The search for neutralizing epitopes that could form the basis for a preventive vaccine has shown that the surface-exposed immunodominant epitopes of the capsid are strongly conformation-dependent, which has precluded detailed epitope analysis. Similarly, immunization with whole, denatured capsid proteins has only identified linear immunodominant epitopes positioned on the inside of the capsid. Reasoning that linear surface-exposed epitopes should exist, but might be cryptic, a set of 66 overlapping synthetic peptides corresponding to the entire HPV16 capsid proteins was used to generate hyperimmune sera. Several antisera against 3 different peptides were reactive with intact HPV16 capsids at titers up to 1:150.000.

J2-225 EFFECTIVE EX VIVO NEUTRALIZATION OF PLASMA HIV-1 USING RECOMBINANT IMMUNOGLOBULIN MOLECULES, Marie-Claire Gauduin, Graham P. Allaway, Paul J. Maddon, Carlos F. Barbas, Dennis R. Burton, and Richard A. Koup. Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

Primary isolates of HIV-1 have been shown to be less sensitive to neutralization by immune sera, monoclonal antibodies and CD4-based molecules than T cell line-adapted strains of HIV-1. We studied two immunoglobulin molecules for ability to neutralize primary isolates of HIV-1. IgG12 is an immunoglobulin molecule created from a combinatorial phage expression library and reacts with the CD4 binding site (CD4-BS) on gp120. CD4-IgG2 is a recombinant molecule in which the variable domains of both heavy and light chains of IgG2 were replaced with the first and second immunoglobulin-like domains of human CD4. Both molecules have been previously shown to effectively neutralize HIV-1 in vitro. Ex vivo neutralizations were performed as follows: IgG12 and CD4-IgG2 were added at 25 µg/ml to wells containing serial dilutions of plasma from HIV-1-infected patients and PHA-stimulated peripheral blood mononuclear cells from seronegative donors. P24 production was measured over 14 days of culture and an end-point titer of HIV-1 in the presence and absence of added antibody was determined. Both IgG12 and CD4-IgG2 were found to reduce the original HIV titer from seven plasma samples with high virus titer (>250 $TCID_{50}$/ml) by up to 625-fold. This is in comparison to soluble CD4 which only reduced viral infectivity by ≤5-fold at the same concentration. In vitro binding and neutralization assays on isolates recovered from plasma confirm the potency and breadth of neutralization by these two molecules. These studies suggest that recombinant antibodies directed at the CD4-BS of HIV-1 gp120 are able to effectively neutralize primary isolates of HIV-1 and may be useful in dissecting the mechanisms of resistance to neutralization by other antibodies.

J2-226 BRUCELLA ABORTUS COUPLED TO V3 LOOP PEPTIDES, GENERATES NEUTRALIZING ANTI-HIV-1 SERUM ANTIBODIES AND MUCOSAL IGA. Basil Golding, John Inman, Paul Beining, Jody Manischewitz, Robert Blackburn and Hana Golding. Div. of Hematology and Viral Products, CBER, FDA, and Lab. of Immunology, NIAID, Bethesda MD 20892.

Previously, we showed that HIV-1 proteins conjugated to *B. abortus* (BA) could generate anti-HIV-1 neutralizing antibodies in mice even after depletion of $CD4^+$ T cells. In this study a 14-mer peptide from the V3 loop of HIV-1(MN) was synthesized (V3) and coupled to BA and KLH. BALB/c mice were immunized twice i.p. with these conjugates at two week intervals. V3-KLH induced mainly IgG1, whereas V3-BA induced all IgG isotypes but IgG2a predominated. Fecal extracts from mice immunized with V3-BA were shown by ELISA to contain IgA antibodies. Sera from these mice bound gp120, expressed on the surface of infected cells. Sera from mice immunized with V3-BA inhibited syncytia formed between $CD4^+$ T cells and chronically infected [HIV-1(MN)] H9 cells. Inhibition of syncytia, formed by other HIV-1 lab. strains correlated with the degree of their homology with the V3 region of HIV-1 (MN). To mimic the efffect of HIV-1, mice were depleted of $CD4^+$ cells using anti-L3T4 at the time of primary or secondary immunization. Following primary immunization, CD4+ T cell depletion abrogated V3-KLH antibody responses, whereas responses to V3-BA were retained and sera from these mice were able to inhibit gp-120-mediated syncytia. In secondary responses, $CD4^+$ T cell-depletion prevented boosting to V3-KLH, but V3-BA increased anti-V3 and syncytia-inhibiting antibodies. These results suggest that: 1. *B. abortus*, can provide carrier function for a peptide and induce both serum and mucosal antibody responses, and 2. that infection with HIV-1 with subsequent impairment of $CD4^+$ T cell function would not abrogate anti-HIV-1 antibody responses if *B. abortus* is used as a carrier to stimulate memory responses.

J2-227 **ROLE OF CIRCULATING IgG IN CLEARANCE OF INTESTINAL VIRUS INFECTION. Barbara L. Haller, Melissa, L. Barkon, and Herbert W. Virgin IV, Center for Immunology and Division of Infectious Diseases, Washington University School of Medicine, St. Louis MO, 63110.**

We evaluated the clearance of reovirus serotype 3 Clone 9 (T3C9) from the small intestine of normal or immunodeficient adult mice after oral inoculation. CB17, 129, and C57BL/6 mice clear reovirus T3C9 completely from the proximal small intestine 11 days after oral inoculation. In contrast, SCID mice on either the C57BL/6 or CB17 background fail to clear virus from intestine and ultimately succumb to infection. Adoptive transfer of immune spleen cells allowed SCID mice to control intestinal infection. To evaluate the role of CD8 T cells, immunoglobulin, and B cells in clearance of intestinal infection, we challenged β2-microglobulin and immunoglobulin knockout mice (β2KO and IgKO respectively) with T3C9. β2KO mice cleared infection with normal kinetics. In contrast, IgKO mice failed to clear intestinal virus efficiently, with significant viral titers in intestine 11 days after infection. To investigate the mechanism of inefficient clearance in IgKO mice, we adoptively transferred antibody to IgKO and SCID mice 3 and 5 days after oral infection with T3C9. Intraperitoneal transfer of reovirus-immune polyclonal rabbit serum, but not pre-immune serum, completely cleared reovirus from intestine of IgKO mice 11 days after infection. The same antibody significantly decreased virus titer in intestines of SCID mice 11 days after infection. Adoptive transfer of a mouse monoclonal IgG2a antibody specific for the reovirus outer capsid protein σ3 also cleared IgKO mouse intestinal reovirus infection. We are currently evaluating other knockout mice and performing cell transfer studies to further dissect mechanisms of intestinal virus clearance. Our studies in IgKO mice challenge the notion that circulating IgG is unimportant for clearance of virus from mucosal surfaces.

J2-228 HOST RESPONSE TO SENDAI VIRUS IN MICE TREATED WITH THE MEL-14 MAB TO L-SELECTIN. Lisa Hyland[1,3], Sam Hou[1,2], and Peter C. Doherty[1]. [1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101, [2]Departments of Immunology and Microbiology, and [3]Pathology, University of Otago,Dunedin, New Zealand.

The B and T cell responses in C57BL/6J(B6) mice treated with the mAb Mel-14 to L-selectin have been analysed following i.n. infection with Sendai virus. Mel-14 treatment caused a 70-90% decrease in the lymphocyte recruitment to the mediastinal (MLN) and cervical (CLN) lymph nodes following infection with Sendai virus. The cellularity of the spleen was unchanged. The clonal expansion of CD8+ CTL precursors in the MLN was slightly delayed, but potent CTL effectors were present in the virus-infected lung by day 10 after infection and the overall magnitude of the response was not compromised. The prevalence of IgA antibody forming cells (AFCs) was greatly increased in both the MLN and the CLN of the mice given the Mel-14 antibody. The IgM response was prolonged and the IgG response, particularly IgG1, was delayed compared to controls. The altered pattern of the antibody response may reflect the limited availability in Mel-14-treated mice of Th cells secreting lymphokines which are involved in Ig class switching, by blocking the entry of CD4+ Th precursor cells into lymph nodes. FACS sorting for L-selectin+, B220+, and L-selectin-, B220+ cell populations from the MLN and the CLN of normal B6 mice 9 days post Sendai virus infection, showed that the AFCs were from the L-selectin-, B220+ cell population, a population which comprised 6-10% of the total cell population.

J2-229 ON THE ROLE OF SOMATIC MUTATIONS FOR THE GENERATION OF VIRUS NEUTRALIZING ANTIBODIES, Ulrich Kalinke, Etienne Bucher, Annette Oxenius, Rolf M. Zinkernagel and Hans Hengartner, Institute of Experimental Immunology, University of Zürich, Schmelzbergstr. 12, CH-8091 Zürich, Switzerland.

Mice infected with vesicular stomatitis virus Indiana (VSV-IND) mount a protective T_h-dependent IgG response. Hybridomas isolated after infection secrete VSV-IND neutralizing IgG antibodies binding with high affinity to the VSV glycoprotein. One group of 10 structurally related antibodies used V_H7183 and J_H2 elements in the variable regions of the heavy chains (VH) and several gene combinations in the variable regions of the light chains (VL). Nucleotide sequence analysis of the VH genes revealed the usage of one particular V_H germline element (VH61-1P) in all clones. This finding allowed the determination of somatically mutated positions in the VH regions. Two VSV-IND neutralizing antibodies expressed VH and VL genes in complete germline configuration whereas the rest of the clones showed somatic mutations which obviously were antigen dependently selected for. However, binding affinities of mutated and unmutated antibodies were comparably high. In order to determine the influence of somatic point mutations on one single antibody we generated a monovalent single chain antibody (FV-CK) of a mutated clone and reversed it stepwise to germline configuration by means of site directed mutagenesis. Surprisingly, already the germline configuration of FV-CK could neutralize VSV-IND, even though the binding affinty was lower than that of the mutated FV-CK. Every single somatic point mutation tested improved the binding avidity although some mutations reduced affinity. Thus, during the course of VSV-IND infection some antibodies are subjected to avidity maturation although this is not required for the generation of high affine, efficiently virus neutralizing antibodies.

J2-230 **BROADLY NEUTRALIZING ANTIBODIES TO HIV-1 IN INFECTED INDIVIDUALS, Marvin S. Reitz, Brynmor A. Watkins, Audrey Louie, Robert C. Gallo and Marjorie Robert-Guroff, Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD 20892**

We have distinguished targets of broadly neutralizing antibodies present in HIV-1 infected individuals by immunoselection in vitro and by the use of chimeric virus. One target of neutralizing antibodies, defined by an escape mutant with an ala to thr substitution at position 582 in gp41, is resistant to human monoclonal antibodies that map to a site closely congruent with that for CD4 binding. Substitution of gly, ser, and val fail to confer resistance. A second, defined by an ala to val substitution at position 281, upstream from the V3 loop, does not involve the same site and does not involve V3. Substitution of thr or ile also confers resistance. Replacement of the V3 loop of HIV-1(MN) into a clone of HIV-1(IIIB) allows the detection of two other broadly neutralizing targets. One recognizes the V3 peptide of MN but is affected by regions outside V3. The other appears to be conformational and outside V3, but its functional recognition is influenced by the V3 loop. All of these sites seem to depend on the overall conformation of the envelope protein rather than a single discrete linear epitope.

J2-231 THE HUMORAL IMMUNE RESPONSE TO LENTIVIRUS (HIV & SIV) GP41 TRANSMEMBRANE GLYCOPROTEIN IS DETRIMENTAL TO THE HOST, W. Edward Robinson, Jr.[1], Murray B. Gardner[2], and William M. Mitchell[3]. Departments of Pathology, [1]University of California, Irvine, CA, 92717-4800, [2]Vanderbilt University, Nashville, TN, and [3]University of California, Davis, CA.

Antibodies against amino acids 579-613 of the HIV transmembrane (TM) glycoprotein have been shown to enhance HIV infection in vitro in the presence of complement. There has been no study demonstrating that enhancing antibodies to this region of HIV, despite increasing levels of infectious virus 10 to 100 fold in vitro, adversely affect disease pathogenesis. In two separate studies reported herein, it is shown that animals which have high levels of antibody against this region of SIV, amino acids 603-622 of the envelope, fair poorly compared to animals with lower antibody levels against this region when subsequently challenged with SIV. When actively immunized with a synthetic peptide from this region of SIV, animals died earlier and failed to clear antigen at two weeks after infection compared to animals that received a control peptide ($p<0.05$). When animals were passively immunized with antibodies from a longterm survivor of SIV infection, those animals that received higher levels of antibody against the TM peptide died within six months compared to longer intervals for those animals that had lower levels of antibody to this region. When taken together, these data suggest that antibody to the TM region of SIV and HIV in general, and to this highly conserved peptide in particular, are detrimental to the host. Therefore, immunization strategies that minimize the immune response against TM or treatment protocols that decrease antibody levels against TM may lead to prolonged survival following exposure to lentiviruses.

J2-232 HUMORAL IMMUNITY AGAINST THE HYPERVARIABLE REGION 1 OF THE PUTATIVE ENVELOPE GLYCOPROTEIN (GP70) IN HEPATITIS C PATIENTS, Elisa Scarselli[1]*, Antonella Cerino[2], Gloria Esposito[1], Mario U. Mondelli[2], and Cinzia Traboni[1], [1]Istituto di Ricerche di Biologia Molecolare P. Angeletti (IRBM). Via Pontina Km 30.600. 00040 Pomezia, Italy. [2]Istituto di Clinica delle Malattie Infettive, I.R.C.C.S. Policlinico San Matteo, University of Pavia, Italy.

The Hepatitis C virus is a frequent cause of chronic liver disease. A proposed mechanism responsible for virus persistence is evasion of the host immune response through a high mutation rate of crucial regions of the viral genome. The portion of HCV genome coding for the amino-terminal part of the putative envelope protein (gp70) undergoes frequent mutation during the course of infection. We have cloned and sequenced the hypervariable region (HVR1) of the virus isolated from an HCV asymptomatic patient at three time points during 18 months follow up. Sequence analysis has allowed the identification of variants of this region and multiple antigenic peptides (MAP), corresponding to three HVR1 variants, sequentially found in the blood stream of the patient, have been synthesized. MAPs have been used as antigens for detection of specific antibodies in ELISA. Our results show that anti-HVR1 antibodies and their cognate viral sequence coexist in the blood stream but a viral sequence becomes undetectable when the specific antibodies reach maximum levels of reactivity. Thus humoral immunity against the HVR1 may play a role for virus clearence. The presence of anti-HVR1 antibodies was also investigated in 100 hepatitis C viremic individuals and 25 non-viremic patients. A high frequency of positive reaction (90%) against at least one of the three HVR1 variants analysed in this study was detected in the viremic patients. Finally, competition experiments show that antibodies cross-reacting with more than one HVR1 variant are produced by HCV infected individuals. This results suggest that complex cross-reactivity exist between HCV isolates for antibodies against the HVR1 region as described for antibodies against the gp120 V3 loop of HIV. We propose as mechanism for viral escape in HCV chronic infections the one described as the "original antigenic sin", observed firstly in influenza, in togavirus, paramixovirus, enterovirus, and recently in HIV infection.

J2-233 SEROLOGICAL RESPONSES TO HPV 16 E7 PROTEIN IN A MOUSE MODEL, Margaret Stanley, Jyoti Parikh, Mark Chambers, Merilyn Hibma and Wei Zhang , Department of Pathology and ICRF Tumour Virus Group, Tennis Court Road, Cambridge, CB2 1QP, UK

We have developed a mouse model to examine the immune response to HPV 16 proteins when these proteins are presented to the immune system via the epithelial route. In this model animals are grafted with keratinocytes expressing HPV E6 and E7 genes using a transplantation procedure which permits epithelial reformation. Animals so grafted when challenged intradermally with E7 either as protein or via a recombinant vaccinia virus exhibit a delayed type hypersensitivity response which is E7-specific and $CD4^+$ T cell mediated. Animals grafted with a sub optimal priming inoculum of cells develop immune non-responsiveness and have an abrogated DTH response when challenged subsequently with a priming cell graft. In the present study we have examined the antibody status in these animals. The E7 protein of HPV 16 was expressed in *E. coli* as a maltose binding fusion protein using the plasmid vector pMalc. After cleavage and affinity purification this protein was used in an ELISA assay to measure antibody levels in 4 groups of mice (1) those not challenged with E7 (2) mice not grafted but challenged with E7 protein in the ear (3) mice primed by grafting with 107 HPV E7 expressing cells and challenged with E7 protein (4) mice primed by grafting with 5×10^5 HPV 16 E7 cells on day 7, grafted again with 10^7 HPV 16 E7 cells on day 14 and challenged with E7 protein in the ear. Mice optimally grafted and challenged (group 3) exhibited high titres of IgG antibodies, particularly elevated levels of IgG_{2a}. Mice sub-optimally grafted (Group 4) exhibited IgG antibody levels comparable to the control group (1). The possible mechanisms of this immune attenuation are discussed.

J2-234 $\alpha\beta$ T CELL-INDEPENDENT B CELL RESPONSE HAS A MAJOR ROLE IN POLYOMAVIRUS CLEARANCE IN THE ABSENCE OF $\alpha\beta$ T CELLS, Eva Szomolanyi-Tsuda and Raymond M. Welsh, Dept.of Pathology, Univ.of Massachusetts Medical Center, Worcester, MA 01655

In *scid/scid* (SCID) mice polyomavirus (PyV) induces an acute hematological disease with 100% mortality by day 16 post infection. PyV does not cause a comparable disease in immunocompetent or nude mice, but it induces a wide array of epithelial and mesenchymal tumors in neonatally infected mice, and mainly mammary tumors in adult, nude mice. In order to determine which component of the immune system is playing a major role in the protection of mice from the PyV-induced acute hematological disease, we performed two types of experiments. (i) Mice with different types of immunodeficiencies (SCID, $\alpha\beta$ knockout (KO), $\gamma\delta$ KO, B cell KO) and/or depleted of certain cell populations ($CD4+$,$CD8+$,asialo-GM_1+ (AGM_1) or $NK1.1+$) in vivo, were infected with PyV. (ii) SCID mice were adoptively transferred with splenocytes from immunocompetent, or from immunodeficient ($\alpha\beta$ KO; B cell KO) mice, or with the same splenocytes in vitro depleted with anti-Thy-1 and/or anti-AGM_1 antibodies, then infected with PyV. Both types of experiments clearly showed that, in the absence of $\alpha\beta$ T cells or of $\alpha\beta$ T cells and NK cells, the presence of B cells protected mice from the PyV-induced hematological disease. This was accompanied by a major reduction in the amount of virus in different organs. At day 4 post infection PyV DNA was detected in the spleens but not in other organs of SCID mice, or of SCID mice adoptively transferred with splenocytes with or without Thy-1 depletion. However, at day 14 post infection, while in SCID mice all four major organs tested (spleen, kidney, liver, lung) contained a strong virus-specific band, no viral signal was detected in SCID mice adoptively transferred with B or with T and B cells. Transfer of immune serum raised in PyV-infected $\alpha\beta$ KO mice,(but not serum from uninfected mice) delayed the onset of acute disease by 4 weeks. Ig fractions responsible for this protective effect are being analyzed. Our result indicate that B cells can mount an effective response against PyV in the absence of $\alpha\beta$ T cells and NK cells.

J2-235 ACTIVE IMMUNITY AGAINST ROTAVIRUS INFECTION IN MICE IS CORRELATED WITH VIRAL REPLICATION AND TITERS OF SERUM ROTAVIRUS IgA FOLLOWING VACCINATION, Richard L. Ward*, Rosemary L. Broome†, and Monica M. McNeal*, *James N. Gamble Institute of Medical Research, Cincinnati, OH 45219, †Department of Gastroenterology, Veterans Affairs Medical Center, Palo Alto, CA 94304.

Using an adult mouse model to study active immunity against rotavirus infection, it was previously shown that oral immunization with some, but not all, animal rotavirus strains induced protection against subsequent infection following oral challenge with the murine rotavirus strain EDIM (Ward et al., 1992). To determine if a specific rotavirus protein could be associated with protection in this model, mice were immunized with a series of 18 reassortants between the fully protective EDIM strain and a partially protective heterologous rotavirus strain (RRV-G). Reassortants that contained genes for EDIM proteins responsible for protection were anticipated to provide complete protection; however, no EDIM proteins were found to be both necessary and sufficient for full protection. Instead, protection was found to be highly correlated with viral shedding ($P = .005$) and with serum rotavirus IgA titers stimulated by the different reassortants ($P < .001$). This indicated that protection was related to the intestinal replication properties of the different reassortants rather than to specific immunogenic properties of EDIM proteins. This conclusion was supported by the finding that the titers of serum rotavirus IgA, but not IgG, stimulated in mice following oral immunization with a series of animal rotaviruses was directly related to protection against EDIM. If these findings can be extended to humans, they suggest that the efficiency of intestinal replication following oral inoculation with a live rotavirus vaccine candidate may be the primary determinant of successful immunization.

J2-236 LONGITUDINAL ANALYSIS OF ANTI-V3 ACTIVITY IN RAPIDLY PROGRESSING AND SLOWLY PROGRESSING HIV-1 INFECTED PERSONS, Michael T. Wong, Stephanie A. Anderson, Ronald Q. Warren, Matthew J. Dolan, Gregory P. Melcher, Maryanne T. Vahey, and Ronald C. Kennedy. Department of Infectious Diseases, Wilford Hall Medical Center, Lackland AFB, TX 78236, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX 78227, and the Department of Interventional Assessment, Military Medical Consortium for Applied Retroviral Research, Rockville, MD 20850.

Of 1200 patients enrolled in the HIV Natural History study at Wilford Hall Medical Center, 55 individuals with adequate serum samples were identified as either rapidly progressing (RP) or slowly progressing (SP) by clinical and surrogate marker criteria. Anti-V3 profiles were determined using synthetic proteins derived from the amino acid sequences of the V3 region of 5 laboratory strains of HIV-1 in standard capture ELISA format. Serum obtained from each patient at multiple different time points was screened against these peptides. The majority of individuals in both groups demonstrated broad recognition, with reactivity to peptides corresponding to the V3 regions of MN, SF2, NY5 and HAN/SC. Less than 50% of individual in each group recognized the V3 peptide derived from IIIB, (p=NS, between groups). As the RP progressed to AIDS there was significant nonspecific narrowing of response, while the SP remained broadly reactivity ($P < .001$). *In vitro* neutralizing activity of the homologous laboratory isolates was determined with cytotoxicity, cytopathic effect and p24 Ag inhibition assays. Although most patient serum was capable of inhibiting p24 Ag production in homologous lab strains while AIDS-free, there was no relationship with the ability to inhibit homologous virus effects on target cells and anti-V3 profiles.

Memory and the Immune Response to Virus

J2-237 BONE MARROW IS A MAJOR SITE OF LONG-TERM ANTIBODY PRODUCTION AFTER ACUTE VIRAL INFECTION, Rafi Ahmed, Mark K. Slifka and Mehrdad Matloubian Department of Microbiology and Immunology, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

Antiviral antibody production is often sustained for long periods after resolution of an acute viral infection. Despite the extensive documentation of this phenomenon, the mechanisms involved in maintaining long-term antibody production remain poorly defined. As a first step towards understanding the nature of long-term humoral immunity, we have examined the anatomical location of antibody producing cells during acute viral infection. Using the lymphocytic choriomeningitis virus (LCMV) model, we show that after resolution of the acute infection, when antiviral plasma cells in the spleen decline, a population of virus-specific plasma cells appear in the bone marrow and constitute the major source of long-term antibody production. Following infection of adult mice, LCMV-specific antibody secreting cells (ASC) peaked in the spleen at 8 days post-infection, but were at this time undetectable in the bone marrow. The infection was essentially cleared by 15 days and the ASC numbers in the spleen rapidly declined while an increasing population of LCMV-specific ASC appeared in the bone marrow. When compared to the peak response at 8 days post-infection, timepoints from 30 days to more than one year later demonstrated greater than a 10-fold reduction in splenic ASC. In contrast, LCMV-specific plasma cells in the bone marrow remained at high numbers and correlated with the high levels of antiviral serum antibody. The presence of antiviral plasma cells in the bone marrow was not due to a persistent infection at this site, since virus was cleared from both the spleen and bone marrow with similar kinetics as determined by infectivity and PCR assays. The IgG subclass profile of antibody secreting cells derived from bone marrow and spleen correlated with the IgG subclass distribution of LCMV-specific antibody in the serum. Upon rechallenge with LCMV, the spleen exhibited a substantial increase in virus-specific plasma cell numbers during the early phase of the secondary response, followed by an equally sharp decline. Bone marrow ASC populations and LCMV-specific antibody levels in the serum did not change during the early phase of the reinfection but both increased about 2-fold by 15 days post-challenge. After both primary and secondary viral infection, LCMV-specific plasma cells were maintained in the bone marrow showing that the bone marrow is a major site of long-term antibody production after acute viral infection.

J2-238 CD4+ T CELL MEMORY PHENOTYPES IN HIV-1 INFECTION, Matthew J. Dolan, Robert Doe, Rex Hensley, Gregory P. Melcher, Maryanne Vahey, Department of Infectious Diseases, Wilford Hall Medical Center, Lackland AFB, TX 78236, and Retrovirology Division, WRAIR.

Both CD29 and CD45RO have been considered markers of "memory" T cells, and associated with responsiveness to soluble and recall antigens. CD4+ lymphocytes staining bright, dim, or negative (equivalent to an isotype control) for CD29 were evaluated in 49 uninfected controls (group 1), 84 HIV-1 positive patients with ≥20% CD4+ T cells (group 2), and 47 HIV-1-infected patients with <20% CD4+ T cells (group 3). Most of these subjects also had 3-color staining for CD4\CD45RO\CD45RA.

The appearance of positive CD29 and CD45RO on HIV-infected and uninfected cells correlated well (R=.82 P<.001). The percentage of cells staining CD4+\CD29+.(bright plus dim) was 43.3 (95%CI 37.3-49.4) in group 1, 28.9 (27.5-30.4) in group 2, and 10.2(8.6-11.9) in group 3. The respective values for these groups that were CD4+\CD29^{bright} was 30.6 (26.9-34.3), 20.7 (19.3-22.2), and 7.4(6.3-8.6). Values for CD4+\CD45RO+ were 33.7 (31.8-35.5), 21.8 (20.5-23.1), and 9.9 (8.5-11.3), respectively. In single factor discriminate function tests, the %CD4+\CD29+ cells best predicted subject group (87% correct), proving to be a better discriminator than %CD4+\CD29^{bright} (77% correct),CD4+\CD29^{dim} (51%), CD4+\CD45RO+ (75%) and CD4+\CD45RO+\CD45RA- (63%).

Overall, no advantage was seen to splitting the CD4+\CD29+ cells into bright and dim positive subsets in the subjects studied for the purpose of stratifying early vs. late HIV infection. Likewise, splitting the CD4+\CD45RO+ compartment into CD45RA± subsets did not improve the ability to distinguish between uninfected and early or late HIV-1 infected patients.

J2-239 FUNCTIONAL AND PHENOTYPIC CHARACTERIZATION OF INFLUENZA VIRUS-SPECIFIC CD8$^+$/CD45RO$^+$ CTLS AND CORRELATION WITH IFN-γ PRODUCTION, Innocent N. Mbawuike[1], Simonetta Di Fabio[2], Kohtaro Fujihashi[3], Hiroshi Kiyono[3], Jerry R. McGhee[2] and Robert B. Couch[1], [1]The Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030 and The Departments of [2]Microbiology and [3]Oral Biology, The University of Alabama at Birmingham, AL 35294.

MHC Class I restricted CD8$^+$ CTL activity plays an important role in the control of influenza virus infection as indicated in studies in mice and humans. Cytokines such as IL-2 and IFN-γ regulate the generation of virus-specific CTL responses. We recently demonstrated a good correlation between the induction of influenza virus-specific CTL activity and the production of IFN-γ by the CD8$^+$ T cells at the single cell level using an IFN-specific ELISPOT assay, secreted IFN-γ by an ELISA, and IFN-γ specific mRNA expression by RT-PCR. Several recent studies have characterized CD4$^+$ and CD8$^+$ T cells by their expression on the surface of distinct D45R isoforms. CD45RA is expressed on naive or virgin T cells, while CD45RO is expressed on memory T cells.

In the present study, PBMC of healthy young adult subjects were stimulated with influenza A virus and then enriched for CD8$^+$ T cells. The CD8$^+$ cells were stained for CD45RO$^+$ (PE) and CD45RA$^+$ (FITC) cells and sorted. CTL activity against virus-infected autologous target cells was determined in a 4 hour ^{51}Cr release assay while IFN-γ production and expression was assessed by ELISPOT and quantitative RT-PCR, respectively. CD8$^+$/CD45RO$^+$ (memory) cells exhibited significant MHC class I CTL while CD8$^+$/CD45RA$^+$ cells exhibited no lytic activity. No activity was exhibited by freshly isolated or unstimulated CD8$^+$/CD45RO$^+$ T cells. Similarly, CD8$^+$/CD45RO$^+$ T cells contained significantly higher numbers of IFN-γ spot forming cells and higher quantity of IFN-γ-specific mRNA than CD8$^+$/CD45RA$^+$ cells. These data support our previous findings that IFN-γ may serve as a useful surrogate marker for influenza virus-specific CTL activity in humans.

J2-240 INDUCTION OF CYTOTOXIC -T- LYMPHOCYTE ACTIVITY BY HIV-GP160 RECOMBINANT CANARYPOXVIRUS : CTL MEMORY WITHOUT ANTIGEN. Yves Rivière[1], Geneviève Janvier[1], Béatrice Fleury[1], Jean Louis Excler[2], Stanley Plotkin[2], Gilles Pialoux[3] and James Tartaglia[4]. 1 UVIC, Institut Pasteur,75724 Paris cedex 15. 2 PMSV,Marcy l'Etoile, France, 3 Hôp.Pasteur,Paris,France, 4 Virogenetics,Troy, USA.

The relationship between the virus-specific cytotoxic response in HIV infected patients and disease progression support the concept that a vaccine candidate should also induce a virus-specific CTL activity. Immunization of uninfected adult volunteers by a HIV-gp160 recombinant canarypox virus was carried out in a phase I trial.Two injections of a recombinant canarypox expressing the HIV-1/MN gp160 were performed at month 0 and 1 and two boosts of recombinant gp160MN/LAI at month 3 and 6 in alum or incomplete freund adjuvant(IFA). HIV-envelope specific cytotoxic activities were detected from CTL lines derived from PBMC stimulated by specific stimulation with autologous HIV infected blasts. CTL lines were obtained from 18 out of 20 donors : seven out of eighteen (39%) were found to present envelope specific cytotoxic activity at months 2, 4, 7 or 12 post immunization ; this activity was characterized as a CD3+,CD8+, MHC class-I restricted cytotoxic activity, and for at least two volunteers, this activity was still present two years after the first canari-pox/env injection.

Because avian poxviruses are incapable of complete replication and undergo abortive replication in mammalian cells , this is an example of the persistence of long term memory CD8+ cytotoxic T lymphocytes in the absence of the priming antigen, indicating that T-cell memory might be independent of continued antigenic exposure.

J2-241 DELETION OF VIRUS-SPECIFIC MEMORY pCTL BY INFECTION WITH HETEROLOGOUS VIRUSES.L.K. Selin, S.R. Nahill, K. Vergilis and R.M. Welsh. University of Massachusetts Medical Center, Worcester, MA.

In studying the kinetics of the CD8$^+$ T cell response in LCMV infection we have observed a profound activation and proliferation of CD8$^+$ T cells with a 10-40 fold increase in total number peaking at day 8-9 post infection. In C57BL/6 mice, most of the viral antigen is cleared by day seven, and after day 9 the total CD8$^+$ number per spleen drops about 10-fold. However, the relative specificity of the viral peptide-specific precursor CTL frequencies (pCTL/f) per CD8$^+$ cell remains remarkably stable between day 7-8 of the acute infection and for many months thereafter. Thus, the decline in the CD8$^+$ T cell number is not a function of the TcR specificities but is rather an across-the-board event.

In contrast, we found that subsequent to the decline of the CTL response to a second heterologous virus infection such that the mouse was in a "resting, immune" state, there often was a reduction in pCTL/f to the first virus. For example, infections with VV or MCMV substantially reduced the pCTL/f to LCMV or PV in all memory compartments, including spleen, lymph nodes, peritoneal exudate cells. Reinfection with the original virus substantially elevated its pCTL/f and restored the pCTL/f that had been reduced by a heterologous viral infection. Analyses of the progression of CTL responses during a heterologous virus challenge of a virus-immune mouse indicated a high frequency of crossreactive CTL appearing early during infection, but as the infection progressed there was a higher proportion of CTL specific only for the second virus. Thus, we believe that when the across-the-board apoptosis of T cells occurs late in the infection, CTL specific for the first virus are diluted by those responding to the second virus. This may cause the reduction in memory to the first virus and may be one of the mechanisms contributing to the waning of secondary immune responses to certain viruses over time if there is no re-exposure to the original infectious agent.

J2-242 **ANALYSIS OF MEMORY CD4 HELPER T-CELL PRECURSOR FREQUENCIES IN SENDAI VIRUS IMMUNE C57BL6 MICE.** **David J. Topham, Christine Ewing, and Peter C. Doherty. Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105.** Little is known regarding the nature of specific CD4+ helper T-cells in mice previously infected with Sendai virus. Knowing the fate and phenotype of memory helper T-cells which arise after virus infection will aid our understanding of T-cell memory and be useful in the design of vaccines which augment the memory response. To estimate the Sendai virus specific precursor frequency in memory mice, CD4+ cells from C57BL6 female mice which had been infected with Sendai virus intranasally (i.n.) more than two months earlier were subjected to limiting dilution analysis. Responder cell populations were enriched for CD4+ cells either by magnetic bead depletion of non-CD4+ cells, or by FACS after staining with anti-CD4 monoclonal antibody. These enriched (>90% CD4+) responders were cultured with Sendai virus-infected, irradiated, T-cell depleted splenic antigen presenting cells (APC). Supernatants from these cultures were tested for activity on the cytokine-dependent CTLL cell line. Duplicate cultures of responders on uninfected APC were used to set the level of rejection (mean CPM + 3x std. dev.). Using this type of analysis we were able to demonstrate a frequency of memory Thp at 1/1600 CD4+ cells, compared to a frequency greater than 1/100000 in naive controls. The memory CD4+ cells were further characterized as CD45RB-low (1/472) , CD44-high (1/294), L-selectin-low (1/364), and CD49d-high (VLA-4-high) (1/102). This is close agreement with other phenotyping studies on CD4+ memory cell specific for soluble antigens.

J2-243 **LOCALIZATION AND CYCLING FOR CD8$^+$ T CELLS,** **Ralph A. Tripp, Sam Hou, Anthony McMickle, James Houston and Peter C. Doherty, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105.** The immune response of influenza A and Sendai-virus-specific, memory CD8$^+$ cytotoxic T lymphocyte precursors (CTLp) have been analyzed in C57BL/6 mice infected intranasally with unrelated or cross-reactive respiratory viruses. The numbers of influenza A-specific memory T cells increased in the regional lymph nodes (LN), spleen and bronchoalveolar lavage through the course of an irrelevant infection (influenza B). Memory T cells showed evidence of enhanced steady-state activation. Profiles of CTLp recruitment were analyzed in association with T cell proliferation and activation to determine whether signaling via the T cell receptor is necessary to induce "bystander" stimulation of the memory T cell pool. The extent of T cell proliferation was addressed by treating mice with low doses of cyclophosphamide (Cy). "Resting" Sendai virus-specific memory T cells were unaffected by Cy treatment, however upon challenge with influenza and treated 5 or 6 days later, the emergence of influenza-specific CTLp was severely diminished. Cell cycle analysis showed that Cy eliminated the majority of CD8$^+$ T cells from the LN and spleen resulting in DNA fragmentation of 12-18% of this lymphocyte subset. A decrease (though smaller) in the numbers of Sendai virus-specific CTLp indicated that some of the cycling cells killed by Cy were memory T cells, presumably activated in a "bystander" manner. The decrease in CTLp numbers for both influenza and Sendai virus-specific CTLp was still apparent 9 days after Cy treatment, long after the viral elimination. Thus, immune responses to unrelated antigens may be a mechanism involved in maintaining the pool of memory T cells.

Molecular Immunology of Neurotropic Virus Infections

J2-244 INHIBITION OF VESICULAR STOMATITIS VIRUS (VSV) INFECTION BY NITRIC OXIDE, Zhengbiao Bi[1] and Carol S. Reiss[1,2,3]. [1]Department of Biology, [2]Center for Neural Science, [3]Kaplan Comprehensive Cancer Center, New York University, New York, N.Y. 10003-6688

The mechanisms of viral clearance from infected neurons are not clearly understood. Since neurons do not express MHC molecules, viral clearance from infected neurons is less likely mediated by viral-specific T cells. Factors such as interferons and Nitric Oxide (NO) which readily diffuse into cells may play significant roles in clearing virus from neurons.

Experimentally VSV can result in an acute CNS infection of mice. Data from our in vitro experiments indicate that NO has inhibitory effect on productive VSV infection.

VSV infection at Neuroblastoma NB41A3 cells was significantly inhibited by 100μM of a NO donor S-Nitro-N-acetylpencillamine (SNAP), while 100μM of the control compound N-acetyl-pencillamine (NAP) had no effect. When VSV infected NB41A3 cells were treated with 500μM of a constitutive NO synthase (cNOS) activator N-Methyl-D-Aspartate (NMDA), a significant inhibition of VSV production was observed. Inhibition by 500μM of NMDA was reversed by 300μM of NOS inhibitor N-Methyl-L-Arginine (L-NMA).

Work is in progress to determine the effects of inducible NOS (iNOS) in a glioma cell line C6 on VSV infection. Levels of NO and expressions of both cNOS in neurons and iNOS in glial cells in the CNS following VSV will be further investigated.

Supported by NIH grant AI18083 to Carol S. Reiss.

J2-245 IDENTIFICATION OF CTL EPITOPES WITHIN THE SURFACE (S) GLYCOPROTEIN OF A MOUSE NEUROTROPIC CORONAVIRUS AND THEIR POSSIBLE ROLE IN VIRAL DEMYELINATING DISEASE, Raymond F. Castro and Stanley Perlman, Departments of Microbiology and Pediatrics, University of Iowa, Iowa City, IA. 52242

Mouse hepatitis virus, strain JHM (MHV-JHM), is a neurotropic coronavirus which causes acute encephalitis and acute and chronic demyelinating encephalomyelitis in susceptible rodents. 40-90% of suckling C57Bl/6 (K^bD^b) mice inoculated intranasally with MHV-JHM at 10 days and nursed by dams immunized against the virus develop a chronic demyelinating encephalomyelitis characterized clinically by hindlimb paralysis, at 3-8 weeks postinoculation. Balb/c ($K^dD^dL^d$) mice treated in the same manner do not develop the chronic demyelinating encephalomyelitis nor the clinical symptoms. Recently, it was shown that lymphocytes isolated from the central nervous system (CNS) of C57Bl/6 mice both acutely and persistently infected with MHV-JHM display a cytotoxic T lymphocyte (CTL) response to the S protein of MHV-JHM. This response was further characterized by identifying the CTL epitopes that are recognized by a bulk population of CTLs from the CNS of MHV-JHM infected C57Bl/6 mice. Three epitopes were identified using synthetic peptides and truncated forms of the S protein in primary CTL assays. The epitopes recognized were amino acids 510-518 (CSLWNGPHL, D^b), 598-605 (RCQIFANI, K^b), and 1143-1151 (NFCGNGNHI, D^b). Thus, the results indicate that cytotoxic T lymphocytes responsive to the S protein of MHV-JHM in C57Bl/6 mice recognize both K^b and D^b-restricted CTL epitopes. CTL lines and clones specific to these peptides and the entire S protein are being developed to test their biological significance in vivo with respect to the acute encephalitis and chronic demyelinating disease caused by MHV-JHM.

J2-246 A PSEUDORABIES VIRUS WITH DELETION AT THE STANDARD RECOMBINATION JUNCTION IS DEFECTIVE IN REPLICATION IN THE CENTRAL NERVOUS SYSTEM, Andrew K. Cheung,[1] Hansi J. Dean,[1] Janice M. Miller[2] and Mark R. Ackermann,[3] Virology Swine,[1] Virology Cattle,[2] and Avian Diseases[3] Research Units, National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA 50010

A recombinant pseudorabies virus (PRV) (LLT$\beta\Delta$2) was constructed which contains a 3.0 kb deletion spanning the standard recombination junction of the unique long and internal repeat sequences replaced by a lacZ expression cassette. This deletion interrupted the large latency transcript gene (LLT) and truncated one copy of the diploid immediate early IE180 gene. Replication and viral gene expression of LLT$\beta\Delta$2 in Madin-Darby bovine kidney cells was similar to that of the parental virus and a virus rescued for the deleted sequences (LLTβres). When inoculated intranasally in 4-week-old or 4-day-old pigs, LLT$\beta\Delta$2 replicated efficiently at the site of inoculation yet caused markedly reduced fatality when compared to the parent or LLTβres viruses. In particular, the LLT$\beta\Delta$2-infected pigs did not exhibit neurological symptoms characteristic of PRV infection. To further examine the pathogenesis of LLT$\beta\Delta$2, 4-day-old pigs were infected intranasally with LLT$\beta\Delta$2 or LLTβres and necropsied at various times post-infection. Virus isolation from the nasal turbinate, tonsils, and trigeminal ganglia was comparable between the two viruses. Although both viruses spread to the brain and induced an inflammatory response in CNS tissues, virus isolation from brain tissues was reduced about 20-fold for LLT$\beta\Delta$2. Abundant PRV antigen was detected in the cerebrum and cerebellum of LLTβres-infected pigs, but only a few antigen positive neurons were observed in the cerebrum of LLT$\beta\Delta$2-infected pigs. While replication of LLTβres in the brain progressed until death at 7 days post-infection, replication of LLT$\beta\Delta$2 in the brain ceased by 9 days post-infection and the pigs exhibited only mild clinical signs. Since LLT$\beta\Delta$2 is capable of spread to the CNS, reduced neurovirulence of LLT$\beta\Delta$2 is likely the result of its decreased ability to replicate in CNS tissues.

J2-247 AGE-RELATED RESTRICTED REPLICATION AND SPREAD OF SEMLIKI FOREST VIRUS IS RELATED TO SYNAPTOGENESIS AND MATURITY OF NEURONAL SYSTEMS

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A marked change in susceptibility to some neurotropic viruses during the first few postnatal weeks has long been recognised in rodents. Infection of neonatal or suckling mice with the neurotropic alphavirus, Semliki Forest virus results in lethal encephalitis. Infection of weaned animals is not lethal. Earlier investigations focusing on changes in specific immunity have shown this not to be the explanation. Infection of 3-4 week old mice with severe combined immunodeficiency does not result in acute rapidly fatal encephalitis. We have studied mortality, neuroanatomical distribution and spread of infection in mice of different ages and the effect of gold compounds on rendering infection of 3-4 week old mice lethal. Neuroanatomical distribution of infection correlates with synaptogenesis. As this is completed in different systems within the first two weeks postnatal, systems no longer transmit virus and infection switches from disseminated to focal and restricted. Complete productive replication and transmission of infection require smooth membrane synthesis which is present in neurones undergoing synaptogenesis, absent in mature neurones but inducible by administration of gold compounds. Infection of neurones undergoing synaptogenesis is productive and virus is transmitted along neuralpathways, infection spreads rapidly around the brain, destroys cells and animlas die of a fulminant encephalitis. In mice infected after 14 days of age replication in mature neurones is restricted, non-productive, cannot be transmitted, does not spread, is non-destructive and non-lethal. As a consequence, in the absence of immune responses virus can persist in isolated CNS cells for life and can even be detected by reverse transcriptase PCR in immunocompetent mice months after infection. In the presence of an immuné response, $CD8^+$ T-cells recognise and destroy infected glial cells leading to demyelination.

J2-248 MICROGLIA-MEDIATED SIV INFECTION, Howard S. Fox, Thomas E. Lane, Lisa H. Gold, Debbie Watry, Dorata Jakubowski, and Marla Streb, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

The CNS is a target for HIV infection, and in individuals with AIDS this can lead to a devastating dementia. Only certain viral variants appear capable of invading the CNS and infecting microglia and brain macrophages. In order to determine whether the virus entering the brain may be particularly pathogenic to the CNS, we isolated microglia from the brains of SIV-infected rhesus monkeys. Transfer of these cells into naive animals indicated that productive SIV infection could indeed be transferred. Furthermore, CNS infection occurred within a relatively short time span, and was associated with viral gene expression in the brain and pathology characteristic of HIV encephalitis. Serial transfer of microglia into additional animals also resulted in successful transfer of infection, neuroinvasion, and neuropathology. Behavioral analysis in a trained group of animals is ongoing.

This result demonstrates that neuropathogenic virions partition into the CNS during natural SIV infection, likely driven by mutational events that occur during the course of infection. Molecular characterization of the microglia-associated virus has revealed that a distinct pattern of sequence changes in the envelope gene occurs concomitantly with this *in vivo* selection. Our approach will allow the dissection of functional neuropathogenic elements present in these viruses.

J2-249 MACROPHAGE-DERIVED NITRIC OXIDE-MEDIATED INHIBITION OF VIRUS REPLICATION, Gunasegaran Karupiah and Nicholas Harris, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Successful elimination of a viral infection is dependent on both innate and acquired immunity. The induction of an anti-viral state by interferons (IFNs) is one of the earliest known responses to viral infection and is an essential element in non-specific host defense mechanisms. IFN-γ-induced nitric oxide (NO) in murine macrophages was previously shown to inhibit the replication of poxviruses and herpes simplex virus type 1 (HSV-1). We now demonstrate that murine macrophages activated as a consequence of vaccinia virus (VV) infection in vivo express inducible nitric oxide synthase (iNOS). The VV-elicited macrophages were resistant to infection with VV and efficiently blocked the replication of VV and HSV-1 in infected bystander cells of epithelial and fibroblast origin. This inhibition was arginine dependent, correlated with NO production in cultures and was reversible by the NOS inhibitor N^{ω}-monomethyl-L-arginine. The mechanism of NO mediated inhibition of virus replication was studied by treating VV-infected 293 cells with the NO-producing compound, *S*-nitroso-*N*-acetyl-penicillamine. Antibodies specific for temporally expressed viral proteins, a VV-specific DNA probe and transmission electron microscopy were employed to show that NO inhibited late gene protein synthesis, viral DNA replication and virus particle formation, but not expression of the early proteins analyzed. Further, we have also identified putative enzymatic targets of inactivation by NO that results in inhibition VV replication. Although antiviral CTL are important for virus elimination, they can only halt further virus spread, and cannot reduce the number of infectious particles already present. The beneficial effect of CTL-mediated lysis is apparent only if infected cells are lysed before assembly of progeny virus. If infectious virus was released from infected cells in solid tissues before the generation of neutralizing antibody or in sites where antibody did not readily penetrate, then recruitment of mononuclear phagocytes, which phagocytose and destroy infectious material and/or become non-productively infected, would definitely help control virus dissemination. In this context, iNOS induction in macrophages may be an important antiviral strategy. In addition, the inhibition of virus replication in infected contiguous cells by iNOS-expressing macrophages at infectious foci would prevent release of mature viral particles after lysis by NK cells and CTL. Since viral early proteins are expressed in such infected cells, their recognition and subsequent lysis by CTLs will not be hindered.

J2-250 MOLECULAR ANALYSIS OF MICROGLIA-MEDIATED SIV INFECTION, Thomas E. Lane, Michael J. Buchmeier, Dorota Jakubowski, Debbie D. Watry, and Howard S. Fox, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Our laboratory is interested in the effects of SIV infection in the central nervous system of rhesus macaques. To enrich for neuroinvasive and neurovirulent viruses, microglia were isolated from infected monkeys and used to infect new, uninfected monkeys. Such microglia-mediated infection resulted in the production of neuropathological changes, including giant cells, macrophage infiltrates and microglial nodules in recipient animals within 4 months. Microglial cells isolated from SIV-infected monkeys produced virus *in vitro* as measured by reverse transcription (RT) and p27 production. Treatment of microglia with recombinant human interferon alpha (rHuIFN-α) resulted in a sharp decrease in viral activity (both RT and p27 production) suggesting that rHuIFN-α is able to modulate viral activity in infected microglia. We have analyzed SIV*env* sequences by PCR amplification directly from microglia DNA preparations from monkeys. Nucleotide sequence analysis results in an enrichment of unique sequences in the V1 region of the SIV *env* gene. The majority (>95%) of nucleotide changes encoded amino acid changes, indicating that these envelope sequences evolved as a result of selection. Moreover, sequential passage of SIV-associated microglia resulted in an increase in potential N-linked glycosylation sites within the V1 region of the *env* gene when compared with the parental virus. These data suggest that sequential passage of microglia-associated SIV may select for neuroinvasive, neurovirulent variants.

J2-251 CNS PERSISTENCE, TROPISM AND GENETIC VARIABILITY OF THEILER'S VIRUS

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Theiler's murine enchephalomyelitis virus, a natural occuring enteric pathogen of mice, is a picornavirus belonging to the *Cardiovirus* genus. Following intracerebral inoculation of 3-4 week old CBA or BALB/c mice, the BeAn strain causes a chronic persistent CNS demyelinating infection in a proportion of the CBA that survive acute infection. BALB/c mice are resistant to chronic demyelinating disease. We have studied the tropism, persistence and genetic variability of BeAn, in CBA and BALB/c mice in the chronic phase of this disease. By *in situ* hybridisation and reverse transcription (RT) PCR and Southern blot analysis, no viral RNA could be detected in the CNS of any BALB/c mice later than day 60 post-infection. In contrast, in a large group of CBA mice studied up until 393 days post-infection, viral RNA could be detected by both techniques in 50% of mice until as late as 268 days post-infection. By employing a combination of, *in situ* hybridisation for viral genome followed by immunocytochemistry for cell phenotypic markers, BeAn RNA was observed predominantly in oligodendrocytes and occasionally in astrocytes during persistent infection, in both brain and spinal cord. In the persistently infected mice, the striking total destruction of the pyramidal layer of the hippocampus, substantia nigra and anterior thalamic nuclei indicated that these were the mice that had had greatest dissemination of virus and highest virus titers during the preceeding acute phase of infection. Direct PCR thermal cycle sequencing of uncloned RT-PCR products, revealed that during persistent infection, loops I and II of the VP1 capsid protein gene did not undergo any genetic variability. Furthermore, no changes were detected in this region in sequenced PCR products amplified from the CNS of mice with severe combined immunodeficiency in which no selective immunological pressure would have been operative.

J2-252 THE ROLE OF CD4+ T CELLS IN THE ENTRY OF CD8+ CTL INTO THE CENTRAL NERVOUS SYSTEM DURING ACUTE INFECTION WITH THE NEUROTROPIC JHMV STRAIN OF MOUSE HEPATITIS VIRUS, Stephen Stohlman, Cornelia Bergmann, and David Hinton, Departments of Neurology, Microbiology and Pathology, University of Southern California School of Medicine, Los Angeles, CA 90033.

The adoptive transfer of CTL specific for an Ld-restricted epitope within the nucleocapsid protein of the JHMV strain of mouse hepatitis virus both protect from acute infection and reduce virus replication in the MHC class 1 positive cells within the CNS. The source of these CTL and the route of their delivery is critical in the outcome of this protection. For example, 10 fold less spleen cells activated in vitro with the pN peptide are required for protection via the direct i.c. route than the i.v. route. In addition, CTL clones are unable to protect via the i.v. route and are very efficient via the i.c. route. These data suggested the possibility that the CD4+ T cells within the polyclonal activated spleen cell population derived from in vitro culture on the pN peptide were facilitating access to the CNS. To examine this question, polyclonal pN-specific T cells were either depleted of CD4+ T cells prior to transfer to infected recipients or untreated cells were transferred to recipients depleted of CD4+ T cells with monoclonal antibody GK1.5. Both of these treatments eliminated the ability of the CTL to reduce virus replication within the CNS, suggesting that CD4+ T cells in the peripheral compartment are required for the entry of CTL into the parenchyma of the CNS during acute CNS encephalomyelitis.

Vaccine Design and Strategies

J2-300 DUAL INFECTION WITH HIV-1 OF DISTINCT ENV SUBTYPES IN HUMANS, A. W. Artenstein, T. C. VanCott, J. R. Mascola, J. K. Carr, P. A. Hegerich, J. Gaywee, E. Sanders-Buell, M. L. Robb, D. E. Dayhoff, S. Thitivichianlert, S. Nitayaphan, J. G. McNeil, D. L. Birx, R. A. Michael, D. S. Burke, F. E. McCutchan, Division of Retrovirology, Walter Reed Army Institute of Research and Henry M. Jackson Foundation, Rockville, MD 20850; Department of Retrovirology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Background. The HIV-1 epidemic in Thailand is largely due to two highly divergent subtypes of virus, B and E. Dual infection with distinct HIV-1 subtypes, which has not been reported previously, would suggest that antiviral immunity evoked by one subtype can be incompletely protective against a second. *Methods:* PCR typing and serologic typing were used to screen a panel of non-random convenience specimens from HIV-1 infected subjects in Thailand. Specimens that showed dual subtype reactivity in these assays were subjected to differential probe hybridization and nucleotide sequence analysis of multiple molecular clones to confirm the presence of dual infection. *Results.* Two individuals were shown to simultaneously harbor HIV-1 of *env* subtypes B and E (Table). Additionally, both subtypes were identified in co-cultured PBMC from one individual. *Conclusions.* These data provide the first evidence of dual HIV-1 infection in humans and reinforce the need for polyvalent vaccines.

Table. Genotypic and serotypic analysis of clinical samples

Subject	PCR type	V3 serotype *		Colony hybridization		
		B	E	#positive	%B	%E
Case 1	B +E	<50	200	247	3	97
Case 2	B + E	1600	3200	86	50	50
Subtype E control	E	<50	3200	88	0	100
Subtype B control	B	1600	<50	105	100	0

*Values represent V3 ELISA serum endpoint titers; <50 indicates no reactivity observed at lowest serum dilution used (i.e. 1:50)

J2-301 RETROVIRAL-MEDIATED INDUCTION OF MHC-CLASS- I RESTRICTED CTL SPECIFIC FOR HEPATITIS B VIRUS (HBV) CORE ANTIGEN, Theresa A. Banks[1], Kay Townsend[1], Joanne O'Dea[1], David Milich[2], Jan Hughes[2], Stephen Chang[1], Douglas Jolly[1], and William T.L Lee[1], Viagene, Inc., 11055 Roselle Street, San Diego, CA 92121; The Scripps Research Institute, La Jolla, CA

Since the cytotoxic T lymphocyte (CTL) response appears critical for achieving recovery from virus infections, interest continues to be focused on the identification of viral antigens recognized by CTL and the means by which such CTL responses can be optimally induced. Ultimately, this information should allow for the rational design of effective vaccines and immunotherapeutics. Previously, Viagene has demonstrated that recombinant retroviral-mediated expression of HIV *env* induces potent antigen-specific CTL responses in mice and macaques. In the present study, HBV core antigen was expressed from a replication-defective recombinant retroviral vector. Intramuscular or intradermal injection of this vector into C3H/He (H-2k) mice induced both humoral and cellular immune responses. Specifically, IgG antibodies to the HBV core were induced as well as $CD8^+$ MHC-class I- restricted CTL. Interestingly, the CTL response is cross-reactive since syngeneic cell lines expressing either HBV core or precore antigen were lysed by the induced CTL. A variety of approaches are currently being used to identify the epitopes which induce these responses.

J2-302 INDUCTION OF CTL BY RECOMBINANT ICP27 PROTEIN OF HERPES SIMPLEX VIRUS-2 FORMULATED IN OIL-IN-WATER EMULSIONS AND ADJUVANTED WITH MPL AND QS21, Carine Bastin°, Daniel Gilles°, Omer Van Opstal°, Patricia Momin° Nathalie Garçon°, Myriam Francotte°, Michel Hendricks*, Martin Comberbach°, Moncef Slaoui° and Pietro Pala, R&D Department, °SmithKline Beecham Biologicals, Rixensart, Belgium; *Service de Génétique Appliquée, CRI/ULB, Nivelles, Belgium.

Infection by herpes simplex virus (HSV) induces in man and in mice cytolytic T lymphocytes (CTL) which recognize the immediate-early protein ICP27.

Because of its early expression during the HSV replication cycle, ICP27 represents a prime target for specific T cell responses susceptible of controlling virus replication.

We have expressed in E. coli a recombinant construct coding for a fusion protein consisting of a fragment of influenza virus non-structural protein-1 (NS1) and the ICP27 sequence of HSV-2. The NS1-ICP27 protein was purified by preparative electrophoresis and formulated in oil-in-water emulsions with monophosphoryl lipid A (MPL) and QS21 adjuvants. Balb/c mice were immunized by two intra-footpad injections of formulations containing 5 µg of NS1-ICP27. Responder cells obtained from draining lymphnodes were re-stimulated in vitro with P815 cells transfected with ICP27 and then tested for cytolytic activity on ICP27-P815 and control P815. The induction of ICP27 specific CTL by different formulations was observed and will be discussed.

J2-303 CHIMERIC CTL EPITOPES: CTL RECOGNITION VERSUS INDUCTION, Cornelia Bergmann, Dept. of Neurology and Microbiology, USC School of Medicine, Los Angeles, CA 90033.

The induction of heterologous cytotoxic T lymphocytes (CTL) using cassettes of multiple conserved T cell epitopes derived from different proteins and/or virus strains is envisioned as a promising vaccine approach. To study the effects of antigen processing on peptide presentation from chimeric epitope precursors we are using a model system comprising two distinct viral epitopes which are immunodominant in the H-2d haplotype: a Dd restricted epitope from the gp160 protein of HIV-1 and an Ld restricted epitope from the murine hepatitis virus nucleocapsid protein (MHV N).

The influence of proximity and flanking sequences of epitopes on antigen presentation was analyzed using vaccinia virus (vv) recombinants in which the epitopes were expressed as chimeras containing the individual epitopes in reverse order or separated by different spacer residues. Whereas individually expressed epitopes were efficiently recognized by protein-specific CTL, recognition of peptides derived from tandem constructs varied significantly with closer epitope proximity and sequential order. Following immunization with the recombinant viruses, the chimeras were all able to induce antiviral CTL specific for the native proteins. However, CTL frequency analysis indicated that the number of responder cells to the same epitope dramatically depends on its context within the chimera and correlates with antigen recognition in vitro. The profound effect of flanking regions on CTL induction suggests that the context of an epitope will require careful evaluation in the design of recombinant multivalent minigene vaccines to induce an optimal T cell mediated immune response.

J2-304 ANALYSIS OF THE ANTIVIRAL CYTOTOXIC T LYMPHOCYTE RESPONSE IN PRIMARY HIV-1 INFECTION, Persephone Borrow[1], Hanna Lewicki[1], Marc Horwitz[1], Beatrice H. Hahn[2], George M. Shaw[2] and Michael B.A. Oldstone[1], [1]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, [2]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294

Human immunodeficiency virus type 1 (HIV-1) Env-, Gag-, Pol-, Nef-, and Tat-specific cytotoxic T-lymphocyte (CTL) activities were quantitated temporally in five patients with sympomatic primary HIV-1 infection. A dominant $CD8^+$-mediated, major histocompatibility complex class I-restricted CTL response to the HIV-1 envelope glycoprotein, gp160, was noted in four of the five patients studied. The level of HIV-1-specific CTL activity in the five patients paralleled the efficiency of control of primary viremia. Patients who mounted strong gp160-specific CTL responses showed rapid reduction of acute plasma viremia and antigenemia, while in contrast, primary viremia and antigenemia were poorly controlled in patients in whom virus-specific CTL activity was low or undetectable. These results suggest that HIV-1-specific CTL activity is a major component of the host immune response associated with the control of virus replication following primary HIV-1 infection. To characterise this response further, a panel of HIV-1-specific CTL clones has been established from acute-phase CTL from one of the patients and their epitope specificity is being determined. Definition of the heterogeneity and cross-reactivity of the primary CTL response to HIV-1 will have important implications for the design of antiviral vaccines.

J2-305 DNA VACCINES: EFFECTS OF DOSING SCHEDULE ON ANTIBODY RESPONSES.

C.M. Boyle, M.J. Morin, and H.L. Robinson, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655

Different schedules of DNA inoculations are being evaluated for their effect on antibody responses to the influenza virus hemagglutinin glycoprotein. Studies are being conducted in a murine influenza virus model. All inoculations consisted of 0.4 ug of a hemagglutinin-H1-glycoprotein-expressing DNA delivered with a DNA gun. One group of animals has been given a single DNA inoculation. A second group has been given monthly inoculations. A third group is receiving biweekly inoculations. The effects of these different immunization schedules on the time course and isotype of antibody responses and the breadth of neutralizing responses will be presented.

J2-306 QUANTITATION OF HUMAN VIRAL EPITOPE SPECIFIC CTL PRECURSORS BY LIMITING DILUTION, Andreas Cerny* ¶, Benno Grabscheid*, Mary A. Brothers¶, Werner J. Pichler# and Francis V. Chisari¶, The Scripps Research Institute, La Jolla CA 92037; *Department of Medicine and #Institute for Clinical Immunology, Inselspital, University Hospital, 3010 Bern Switzerland;

Immunological memory after viral infection is thought to be mediated by an expanded population of immune effector cells. Detection of cytotoxic T cells (CTL) specific to viral antigens in the peripheral blood of immune donors usually requires an in vitro restimulation of large cell numbers using viral antigens or synthetic peptides containing HLA class I binding motifs. We have used this approach for the in vitro expansion of in vivo sensitized CTL in patients with hepatitis B (HBV) and hepatitis C virus (HCV) infection. This has led to the definition of multiple HLA-A2 restricted CTL epitopes for both viruses. This type of culture systems yields only limited quantitative information. We herein describe a protocol for the quantitation of viral epitope specific CTL in the peripheral blood. The method uses virus derived HLA-A2 binding synthetic peptides derived from influenza virus, HCV or HBV and r-hu IL-2 added to peripheral blood monunuclear cells in a limiting dilution format. For optimal sensitivity and fulfillment of single hit criteria cultures have to be maintained for 21 days including restimulation with autologous antigen presenting cells before testing in a split well CTL assay against HLA-A2 matched target cells sensitized with peptide or medium alone. HLA-A2 restricted effector cells specific for influenza matrix M58-68 could be detected in the blood of normal donors in the order of one precursor per 10^{-5} PBMC. Similar CTL precursor frequencies to HBV (HBV core 18-27) and HCV (HCV core 131-140, NS3 1406-1415) derived peptides could be detected in patients chronically infected with the corresponding virus. CTL effector cells detected derive from the CD45 RO positive subset. Uninfected subjects had CTL precursor frequencies which were 10 to 100 times lower. In summary, we describe a method for the quantitation of viral epitope specific, class I restricted CTL precursors in the peripheral blood using a limiting dilution system. This will serve as a tool to prospectively assess the human CTL response to selected, defined epitopes during the course of viral disease and after vaccination.

J2-307 INHERENTLY IMMUNOGENIC CO-ARRAYS OF DEFINED SIZE AND VALENCE BEARING T CELL AND B CELL EPITOPES. Claire Coeshott, Cheryl Cook, Catherine McCall, Christie Ohnemus, James Blodgett, Lisa Allen and Vidal de la Cruz, Cortech Inc., Denver, CO 80221.

It has been shown that a T-independent, IgM anti-hapten antibody response in mice may be induced specifically by treatment with large multivalent hapten-dextran arrays of defined size and valence[1]. Most vaccines of clinical importance require a T-dependent antibody response. Our research has therefore been directed towards the preparation of inherently immunogenic, multivalent arrays that can stimulate IgG antibody responses. Multivalent constructs were prepared in which a controlled number of molecules either of the hapten, fluorescein, or the *Plasmodium berghei* B cell epitope, $(DPPPPNPN)_2$, were co-arrayed with a controlled number of copies of a T cell peptide epitope (chicken ovalbumin 323-339) on size-fractionated dextran (MW 500 kd). IgG antibodies were raised in Balb/c mice to fluorescein with one immunization of 10ug of the co-array. IgG antibodies were also detected to the *P. berghei* epitope two weeks following a second dose of the co-array. Adjuvants were not required to produce these antibody responses. These data have implications for future vaccine development whereby multiple B and T epitopes could be arrayed on the same backbone in a configuration designed to meet the minimal requirements for stimulation.

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J2-308 PROTECTION AGAINST INFLUENZA IN MICE BY VACCINATION WITH A VENEZUELAN EQUINE ENCEPHALITIS VIRUS VECTOR EXPRESSING THE HA PROTEIN,

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A full-length cDNA clone of Venezuelan equine encephalitis virus (VEE) has been altered to contain two strongly attenuating mutations and a second subgenomic RNA promoter immediately downstream of the structural gene region. Expression of the influenza HA protein from this second promoter in baby hamster kidney (BHK) cells was approximately 50% of the level in influenza virus-infected cells, as measured by immunoprecipitation. Four-week-old CD-1 mice were inoculated subcutaneously with 2×10^4 pfu of the HA vector, vector alone or diluent. Expression of HA mRNA was detected in the draining lymph node of HA vector-inoculated mice by *in situ* hybridization, consistent with the organ tropism of VEE. Mice were challenged three weeks after immunization by intranasal administration of 10^5 EID_{50} of influenza virus. All 24 control mice suffered severe disease and 50% died. Only one of 12 HA vector-inoculated mice died, and another exhibited signs of disease for one day and recovered. The geometric mean ELISA titer of anti-HA serum IgG in the HA-vector inoculated mice was 246, while only three control mice had measurable serum reactivity, and that was at the lowest dilution tested, 1:50. In a parallel experiment, no influenza infectivity was detected in the lungs of 12 HA vector immunized mice at 4 days post-challenge. In contrast, 8/12 PBS-inoculated mice and 5/12 inoculated with vector alone were positive for influenza infectivity and had geometric mean titers of 3.04 and 1.93×10^6 pfu/gm, respectively. This vector also has been used to express the HIV MA/CA protein in a form recognized by patient sera and a specific antibody on Western blots. These experiments demonstrate the feasibility of using vectors based on attenuated VEE cDNA clones for protective immunization against heterologous human and animal pathogens.

J2-309 MODULTION OF IMMUNE RESPONSES TO DNA VACCINES

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Inoculation of mice with plasmid vectors carrying a microbial gene under the control of an appropriate promoter results in a full spectrum of immune responses to the vector-encoded antigen. Using a murine rabies model a plasmid termed pSG5rab.gp expressing the full-length rabies virus glycoprotein regulated by an SV40 promoter was shown to induce upon intramuscular inoculation a rabies virus specific T helper cell response of the Th1 type, cytolytic T cells and virus neutralizing antibodies resulting in protection against a subsequent challenge with live rabies virus given either peripherally or directly into the central nervous system. A response comparable in magnitude was also induce upon inoculation of a vector expressing a secreted form of the rabies virus glycoprotein.
The immune response to the DNA vaccine could be modulated by co-injection of the rabies virus glycoprotein-expressing vector with plasmids expressing mouse cytokines. Inoculation of mice with the pSG5rab.gp vector and a vector expressing granulocyte/macrophage colony stimulating factor (GM-CSF) enhanced both the T helper and the B cell response to rabies virus thus improving vaccine efficacy. Co-inoculation with vectors expressing interferon-g failed to improve the response. Co-inoculation of the antigen-expressing vector with a plasmid encoding mouse IL-4 caused a reduction of both the T helper cell response and the B cell response to rabies virus.

J2-310 DNA VACCINES: DOSE/RESPONSE ANALYSES FOR DIFFERENT ROUTES OF INOCULATION.

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Dose/response curves have been used to compare different routes of immunization with plasmid DNA encoding the H1 hemagglutinin glycoprotein of influenza virus. Routes of inoculation included intramuscular, intradermal and gene gun delivery of DNA. From 100 to 0.1 ug of DNA was inoculated by intramuscular and intradermal routes. From 0.4 ug to 0.0004 ug of DNA was inoculated by gene gun. Each route was evaluated for single and boosted immunizations. Antibody titers were followed over a 20 week period, following which animals were evaluated for protection against a lethal challenge. Each of the routes raised both antibody and protective responses. Gene gun-delivery of DNA required 250 to 2,500 times less DNA to raise responses than the intramuscular and intradermal inoculations. Boosts did not have much of an effect on antibody titer or protection except at low dose inoculations (4 ng and lower for the gene gun). For each of the routes, antibody responses showed good persistence over the 20 weeks of the experiment.

J2-311 HPV16 E7 TRANSGENIC SKIN IS NOT REJECTED IN E7 IMMUNISED MICE.

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HPV associated cervical cancer cells express HPV16E7 protein and antibody to HPV16 E7 can be detected in the blood of cancer patients, yet the tumours are not rejected. A mouse transgenic for the E7 protein of HPV16, and expressing E7 protein in the skin, has recently been described (1) and these mice develop spontaneous humoral immunity to E7 protein similar to patients with cervical cancer(2). To determine whether immunisation could induce immunity to E7 sufficient to allow tumour rejection, we firstly demonstrated that immunisation of $H\text{-}2^b$ mice with HPV16E7 protein with Quil A as adjuvant could induce cytotoxic T cells able to kill HPV16 E7 expressing tumour cells *in vitro*. We then used similar immunisation with E7/Quil A to induce E7 specific immunity in FVB ($H\text{-}2^q$) mice. $H\text{-}2^q$ skin grafts expressing E7 were not rejected by E7 immunised $H\text{-}2^q$ mice, though immunisation induced antibody to E7, and similar grafts were rejected, as expected, across an alloantigen mismatch in $H\text{-}2^b$ mice. We conclude either that HPV16 E7 lacks a Tc epitope in the context of $H\text{-}2^q$, or that expression of E7 in the skin from the E7 transgenic mice is insufficient for recognition by primed effector cells, and further experiments will address this distinction.

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J2-312 INDUCTION OF CTL RESPONSES USING HYBRID Ty-VIRUS-LIKE PARTICLES, Tim French, Steve Harris, Daniel West, Jeremy Myhan, Nichola Meyers, Michele Hill-Perkins, Sarah Woodrow and Guy Layton. British Biotech Pharmaceuticals Ltd, Watlington Rd, Oxford, OX4 5LY, UK.

CD8+ cytotoxic T-lymphocytes (CTL) play a major role in protection against intracellular pathogens and also in destroying tumour cells. The ability to induce specific CTL responses by vaccination would therefore be of great therapeutic value. However, using simple monomeric protein immunogens and existing adjuvants, the induction of CTL responses has proved difficult. In our attempts to develop improved vaccines, we have been evaluating particulate, multivalent antigen presentation systems, one of which utilises the p1 protein encoded by the TYA gene of the yeast retrotransposon (Ty). Coding sequences from proteins of interest are fused to the 3' end of a truncated TYA gene. The expression of these genes in yeast leads to the production of p1 fusion proteins which spontaneously assemble into hybrid virus-like particles (VLPs) which are easily purified.

We have previously shown that VLPs carrying the V3 loop of HIV-1 gp120 (V3-VLPs) are potent inducers of V3-specific CTL responses in mice. These CTL were CD8+ and H-2d-restricted. The use of alum adjuvant inhibited CTL induction but augmented T helper cell priming. We have now demonstated that hybrid VLPs carrying sequences from several other microbial proteins can prime for rapid and potent CTL induction in mice; for example, Sendai and influenza virus nucleoproteins, Malaria circumsporozoite protein and human papilloma virus16 E7 protein.

The mechanism of induction, the kinetics and the protective capacity of hybrid VLP-induced CTL responses are now under investigation.

J2-313 EFFECT OF PREVIOUS INFLUENZA INFECTION AND/OR IMMUNIZATION WITH DNA ON THE ABILITY OF MICE TO RESIST INFLUENZA CHALLENGE, Arthur Friedman, Douglas Martinez, John J. Donnelly and Margaret A. Liu, Department of Virus and Cell Biology Research, Merck Research Laboratories, West Point, PA 19486

Mice infected with the laboratory strains of A/PR/8/34 (H1N1) or the mouse adapted A/HK/68 (H3N2) show complete protection against challenge with a different strain of Influenza A. Humans, however, undergo multiple influenza infections as previous infections appear to provide weak or short-lived protection against the continual antigenic change of strains. We have previously shown that immunization of naive mice with DNA encoding the conserved internal antigen nucleoprotein (NP) provides protection against both H1 and H3 strains of A/influenza. Although such mice became infected they were resistant to weight loss and death: this differed substantially from A/PR8 and A/HK recovered mice which were resistant to subsequent infection. To produce a more representative model of human infection, we infected the lungs of mice with currently circulating strains of human influenza. Mice that had been given lung infections with A/Beijing/92 were susceptible to subsequent infection with the A/HK/68 strain although they were resistant to weight loss and death. Other strains such as A/Beijing/89 or A/Georgia/93 provided only marginal protection against weight loss and death against A/HK challenge. Mice that were immunized with NP DNA had greater resistance to weight loss and death after A/HK/68 challenge than mice previously infected with A/Bei/89 and A/Ga/93, and were similar to mice that had been previously infected with A/Bei/92. Thus, infection with different virus strains provide various levels of cross strain protection and the level of protection provided by immunization with DNA can exceed that induced by live influenza infection.

J2-314 **CONSTRUCTION OF A RECOMBINANT VACCINIA VIRUS EXPRESSING HPV TYPES 16 AND 18 E6 AND E7 FOR IMMUNOTHERAPY OF CERVICAL CARCINOMA.**

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*Cantab Pharmaceuticals Research Ltd, Cambridge, U.K., †Patterson Institute for Cancer Research, Christie Hospital, Manchester, U.K., and ‡Dept. of Medicine, University of Wales College of Medicine, Cardiff, U.K..

Cervical carcinoma is strongly associated with infection by human papillomavirus (HPV) types 16 or 18, and continued expression of the E6 and E7 gene products. This provides an opportunity for an immunotherapeutic approach to the treatment of cervical carcinoma by activation of immune reponses directed against these virally encoded tumour specific antigens. We have constructed a recombinant vaccinia virus expressing E6 and E7 from HPV16 and 18 with the aim of inducing E6 and E7 specific HLA class I restricted cytotoxic T lymphocytes (CTL).

The sequences have been inserted into the Wyeth vaccine strain of vaccinia virus at a single locus in the form of two separate fused E6/E7 reading frames, each under the control of an early vaccinia promoter, and each modified to inactivate the Rb binding site. The virus has been characterised with respect to its ability to synthesise the expected HPV proteins, its genetic stability, and growth and virulence in a mouse model prior to use in human clinical trials.

Analysis of HPV16 E7 specific CTL from C57BL/6 mice immunised with this recombinant virus show the response to be equivalent to that generated by a control vaccinia recombinant expressing non-modified HPV16 E7 alone, with similar recognition of the defined immunodominant H-2D^b restricted epitope, E7 residues 49-57.

J2-315 HOST RESPONSE TO SENDAI VIRUS IN MICE LACKING CLASS II MHC GLYCOPROTEINS, Sam Hou[1], Lisa M. Hyland[2] and Peter C. Doherty[3], [1]Department of Microbiology, [2]Department of Pathology, University of Otago, Dunedin, New Zealand, [3]Department of Immunology, St Jude Children's Research Hospital, Memphis, TN 38105.

The development of Sendai virus-specific cytotoxic T lymphocyte (CTL) effectors and precursors (p) has been compared for mice that are homozygous (-/-) for a disruption of the H-2I-A^b class II major histocompatibility complex (MHC) glycoprotein, and for normal (+/+) controls. The generation of CD8+ CTLp was not diminished in the (-/-) mice, although they failed to make virus-specific IgG class antibodies. While the cellularity of the regional lymph nodes was decreased, the inflammatory process assayed by bronchoalveolar lavage (BAL) of the infected lung was not modified and potent CTL effectors were present in BAL populations recovered from both groups at day 10 after infection. There was little effect on virus clearance. As found previously with CD4-depleted H-2^b mice, the absence of a concurrent class II-MHC-restricted response does not compromise the development of Sendai virus -specific CD8+ T cell-mediated immunity.

J2-316 CONJUGATION OF A PEPTIDE FROM THE V3 LOOP OF HIV gp120 TO THE VACCINE CARRIER *BRUCELLA ABORTUS* INDUCES A SPECIFIC, MHC-RESTRICTED CYTOTOXIC RESPONSE AGAINST HIV gp120. Cheryl K. Lapham[1], Hana Golding[1], John Inman[3], Robert Blackburn[1], and Basil Golding[2]. [1]Divisions of Viral Products and [2]Hematology, CBER, FDA, and [3]Laboratory of Immunology, NIAID, NIH, Bethesda, MD.

In previous studies we showed that immunization of mice with inactivated *Brucella Abortus* (BA) conjugated to HIV-derived proteins or peptides induces the secretion of virus-neutralizing antibodies, mainly of the IgG2a isotype. In addition, BA was shown to activate human CD4+ and CD8+ cells to secrete IFN-γ. Since these are both characteristics of a Th1-type immune response, which is associated with the development of cell-mediated immunity, we determined whether BA would also induce a CTL response. As a model antigen we conjugated an 18 amino acid peptide from the V3 loop of HIV gp120 that contains both B and T cell epitopes (BA-V3). A 10 amino acid fragment of this peptide has been shown to be the minimal CTL determinant presented by D^d. We found that two *in vivo* immunizations with 10^8 BA-V3 organisms followed by *in vitro* stimulation with peptide induced a specific CTL response in neonates as well as adults. Conjugation to BA was required, since there was no induction when BA was mixed with peptide. Targets pulsed with peptide as well as those infected with a vaccinia virus encoding HIV gp120 were recognized, demonstrating recognition of naturally-processed peptide. In addition, MHC-incompatible cells infected with vaccinia viruses encoding D^d but not K^d were lysed, showing Class I restriction. Since most antigens presented by class I MHC are synthesized intracellularly, we are currently studying the processing mechanism of this immunogen. Pretreatment of the mice with anti-L3T4 prior to immunization caused a severe depletion of CD4+ lymphocytes in the spleen, yet did not decrease the CTL induction. Therefore, BA can induce non-CD4+ cells to produce the cytokines required for CTL induction. Based on these results, we conclude that *Brucella Abortus* stimulates a cellular as well as humoral immune response, even in the relative absence of helper cells. It may be a particularly useful vaccine carrier in HIV-1-infected children or adults with impaired CD4+ T cell function.

J2-317 RABIES VIRUS GLYCOPROTEIN EXPRESSED IN A VACCINIA VIRUS RECOMBINANT VACCINE CROSS PROTECTS MICE AGAINST UNIQUE GENETIC VARIANTS OF RABIES VIRUSES ISOLATED WORLDWIDE, Donald L. Lodmell [1], Jean Smith [2], and Larry C. Ewalt [1]. [1] Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT 59840; [2] Rabies Laboratory, Viral and Rickettsial Zoonoses Branch, Division for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333.

Rabies today occurs primarily in underdeveloped Third World countries where canine rabies is uncontrolled. Rabies also is epizootically active in several species in most areas of the world. Thus, vaccination of animals, both wild and domestic, as well as postexposure treatment of humans remains a global concern. Unfortunately, in those countries in which people most need post-exposure prophylaxis, the best vaccines are expensive and in limited supply, whereas available vaccines are of questionable immunogenic efficiency, are often contaminated and may produce neurological complications. The goal of this study was to determine whether a rabies vaccine for global use is feasible. To this end, mice were vaccinated with either the glycoprotein of the fixed CVS rabies virus expressed in a recombinant virus, or the human diploid cell vaccine (HDCV). Vaccinated mice were then challenged with 17 rabies virus strains that were representative of various reservoirs of rabies worldwide (domestic dogs in Asia, Africa and Latin America and wild carnivores and bats in North America). The 17 strains of virus also were representative of the 11 different genetic subgroups of rabies virus as determined by a >/= 10% difference in the nucleotide sequence of a 200 bp region of their nucleoprotein gene. Protection studies showed that either 1×10^7 PFU of recombinant virus administered by tailscratch, or 0.05 ml of IM-administered HDCV, protected > 99% of the mice that had been infected intraplantarily with 200 $MFPLD_{50}$ of the 17 viruses representing every genetic subgroup. Mice also were protected against varying concentrations of these same viruses which were present in host brain samples, but had not been passaged in mice prior to use. Cross-neutralization studies with antisera prepared against each virus determined that each antiserum completely neutralized all 17 viruses. These results indicate that a singular rabies vaccine for global use is possibile, that tailor-made vaccines for specific areas of the world are not necessary, and that possible antigenic differences between the fixed rabies viruses that are used to prepare vaccines and the antigenic variability of the wild-type street viruses prevailing worldwide should not result in vaccine failures.

J2-318 **MECHANISMS OF INDUCTION OF ANTIGEN SPECIFIC CYTOTOXIC T LYMPHOCYTES USING SUBUNIT VACCINES.** Raphael J. Mannino, Mario Canki, Yvette Edghill-Smith, Eleonora Feketeova, and Susan Gould-Fogerite. Dept. of Laboratory Medicine and Pathology, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2714

The importance of cytoxic T lymphocytes in defense against acute and chronic viral infections is gaining increasing recognition. Our approach to investigating the structure-function relationship between immunogens and their in vivo ability to elicit cytotoxic T lymphocyte responses has been to formulate simple, well-defined structures that vary in their ability to introduce associated antigens directly into the cytoplasm of antigen presenting cells.

We have introduced methods for the preparation of unique, lipid-matrix based immunogens, which are highly effective in mice and monkeys for stimulating strong CD8+ cytotoxic T cell responses, (CTL). Antigens used have been proteins or peptides derived from influenza, parainfluenza, and HIV viruses, and whole formalin-fixed SIV. CTL can be induced by parenteral as well as oral administration.

Comparing the physical and chemical nature of our formulations with those from other laboratories which have reported the use of subunit preparations to induce CD8+ CTL, leads us to propose that a minimal immunogenic formulation capable of eliciting CD8+, MHC Class I restricted cytotoxic T lymphocytes includes: I) a peptide that represents a MHC Class I epitope; ii) a component that enhances the affinity of the immunogen for MHC Class I positive antigen presenting cells ; iii) properties that can compromise the integrity of a lipid bilayer, facilitating delivery of the antigen directly into the cytoplasm for class I presentation.

The ability of our lipid matrix-based vaccines to stimulate CD8+ responses to peptides, glycoproteins, and even whole fixed viruses, makes them attractive candidates for diseases where clearance of infected cells is important in protection and recovery.

J2-319 **LONG-TERM PROTECTION FOLLOWING VACCINATION WITH A gH-DELETED HERPES SIMPLEX VIRUS TYPE 1 .** C.S.McLean[1], I.A.Duncan[1], S.E.Chisholm[1], S.L.Bolt[1], M.E.G.Boursnell[1], S.C.Inglis[1], A.C.Minson[2] and J.G.Shields[1]. [1]Cantab Pharmaceuticals Research, 184 Cambridge Science Park, Cambridge CB4 4GN, U.K. [2]Division of Virology, University of Cambridge, U.K.

Viruses which lack an essential gene and thus can only complete one round of replication have the potential to be used as vaccines. We have previously reported the ability of a gH-deleted Herpes Simplex Virus Type 1(HSV-1) to protect mice and guinea-pigs from subsequent challenge with wild-type HSV. This virus, which we have called DISC (Disabled Infectious Single Cycle) virus, can infect normal cells but the absence of gH in the progeny virus prevents further rounds of infection.

As DISC HSV clearly has potential as a vaccine, it is important to determine the durability of the immune response elicited by this virus. We have investigated the ability of DISC HSV-1 to protect mice from a wild-type virus challenge six months post vaccination using the ear model of HSV infection. Two immunisations on day 0 and day 21 resulted in a considerable reduction in virus titres in the challenged ears, and an almost complete absence of virus in the dorsal root ganglia. HSV-specific antibody titres as determined by neutralisation and ELISA were maintained for the six months period. It was possible to demonstrate an HSV-specific cytotoxic T-cell response in the DISC HSV-1 vaccinated mice following challenge; this CTL activity was similar to that observed in mice vaccinated with wild-type virus and challenged after the same time period. Animals vaccinated with inactivated virus or control mock-vaccinated mice showed a low level of CTL activity typical of a primary CTL response following challenge.

These results indicate that an effective cell-mediated and humoral anti-HSV immune response can be maintained for at least six months following vaccination with DISC HSV-1.

J2-320 **COMPARISON OF FORMULATIONS FOR INDUCTION OF CTL RESPONSES USING SYNTHETIC PEPTIDES,** Douglas F. Nixon, Pei-de Chen, Peter J. Kuebler, Catarina Hioe, Tim Zamb, J. Paul McGee, Derek T. O'Hagan, Howard Qiu, Joseph Li, Chang Yi Wang and Wayne C. Koff, United Biomedical, Inc., Hauppauge, NY 11788.

Objective:
To assess the ability of peptide epitopes presented in three different formulations to stimulate CTL.

Methods:
The immunogenicity of two CTL epitopes, influenza NP147-158 and plasmodium berghei CS protein 252-260 were studied in BALB/c mice. Peptides were formulated as a) a lipopeptide - peptide conjugated to tripalmitoyl-S-glycerol cysteine (Pam_3Cys) and dissolved in a 1% DMSO/glycerol solution, b) microparticles prepared with poly (D,L lactide-co-glycolide) using a solvent evaporation technique. The microparticles were administered as a suspension in phosphate buffered saline or c) an emulsion prepared with egg lecithin and 10% soya oil in water. 100μg of peptide or controls (the weight equivalent of blanks) were administered to groups of 3 mice intra-peritoneally or sub-cutaneously at 1,10 and 20 days. 7 days following the last immunization splenocytes were cultured in vitro in the presence of appropriate peptide or control with rat con A supernatant as a source of growth factors. CTL activity was measured in a standard 4 hour chromium release assay and results expressed as % specific lysis.

Results:
CTL could be elicited in vivo with all three formulations. At an effector:target ratio of 100:1 the plasmodium berghei peptide encapsulated in microparticles gave 47% lysis on peptide pulsed target cells. Levels of lysis were similar for the peptide in emulsions. The lipopeptide P3C-CS252-260 gave a level of lysis of 82% at an E:T ratio of 100:1.

Conclusion:
These results demonstrate that peptides administered in a variety of formulations can induce a systemic CTL response in vivo. Peptide vaccines using such formulations could be used to stimulate CTL responses as part of a prophylactic vaccines or as immunotherapeutics.

J2-321 IMMUNOGENICITY STUDIES IN ADULTS AND CHILDREN WITH TRYPSIN-MODIFIED INACTIVATED POLIOVIRUS VACCINE

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* National Institute for Public Health and Environmental Protection, Bilthoven, the Netherlands (RIVM)

A pilot enhanced-potency inactivated poliovirus vaccine (E-IPV) with assumably improved immunogenicity containing trypsin-treated type 3 poliovirus (strain Saukett) together with the regular type 1 and type 2 components was subjected to standard safety and potency tests in the laboratory and taken through Phase I and II clinical trials. In Balb/c mice, the trypsin-modified E-IPV (TryIPV) was found to induce antibodies targeted outside the trypsin-sensitive BC-loop of capsid protein VP1, as previously shown for trypsin-treated type 3 poliovirus given alone. Trypsin used to modify the type 3 component at the bulk phase was removed by the vaccine manufacturer (RIVM) in the regular purification process. Absence of trypsin in the final product was further confirmed by immunizing mice and rabbits with 10-fold concentrated type 3 component of TryIPV. Assays for trypsin antibodies using EIA and Western blot techniques were negative. In the clinical phase I trial six adult volunteers with existing immunity to poliovirus were given increasing doses of TryIPV. Already one tenth of the regular dose induced a booster effect in neutralizing antibodies to both intact and trypsin-treated type 3 poliovirus. No unexpected side-effects were recorded. Phase II trials comprised 50 adult volunteers with at least 5 years since the last dose of poliovirus vaccine and 50 children who were due to receive the third dose of the regular immunization schedule at about 2 years. In both groups, 25 individuals received TryIPV and 25 were injected with the regular enhanced potency IPV (E-IPV). Serum specimens drawn before injection and one month after were tested for neutralizing antibodies using standard microneutralization assays (all serotypes) and the RACINA test (intact and trypsin-treated type 3 poliovirus). In all volunteers TryIPV was at least as immunogenic as the regular E-IPV according to all assays. No statistically significant differences in side effects were reported.

J2-322 ATTENUATED RECOMBINANT POLIOVIRUSES AS VACCINE VECTORS, Patricia A. Reilly, Nora Mattion, Sally T. Ishizaka, Susan DiMichele, Susan Fantini, Elena Camposano, Joan Crowley and Carolyn Weeks-Levy, Viral Vaccine Research, Lederle-Praxis Biologicals, Pearl River, NY 10965

The attenuated Sabin strains of poliovirus have been used for many years to elicit protective immunity to poliovirus. Oral vaccination with replicating polioviruses generates both mucosal and systemic immunity. therefore, use of recombinant polioviruses expressing heterologous antigens as vaccine delivery vectors should provide a system for generating protective immunity to those antigens. cDNA copies of the poliovirus genome has been used to construct vectors containing a multiple cloning site for insertion of heterologous genes. Genes have been inserted in-frame with the viral open reading frame and are translated as part of the polyprotein. Incorporation of an engineered viral protease recognition site at the junction of the heterologous protein and the polyprotein results in cleavage of the protein during viral replication. Recombinant polioviruses expressing antigens from herpes simplex virus and rotavirus are being characterized. Expression of the foreign protein can be demonstrated in infected cells. Transgenic mice carrying the human poliovirus receptor gene are being used to characterize the immune responses to both poliovirus and the expressed antigen.

J2-323 DNA VACCINES: LONGEVITY OF AN ANTI-INFLUENZA RESPONSE. J.C. Santoro[1], E.M. Fynan[2], and H.L. Robinson[1], [1]Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655; [2]Department of Biology, Worcester State College, Worcester, MA 01602

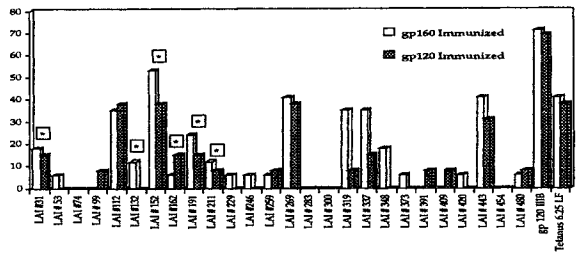
A murine/influenza virus model has been used to evaluate the longevity of antibody and protective responses raised by gene gun delivery of a hemagglutinin-expressing DNA. Mice were immunized and boosted at one month with 0.4 ug of an H1 expressing plasmid DNA (pCMV/H1). Antibody responses and protection against a lethal challenge were followed over the next year. Antibody responses had good longevity exhibiting comparable titers at one year post boost as at 10 days post boost. Protection against the lethal challenge was complete at 10 days, 1 month and four months post boost, but only partial at one year.

J2-324 A TRANSGENIC MOUSE MODEL FOR IDENTIFING HTLV-1 T-CELL EPITOPES: GENERATION OF HLA-B*3501-RESTRICTED CTL DIRECTED AGAINST SYNTHETIC PEPTIDES AND NATURALLY PROCESSED VIRAL ANTIGENS, Christian Schönbach*, Ai Kariyone#, Kiyoshi Nokihara^+, Karl-Heinz Wiesmüller$ and Masafumi Takiguchi*, Departments of Tumor Biology* and Immunology#, Institute of Medical Science, University of Tokyo, Tokyo ^Tokyo University of Agriculture and Technology, Tokyo +Biotechnology Instruments Department, Shimadzu Corp., Kyoto, Japan $Natural and Medical Science Institute at the University of Tübingen, Reutlingen, Germany

The majority of human T-cell leukemia virus type-1 (HTLV-1), HLA class I-restricted T-cell epitopes have been identified by cloning HTLV-1 patient-derived T cells. Here we describe for the first time a rapid method (reverse immunogenetics) for identifing T-cell epitopes, together with a transgenic mouse model as a guide for testing the cellular immune response to a mixture of the lipohexapeptide immunoadjuvant Pam_3Cys-Ser-$(Lys)_4$ and synthetic HTLV-1 peptides which seem suitable for vaccine design. HTLV-1 amino acid sequences were searched for eight to 14mer patterns carrying the anchor residues of the HLA-B*3501 peptide motif at positions two and eight to fourteen. 65 candidate peptides were synthesized according to the matched sequence patterns. Their HLA-B*3501 affinity was quantitatively analyzed in an indirect immunofluorescence peptide binding assay using RMA-S-B*3501 cells. 40 peptides which bound to HLA-B*3501 were mixed with Pam_3Cys-Ser-$(Lys)_4$ and used to immunize HLA-B*3501 transgenic C3H.He (H-2*K*) mice. One week after *i.p.* immunization, lymphocytes were cultured and repeatedly stimulated with peptide. Nine out of 30 tested peptides mounted CTL responses after two or three *in vitro* stimulations. Three peptides derived from env-gp46 and pol proteins induced a specific CTL response against peptided loaded RMA-S-B*3501 cells only. Bulk CTL generated by six peptides derived from env-gp46 (PNVSVSSSSTPLLY, SPPSTPLLY, VPSPSSTPLL, VPSPSSTPLLY), pol (QAFPQCTILQY) and gag-p19 (CPINYSLLASL) protein sequences of four HTLV-1 strains killed peptide pulsed RMA-S-B*3501 and P815-B*3501 target cells infected with the corresponding vaccinia virus-HTLV-1 recombinant. The latter peptides therefore represent T-cell epitopes and vaccine candidates for our transgenic mouse model.

J2-325 GP120 PEPTIDE RECOGNITION BY PBMCS FROM H I V INFECTED PATIENTS IMMUNIZED WITH EITHER GP160 OR GP120. K.V. Sitz, S. Ratto^, A.M. Scherer^, L. Loomis^ R.R. Redfield, R.N. Boswell, D.S. Burke and D.L. Birx, Division of Retrovirology, WRAIR and ^the Henry M. Jackson Foundation, Rockville, MD 20850 We tested the PBMCs of patients participating in two vaccine therapy trials for their ability to recognize overlapping peptides of the gp120 LAI sequence. Seventeen patients participating in a Phase I gp160 protocol and 13 patients participating in a Phase I gp120 protocol had their PBMCs isolated by Ficoll separation of heparinized venous blood. The fresh PBMCs were plated, in triplicate, into 96 well plates containing peptides overlapping the LAI sequence of gp120, pulsed on day 7 with tritiated thymidine and harvested and counted on day 8.

Results: The percentage of patient's PBMCs from each trial with an LSI $\geq$ 5 to each peptide are depicted below.



Conclusions: The PBMCs of HIV-infected volunteers who have been multiply immunized with either gp160 or gp120 proliferate to multiple peptides within the gp120 molecule. Reactivity from the end of C1 through early C2 (LAI #112-211) is particularly prominent and contains previously undescribed Th epitopes (asterisks). Conspicuously missing is reactivity to the V3 loop peptide (LAI #300). Although the percent reactivity to the entire gp120 molecule is similar between the immunization groups, there is differential recognition of some of the individual peptides, particularly peptides in early C3 (LAI #319-348).

J2-326 A COMPARISON OF IMMUNIZATION WITH PLASMID DNA AND VACCINIA RECOMBINANTS CONTAINING THE NUCLEOPROTEIN GENE OF INFLUENZA IN MHC CLASS I DEFICIENT MICE, [1]Patricia R. Slev, [2]Bradley S. Bender, [1]Wayne K. Potts, [1]Department of Pathology, [2]Geriatric Research, Education and Clinical Center, University of Florida, Gainesville, FL 32610

The efficacy of types of immunizations were evaluated in the context of protection during an influenza infection (H1N1) in class I deficient mice and controls. Genetic immunization (an expression plasmid containing the nucleoprotein gene of influenza A) was administered by intramuscular injection. The second and third types of immunization used vaccinia recombinants also containing the nucleoprotein gene of influenza A (VAC-NP) and were administered either by an intramuscular (IM) or intranasal (IN) route. The fourth group (controls) were inoculated with H3N2 (IN) thereby providing heterotypic CTL immunity in the context of a natural infection without the confounding effects of humoral immunity against surface antigens. All four types of inoculations have been shown to protect normal (class I expressing) mice from a lethal challenge with influenza, presumably mediated by class I restricted cytotoxic T cells. The two groups inoculated via the intranasal route may gain additional protection by activating the mucosal immune system (*IgA*). None of these types of inoculations has been evaluated in the context of class II restricted cytotoxic T cells, the only CTLs found in class I deficient mice. For all four types of inoculations, MHC class I deficient mice lost significantly more weight than the class I expressing control groups (seven mice per group) indicating the importance of class I restricted T-cells in protection. Within the class I expressing groups, there was no significant difference between the four types of inoculations; within the class I deficient groups the VAC-NP IM immunized mice lost significantly more weight than the H3N2 group;the other two groups, VAC-NP IN and genetically immunized groups had intermediary results. These data lend support for a protective role for mucosal immunity. Results on both class I and class II CTL activity for the four types of inoculations will also be presented.

J2-327 PROTECTIVE ANTIVIRAL CELL MEDIATED IMMUNITY USING RECOMBINANT *LISTERIA MONOCYTOGENES* AS A LIVE VACCINE VEHICLE. Slifka M.K., Shen, H., Matloubian, M., Jensen, E.J., Miller, J.F., and Rafi Ahmed, Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles, CA 90024

The intracytoplasmic lifecycle of *Listeria monocytogenes* (LM) enables it to be a convenient vaccine vehicle for the introduction of foreign proteins into the MHC class I pathway of antigen presentation. Taking advantage of these properties, we have inserted the nucleoprotein (NP) gene from lymphocytic choriomeningitis virus (LCMV) into the LM chromosome by site specific homologous recombination. Infection of mice with recombinant LM expressing LCMV-NP elicited a virus-specific CTL response. We were able to recover LCMV-NP specific CTL precursers from recombinant LM vaccinated mice as shown by vigorous secondary CTL responses after in vitro stimulation. In contrast to mice immunized with wild type LM, mice vaccinated with NP-recombinant LM were protected against challenge with immunosuppressive LCMV variants. Protection was demonstrated by reduced viral titers or complete clearance of LCMV from serum and various organs including, spleen, liver, lung, kidney, and brain. The kinetics of the LCMV challenge indicate that mice vaccinated with recombinant LM were able to arrest viral growth early in the infection due to a strong CTL response and did not exhibit the immunopathology associated with infection of naive mice. Since LM not only delivers antigens into the MHC class I pathway but also induces IL-12 production, it has the potential to function simultaneously as a vehicle for expressing foreign antigens and as an adjuvant promoting cell mediated immunity.

J2-328 MHC CLASS-I RESTRICTED CYTOTOXIC T LYMPHOCYTE RESPONSES TO EPSTEIN-BARR VIRUS IN CHILDREN, John L. Sullivan, Hisashi Tamaki, Brian L. Beaulieu and Mohan Somasundaran, Department of Pediatrics and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605

EBV-specific CTL responses were evaluated in nine children aged 20-35 months. Our aim was to assess the capability of latently infected young children to generate EBV-specific CTL responses. Autologous EBV-transformed B lymphoblastoid cell lines (BLCL) were used to restimulate EBV-specific, memory CTL precursors in vitro. Recognition of individual EBV gene products by bulk CTL lines was evaluated by combining CTL lines with B cell blasts infected with recombinant vaccinia constructs expressing single latent genes (Epstein-Barr nuclear antigen [EBNA] 1, 2, 3A, 3B, 3C, leader protein [LP], latent membrane protein [LMP] 1 and 2A) in chromium release assays. We were fortunate in identifying one child from whom cryopreserved PBMC samples were available before and during EBV seroconversion. EBV-specific CTL activity was demonstrated concurrent with initial detection of virus in the peripheral blood by EBV-DNA PCR, in the absence of detectable serum antibody. CTL lines from all nine children recognized one or more EBV latent gene product(s). All children demonstrated CTL responses against one or more EBNA 3 proteins (3A, 3B, 3C), and EBNA 3C was recognized most frequently. No CTL responses were detected against the EBV latent proteins EBNA 1, 2, LP or LMP 1. The EBV-specific CTL lines expressed CD3/CD8 and mAb blocking experiments demonstrated that the majority of target cell lysis was inhibited by antibody against MHC class-I but not antibody against MHC class-II. These results represent one of the first reports characterizing EBV-specific CTL responses in young children. The striking similarity between EBV-specific CTL responses described here in young children and those reported for adults suggests that the EBNA 3 family of proteins and LMP 2A should be considered for inclusion in candidate EBV vaccines.

J2-329 HUMAN AND RHESUS MACAQUE CTL RECOGNIZE SIMILAR REGIONS OF HIV AND SIV GAG PROTEINS. Cécile A.C.M. van Els[1], Carel A. van Baalen[2], Marlinda Dings[3], René P.C. Keet[3], Jonathan L. Heeney[4], Ab D.M.E. Osterhaus[2]. [1]Lab. of Vaccine Development and Immune Mechanisms, RIVM, Bilthoven, [2]Dept. of Virology, Erasmus University, Rotterdam, [3]Municipal Health Service, Amsterdam, [4]Lab. of Viral Pathogenesis, BPRC-TNO Rijswijk, The Netherlands.

The identification of CTL epitopes is essential for subunit vaccine design against AIDS. A major portion of the anti-viral CTL responses after infection with HIV in humans or SIV in rhesus macaques is made up by anti-gag specificities. Here, effector cell populations were established from HIV seropositive individuals by specific stimulation of PBL with pfa fixed autologous B-LCL infected with rvv gag. Expanded cultures were then tested for CTL activity against autologous B-LCL pulsed with overlapping 20-mer peptides derived from p24. Two individuals (008 and 617) had CTL against p24.14 (a(mino) a(cids) 263-282, KRWIILGLNKIVRMYSPTSI), two others (038 and 157) recognized p24.17 (aa293-312, FRDYVDRFYKTLRAEQASQD). Similarly we found two SIV infected rhesus macaques reacting against the analogous aa sequences of SIV; i.e. p26.14 (8653) and p26.17 (1VY). This led us to further fine map the human and rhesus CTL reactivities using sets of 9 aa overlapping 10-mers and to test for HIV-1/SIV crossreactivity. Both two p24.14 epitopes (possibly restricted by HLA-A2) and the p26.14 epitope were non-overlapping, and all three epitopes were non-crossreactive. Finemapping of the p24.17 epitopes is in progress. The SIV p26.17 epitope was also non-crossreactive, despite only one aa difference (position 6, S→T) between the SIV and HIV sequence. Others have also mapped CTL epitopes to the same regions of p24 in humans (Johnson JI 147,1512,1991, Buseyne JV 67,694,1993) and to p26.17 in cynomolgus macaques (Gotch JMPrim 22,119,1993). In conclusion, multiple non-cross reactive CTL epitopes are clustered within corresponding regions of HIV and SIV gag. CTL hotspots may be important for inclusion into vaccines.

J2-330 EVALUATION OF CELLULAR IMMUNE RESPONSES TO ADENOVIRUS VECTORS IN THE COTTON RAT. Soonpin Yei,[1] Gary Kikuchi,[2] Ke Tang[1] and Bruce C. Trapnell.[1] Departments of Virology[1] and Immunology,[2] Genetic Therapy, Inc., Gaithersburg, Maryland 20878

Replication deficient recombinant adenovirus (Av) vectors are efficient gene delivery vehicles currently being developed for a variety of *in vivo* gene therapy strategies such as for the fatal pulmonary component of cystic fibrosis. The cotton rat (Sigmodon hispidus) is one of the most widely accepted animal models for studying these Av vectors because wild type human adenovirus replicates in cotton rats and because of the histopathologic similarities of infected respiratory epithelial tissues from humans and cotton rats. Despite this, methods for studying immunologic responses in the cotton rat have not been developed. Importantly, recent studies in the cotton rat (*Gene Ther.* 1:192-200; 1994) in our laboratory suggest that a dose-dependent specific immune response to Av vectors can limit expression of the transgene. In this context, we have established methods to evaluate cytotoxic lymphocyte (CTL) responses to Av vectors in the cotton rat. To accomplish this, a CTL target cell line was established consisting of primary cotton rat lung fibroblasts (CRLF). Splenocytes from cotton rats exposed previously to an Av vector were harvested, cultured *in vitro* with irradiated, Ad*dl*327-infected CRLF. Cultured (effector) splenocytes were then incubated with ^{51}Cr-labelled CRLF (target) cells at effector:target (E:T) ratios of 100, 50 and 10. In parallel, splenocytes from naive cotton rats served as negative controls. Results demonstrated vector-specific CTL lysis of target cells significantly greater than controls: 80.3±1.3% vs 6.2±0.5%, 49.6±1.6% vs 5.7±0.4%, and 22.8±3.5% vs 4.8±0.5% (Mean±S.E.M., n=3; $p<0.01$, all comparisons) at E:T ratios of 100, 50 and 10, respectively. Thus, a specific CTL response does develop in cotton rats following *in vivo* Av vector administration. This observation will be useful in guiding vector modifications in the rational development of improved vectors for clinical use.

Molecular Pathogenesis of Viral Infections

J2-331 ENTEROVIRUS-IMMUNE CELL INTERACTIONS: IMPLICATIONS IN ENTEROVIRUS-INDUCED DISEASES. Daniel R. Anderson, Janet E. Wilson, Greg A. Perry, Graeme Dougherty, Bruce M. McManus. Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C. V6Z 1Y6

Background: Intriguing associations have been established between many different virus families and virtually every tissue and cellular element of the immune system. Recently, virus-immune cell associations have become an important issue in the study of the pathogenesis of virus-induced diseases, and for coxsackieviruses we determined by in situ hybridization a particular early and late post-infectious localization of virus in splenic and lymph node follicles.

Experimental Designs: We have further examined virus-immune cell interactions after *in vitro* $CVB3_m$ infection (multiplicity of infection of 5) of immune cells derived from *un*infected adolescent C3H/HeSnJ mice. After a 12 hour incubation, fluorescence-activated cell sorted immune cell subsets (FACS, >99% purity) were evaluated by confocal laser microscopy and plaque assay.

We have also evaluated the effect of virus infection on the humoral immune response to $CVB3_m$ infection in adolescent C3H/HeSnJ mice. Antigen presenting cell, T-helper cell and B-cell function were evaluated utilizing a sheep red blood cell (sRBC) plaque assay. Mice were injected intraperitoneally (ip) with 10^5 plaque forming units of $CVB3_m$ at day 0 and with 10^7 sRBC's at days 0, 2, 3 and 4 post-$CVB3_m$ infection. Splenocytes were harvested 4 days post-sRBC injection, mixed with target sRBC's and guinea pig complement and incubated. Plaques were then quantitated.

Results: $CVB3_m$ was associated with 12.9% to 17.4% of CD-8 positive T-cells and with 11% to 26% of adherent splenocytes. After mitogen (LPS and Con A) stimulation, B-cells and adherent cells were demonstrated to be permissive for viral replication. A 248% and 738% increase in virus titer was observed in B-cells and adherent cells. Likewise, unstimulated B-cells and CD-8 positive T-cells were permissive to infection. Under non-stimulated conditions, an average of 1% of virus is cell-associated (plaque-forming virus) with CD-4 and CD-8 T-cells, B-cells and adherent cells, while an average of 5% of virus was associated with LPS-stimulated B-cells and adherent cells.

In the second experiment the combined function of antigen presenting cells, T-helper cells and B-cells (humoral immune response) to a third party antigen (sRBC) during the acute phase of enterovirus induced disease was increased at day 0, and decreased at days 2, 3 and 4 post-$CVB3_m$ infection relative to *un*infected animals.

Conclusions: These data indicate that $CVB3_m$ associates with and replicates in murine splenic immune cells, and that infection of susceptible mice with $CVB3_m$ modifies the immune response to a third party antigen during the acute stages of disease. An understanding of such $CVB3_m$-immune cell interactions is critical for gaining insight into the pathogenesis and persistence of enteroviral infection both within and outside of the immune system.

J2-332 HUMAN γδ T CELL-MEDIATED RECOGNITION OF NONVIRAL CELL SURFACE ANTIGENS INDUCED BY ACUTE VIRUS INFECTION, **Jack F. Bukowski, Craig T. Morita, and Michael B. Brenner,** Department of Rheumatology/Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Lymphocytes bearing γδ T cell receptors (TCR) have reactivities distinct from those expressing the αβ TCR. Previous studies have shown that γδ T cells are overrepresented in the peripheral blood and broncheoalveolar lavages of humans infected with such viruses as human immunodeficiency virus (HIV) and and Epstein-Barr virus (EBV). Similarly, in the mouse, intranasal infection with influenza or Sendai viruses induces the accumulation of γδ T cells in the lung. We report here that coculture of human peripheral blood mononuclear cells (PBMC) from herpes simplex virus (HSV)-seropositive individuals with autologous HSV-infected cells results in the TCR variable region-determined proliferation and expansion of γδ T cells. These Vγ2Vδ2 T cells do not lyse mock-infected targets, but do lyse cells acutely infected with HSV or with an unrelated virus, vaccinia virus. This γδ T cell-mediated lysis is MHC-unrestricted, blocked by antibodies to the TCR, and is not specific for the virus used to induce γδ T cell proliferation. It thus appears that the process of acute virus infection induces or modifies a nonvirally-encoded cell surface moiety which is then capable of being recognized by γδ T cells in a TCR-dependent manner. Such antiviral γδ T cells may play a role in the immunity or pathogenesis of acute virus infection.

J2-333 PATHOGENICITY OF MURINE CYTOMEGALOVIRUS MUTANTS, Victoria J. Cavanaugh, Richard M. Stenberg, and Ann E. Campbell, Department of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk, VA 23507

Understanding the pathogenic mechanisms of virus infections depends upon identification of viral gene products involved in host-virus interactions in vivo. Many viral gene products which affect pathogenesis in vivo are nonessential for virus replication in vitro. Therefore, we constructed insertion or deletion mutants in the Hind III J and I regions of murine cytomegalovirus (MCMV) to assess the importance of these genes in in vivo replication of virus as well as interaction with immune regulatory and effector cells. Three mutants replicated in NIH 3T3 fibroblasts as efficiently as wild-type virus, thus confirming the nonessential nature of the genes. Deletion of 2.8 Kb in Hind III J and of 7.7 Kb in Hind III J and I resulted in mutants with reduced replication in target organs in vivo. An insertion in Hind III J had no significant effect on replication in mouse salivary gland, but curtailed growth of the virus in footpad fibroblasts and subsequent priming of MCMV-specific CTL precursors in the draining lymph node. In addition, the 7.7 Kb deletion mutant was unique in its failure to replicate in a permissive, differentiated macrophage cell line. Current studies aim to further define the role of this gene region in MCMV pathogenesis.

J2-334 CHARACTERIZATION OF A CELL MUTANT RESISTANT TO VACCINIA VIRUS KILLING

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We have isolated a CHO mutant clone, VV5-4 that is resistant for vaccinia virus (VV) cytotoxicity. Vaccinia virus can infect VV5-4 to a similar extent as parental CHO cells. In addition, a marker gene β-gal expressed from a viral immediate-early promoter is expressed in infected VV5-4 cells. However, viral intermediate and late gene products are not detected in infected VV5-4 cells. It therefore suggests that in VV5-4 progression of VV life cycle is blocked at the the uncoating II or replication stage. Furthermore, host protein synthesis in VV5-4 cells is not shut-off after VV infection suggesting uncoatingII/replication is important for determining cell fate.

Since this mutant clone VV5-4 was isolated by retroviral insertional mutagenesis. A cellular gene that was integrated by single retrovirus was isolate and codes for a 5Kb transcript in Northern blot analysis. A 2 Kb cDNA was isolated and codes for a novel polypeptide of 800 amino acids that show no homology with known proteins.

Experiments are in progress to understand the role of this cellular gene in VV infection. In addition, a two-hybrid system will be employed to identify any viral gene product interacting with this cellular target

J2-335 **VIRAL INFECTIVITY AND PATHOGENICITY VARIATIONS SEEN IN TWO ANTIGENICALLY INDISTINGUISHABLE INFLUENZA STRAINS,** Alexander D. Diehl*†, Mary Beth Graham, M.D.*, Vivian L. Braciale, Ph.D.*, and Thomas J. Braciale, M.D., Ph.D.*, *The Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22908, †and program in Molecular and Cellular Biology, Washington University, St. Louis, MO 63110.

Using our laboratory's standard strain of A/Japan/305/57 in *in vitro* assays of viral infectivity of the mouse B-lymphoma A20-1.11, we have found that although infection sensitizes the A20 cells for recognition by both Class I and Class II restricted CTL's and leads to high surface expression levels of hemaglutinin (HA), the infected A20 cells grow through the infection, HA expression declines, and few of the infected cells die. In contrast, using a second strain of A/Japan/305/57 from a different passage history, we find that not only are the infected A20 cells sensitized for lysis by both Class I and Class II restricted CTL's, exhibiting even higher levels of HA surface expression, but in addition, most cells in the infected population die with characteristics of apoptosis. The two virus strains appear to be highly related because in various assays we have found them antigenically indistinguishable in both their T- and B-cell epitopes.

Using these two virus strains in an *in vivo* dose response assay, we have found that our laboratory's standard strain will kill all mice infected with a 10^{-2} dilution of virus whereas infection of mice with the second strain of influenza at the same dilution (an equivalent amount of HAU's) leads to no mortality and little apparent morbidity in the infected animals. We are investigating the relationship between the *in vitro* and *in vivo* viral effects further as well as examining the molecular basis for the difference in cellular pathology.

J2-336 **ENHANCING VIRAL YIELDS OF HUMAN BONE MARROW CELL INFECTED WITH DENGUE HEMORRHAGIC FEVER VIRUS STRAIN** Chwan-Chuen King and Chia-Chi Ku, Inst. of Epidemiology, National Taiwan University,Taipei,Taiwan, R.O.C.

Many viruses (parvovirus, retrovirus, hepatitis virus, herpesvirus and flavivirus) are involved in infection of hematopoietic cells. Disease induced by dengue(DEN) virus infection were frequently associated with bone marrow(BM) abnormalities. Since neutrophilia and thrombocytopenia were common in dengue patients,we are investigating whether BM may be accompanied with abnormal hematopoiesis. Using Ficoll-Hypaque separation, mononuclear cells were prepared from the total BM cells. $1.0x10^6$ cells/well of both adherent and nonadherent cell types were infected with two different DEN-2 virus strains : (1) Thiland strain isolated from the dengue hemorrhagic fever(DHF) patient and (2) Taiwan strain isolated from the dengue fever(DF) patient at MOI=1 and collected the suprnatants at various time points for assay. The preliminary data showed that : (a) adherent cells were infected with DEN2 and DHF virus strain replicated much more efficiently ($2.3x10^4$ PFU/ml on day 3 p.i.) than DF strain; (b) nonadherent cells were 10 times less efficient to produce viral yields than adherent cells (DHF and DF strains peaked at $6.3x10^3$ PFU/ml and $1.5x10^2$ PFU/ml on day 3 p.i., respectively); (c) addition of GM-CSF to nonadherent cells increased virus infectivity and productivity. In conclusion, DEN viruses were able to infect BM cells and the more virulent virus strain isolated from DHF patients had better replication capability in human bone marrow cells. Future studies on cytokines and immunologic evaluation are in progress.

J2-337 INTERLEUKIN-12 PROMOTES RESISTANCE TO EXPERIMENTAL HERPES VIRUS INFECTION, Michael J Mulqueen, Jackie Carr, Jane Rogerson, Alan Lamont*, Vivien Gibson* and Robert F G Booth, Departments of Virology and *Anti-Inflammation, Roche Products, Welwyn Garden City, Herts, U.K., AL7 3AY

The therapeutic and prophylactic antiviral efficacy of interleukin-12 (IL-12) was studied using murine models of herpes simplex virus (HSV) and murine cytomegalovirus (mCMV) infection. Therapeutic intraperitoneal administration of IL-12 commenced 6 hours after mice were infected with HSV, and was continued daily for a total of 5 days. IL-12 therapy improved the survival rates of mice with systemic HSV infection, compared to that of placebo treated infected mice. Subcutaneous administration of IL-12 also improved the rate of survival in systemic HSV infection, although higher doses were required to give comparable effects. Prophylactic IL-12 produced the greatest effect on survival of an otherwise lethal systemic infection. Intraperitoneal administration of IL-12 for 2 days prior to and 3 days following systemic infection with HSV permitted >80% of mice to survive the infection. These surviving mice proved resistant to subsequent re-infection with HSV. Such resistance was apparently specific for HSV infection, since a second group of survivors succumbed to a lethal infection of mCMV. Infectious virus could not be recovered from 5 day cultured lumbar ganglia explants, dissected from survivors of IL-12 prophylaxis, suggesting that IL-12 treatment reduced the establishment of latent HSV infection. Mice treated with IL-12 show enhanced Natural Killer (NK) cell activity *in vitro*, while mCMV infection appeared to suppress normal NK cell function. One action of IL-12 may therefore be to enhance NK cell mediated clearance of the virus. However, IL-12 therapy was also effective in mice carrying the *beige* mutation, which reduces NK cell activity, indicating that IL-12 has additional activities *in vivo*.

J2-338 **RABBITPOX PS/HR (VV-WR-B5R ORF) GENE PRODUCT INHIBITS THE GENERATION OF AN INFLAMMATORY RESPONSE IN VIVO.** Gregory J. Palumbo[1], Richard Stern[2], Wayne C. Glasgow[3], Luisa Martinez[2], R. Mark Buller[4], and Richard W. Moyer[2].
[1]Departments of Microbiology and Immunology, University of Oklahoma Health Science Center, Oklahoma City, OK 73190, [2]Immunology and Medical Microbiology, University of Florida Health Science Center, Gainesville, FL 32610, [3]Laboratory of Molecular Biophysics, NIEHS, Research Triangle Park, NC 27709, [4]Molecular Microbiology and Immunology, St. Louis University, St. Louis, MO 63104.

Poxviruses have recently been found to possess a number of gene products whose function is to modify host responses. Recent evidence suggests that a subclass of poxvirus-encoded host response modifiers interferes with host defense responses, and thus can be termed immune or inflammatory response modifiers (***irms***). Several poxvirus-encoded ***irms*** possess homology to mammalian proteins which are associated with host defenses, while other poxvirus ***irms*** have little sequence homology to immune-effector molecules or their receptors. The Ultrecht strain of rabbitpox (RPV) virus induces a large reddish pock on the chorioallantoic membrane (CAM) of the chicken embryo. RPV mutants lacking the SPI-2/crmA/38K gene trigger an inflammatory response by 36-48 hr after inoculation, similarly to that found for cowpox virus (Brighton red strain) mutants. RPV mutant viruses with a deletion of the PS/HR gene, a gene which has recently been associated with virus host range and virion maturation, elicited an inflammatory response on the chicken embryo CAM at 48-72 hr after virus inoculation. Thus, another poxvirus-encoded gene product has been identified that has ***irm*** functional activity which would not have been predicted by sequence analyses.

J2-339 MOLECULAR LATENCY OF MCMV AND ANALYSIS OF LATENT CELL TYPES. Jessica L. Pollock and Herbert W. Virgin IV, Center for Immunology and Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110

Murine cytomegalovirus (MCMV) follows three stages of infection in the immunocompetent host. Following the initial acute infection, a persistent infection remains in which lytic virus resides in the acinar cells of the salivary glands. Finally a third stage of infection exists which is characterized by a lack of detectable infectious virus. It is not known whether MCMV remains persistent at some low level during this third stage or establishes molecular latency. To test this, spleens, kidneys and salivary glands from mice 2-8 months post infection were evaluated by two sensitive assays. Sensitivity was tested using virus co-cultured on murine embryo fibroblasts (MEFs) for 14 days. By this method, 1 pfu MCMV was reproducibly detected in the presence of sonicated spleen, kidney and salivary glands diluted 1:10. SCID mice were used as a second detection assay for MCMV. Using 46 SCID mice and different doses of virus, the LD_{50} of MCMV in SCID mice was 2-3 pfu. MCMV infections were detected when 10-25 pfu were added to sonicated spleens, kidneys, and salivary glands prior to injection into SCID mice. Using both co-culture with MEFs and injection of tissue into SCID mice, we have tested a total of 34 spleens, 34 kidneys, and 37 salivary glands and have detected no persistent virus. While no preformed virus existed in these tissues, reactivation from explanted spleen, kidney and peritoneal exudate cells occurs in culture after 10-40 days. In addition, lytic MCMV was detected in SCID mice 28 days after injection with $2(10)^7$ kidney cells from latently infected mice. Following identical transfers of latent spleen cells, MCMV was not detected in SCID mice although explants from the same organs reactivated in vitro. Using FACS sorting, panning, magnetic bead separation, and reactivation assays, individual cell types have been analyzed for the presence of MCMV genome and the ability to reactivate virus.

J2-340 STRAIN-DEPENDENT DIFFERENCES IN THE EFFECT OF *IN VIVO* IFN-GAMMA NEUTRALISATION ON CYTOKINE PROFILES IN INFLUENZA-INFECTED MICE.

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The effect of *in vivo* neutralisation of IFN-γ on cytokine production during influenza infection was compared in C57/BL6 and BALB/c mice. Similar cytokine profiles were obtained on *in vitro* restimulation of MLN or spleen cells from virus-infected mice of each strain. At days 7 and 10 postinfection, MLN or spleen cells from both strains of mice produced IL-2, IL-6, IL-10 and IFN-γ, but little or no IL-4 or IL-5. However, following *in vivo* treatment with anti-IFN-γ antibody, production of IL-4 and IL-5 was induced in MLN and spleen cells of BALB/c mice whereas those from C57/BL6 mice showed little change in cytokine profile. An increase in IL-6 and IL-10 production was also observed on *in vitro* restimulation of splenocytes from BALB/c mice. However, significant amounts of IL-2 and IFN-γ were still secreted in these cultures. *In vitro* neutralisation of IFN-γ in the restimulated cultures had little effect on the production of other cytokines, regardless of whether this cytokine had also been neutralised *in vivo* .Despite the change in cytokine profile, anti-IFN-γ treatment had no effect on the kinetics of viral clearance in BALB/c mice. A similar situation was seen for C57/BL6 mice. Congenic strains of mice are currently being used to determine whether the effect on cytokine profiles is associated with the MHC haplotype .

J2-341 VACCINIA VIRUS REPLICATION IS INDEPENDENT OF THE HSP70 RESPONSE TO VIRUS INFECTION.

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Heat shock proteins (HSP) are expressed in all organisms in response to a variety of stresses, including virus infection. However, since viruses are not known to encode HSP genes, this represents expression of cellular HSPs. We have found a dramatic induction of the 72kDa HSP in the ovaries of VV-infected mice, and using primary ovarian fibroblasts, demonstrated HSP72 mRNA within virus-infected cells. The physiological role of HSPs expressed during virus infection is unknown, although HSPs act as molecular chaperones to facilitate protein folding and assembly in unstressed cells, and a number of bacterial HSPs are essential for bacteriophage replication and morphogenesis. In order to determine whether HSP72 expression was beneficial to VV replication, we constructed a recombinant VV which encoded murine HSP72, but found that this virus grew to similar titres as control viruses, both *in vitro* and *in vivo*. In particular, kinetic studies of virus growth in an HSP72-negative cell line showed no difference from control viruses, and the presence of HSP72 did not influence the virulence of infection in immunocompromised nude mice. These results are interesting given that HSP70 proteins act as molecular chaperones and that HSP72 can be found in association with VV proteins. We suggest that this association may simply reflect the inherent affinity of HSP70 for non-native proteins, and the close physiological proximity of HSP and nascent viral proteins within the virus infected cell. Our results indicate that the functional significance of these interactions are minimal with respect to the outcome of virus infection. Furthermore, although the expression of HSP72 in VV-infected mice coincides with the peak anti-viral cellular immune response, HSP72 does not appear to be immunologically significant, as we cannot demonstrate any immunological responses to HSP72 during the course of VV infection.

J2-342 CONSTRUCTION OF A SIMPLIFIED FORM OF RABIES VIRUS GLYCOPROTEIN. Boguslaw Wojczyk, William H. Wunner, and Steven L. Spitalnik, Department of Pathology and Laboratory Medicine, University of Pennsylvania and The Wistar Institute, Philadelphia, PA 19104

Rabies virus glycoprotein (RGP) is the only glycoprotein on the viral surface, is the target of viral neutralizing antibodies, and is the viral protein that interacts with the host cell receptor. RGP of the ERA strain is a 505 amino acid Type I membrane glycoprotein with 3 potential N-glycosylation sites at Asn37, Asn247, and Asn319. Due to RGP's central role in the immunology and biology of rabies, it is important to determine its three-dimensional structure. Towards this end we produced a recombinant form of RGP that may be more amenable to analysis by x-ray crystallography. To avoid the use of detergents, we constructed a soluble 434 amino acid form of RGP lacking a transmembrane domain (RGPT434). RGPT434 was secreted by transfected cells and was appropriately glycosylated, assembled, antigenic, and immunogenic. Since N-glycosylation was required for RGPT434 secretion, we minimized the number of N-glycans by site-directed mutagenesis. Interestingly, RGPT434 was secreted only when Asn319 was N-glycosylated. In contrast, full-length RGP was expressed at the cell surface if any of the three potential sites was N-glycosylated. Soluble inhibitors of N-glycosylation were used to simplify the structures of the N-glycans on RGPT434. Although inhibiting alpha-glucosidases with castanospermine blocked secretion, inhibiting any further processing with deoxymannojirimycin had no effect on secretion. Finally, to simplify the purification process, a tail of 6 His residues was added to the COOH-terminus of RGPT434. This modification did not affect secretion, antigenicity, of assembly of the recombinant glycoprotein, but did allow purification using Ni+2-agarose chromatography. In summary, we constructed a soluble form of RGP with a minimal number of homogeneous N-glycans and an "epitope" tag. This form of RGP is structurally similar to the extracellular domain of the full-length protein and may be amenable to analysis by x-ray crystallography.

Emerging Viral Infections

J2-343 WIDESPREAD ENZOOTIC OF A NOVEL HANTAVIRUS OF THE HARVEST MOUSE IN NORTH AMERICA. Bruce Anderson, Terry Yates, Norah Torrez-Martinez, Wanmin Song, Brian Hjelle. University of New Mexico, Albuquerque, N.M.

We recently identified a new species of hantavirus (HMV) associated with the harvest mouse *Reithrodontomys megalotis* (Hjelle B et al, *J. Virol.* 1994, in press). An Arizona woodrat (*Neotoma mexicana*) was found to be infected with HMV, presumably through "spillover". HMV is most closely related to the Four Corners hantavirus (FCV) of deer mice (genus *Peromyscus*). The nucleocapsid gene and protein of HMV differ from those of FCV by 24% and 15% of residues, and the 1896 nt S genome is shorter by 163 nt. We surveyed 174 *Reithrodontomys* animals captured in the U.S. and Mexico for hantavirus antibodies; 27 (15.6%) were positive. S segment cDNAs were amplified and sequenced from seropositive animals captured in California (4), Arizona (3), New Mexico (1), and Mexico (2). A monophyletic clade of HMV-like agents was identified at all sites, although an *R. megalotis* infected with an FCV-like virus was also identified in the state of Zacatecas, Mexico. Nucleotide sequence distances among members of the HMV clade were up to 15.5%, but amino acid distances were less than 2%. HMV is enzootic in harvest mice throughout much of North America, and can also infect wood rats.

J2-344 GENETIC IDENTIFICATION OF A NOVEL HANTAVIRUS IN A HARVEST MOUSE (*REITHRODONTOMYS MEXICANA*) IN COSTA RICA. Brian Hjelle, Norah Torrez-Martinez, Wanmin Song, Bruce Anderson, William L. Gannon, and Terry Yates. University of New Mexico, Albuquerque, N.M.

Phylogenetic relationships among hantaviruses strongly parallel those of their corresponding rodent hosts. Hantaviruses of closely-related murid rodents are closely related to each other. We recently identified a new species of hantavirus (HMV) of the harvest mouse *Reithrodontomys megalotis* in California and Arizona (Hjelle B et al, *J. Virol.* 1994, in press). During a serosurvey of harvest mice in the Americas, 5 *R. mexicana* animals from Costa Rica were tested for hantavirus antibodies by recombinant nucleocapsid antigen western blot. One was positive. S segment cDNA was amplified from the kidney RNA of that animal. The entire S segment has been cloned, and the sequence of 978 nt of the nucleocapsid gene has been determined. The nucleocapsid gene and protein differed from prototype California and Arizona HMVs by ~22% of nt and 10.8% of aa residues. Compared with the deer mouse hantavirus of North America, the Costa Rican *R. mexicana* virus nucleocapsid gene sequence differed by 25% of nt and 20% of aa. We believe that the Costa Rican sequence is derived from a new hantavirus, most closely related to that of *R. megalotis*. The relatively close relationship between the virus identified in an *R. mexicana* animal and that of *R. megalotis* supports the hypothesis that hantavirus speciation is linked to the speciation of the corresponding host rodents, and that hantaviruses are in equilibrium with their rodent hosts.

J2-345 NUCLEOTIDE SEQUENCE DIVERSITY AMONG NEWCASTLE DISEASE VIRUS DEMONSTRATES THAT ISOLATES FROM RECENT DISEASE OUTBREAKS ARE RELATED TO VIRUSES OF PSITTACINE ORIGIN, Bruce S. Seal, Southeast Poultry Research Laboratory, U.S.D.A., A.R.S., 934 College Station Rd., Athens, GA 30605

Newcastle disease virus (NDV; avian paramyxovirus 1) is represented by three pathotypes. Lentogenic isolates are not virulent in chickens and require trypsin for fusion protein cleavage during replication in cell culture. Mesogenic viruses are of intermediate pathogenicity and the virulent velogenic isolates may be neurotropic or viscerotropic. Recently, neurotropic velogenic viruses were isolated from an outbreak of Newcastle disease in cormorants and turkeys. These viruses did not react with strain differentiating monoclonal antibodies in diagnostic hemagglutination inhibition tests. The cormorant and turkey NDV isolates had the fusion protein cleavage sequence ^{109}SRGRRQKRFVG^{119} as opposed to the consensus sequence ^{109}SGGRRQKRFIG^{119} of known velogenic NDV isolates. The R for G substitution at position 110 may be unique for the cormorant and turkey isolates. Nucleotide sequencing and analysis of the conserved matrix protein gene coding region was completed for isolates representing all pathotypes. Phylogenetic relationships demonstrated that the isolates from recent outbreaks of Newcastle disease, and an isolate thought to have caused the major outbreak in California during the 1970's, are related to viruses of psittacine origin. These viruses are phylogenetically distinct from the three groups originally proposed (Toyoda et al. Virology 169:273, 1989) for avian paramyxovirus 1 isolates, forming a fourth viral lineage.

Self-Non-Self and Viruses

J2-346 HTLV I TAX-SPECIFIC CTL FROM HAM/TSP PATIENTS CROSS-REACT ON A SELF MYELIN-ASSOCIATED GLYCOPROTEIN-DERIVED PEPTIDE

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HTLV I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a slowly progressive neurological disease characterized by perivascular mononuclear infiltrates in the CNS. HTLV I-specific $CD8^+$ CTL are found in PBL and CSF of infected patients with HTLV I-associated neurological disease but not in HTLV I seropositive individuals without neurological involvement. Previous studies have shown that in $HLA\text{-}A2^+$ patients, HTLV I-specific $CD8^+$ CTL restricted by HLA-A2 recognize a peptide derived from the HTLV I Tax protein (Tax 11-19 LLFGYPVYV). In the present study, we have analyzed the potential of these Tax-specific CTL to recognize additional peptides. Our results demonstrate that a subpopulation of high affinity $CD8^+$ Tax 11-19 specific CTL clones cross-react on a self peptide derived from the sequence of myelin-associated glycoprotein (MAG 556-564 VLFSSDFRI) presented by HLA-A2. These observations suggest that the demyelination process in HAM/TSP may be due, in part, to virus-specific CTL recognition of a self myelin component that is independent of HTLV I infection.

J2-347 DIFFERENT IMMUNOGENICITY OF TWO OVERLAPPING K^d RESTRICTED $CD8^+$ CTL INFLUENZA HEMAGGLUTININ EPITOPES: IMPLICATIONS OF LIMITED T CELL REPERTOIRE FOR A SELF-MIMIC EPITOPE, Wuxiong Cao, Brenda A. Myers-Powell, and Thomas J. Braciale, The Beirne B. Carter Center for Immunology Research and the Departments of Microbiology and Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908

One of the two class I MHC ($H\text{-}2K^d$)-restricted immunogenic sites identified on the influenza strain A/Japan/57 (H2N2) hemagglutinin (HA) encompasses two distinct partially overlapping epitopes, mapping to residues 204-212 and 210-219. When we investigated the magnitude of the CTL responses of BALB/c mice to the two overlapping epitopes, we found that while the NH_2-terminal nonamer epitope is immunodominant, eliciting vigorous CTL responses in A/Japan/57-immunized BALB/c mice, the CTL responses to the COOH-terminal decamer epitope are weak and variable. The C-terminal epitope subdominance seems to be due to factors other than inefficient processing of the epitope in vivo because CTLs generated by priming mice with recombinant Sindbis viruses expressing only one of the HA 204-219 subsites displayed patterns of responsiveness similar to that of influenza virus primed CTLs. Limiting dilution CTL assays showed that the CTL precursor frequency (pCTL) of the N-terminal epitope is at least ten fold higher than the pCTL of the C-terminal epitope, implying that the low and variable pattern of C-terminal specific responsiveness was due to the limited T cell precursors in the C-terminal specific CTL repertoire. This was further confirmed by the limited heterogeneity in the cross reactivity patterns displayed by the C-terminal specific CTL for an Ig VH fragment and the HA 210-219 epitope of influenza strain A/AA/57 in short term bulk cultures, and the FACS analysis of TCR V_β chain usage. Taking these together with our previous observation that some JHA 210-219 specific CTLs can also cross-recognize an Ig VH fragment, these studies had provided a strong evidence that Ig gene products may influence T lymphocyte function and repertoire development.

J2-348 INVOLVEMENT OF CD4+ CELLS IN AUTOIMMUNE ANAEMIA AND HYPERGAMMAGLOBULINAEMIA INDUCED BY LYMPHOCYTIC CHORIOMENINGITIS VIRUS, Jean-Paul Coutelier, Sally J. Johnston, Mohammed El Azami El Idrissi, Charles J. Pfau, International Institute for Cellular Pathology, Catholic University of Louvain, Brussels, Belgium and Rensselaer Polytechnic Institute, Troy, NY.

Development of pathology varies widely between different strains of mice after intracerebral inoculation with the so-called 'docile' isolate of Lymphocytic Choriomeningitis (LCM) virus. The C3HeB/FeJ and B10.BR/SgSnJ mouse strains have been of special interest because they display autoimmune hemolytic anaemia with varying degrees of apparent immunological involvement. In this study, we examined the role of CD4+ T helper cells in this autoimmune response by treating mice with the CD4-specific GK1.5 monoclonal antibody. We also determined if polyclonal activation of B lymphocytes, induced either by LCM virus or by lactate dehydrogenase-elevating virus, another well known B cell activator, correlated with the development of anaemia in these mice. Our results strengthened the central role of the immune system in the anaemia in C3H mice by showing that depletion of CD4+ cells largely, if not completely, abrogated this anti-erythrocyte autoimmune reaction. As reported by others, we found that the anaemia was more mild in B10.BR mice than in C3H mice. However, we could not confirm the difference in the degree of B lymphocyte polyclonal activation between these mice. Furthermore, lactate dehydrogenase-elevating virus had no apparent effect on erythrocytes, even though this virus also induced a sharp increase in plasma IgG levels.

J2-349 ANTI-HLA CROSS REACTIVE AUTOANTIBODIES IN HIV-INFECTED INDIVIDUALS: CORRELATION WITH DISEASE PROGRESSION IN MACS STUDY PATIENTS.

Hana Golding, Robert A. Kaslow, Robert Blackburn, and the MACS study. Division of Virological Products, CBER, FDA, and DMID, NIH, Bethesda,MD, 20892.

We have previously described the identification of homologous regions in the C-terminus of HIV-1 gp41 and in the N-terminus of HLA class II beta chains. Forty percent of patients infected with HIV-1 virus were shown to have antibodies which bind to the homologous sequences, as well as to native HLA class II molecules. Affinity purified cross-reactive antibodies (CrAb) were shown to have direct blocking effects on normal T cell responses to recall antigens, and could mediate ADCC of HLA class II^+ cell lines.

In order to determine the contribution of such antibodies to disease progression, we obtained longitudinal plasma samples from patients in the MACS study. In a first study, it was found that the presence of high titers CrAbs correlated with a more rapid disease progression ($P = 0.027$ by Fisher two tail analysis)

In a second, 7 year-longitudinal study of 12 progressors and 12 stable patients we found: (1) The production of CrAb was seen in 70 - 80% of rapid progressors, while the true stables produce only infrequent low-titers CrAb. (2) In rapid progressors, production of CrAb preceded by 2-3 years the marked drop in CD4 counts. (3) CrAb production did not correlate with the degree of hyperglobulinemia in these patients. (4) The presence of CrAb during the asymptomatic stage correlated with early loss of T-helper responses to recall antigens.

We are currently establishing whether periodic measurements of CrAb in patients sera could be valuable in predicting a drop in CD4 counts and disease progression.

J2-350 ENDOGENOUS RETROVIRUS (EV) ANTIGENS REDUCE THE CELL MEDIATED IMMUNE RESPONSE TO AN EXOGENOUS RETROVIRUS

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Endogenous retroviral genes (*ev*) are well characterized in chickens. Our laboratory has established chicken lines with single and multiple *ev* loci and, compared to *ev* negative birds, these lines exhibit a delayed and suppressed titer of neutralizing antibody in responses to avian leukosis virus (ALV) challenge. To address the effects of *ev* genes on the cell mediated immune response we have developed an *in-vitro* assay to monitor the *in-vivo* induction of MHC restricted cytotoxic T cells (CTL) induced by ALV. Using this assay, we find the CTL response in birds expressing the *ev*-21 locus is reduced 85 to 95% compared to *ev* negative birds having nearly identical (eighth backcross) genetic backgrounds. Other *ev* loci (1,6,10 and 11) also reduce the CTL response to ALV in MHC matched chickens with different genetic backgrounds.

J2-351 ROLE OF ENTEROVIRUSES IN INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

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Coxsackie B virus infections have been associated with clinical IDDM in several previous studies but their initiating role in the slowly progressing beta-cell damage was for the first time directly implied in our recent prospective, nationwide DiMe study. Siblings of the index IDDM cases who progressed to clinical IDDM experienced enterovirus infections more frequently than nonprogressing siblings during prospective follow-up period. Enterovirus infections were initially demonstrated by detecting significant increases in serum class-specific cross-reactive enteroviral antibodies by using radioimmunoassay (RIA) with the heavy-chain capture principle. Causal association between these infections and the pathogenesis of IDDM was suggested by temporal association of seroconversions of viral antibodies with increases in islet cell antibody levels which are considered to be markers of β-cell damage. The possibility of the existence of specific diabetogenic strains of enteroviruses was then considered. To identify the serotypes responsible for the infections, successive sera from subjects exhibiting seroconversion of autoantibodies coinciding with an increase in enteroviral antibodies were anlyzed by plaque neutralization assay using a set of different enteroviruses. The results indicate that several enterovirus serotypes may be associated with the progressive prediabetic process. Not only different coxsackie B but also coxsackievirus A9 -infections were found to coincide with seroconversions of islet-cell and insulin autoantibodies. It is known that an immunogenic region of GAD 65, one of the major islet-cell antigens, shows a high degree of homology with the 2C protein of CBV-4. Studies on potential immunological cross-reactions between enteroviruses and two significant autoantigens, HSP60 and GAD65, are in progress. This is carried out by using immunohistochemistry in infected cell lines and in frozen sections of human pancreas with antibodies induced by synthetic peptides and natural and expressed viral proteins.

Late Abstracts

SV40 LARGE T ANTIGEN AND P53 RELATIONSHIP IN PLEURAL MESOTHELIOMA

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The role of p53 alterations in human malignant pleural mesotheliomas (MPM) is unclear. Immunostaining (IPOX) of snap frozen MPM specimens revealed p53 expression in 31 of 52 (60%). p53 detection by IPOX is usually associated with mutated p53. SSCP for exons 5-9 revealed p53 alterations in 2 of 25 specimens, and LOH at exon 4 was seen in only 2 of 12 evaluable specimens. Thus, most of these specimens appeared to contain wild-type (wt) p53. We recently reported that SV40 induces mesotheliomas in rodents, and that SV40-like sequences are present and expressed in MPM. Of note, SV40 large T antigen (Tag) binds and inactivates wt p53 in vitro, abolishing its tumor suppressor function. We theorized that the immunohistochemical persistence of p53 may result from its physical binding with Tag. Tag was detected by IPOX in 32 (62%) of these MPM specimens. Expression of Tag was significantly associated with coexpression of wt p53 ($p_2 = 0.008$, Fisher's exact text), and preliminary experiments suggest co-precipitation of p53 and Tag. Finally, we demonstrate that WAF-1 is not detectable in MPM expressing wt p53 suggesting that p53 is inactive. We speculate that Tag expression in MPM binds to and inactivates p53 contributing to the malignant phenotype.

HLA-A2 RESTRICTED CTL RESPONSE TO THE HPV E7 PROTEIN IN THE PERIPHERAL BLOOD OF CIN PATIENTS, Carys Wyn Evans, Thomas Grubert, Stefan Bauer, Herrman Wagner and Grayson B. Lipford, Institute for Medical Microbiology, Technical University of Munich, Munich 81675, F.R.G.

Human Papilloma Virus (HPV) infection is involved in 90% of cervical carcinomas and possibly 95% of cervical intraepithelial neoplasia (CIN). At present it is unclear whether patients infected with the transforming HPV types can mount efficient T cell responses. To address this issue we examined the CTL response of peripheral blood lymphocytes from patients attending a cervical neoplasia outpatient clinic. All were diagnosed HPV positive with various stages of CIN and nine patients were selected for HLA-A2 expression by FACS analysis. PBMC were stimulated with CaSki, a cervical carcinoma cell line demonstrated to be HPV type 16^+ and HLA-$A2^+$. Culture media consisted of RPMI-10% human serum supplemented in three cases with 15 U/ml IL-7, in another three cases with 3 U/ml IL-2 and 15 U/ml IL-7, and in the final three cases with 10 U/ml IL-2 and 15 U/ml IL-7. The CTL were screened for reactivity to CaSki and in addition the cervical carcinoma cell line C33A (HPV^-, HLA-$A2^+$) transfected with either the HPV type 16 E6 or E7 genes. One patient's cells maintained with 10 U/ml IL-2 and 15 U/ml IL-7 showed HPV E7 protein specific cytotoxicity in a HLA-A2 restricted fashion. These CTL lysed the CaSki stimulator cell line but not the NK sensitive target K-562. The CTL efficiently lysed a C33A-E7 clone but in contrast a vector only transfectant was not lysed. The lysis of C33A-E7 was blocked with α-CD3 and α-CD8 antibodies at 66% and 41% respectively. FACS analysis determined that this CTL population was 92.8 % $CD3^+CD8^+$ and 7.3% $CD3^+CD4^+$. Limited E6 recognition was also observed but has not been fully characterized. To our knowledge this report represents the first demonstration of HPV E7 responsive CTL isolated from the peripheral blood of HPV infected patients.

HIV-1 SPECIFIC CTL CORRELATE WITH CLINICAL COURSE OF HIV-1 INFECTION

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Objective: Clinical course of HIV-1 infection may be influenced by an effective host immune response against HIV-1 and/or by viral properties. To investigate the cellular immune response to HIV-1 we analyzed CTL activity against different HIV-1 proteins in long-term asymptomatic HIV-1 infection as well as during rapid progression to AIDS.

Methods: 10 Longterm asymptomatic individuals with CD4 counts >500 cell/μl after more than 8 years of infection were selected from The Amsterdam Cohort Study on AIDS versus 10 subjects who progressed to AIDS <5 years. CTL activity was measured on ^{51}Cr labelled HLA matched or autologous B-LCL, infected with rVV expressing HIV-1 Ag. Both bulk and limiting dilution CTL assays were performed longitudinally with PBMC after Ag-specific stimulation. Sequences of CTL epitopes were determined in homologous virus isolates.

Results: Different kinetics of anti-Gag CTL responses were observed in rapid progressors. In any case CTL responses disappeared during progression to AIDS. In long-term asymptomatic subjects persistent CTL responses were observed together with low viral load.

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

Variation in T-cell epitopes of HIV-1: sequences detected in viral RNA

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We have previously shown that proviral variants found in the peripheral blood mononuclear cells (PBMC) of HIV-1 infected individuals can interfere with recognition by autologous cytotoxic T-lymphocytes (CTL). Abolition of recognition could be caused by interference with the processing of viral peptides, by a failure of the variant peptides to bind to MHC class-I molecules or by a failure of the MHC class-I/peptide complex to efficiently activate the TCR (T-cell receptor). These mechanisms could allow the virus to escape the immune surveillance by CTL.

Most studies so far have addressed the question of viral variation in T-cell epitopes by analysing proviral DNA, extracted from the PBMC of HIV-1 infected individuals. However, the analysis of virion-associated RNA offers several advantages. Sequences found in viral RNA are likely to be functional, at least up to the point of packaging into virus particles. Furthermore, RNA sequences represent virus which is currently actively produced.

Here, we present the sequence variation in two HLA-B8 restricted epitopes: p17-3 gag and amino acids 18-26 of the pol gene. Both proviral DNA from PBMC and viral RNA sequences from plasma are discussed. The ability of viral variants to bind to MHC class-I molecules was assessed and the recognition of variant peptides by CTL was tested. Variants were also tested for their ability to antagonize recognition of the index sequences. We show that viral variants with the ability to interfere with recognition by CTL are not only found in proviral DNA but also in virion associated RNA.

GENOTYPIC AND PHENOTYPIC VARIATION AMONGST ENTEROVIRUS ISOLATES,

Tom A. McKee, Jasper D David & Shuo Shen, Division of Virology, Department of Pathology, Cambridge University, Cambridge UK.

Enteroviruses are a large group of positive stranded RNA viruses known to be responsible for a number of distinct disease entities. Recombination is thought to be capable of generating new enterovirus strains that cause significant morbidity. For example, enterovirus 70 which was responsible for a pandemic of haemorrhagic conjunctivitis and poliomyelitis is thought to have originated by recombination between a coxsackie B like virus and another unidentified enterovirus. We are studying a group of echovirus 11 isolates from an outbreak of disease in southern India. Sequence analysis within the 5' untranslated region reveals that these isolates fall into two groups that differ by ~20% (equivalent diversity to that seen between between published sequences of poliovirus 1 and coxsackie A 9 virus).

These two groups of viruses also differ in their cell tropism. Isolates defined as group 1 by their 5'UTR sequence grow equally well on HT29 cells (a human colon carcinoma cell line) and Vero cells. Isolates of group 2, with one exception, grow only on HT29 cells. Analysis of the structural proteins of these isolates revealed differences in migration that correlated with their cellular tropism. Thus, significant genotypic and biological diversity exists amongst these virus isolates. One virus isolate had the 5' untranslated region sequence of a group 1 virus but the protein profile and cellular tropism of a group 2 virus. The best explanation of these findings is that this anomolous isolate is a natural recombinant between the parenteral strains. Both the ease with which viable recombinants are generated and the diversity present within this one enterovirus serotype increase the potential for the production of novel pathogenic enterovirus strains.

DOMINANT SUSCEPTIBILITY TO POLYOMA TUMORS IN INBRED WILD MICE, Sharon R. Nahill, Yupo Ma, John Carroll and Thomas L. Benjamin, Department of Pathology, Harvard Medical School, Boston, MA 02115

Polyoma virus (Py) is a mouse DNA tumor virus which, under appropriate conditions, causes tumors in a wide variety of cell types. Generation of tumors is a function of both the viral and host genomes. Lukacher et al. have recently described a dominant gene, Pyv^s, carried by the C3H/Bi mouse strain, which confers susceptibility to PY-induced tumors. Mapping and immunological analyses indicate that Pyv^s is the mouse mammary tumor virus 7 superantigen (Mtv 7 *sag)* gene, which deletes T cells required for Py tumor immunosurveillance in H-2^k mice. To determine the generality of endogenous superantigens as determinants of susceptibility and to reveal potentially novel mechanisms of susceptibility, we have looked for dominant susceptibility (DS) gene(s) in newly established and genetically diverse inbred wild mouse strains, Czech II and Peru/atteck (Peru). Both strains are susceptible to PY as 100% of infected animals develop a full profile of tumors. Crosses between C57BR, whose resistance is contributed by the major histocompatibility (MHC) locus, and susceptible Peru or Czech II, yield F1 progeny which are fully susceptible, indicating a dominant inheritance pattern of susceptibility. The incidence of tumor-bearing backcross animals [((Peru x C57BR) x C57BR) and ((Czech II x C57BR) x C57BR)] suggests that DS is due to at least one, but not more than two genes. Amplification of genomic DNA from the Czech II and Peru mice by PCR using primers specific for MTV 7 *sag* indicates that both strains are negative for proviral MTV 7 *sag*. Furthermore, the mechanism of DS in these mice may be independent of all MTV *sag* as PCR using primers specific for the highly conserved region of MTV *sag* is unable to amplify MTV DNA from Peru or Czech II genomic DNA. These results indicate that, like the C3H/Bi, the Peru/atteck and Czech II contain gene(s) which overide the resistance to PY-induced tumors contributed by the MHC of the C57BR parent and which may cause tumors via a novel, MTV *sag*-independent mechanism. We have initiated efforts to map the DS in Peru and Czech II mice using PCR and primer pairs flanking simple sequence length polymorphisms.

IMMUNOLOGICAL RECOGNITION OF FMDV AND ITS PROTEINS IN THE BOVINE, R. Michael E.Parkhouse, Jana Baron, Amanda Childerstone, Trevor C. Collen, Tim Doel, Michael Flint, Mildred Foster-Cuevas, Mercedes Garcia-Valcarcel, Martin Ryan and Haru Takamatsu. Department of Immunology, Institute for Animal Health, Pirbright Lab., Woking, Surrey. GU24 ONF. U.K.

Systematic longitudinal studies of serological and cellular immune responses to FMDV have been done in defined clinical groups of cattle (infected versus vaccinated and recovered virus negative versus persistent.) The principal findings were:

1. **RECOGNITION OF INTACT VIRUS**

a) Humoral: Unlike clasical neutralising antibodies, total anti-FMDV antibodies, as measured in an ELISA capture assay, were cross reactive.

b) Cellular: Proliferative (CD4) T cell responses of peripheral blood mononuclear cells (PBMC) were low or undetectable during primary responses to vaccine or virus, and frequently low during secondary responses. For good T cell proliferation in vitro, multiple immunisation is required. This may reflect preferential stimulation of the Th2 CD4 T cell subset. Interestingly, when CD4 responses were observed, CD8 T-cell responses were also detectable.

2. **RECOGNITION OF INDIVIDUAL VIRAL PROTEINS**

a) Expression Cloning: Structural and non-structural protein pseudogenes were cloned from cDNA by PCR, expressed in pGEX-3XUC, and purified by SDS-PAGE.

b) Humoral: Structural and non-structural proteins were recognised by infected animals. A good anamnestic anti-non-structural response was only observed when the boosting serotype differed from the serotype stimulating the primary response.

c) Cellular: Both structural and non-structural proteins were recognised and some were cross reactive. Interestingly, VP1 was strain specific, and the polymerase (3D) was the most immunogenic and cross reactive.

d) A construct comprising 3D and the immunodominant VP1 epitopes was prepared and tested.

STUDIES OF GENOMIC DETERMINANT OF IMMUNOSUPPRESSION IN FIS-2; FRIEND IMMUNOSUPPRESSIVE VIRUS

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FIS-2 is a low leukemogenic, but relatively strong immunosuppressive variant of Friend Murine Leukemia Virus (F-MuLV). This variant was originally isolated from T-helper cells of FLC-infected adult NMRI mice. Compared to F-MuLV, FIS-2 suppresses primary antibody response more efficiently in infected mice. Some of the FIS-2 infected adult NMRI mice developed a disease resembling the acquired immuno-deficiency syndrome induced by HIV. Restriction mapping and nucleotide sequence analysis of FIS-2 show a high degee of homology between this variant and the prototype F-MuLV clone 57.

In this study we have attempted to localize the genomic determinant of FIS-2 which is responsible for induction of a strong suppression of primary antibody response. Six chimeric viruses of FIS-2 and F-MuLV were constructed. The primary antibody response of the mice infected with these chimeric viruses were investigated. The results of these experiments will be presented.

IMMUNE RESPONSES TO BACULOVIRUS-EXPRESSED EQUINE HERPESVIRUS 1 GLYCOPROTEINS IN A MURINE MODEL,

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In common with other herpesviruses, the envelope glycoproteins of equine herpesvirus 1 (EHV-1; equine abortion virus) are major determinants of the infectious process and pathogenicity, and are inducers of humoral and cell-mediated immune responses. As such, they are candidates for components of subunit vaccines against EHV-1. To generate useful amounts of individual EHV-1 glycoproteins, we have constructed recombinant baculoviruses capable of expressing glycoproteins C, D, H (gC, gD, gH) in insect cells, and have evaluated the recombinant products as immunogens in a murine model of EHV-1 infection. All three glycoproteins induced serum (ELISA) antibodies to EHV-1, and EHV-1 gC and gD also induced neutralizing antibody responses. Following intranasal challenge with infectious EHV-1, protective immunity, as demonstrated by acelerated clearance of virus from respiratory tissues to below detectable levels, was evident in mice immunized with either recombinant gC or gD. In contrast, gH-immunized mice did not develop detectable neutralizing antibody, and did not clear challenge virus more rapidly than controls. Delayed type hypersensitivity and lymphoproliferation responses to EHV-1 antigen were observed for each of the EHV-1 glycoproteins, and in experiments with gD-immunized mice, a role for cell mediated immunity in protection was confirmed by adoptive transfer and T-cell depletion experiments. The data provide support for the potential of glycoproteins C and D as a subunit vaccine against EHV-1.

INTERFERON-γ RECEPTOR DEFICIENT MICE GENERATE ANTIVIRAL TH-1 CHARACTERISTIC CYTOKINE PROFILES BUT ALTERED ANTIBODY RESPONSES. Virgil E.C.J. Schijns*, Bart L. Haagmans*, Eric O. Rijke†, Sui Huang‡, Michel Aguet‡, & Marian C. Horzinek*. **Virology Division, Veterinary Faculty, Utrecht Univ., 3508 TD Utrecht, †Intervet Int. B.V., Boxmeer, The Netherlands and ‡Inst. Mol. Biol., Univ. Zürich, Switzerland.*

The lymphokine IFN-γ is a pleiotropic immunomodulator and possesses intrinsic antiviral activity. We studied its significance in the development of antiviral immune responses using IFN-γ receptor deficient (IFN-γR$^{-/-}$) mice. After inoculation with live attenuated pseudorabies virus (PRV) the mutant mice showed no infectivity titers in various tissues and transient viral Ag expression only in the spleen similar as in wild-type mice. However, the absence of the IFN-γR resulted in increased proliferative splenocyte responses. The PRV-immune animals showed a normal IFN-γ and Il-2 production, without detectable Il-4, and with decreased Il-10 secretion in response to viral Ag or con A. Immunohistochemically, an increased ratio of IFN-γ/Il-4 producing spleen cells was found. After immunization with either live attenuated or inactivated PRV, IFN-γR$^{-/-}$ mice produced significantly less antiviral antibody (Ab), and more succumbed to challenge infection than the intact control animals. The reduction in Ab titers in the mutant mice correlated with lower protection by their sera in transfer experiments. These findings are in line with the strong enhancing effect of exogenous IFN-γ on rabies virus- and PRV-specific IgG responses. Our data demonstrate that a physiological IFN-γ system is surprisingly not critical for the generation of antiviral Th-1-type and the suppression of Th-2-type cytokine responses. The lymphokine, however, is an important mediator in the generation of protective antiviral Ab.