# MOLECULAR ASPECTS OF VIRAL IMMUNITY

**Organizers:** Carol S. Reiss, Barry T. Rouse, Rafi Ahmed, Peter C. Doherty and Thomas J. Braciale  
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Molecular Aspects of Viral Immunity

Keynote Address

52-001  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

52-002  THE ROLE OF T CELL MEDIATED CYTOTOXIC ACTIVITIES TO CONTROL INFECTIONS, TUMOR GROWTH AND ALLOGRAFT SURVIVAL
HANS HENGARTNER1, DAVID KAGI2, BIRGIT LEDERMANN2, PIERRE GOLSTEIN3, MANFRED SCHULZ2, HENK-JAN SCHUURMAN2, ECKHARD PODACK4, KURT BURKIZ, ROLF ZINKERNAGEL1
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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 Ca²⁺ by directed exocytosis of perforin; however residual cytolytic activity in the absence of Ca²⁺ 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.

52-003  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/B71 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.
Molecular Aspects of Viral Induced Inflammatory Disease

J2-005 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-\(\gamma\) expression is pivotal for induction of these changes. At later times during infection, when T cells are expanding, interleukin (IL)-2 expression is increased with transforming growth factor-\(\beta\) expression is 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 and IL-2 production is lower 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-\(\alpha\) 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 \(\times 0.80\). Studies carried out in the CD8\(^+\) T cell-deficient p2-microglobulin (p2-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. 32M\(-/-\) 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, 6:30-538.

2. König, T.M., H. Schorle, M.F. Bachmann, H. Hengartner, R.M. Zinkernagel, and I. Horak. Science 1993, 262:1059-1061.
Molecular Aspects of Viral Immunity

**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. Glialosis 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 HEPATOCYTOE 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 cytotoxic effect of the CTL is minimal in comparison with the cytotoxic 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 those 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 that 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.

**J2-009** Inhibition of antigen presentation to CD8+ T lymphocytes by herpes simplex virus (HSV) immediate early (IE) protein ICP47. David C. Johnson1, Ian A. York1, Frank L. Graham2, David W. Andrews3, Roman Tomazini1 and Stanley B. Riddell4. Departments of Pathology1, Biology2 and Biochemistry3, McMaster University, Hamilton, Ont. Canada L8N 325, +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-IESSMHC, 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 proteins. 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 in lymphocytes. The protein is in progress. In addition, radiolabelled ICP47 binds to a single cellular protein of ~65 kDa on ligand blots. These proteins are good candidates as cellular target 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 responses 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.
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J2-010 SUSCEPTIBILITY TO POLYOMA VIRUS-INDUCED TUMORS IS CONFERRED BY AN ENDOGENOUS MMTV SUPERANTIGEN.
Aron E. Lukacher, Yupo Ma, John P. Carroll, Sara R. Abromson-Leeman, Joseph C. Laning, Martin E. Dorf, and Thomas L. Benjamin.

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/BlDa mouse and the highly resistant but MHC-identical (H-2k) C57Bl/6J mouse, polyoma tumor susceptibility is conferred by a single autosomal dominant gene, which we have designated Pyv. Pyv does not encode a cell receptor 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 C57Bl/6J mice fully susceptible to polyoma-induced tumors, indicating an immunological basis for this strain's resistance. We hypothesized that Pyv encodes an Mtv superantigen (SAG) that confers susceptibility to C3H/BlDa mice by deleting precursors of polyoma-specific T cells. We found that tumor susceptibility in (C3H/BlDa x C57Bl/6J)F1 crosses backcross mice cosegregated with Mtv-7. Inheritance of Mtv-7 showed perfect concordance with absence of peripheral V86* T cells. Genotyping of backcross mice using markers of simple sequence repeat polymorphisms flanking Mtv-7 showed no evidence of recombination between Pyv and Mtv-7. Strongly biased usage of V86 by (a) polyoma-specific CD8+ CTL from virus-infected C57Bl/6J mice and by (b) CD8+ T cells infiltrating a polyoma tumor in a virus-immune C57Bl/6J host further prove that T cells bearing this Mtv-7 SAG-reactive V86 domain are critical anti-polyoma tumor effector Cells. These results indicate identity between Pyv 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?
Roel M. Zinkernagel, B. Odermatt, D. Moskopidis, M. Battagay, D. Kagi, M. van der Broek, H. Hengartner.

Infection of mice with lymphocytic choriomeningitis virus (LCMV) causes a transient to long lasting 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 disappearance 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 augmented 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, Mariet C.W. Feltkamp, Maaike E. Reissing, Rienk Offringa, Howard M. Grey, Alessandro Sette, Cornels J.M. Melief, and W. Martin Kast.

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 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 indicate 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 *0401 and A *0401). 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 immunogenecity studies in HLA-A transgenic mice. Therefore, 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.

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Molecular Aspects of Viral Immunity

**CLASS II PRESENTATION OF AN ENDOGENOUSLY SYNTHESIZED GLYCOPROTEIN.** Carol S. Reiss,1,2,3 Shirley M. Bartold,1 Miriam Stein,1 and Stephanie Diment4,5 Biology Department1, and Center for Neural Science5, New York University, New York NY 10003, Kaplan Comprehensive Cancer Center5 and Pathology Department6, New York University Medical Center, New York NY 10016.

In contrast to Class I presentation which is well characterized to use peptide fragments of proteins synthesized in the cytoplasm, a large polyprotein which is cleaved proteolytically in co- and post-translational reactions to yield all mature viral proteins. 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 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 bystander assay, Gpt-infected cells are incapable of sensitizing 5'Crlabeled 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 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.

Investigations of the characteristics of the enzymes contributing to the degradation of the Gpt have shown that a reducing environment is essential, as diazomethane treatment of cells prevents degradation. Lysosomotropic drugs (eg. NH4Cl 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 pH environment inhibits the enzyme activity. Inhibitors of enzyme classes are consistent with a trypsin-like, and not cysteine-, cathepsin B-, or chymotrypsin-like class.

Supported by NIH grant AI 18083 to CSR.

**Humoral Immunity in Viral Recovery and Protection**

**IMMUNE RESPONSE TO CARDIOVIRUS VACCINE VECTORS.** Ann C. Palmenberg1, Lee Martin1, Jorge Ostroski1, Ralf Altmeyer2, Nicolas Escriv1, Marc Giraud1, and Sylvie van der Werf2. 1Institute for Molecular Virology and Department of Animal Health & Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706; 2Unité de Virologie Moléculaire, 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 marine 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 animals. The glycoprotein studied in detail is a truncated form of the glycoprotein of vesicular stomatitis virus (VSV) which can enter either the exogenous or endogenous pathways for presentation to CD4+ and CD8+ T cells. Investigations of the intracellular sites involved, the proteolytic processes involved in the epitope generation, will be discussed.

The peptides generated are not secreted; using an innocent bystander assay, Gpt-infected cells are incapable of sensitizing 5'Crlabeled 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 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.

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Supported by NIH grant AI 18083 to CSR.

**Local Immunity and Virus Infection of Privileged Sites**

**PROGRESS TOWARDS THE DEVELOPMENT OF A LIVE ATTENUATED RESPIRATORY SYNCCYITAL VIRUS (RSV) VACCINE.** Brian R. Murphy1, James E. Crowe, Jr1, Thuong T. Bu1, William R. Elkins1, Cai-Yen Firestone1, Robert M. Chanock1, Michael D. Lubeck2, Ruth Karron3, Mary Lou Clements4, and Peter F. Wright1. 1National Institutes of Health, Bethesda, MD 20892-0702, 2Wyeth Ayerst Research, Radnor PA 19101-8299, 3Johns Hopkins University, Baltimore, MD 21205-1901, 4Vanderbilt University School of Medicine, Nashville, TN 37232-2581, 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 (cpRSV) 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 cpRSV 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 vivo 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 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 possessively-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 infected 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.

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DS-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 striking enhancement of susceptibility 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 cytokines, to predominantly CD4+ T cells producing Th2 cytokines. In vivo transfer of these cell lines into naive 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. Rouse1, John S. Babu1, Donna M. Bouley2, Johnson Thomas2, and Sally M. Atherton3. 1Department of Microbiology, University of Tennessee, Knoxville, TN 37996-0845 and 2Department 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 neurosensortern 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, leucocyte 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, non-specific 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 suggestions by which the pathologi- cal 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 (immunocomplexes) 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.
Molecular Aspects of Viral Immunity

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 APC 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 persistent. 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.

ESTABLISHMENT AND MAINTENANCE OF T CELL MEMORY TO RESPIRATORY VIRUSES, Peter C. Doherty, Sam Hou, Christine Ewing, David Toplum, Anthony McMickle, James Houston, and Ralph Trpp, 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.

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 naïve 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 IFNγ production of the naïve 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 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 TGFB 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 T cell 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 Aspects of Viral Immunity**

**J2-022** REVERSIBLE CONFORMATIONAL CHANGES OF THE RABIES GLYCOPROTEIN THAT MASK OR EXPOSE EPITOPES INVOLVED IN VIRULENCE, Anne Flamand, Patrick Cottin, Yves Gaudin, Florence Lafay, Hélène Raux and Christine Tuffereau, Laboratoire de Génétique des Virus, CNRS, 9198 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 J2-022 surface which constitute the spikes visible by electron microscopy. Upon exposure to slightly acidic pH, the glycoprotein undergoes a 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 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 conformation: the higher the pH, the more spikes are in their native configuration. 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 primarily by mononuclear phagocytes recruited to the CNS and activated by pro-inflammatory cytokines produced by TMEV-specific Th1 cells.

**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. Kerspu, Northwestern University Medical School, Chicago, IL 60611

Theiler's murine encephalomyelitis viruses (TMEV) are endemic enteric pathogens of wild and colony-raised 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, partial 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 the results of this study, 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- IL-4 and anti-CD4 antibodies; (b) disease susceptibility correlates temporally with the development of TMEV-specific, MHC-class Il-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-α) are predominantly produced in the CNS. We have mapped the predominant Th1 epitope on the virus to amino acids 74-86 of the VP2 cap lipid. A Th1 line specific for VP274-86 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-toleration 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 antagonizes virus-specific Th1 responses. Case studies have also been observed for poliovirus (5). Structural flexibility of external proteins could have important implications in virus-host interactions.

**J2-024** MOLECULAR PATHOGENESIS OF PRION DISEASES, Stanley B. Prusiner, University of California, San Francisco, CA 94134-0515

The concept that prions are novel pathogenic agents 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 consist of inherited, sporadic and infectious neurodegenerative disorders. The inherited human prion diseases are genetically linked to mutations in the PrP gene that result in non-competitive amino acid substitutions. Transgenic (Tg) mice expressing both SHa and mouse (Mo) PrP genes were used to demonstrate that the "species barrier" for scrapie 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 never been synthesized. Similar chimeric human/hamster (Hu/Ha) PrP transgenes were constructed which differ from MoPrP by 9 amino acids between residues 96 and 167. All of the Tg(Mh/Ha) mice developed neurologic disease -200 days after inoculation with brain homogenates from three patients who died of Creutzfeldt-Jakob disease (CJD). Inoculation of Tg(Mh/SHa) mice with CJD prions produced a long incubation period, inoculation with Mo prions produced MoPrP disease. The patterns of Hu/Ha and MoPrP accumulation in the brains of Tg(Mh/Ha) mice were different. About 10% of Tg(Mh/Ha) mice expressing Hu/Ha and non-Tg mice developed neurologic disease >500 days after inoculation with CJD prions. The different susceptibilities of Tg(Hu/PrP) and Tg(Mo/PrP) 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(Mh/Ha) 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 days to 45 days 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 122 in Hu/Ha) produced spontaneous, fatal neurodegeneration between 150 and 300 days of age in two lines of Tg(MoPrP-A) mice designated 21SS and 2247. Genetic crosses of Tg(MoPrP-A) mice resulted in the mouse designated Tg(Mh/Ha) mice which have retained the ability to pass the disease to homozygous offspring. Another line of mice designated Tg(MoPrP-A) overexpress the mouse transgene -32-fold and develop fatal neurodegeneration between 200 and 400 days of age. Tg2862 mice exhibited the most severe spongiform degeneration and had numerous, large granular amyloid plaques. While mutant MoPrP-P20(S15) clearly produces neurodegeneration, wtMoPrP-P20(S15) produced a phenotype that was less severe than that of the wild-type prion. 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(Mh/Ha) mice, to modify the pathogenesis of the dominantly inherited prion diseases.

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GENERA TION OF VIRAL PEPTIDES RECOGNIZED BY MHC CLASS I-RESTRICTED T CELLS, Jack R. Bennink, Luis Anton, Heidi T. Link, and Jonathan W. Tweedie, Laboratory of Viral Diseases, NIAID, Bethesda, MD 20892-4440.

CD8+ lymphocytes (TCD8+) play an important role in host immunity to viruses and other intracellular parasites. Virus-specific TCD8+ 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. To begin to characterize the nature of proteins involved in the generation of antigenic peptides from cytoplasmic 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 NH2-terminal signal sequence. Peptides were generated much more efficiently from the COOH-terminus of the 17 residue precursor than from the NH2-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.

DEVELOPMENT OF SYNTHETIC PEPTIDE VACCINES, Jay A. Berzofsky, Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute, NH, 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+ cytototoxic T lymphocyte (CTL) responses to peptides attached to these helper regions. These synthetic peptide vaccine constructs consisting of the P18 peptide from the V3 loop of HIV-1 IIB or MN, elicited both neutralizing Ab and CTL in multiple strains of both mouse and human. The cluster peptides inducing helper T cells were essential for elicitation of Ab and CTL to the P18 segments of both IIB 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 elicited 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 marine 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.

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 challenge1. HA DNA vaccines generated neutralizing antibodies in mice, ferrets and primates, and provided protection in mice 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 γIFN 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 (Th1) and cytotoxic T cells were generated. Cross-strain protection due to cellular immunity was demonstrated.

1Science, 1993 259:1745-1749, 2DNA Cell Biol, 1993, 12:777-783.
Molecular Aspects of Viral Immunity

HIV and SIV Infections and the Immune Response

J2-028 FACTORS DETERMINING THE NEUROPATHOGENESIS OF SIV INFECTION IN MACAQUES. Opena N. Naryayu, Edward B. Stephena, Sanjay V. Jooq, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, Kansas 66160-7424.

The profile of a neuroviral disease 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 consequent 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 brain blood 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 developing 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. Horwitz1,2, Gyorgy Fejer1, Ildiko Gyorgy1, JoAnn Tufariello1 and Shimon Efrat1. 1Departments of Microbiology and Immunology, 2Pediatrics and 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 transcribed 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) have been transplanted allogeneically to H-2" 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 rat 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. Nichol1, S. Mozranov1, V. Chizhikov1, C.F. Spiropoulou2, H. Feldmann1, E. Rakov1, M. Brown2, A. Sanchez2, W. Thayer4, J.E. Childs1, C. Fujita1, S. Trappier1, E. Tkachenko5, J. Pilaski4, L.T. de Souza2, L.B. Iverson2, J.E. Rowe2, S. St. Jee2, C.J. Peters3,1, Special Pathogens Branch, Division of Viral and Rickettsial Disease, Centers for Disease Control and Prevention, Atlanta; 1Department of Microbiology and Immunology, Emory University, Atlanta; 4Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia; 2Medical Institute of Environmental Hygiene, Heinrich-Heine Universitat Dusseldorf, Germany; 3Instituto Adolfo Lutz, Sao Paulo, SP, Brazil; 5FSP, University of Sao Paulo, Sao Paulo, SP, Brazil; 6Department 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 phyllogenetic 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 Hantavirus 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-tier 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 3.3 K promoter. The 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.
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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 choriomeningitis 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 ifo/ifo mice, which have a mutation in the fng 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
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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 a cellular immune response 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.
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HIV-1-specific CTL response and TCR usage in HIV-1 infection
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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/mm3.

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.
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New Strategies for Molecular Understanding of Viral Pathogenesis

J2-100 IRF-1 TRANSGENIC MICE SHOW INCREASED SUSCEPTIBILITY TO COXSACKIEVIRAL MYOCARDITIS. Karen Attken, Joseph Penninger, Tak Mak, Fayeay Dawood, Weng-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-β 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 inoculated with 10^8 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 IMMUNOCOMPROMISED MICE. Andreas Henke1, Axel Stelzner2, J. Lindsay Whitton3. The Scripps Research Institute, Dept. of Neuropharmacology, La Jolla, CA 92037; Institute of Virology, Friedrich Schiller University, 07745 Jena, Germany3

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 myocyte 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 b2M ko) mice and causes a lethal disease after i.p. infection 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.

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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 achieved 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 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 IFNγ 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 IFNγ levels went up for young animals but remained constant for the elderly mice.

J2-103 MICE LACKING p56lck ARE RESISTANT TO COXSACKIEVIRUS B3 INDUCED HEART DISEASE. Tamara Martino, Karen Attken, Joseph Penninger, Jano Nikhilandduhan, Tak Mak, Fayeay Dawood, Weng-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 p56lck are resistant to CVB3 induced heart disease. The 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.

We conclude that T-cell activation is critical to produce substantive myofiber necrosis after CVB3 infection, that NK-cells play a fundamental role in protecting the mice from severe virus infection, and that lck transgenic mice serve as an important model for understanding the pathogenetic mechanisms involved.

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J2-104 CD4+ CTL FROM 62m-/- MICE FOLLOW SIMILAR KINETICS TO CD8+ CTL FROM NORMAL MICE
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Mice genetically engineered to be deficient in IL-2 microglobulin (62m-/-) 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 62m-/- mice.

We now show that CD4+ T cells from 62m-/- 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 62m-/- mice. However, 62m-/- mice show an earlier rise in percentage and absolute numbers of CD4+ T cells by 7 days after infection. CD4+ T cells from 62m-/- 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 62m-/- mice and have a later peak than CD8+ CTL from normal mice. These changes may explain the lower mortality and longer time course of immunopathologic meningitis in 62m-/- mice.

In conclusion, these data suggest that CD4+ T cells from 62m-/- 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 62m-/- 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 CD40-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. These results indicate that the CD40 ligand (CD40L) is an essential mediator of T cell help required for the late stage antibody response.

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 virus deficient in immunomodulatory 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 (Th) cells of the Th1 subset and, to a lesser extent, of the Th2 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 immunomolecular barriers to gene therapy of CF should facilitate the design of rationale strategies to overcome them.
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**J2-108** PERSISTENT ASYMPTOMATIC INFECTION OF MICE WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV) AND ESCAPE FROM HOST IMMUNE RESPONSES.  
Chen Even, Robert R. Rowland, Grant W. Anderson, Gene A. Palmer and P.G.W. Flaggeman. Department of Microbiology, University of Minnesota, Minneapolis, MN 55455.

LDV establishes a lifelong 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 lymphoid tissues. In addition, large amounts of viremia or viremia debris accumulated in the germinal centers of the spleen and lymph node 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.

**J2-110** A SPECIFIC Th1/Th2 CYTOKINE PATTERN IS RAPIDLY ELICITED FOLLOWING INFECTION WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS, Franziska B. Grieder, Han Nguyen, Xia-Di Zhou, Shen-Jue Chen, and William C. Gause, Department of Microbiology and Immunology, School of Medicine - USUHS, Bethesda, MD 20814-4799.

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 (V3013) (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-2, IL-4, IL-6, and IL-10 gene expression were evident that peaked at 72 hours post-infection. In both strains elevations of TNF-α, IL-12, IFN-γ, IL-2, IL-4, IL-6, and IL-10 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 infection of V3000, elevations were delayed until 24 hours following infection with V3013. 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 altering 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-γ 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.

**J2-109** ADULT NUDE MICE ARE RELATIVELY RESISTANT TO INFECTION WITH MURINE ROTAVIRUS BUT DO NOT DEVELOP IMMUNITY AFTER A PRIMARY INFECTION, Manuel A. Franco, Juan Ludert and Harry B. Greenberg, Department of Gastroenterology, Stanford University School of Medicine, Stanford CA 94305.

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 rotavirus. 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 naïve 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 naïve nude mice, both shed very low but equal levels of rotavirus in the stools. Interestingly adult naïve heterozygous littermates shed more virus than the nude. The factors responsible for this relative resistance of adult nude mice to rotavirus infection as well as the mechanism for viral clearance in nude mice will be discussed.

**J2-111** SEVERE AND PROLONGED INFLAMMATORY RESPONSE RESULTS IN LOCALIZED POXVIRUS INFECTION OF C5-DEFICIENT MICE, Ginsh J. Kotwal and Cathie G. Miller, Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville KY 40292 USA.

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, 335:176-179; Kotwal et al., UCLAA Synp. 1990, 121:149-160). The great majority of the viral genes encoded proteins actively mediating the evasion of host defense are secretory proteins termed "virokinins". 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 virokinin to be reported that has structural similarity to human/mouse complement control proteins, with the greatest similarity to the human complement C4d binding protein (hC4d-BP) and the first protein to have a postulated role in the viral evasion of host defense (Kotwal and Moss, Nature 1988, 335:176-179). Bioactive VCP, purified from serum-free medium has been shown to be more potent than the hC4d-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 homolog of VCP (Jayaraman and Kotwal, 1993 Mol. Immunol. 30:20). 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 possibility 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 immune response is a critical factor in regulating poxvirus pathogenesis.
Molecular Aspects of Viral Immunity

ROLE OF CYTOKINES IN VIRAL PATHOGENESIS.
I.A. Ramshaw, J. Ruby, D.P. Sharma, A. Ramsay. KH Leong and M Ralph 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 to date fall into two categories; either altering viral pathogenesis or selectively stimulating specific immune responses. The expression of IL-2, IFN-γ, IL-10, and IL-4 producing cells were comparable for each strains of mice, while more elevated 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.

In mice infected with lymphocytic choriomeningitis virus (LCMV), interferon-12 (IL-12) dosage 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. These results demonstrate a unique TNF-related immunotoxicity and have significant implications concerning detrimental consequences of in vivo TNF and/or IL-12 for protective anti-viral responses.

LOSS OF MEMORY B CELL RESPONSE TO CYTOMEGALOVIRUS DURING INFECTION WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS WITHIN THE SPLEEN. Raymond R.R. Rowland*, Peter G. W. Plagemann, and Ronald Jessen. 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 elevation of circulating glucocorticoid levels and decreases in thymic mass. These changes are also reversed by anti-TNF. The results demonstrate a unique TNF-related immunotoxicity and have significant implications concerning detrimental consequences of in vivo TNF and/or IL-12 for protective anti-viral responses.

LOSS OF MEMORY B CELL RESPONSE TO CYTOMEGALOVIRUS DURING INFECTION WITH LACTATE DEHYDROGENASE-ELEVATING VIRUS. 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.

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**J2-116** VIREUS-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-ω65 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-ω65 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'-tetramethyl indocarbocyanine perchlorate- or PKH26-GL-labeled bone marrow cells 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 to 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 activation with acute infection. Infection does not effect B cell survival in 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. David Segal, Janet Ruby, Alistair Ramsay and Ian Ramshaw, Department of Cell Biology, John Curtin School of Medical Research, PO Box 334, Canberra, ACT, 2601 Australia.

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 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 vivo 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. Susceptible 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 derived from 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-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 around 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.
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, B2M-/- mice, lacking CD8+ T cells, have dramatically elevated NK cell responses on day 7 post-infection. The B2M-/- 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)-sensitive and interleukin (IL)-2-independent, whereas day 7 NK cell responses in B2M-/- 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-β (TGF-β) 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 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-β, evaluated in biological and ELISA assays, was induced maximally at days 7 to 9 post-infection. The kinetics of TGF-β production by cells from infected B2M-/ mice was similar to that of normal mice. However, cells from B2M-/- 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 B2M-/- context modulates responsiveness of NK cell subsets to other late cytokines. Studies are in progress to distinguish between these two possible mechanisms.

**Molecular Aspects of Viral Immunity**

**J2-120** IL-4 and TGF-β DURING VIRAL INFECTION OF B2M-/- AND B2M-/- PROTOGLOBULIN-DEFICIENT (B2M-/-) 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, B2M-/- mice, lacking CD8+ T cells, have dramatically elevated NK cell responses on day 7 post-infection. The B2M-/- 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 B2M-/- 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-β (TGF-β) 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-β, evaluated in biological and ELISA assays, was induced maximally at days 7 to 9 post-infection. The kinetics of TGF-β production by cells from infected B2M-/- mice was similar to that of normal mice. However, cells from B2M-/- 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 B2M-/- 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 Alcami, 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 hyperthermia 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*, Hidero 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 21203.

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 viral and avirulent strains of MV on the thymus. SCID-hu mice were infected by direct inoculation of the graft with 10⁴ PFU of either a wild type strain of MV(Chicago-1,Chi-1) or an attenuated 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 (105.7 PFU/third of implant), and Mor on d21 (103.2 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 polikaryosis and decreased cellularity. By d4, 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 hemagglutinin(HA) and Abs for either human cytokeratin(HE1/HE3) or CD15 co-localized MV predominantly to epithelial and monocytic cells. Additionally, expression and correlated with virus replication in the brain, the organ that controls body temperature.

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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.
It is becoming increasingly evident that many poxviruses employ mechanisms of immune evasion. The downregulation of CD4 by myxoma virus could be masking of the epitope via viral antigens. Analysis of CD4 levels in the presence of cytosine arabinoside indicates that late in 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, 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 p56lck associates with the cytoplasmic domain of CD4 we have also examined the association of p56lck with CD4 as well as steady state levels of p56lck 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 lymphotropic viruses.

### Molecular Aspects of Viral Immunity

#### J2-123

**CD4 SURFACE EXPRESSION IS DOWNREGULATED IN A RABBIT T CELL LINE INFECTED WITH MYXOMA VIRUS.** Michele Barry1, Siow Fong Lee2, Judy Hannon2, Lynn Bosshkov2 and Grant McPadden1. 1Dept. Biochemistry, U. of Alberta, Edmonton, Alberta, Canada, T6G 2H7, and 2Dept. of Laboratory Medicine and Pathology, U. of Alberta Hospital, Edmonton, Alberta, Canada.

It is becoming increasingly evident that many poxviruses utilize mechanisms of immune evasion. 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 (KL-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, 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 p56lck associates with the cytoplasmic domain of CD4 we have also examined the association of p56lck with CD4 as well as steady state levels of p56lck 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 lymphotropic viruses.

#### J2-124

**DETECTION OF HIV-SPECIFIC CELL MEDIATED CYTOTOXICITY IN THE PERIPHERAL BLOOD OF INFECTED CHILDREN.** Florence Buseyne1, Stéphane Blanche2, Claude Griscelli2 and Yves Rivière1. 1Unité de virologie et d'immunologie cellulaires, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15. 2Unité d'hématologie et d'immunologie, Hôpital Necker, 149 rue de Sèvres, 75434 Paris cedex 15.

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 children had detectable HIV-specific CTL which were CD4+CD8+. We previously showed that among children with a slow disease progression, fresh CTL were more frequent in the P2A(paucysymptomatic) group than in the P1(asymptomatic) and the P2B-8 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. Campbell1, Laura K. Hanson2, Lei Sun2, Jacqueline S. Slater1, Richard M. Stenberg1 and Thomas R. Jones2. 1Department of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk, VA, 23507 and 2Molecular 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 flattening this HCMV region 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 IR51-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: US1 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 anaesthetised this 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-2b) mice was directed. This region of the E6 molecule did not contain the predicted amino acid motif previously identified by 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 contains 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.
Molecular Aspects of Viral Immunity

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

MMTV encodes a superantigen (sag) gene, whose gene product causes the activation 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 in the sag gene. Young HYB PRO/Cla mice (< 10 weeks of age) showed no deletion of their cognate βV14 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 were infected with transgene-encoded MMTV because they had the typical slow deletion of βV14 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 M-Mtv and 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' and Charles M. Rice', 1 Department of Pathology, Berne Carter Center for Immunology, University of Virginia, Charlottesville, VA 22901; 2Department 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 antigens by HA-specific CTL. vHCV1 expresses the structural proteins 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 transected B cell lymphoma line (KG) was infected with vHCV1 or vHCV2 or vS9 as a negative control, cells infected with vHCV1 showed a dramatic reduction of HA recognition by HA-specific CTL in contrast to vHCV2 or vS9 infected KG cells. The level of inhibition was reproducibly in a range of 40% - 60% relative to uninfected KG 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 (Mø) is thought to play an important role in human and murine cytomegalovirus (MCMV) infection. We therefore examined Mø activation during MCMV infection. Intrapерitoneal challenge with 106 pfu of MCMV or 106 pfu of herpes simplex virus type-1 (HSV) resulted in an inflammatory infiltrate consisting largely of MHC class II positive Mø in CB17 SCID mice on day 7 post infection. Increasing doses of MCMV resulted in decreased Mø class II expression, with class II expression absent 7 days after infection with 106 pfu of MCMV. Limiting dilution analysis demonstrated that fewer than 1:250 Møs 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 106 pfu of MCMV induced Mø class II expression, suggesting that the specific immune response overcomes the lack of class II. Consistent with this, Mø from SCID mice reconstituted with MCMV immune spleen cells and challenged with 106 pfu of MCMV, express high levels of class II. The deficiency in class II expression was MCMV specific, since several HSV-1 strains induced Mø class II expression. MHC class I and ICAM-1 levels were increased in the class II negative Mø, making general inhibition of Mø cell surface protein expression unlikely. It was administration of recombinant mouse IFNγ induced high levels of class II on Mø from SCID mice challenged with either HSV or uv inactivated MCMV, but not live MCMV. This suggests that MCMV infection inhibits IFNγ induced Mø class II expression in the absence of specific immunity. This inhibition of one component of IFNγ dependent Mø 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 Hegel1, Christine Ellinger', Jos Pool', Els Gouw1 and Ulrich H. Koszinowski1, 1Department of Virology, University of Heidelberg, Germany, and 2Department 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 (gp-100) are not affected. Steady state levels of MHC class I complexes and unassembled MHC class I heavy chains are found largely decreased, whereas free α2-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, interferon (IFNγ) and tumor necrosis factor (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.
HEPATITIS B VIRUS (HBV)-SPECIFIC T CELL RESPONSE IN CHRONIC HBV INFECTIOH, Cheng-po Hu, Pei-yun Shu, Ching-hung Shen, Mei-yun Lin, Jaw-ching Wu, and Chung-ming 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 HBsAg in asymptomatic HBsAg carriers was significantly stronger than that in patients of CHB with acute exacerbation. In addition, the frequency of HBsAg-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 that HBsAg-specific and HbcAg-specific 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 HBsAg-specific response and some of them have HBsAg-specific response.

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ALTERATION IN LANCERHANS 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 epithelial cells and Langerhans cells respectively. In this study we investigated the effect of flavivirus infection on the expression of MHC in vivo. 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 epithelial cells and the Langerhans cell population using flow cytometry. These increases were detectable as early as 16 hours after infection. A significant increase 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 infection in vitro culture of Langerhans cells. These results, together with the reduction in Langerhans cell numbers, may represent the in situ maturation and consequent migration of these cells as a consequence of virus-induced cytokines within the skin microenvironment.

EARLY INTERFERON-INDEPENDENT INDUCTION OF CD54 BY FLAVIVIRUS INFECTION OCCURS IN QUESCENT 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 genetic variability makes it unlikely that the early increase in CD54 expression was due to a nonspecific viral effect. The closely related flavivirus, Kunjin, induced increased CD54 expression on HEF, while the ability of flaviviruses to modify expression of cell-surface molecules to the Langerhans cell population using flow cytometry. These increases were detectable by 4-5 fold within 24 h of WNV infection on HEF. 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 2 h 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-α. Increased CD54 expression on HEF by WNV was also cell-cycle dependent. CD54 increased only in quiescent, contact-inhibited infected HEF in G0 phase. In contrast, induction of CD54 by type 1 interferon in HEF 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, Semliki 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-α 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.
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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 C-G-K-K-Y-K-L-K, 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 2, 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 down-regulation 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 YT-1 I DNA-BINDING PROTEIN ADPRESSOR INTERFERON-INDUCED MHC EXPRESSION BY INDUCING SINGLE-STRANDEDNESS IN THE PROMOTER

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YT-1 is a member of a newly defined DNA and RNA binding protein, the Y box factors. These proteins have been shown to affect gene expression at both the transcriptional and translational levels. Recently, we showed that YT-1 induces transcription of human major histocompatibility (MHC) genes (Tang et al., 1994, J. Exp. Med., 179:1603-1611). Expression of MHC genes is essential for the development of antigen-specific immune responses. In addiition to our observations, others have shown that YT-1 can activate expression of reporter genes through EIV and HTLV-I promoters (Kashanchi et al., 1994, J. Viral, 68, 551-565). YT-1 is a host protein that potentially can be used by viruses to activate viral expression, while repressing IFN-y induced MHC expression.

We have characterized the DNA binding properties of purified, recombinant YT-1 on the MHC class II DRA promoter. We demonstrate that YT-1 specifically binds single-stranded templates of the DNA promoter with a much greater affinity than the double-stranded template. Most importantly, we also demonstrate that using human lymphoblast (MKN) and K562 cells, which express YT-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 Y box binding results in single-stranded regions within the promoter, preventing, leading to a decrease in the translation of IFN-y induced MHC expression.

J2-187 EXPRESSION OF HPV-16 E5 PROTEIN IN KERATINOCYTES LEADS TO POST-TRANSCRIPTIONAL LOSS OF MHC-I AND TAP-1.

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Dept. of Microbiology & Immunology, University of Rochester, Rochester, NY 14662, USA, Institute of Pathology, Section of Molecular Pathology, Free University Hospital, Amsterdam, Netherlands, Center, such as lysine to arginine or glutamate at position 2, 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 down-regulation 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.

Viral proteins can be recognized by cytotoxic T-cells when presented at the cell surface as small peptides bound by major histocompatibility complex class I molecules. These peptides are translated into the lumen of the endoplasmic reticulum (ER) by the peptide transporter, encoded by the TAP-1 and TAP-2 genes. Subsequent complex formation between peptide, class I and microglobulin in the ER results in stable cell surface expression of the trimeric MHC-I molecule. In previous studies we showed that in HPV-16 positive cervical carcinomas there was a loss of MHC-I 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-I is mediated by an HPV-16 encoded protein. Human keratinocytes were transfected with various HPV-16 constructs including pAT16, the full length genome, pAT16ESX, the full length genome with a premature stop codon in E5, pUC.EET16, the B6 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 blot experiments respectively. Western blots showed very low steady state levels of TAP-1 and MHC-I heavy chains in the cells with pAT16 as well as those containing E5 alone, which was marginally increased by γ-IFN. While the primary keratinocytes, pAT16ESX and pUC.EET16 lines showed comparable TAP-1 and MHC-I 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 EIA 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 EIA epitopes was shown to confer susceptibility to syngeneic Ad5 E1-specific CTLs in vitro, which may influence the tumorigenicity of Ad5 E1- and Ad12 E1-transformed cells in vivo.
**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, rabies, buffalopox, elephantpox and camelpox viruses. The B8R open reading frame of vaccinia virus contains the soluble cytokine receptor BCRFI. 14.7K provides protection against TNF-induced activation of phospholipase A2 and 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 and both 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 vivo protection of virally infected cells against TNF cytosis. The specific immune response is altered by another E3 protein, gp28K. gp28K prevents CTL recognition in vivo and was expected to alter the cellular infiltration caused by CTLs and perhaps even prevent in vivo CTL priming. Surprisingly, when B10 mice were immunized with a mutant lacking gp28K, 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 immunodominant CTL epitope in EIA where used, a second, Toll-like receptor responsive epitope in EIB 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 analogues that are recognized by CTLs does not prevent the generation of Ad specific CTLs.

**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 lab is investigating 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 (CTLs). In vitro the E3 proteins 10.4K/14.7K and 14.7K provide protection against TNF induced activation of phospholipase A2 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 vivo protection of virally infected cells against TNF cytosis. The specific immune response is altered by another E3 protein, gp28K. gp28K prevents CTL recognition in vivo and was expected to alter the cellular infiltration caused by CTLs and perhaps even prevent in vivo CTL priming. Surprisingly, when B10 mice were immunized with a mutant lacking gp28K, 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 immunodominant CTL epitope in EIA where used, a second, Toll-like receptor responsive epitope in EIB 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 analogues that are recognized by CTLs does not prevent the generation of Ad specific CTLs.

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

Julian A. Symons, Antonio Alcamì and Geoffrey L. Smith.

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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 dsDNA 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-10, 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 IFNαβ inhibitor was mapped and the gene expressed from recombinant baculovirus. The inhibitor blocked the binding of 125I-IFNα to U937 cells and binding of 125I-IFNα to supernatants from baculovirus and VV-infected cells demonstrated that the inhibitor functioned as a soluble receptor for IFNαβ. Direct binding of 125I-IFNα to VV WR supernatants revealed that the soluble IFNαβ receptor had a high affinity for type I IFN. Deletion of 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 IFNαβ receptor bound human and bovine IFN with high affinity but murine IFN with relatively low affinity. Interestingly, the soluble IFNαβ receptor is highly conserved in various virus. Given the importance of IFN in antiviral defense it is likely that the soluble IFNαβ receptor plays an important role in the virulence of the orthopoxviruses.

**STRUCTURE-FUNCTION ANALYSIS OF THE EBSTEIN-BARR VIRUS INTERLEUKIN-10 HOMOLOGUE.**

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The BCRFI 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 BCRFI and this has led to it being termed viral interleukin-10. The BCRFI 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 BCRFI 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 bacitally 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 human interleukin-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.
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, Miriam Stein, Stephanie Diment, and Carol S. Reiss, Dept of Biology, Center for Neural Science, and Pathology Dept, New York University, New York NY 10003, Kaplan Comprehensive Cancer Center and Pathology Dept, 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 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 (\(\alpha\)-mannosidase 11, 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.

Supported by NIH grant AI18083 to CSR.

**J2-201 IDENTIFICATION OF 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 SRYWAIART 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 molecule. Stable class I molecules, associated with \(\beta_2\)-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\(^{a}\)), 147-155 (H2-K\(^{b}\)) and 366-374 (H2-D\(^{b}\)). The frameshift was introduced 26 amino acids upstream of the H2-K\(^{a}\) epitope. The mutated genes were then recombined with vaccinia virus and used for presentation using CTL restricted to each of the epitopes described above. We found that, whilst presentation of the H2-K\(^{a}\) epitope was unaffected by the frame shift, the epitope proximal to the frameshift (H2-K\(^{b}\)) 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.
**Molecular Aspects of Viral Immunity**

**J2-204** THE EFFECT OF ACUTE LCMV INFECTION ON THE IMMUNE RESPONSE OF MICE TRANSGENIC FOR A K\(^\beta\)/OVALBUMIN 257-264-REACTIVE T-CELL RECEPTOR, Eric A. Butz, Francis R. Carbone, and Michael J. Bevan, Howard Hughes Medical 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 (SINIFKEKL) in the context of H-2 K\(^\beta\). 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 bearing 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-SINIFKEKL 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 NPS(330-337) K\(^\beta\) EPITYPE 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-2 K\(^\beta\)-restricted epitope derived from the virus nucleoprotein, NPS(330-337) (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\(^\beta\) has shown that the side chains of peptide residues Phen, Gly, Asn, and Ala are available for recognition by the TCR. Notably, residues Gly and Asn are not present 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 that generally appeared to be dominant recognition of Gly and Asn. However, individual hybridomas exhibited distinct patterns of fine specificity for residues Phen and Ala. 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 and Asn in governing TCR-SEV-9/K\(^\beta\) 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. Zaias\(^a\), and Stephen J. Forman, Departments of Hematology and BMT, and Pediatrics\(^a\), and the Division of Immunology\(^a\), City of Hope National Medical Center and the \(*Beckman Research Institute, Duarte, CA 91010\)

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 pursued that strategy in a transgenic murine model of HLA-A2.1 recipients. Seropositive volunteers against CMV. These epitopes will be used to boost responses which are lymphokine-dependent because of adjuvants are effective at eliciting murine Class I restricted responses to the protein. Current studies are evaluating the recognition properties of the epitope recognized by these CTL, and that differed between acute LCMV infection. T-cells bearing 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 a single H2-K\(^\beta\)-restricted epitope derived from the virus nucleoprotein, NPS(330-337) (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\(^\beta\) has shown that the side chains of peptide residues Phen, Gly, Asn, and Ala are available for recognition by the TCR. Notably, residues Gly and Asn are not present 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 that generally appeared to be dominant recognition of Gly and Asn. However, individual hybridomas exhibited distinct patterns of fine specificity for residues Phen and Ala. 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 and Asn in governing TCR-SEV-9/K\(^\beta\) recognition and suggest a structural basis for the diversity of the TCR repertoire selected by this epitope.

**J2-207** DEFINITION OF A HUMAN T CELL EPITOME FROM INFLUENZA A NON-STRUCTURAL PROTEIN 1 USING HLA-A2.1 TRANSGENIC MICE, Victor H. Engelhard, Stephen Man, Michael H. Newberg, Victoria L. Crozer, C. John Luckey, Noelle Williams, Ye Chen, Eric L. Haezko, and John P. Rudge, 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/S4-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/67/ 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.
characterization of antigen presenting cells from mediastinal lymph nodes and lungs of influenza-infected mice, Ann Marie Hamilton-Easton and Maryna Eichler, 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 macropathogens, it is believed that priming of naive T cells occurs in the draining lymph nodes. Kinetic 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 APCs were capable of stimulating virus-specific CTLs, 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 bronchoalveolar 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.

processing, presentation and recognition of a viral CTL epitope expressed in a cellular protein, T-M Fu, S. Alam, M. J. Tevethia, R. K. Verner, and S. S. Tevethia, Department of Microbiology and Immunology and Department 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-2Kb-restricted CTL epitope from herpes simplex virus type 1 (HSV-1) glycoprotein B (gB) epitope, residues 498-505 (SIIEFARK), was cloned into the mouse dihydrofolate reductase protein (DHFR) at amino acid position 87. The recombinant DHFR was expressed within a vaccinia virus recombinant. To distinguish the recombinant DHFR proteins from the endogenous DHFR, an antibody epitope (recognized by monoclonal antibody PA901), derived from SV40 T antigen residues 684-698, was tagged to the C-terminus of recombinant DHFR proteins. In vivo expression of recombinant DHFR was assessed by immunoprecipitation with the monoclonal antibody PAb 901. The H-2Kb cells infected with recombinant vaccinia virus expressing the recombinant DHFR were tested for their APC capacity of these populations from both naive and infected mice.

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processing, presentation and recognition of a viral CTL epitope expressed in a cellular protein, T-M Fu, S. Alam, M. J. Tevethia, R. K. Verner, and S. S. Tevethia, Department of Microbiology and Immunology and Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

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**Molecular Aspects of Viral Immunity**

**J2-212 PEPTIDE-BINDING SPECIFICITY OF RAT MHC CLASS I RT1.A MOLECULES CORRELATE WITH THE TRANSPORT CAPACITY OF THE NATURALLY ASSOCIATED TAP ALLELES.** Etnene Joly, Andrea Gonzalez, Carol Clarkson, Jonathan C. Howard and Geoffrey W. Butcher. Laboratory of Immunogenetics, Department of Immunology, The Babraham Institute, Cambridge CB2 4AT, UK.

Tap transporters from rats can be divided into two allelic groups, depending on their capacity to provide the RT1.Aa molecule with an appropriate level of suitable peptides. Recent results suggest that this might correlate with the RT1.Aa molecule requiring arginine-ended peptides (Powis et al., manuscript submitted), which the Tapb allele of the transporter is unable to translocate across the ER membrane efficiently. RT1.A alleles are naturally linked with the Tapb or the Tapb allelic group. We have set out to characterise various alleles for the RT1.A molecule, and find that, for the majority of TAPβ-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 TAPβ-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.

1. Powis S.J. et al. 1992 Nature, 357: 211-215.
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 Saverton, Pefang Sun and Annalisa Zanini (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 challenged 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 vivo protection, or are a simple by-product of the cellular immune response.

1. Joly E. et al. 1994 Immunogenetics, 40: 45-53
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

**J2-214 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 Laloo, Andrew Leigh Brown*, Frances M. Goutch 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 haemophilic 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.** Eric G. Pamer, Mercedes S. Villanueva, Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT 06520

*Listeria monocytogenes* is a Gram positive bacterium that infects macrophages and secretes proteins into host cell cytosol. The murine 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-2Kd 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.

**J2-215 QUANTITATIVE ANALYSIS OF MHC CLASS I ANTIGEN PROCESSING EFFICIENCY.** Eric G. Pamer, Mercedes S. Villanueva, Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT 06520

*Listeria monocytogenes* is a Gram positive bacterium that infects macrophages and secretes proteins into host cell cytosol. The murine 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-2Kd 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.
Molecular Aspects of Viral Immunity

**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 IBF3, IBF1, and RF but not MN strains of HIV-1. Using vaccinia vectors expressing truncated versions of the HIV-1 envelope, the clones were found to recognize an epitope within amino acids 237-354, but not including 312-326 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 position 305 and another at position 314 were found in the epitope for this particular CTL.

**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 BWS147 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, whereas LMP2 is underepressed. 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, Hisnly Chang, Phyllis Hao, Stephen J. Forman, and Don J. Diamond. Department of Hematology and BMT and the "Division of Immunology, City of Hope National Medical Center and the 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 immunoluted 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 HMC 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 chromosome 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 pp41-specific CD8+ CTL which has a defined minimal cytotoxic epitope will only recognize and kill a subset of B26 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 pp41-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. Tyndall,§ Young S. Hahn,§ and Thomas J. Braciale,*§ Beime B. Carter Center for Immunology Research, Departments of Microbiology and Pathology, University of Virginia Health Sciences 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 HMC class I K* gene, we show that processing of the influenza virus hemagglutinin (HA) glycoprotein for presentation to a panel of K* restricted CTL is dramatically inhibited by the disruption of Tap function. Residual and highly inefficient K* charging in the RMA-S/K* cell can be elicited both with influenza virus as well as recombinant Sindbis virus expression vectors carrying influenza HA gene fragments encompassing known K* epitopes. Sindbis vectors carrying HA gene fragments with or without a signal sequence sensitize RMA-S/K* 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.
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**The Flanking Region of Epitope Influences the Transport of Epitope by TAP**

Bing Yang, Thomas J. Braciale Beime Carter Center for Immunology Research, Department of Microbiology and Department of Pathology, University of Virginia, Charlottesville, VA 22908

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 radio labeled synthetic peptide which can be N-linked 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 "147-155, HA204-212 and HA210-219" was found to be similar. An epitope corresponding to antigenic peptides restricted to the murine Kd molecule was measured by inhibition of labeled 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 radio labeled synthetic peptide which can be N-linked 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 "147-155, HA204-212 and HA210-219" was found to be similar. An epitope 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 "147-155, HA204-212 and HA210-219" was found to be similar. An 11 amino acid peptide corresponding to the sequence 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).

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

JY-222 DENGUE VIRUS VIRULENCE AND ITS INTERACTIONS WITH MACROPHAGES AND LYMPHOCYTES, Yun-Chi Chen, Chia-Chi Ku, Li-Jung Chen, 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 genotype found in DHF outbreaks globally. Current hypothesis 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 lymphocytes (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 -- PLO46 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 preliminary data showed that DHF-associated DEN-2 strain had higher viral yield in certain age of M/M and a mononuclear cell line (HL-CZ) than Taiwanese DF-associated DEN2 strain. In addition, this DHF-DEN2 strain was more likely to infect the promonocyctic (HL-CZ) than well differentiated monocytic (MCF-7) and lymphocytic (RIP) 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 determinant for dengue virus infection and replication, and (3) DEN virus strain virulence -- a more important factor than lymphocyte activation status -- seems to determine whether this strain would infect human PBLs. Further studies should be focused on searching for detailed mechanisms of virus and immune cell interactions.

JY-224 BYPASSING A BLOCK IN ANTIGEN PROCESSING FOR CLASS I-RESTRICTED CYTOPHAGIC CELL RECOGNITION, Amy J. Yellen-Shaw and Laurence C. Eisenlohr. Thomas Jefferson University, Philadelphia, 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 serine-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 the epitope itself critically affects 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 expression 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-2) 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.
Molecular Aspects of Viral Immunity

**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 (DHF) involves how could virus efficiently infect target cells and spread into blood stream. Two major hypotheses have been proposed: (1) antibody dependent 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-60) by infecting HL-60 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 than PL046 (2.5*10^3pfu/ml on day3 vs 2*10^3pfu/ml on day4 for m.o.i.=0.01). This phenomena paralleled well with the severity of clinical syndrome. 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 outside 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 protein 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.** Ex vivo neutralizations were performed as follows: IgG12 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 (MN) was synthesized and coupled to BA (V3-KLH antibody responses, whereas responses to 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-I (MN)] H9 cells. Sera from mice immunized with V3-BA inhibited syncytia-medicated syncytia. In secondary responses, CD4 T cell-depletion abrogated anti-HIV-1 antibody responses if 8. abortus is used as a carrier to stimulate memory responses.

**J2-226 BRUCELLA ABORTUS COUPLED TO V3 LOOP PEPTIDES, GENERATES NEUTRALIZING ANTI-HIV-1 SERUM ANTIBODIES AND MUCOSAL IGA, Basil Golding, John Inman, Paul Beling, Jody Manischewitz, Robert Blackburn and Hana Golding.** V3-KLH induced mainly IgG1, whereas V3-BA induced all IgG isotypes but IgG2s predominated. Fecal extracts from mice immunized with V3-BA were shown to be cryptic, a set of 66 overlapping synthetic peptides corresponding to the entire HPV16 capsid protein 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. 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 gp120-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.
IgKO respectively) with T3C9. &?KO mice cleared infection with microglobulin and immunoglobulin knockout mice and B cells in clearance of intestinal infection, we challenged the adult mice after oral inoculation. CB17, 129, and C57BU6 mice infection. To evaluate the role of CD8 T cells, immunoglobulin, IgKO and SCID mice 3 and 5 days after oral infection with clear reovirus T3C9 completely from the proximal small intestine after infection. To investigate the mechanism of inefficient monoclonal IgG2a antibody specificity for the reovirus outer capsid mucosal surfaces.

ANTIJ3ODIES, Ulrich Kalinke, Etienne Bucher, Annette Oxenius, that circulating IgG is unimportant for clearance of virus from virus efficiently, with significant viral titers in intestine 11 days after infection. Our studies allow the determination of somatically mutated positions in the VH regions. Two VSV-IND neutralizing antibodies expressed mutated and unmutated antibodies were comparably high. In order to determine the influence of somatic point mutations on one single VH andVL 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 monoclonal 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 affinity 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 somatic antibodies are subjected to avidity maturation although this is not required for the generation of high affine, efficiently virus neutralizing antibodies.

J2-238 HOST RESPONSE TO SENDAI VIRUS IN MICE TREATED WITH THE MEL-14 MAB TO L-SELECTIN. Lisa Hyland, Sam Hou, and Peter C. Doherty. 1Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101, 2Departments of Immunology and Microbiology, and 3Pathology, University of Otago, Dunedin, New Zealand. The B and T cell responses in C57BL/6J 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 ~10% of the total cell population.

J2-230 BROADLY NEUTRALIZING ANTIBODIES TO HIV-1 IN INFECTED INDIVIDUALS, Marvin S. Reitz, Brynmore A. Watkins, Audrey Louise, 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(NN) into a clone of HIV-1(IIIB) allows the detection of two other broadly neutralizing targets. One recognises the V3 peptide of NN 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 EXPRESSED IN E. COLI. W. Edward Robinson, Jr., Murray B. Gardner, and William M. Mitchell. Departments of Pathology, University of California, Irvine, CA, and University of California, Davis, CA.

Antigens 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, fairly 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 long-term 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 HYPERVARIEABLE REGION 1 OF THE PUTATIVE ENVELOPE GLYCOPROTEIN (GP170) IN HEPATITIS C PATIENTS. Elisa Scarnecchi, Antonio Cerino, Gloria Esposito, Mario U. Mondelli, and Cinzia Traboni. Istituto di Ricerche di Biologia Molecolare P. Angeletti (IRBM), Via Pontina Km 30 600, 00040 Pomezia, Italy, 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 (gp170) 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 clearance. 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 reactions (98%) 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, paramyxovirus, 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, Departments of Pathology and Genitourinary 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 10^7 HPV E7 expressing cells and challenged with E7 protein (4) mice primed by grafting with 5 x 10^7 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 IgG2a. 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  aB T CELL-INDEPENDENT B CELL RESPONSE HAS A MAJOR ROLE IN POLYOMA VIRUS CLEARANCE IN THE ABSENCE OF aB 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, a2 knockout (KO), a2 KO, B cell KO) and/or depleted of certain cell populations (CD4+ , CD8+, asialo-GM1+ (AGM) or NK1.1+) in vivo, were infected with PyV. (ii) SCID mice were adoptively transferred with splenocytes from immunocompetent, or from immunodeficient (a2 KO; B cell KO) mice, or with the same splenocytes in vitro depleted with anti-Thy-1 and/or anti-AGM, antibodies, then infected with PyV. Both types of experiments clearly showed that, in the absence of aB T cells or of aB 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 Th1 depletion. However, at day 14 post infection, while in SCID mice all four major organs tested (spleen, 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 a2 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 aB T cells and NK cells.
Memory and the Immune Response to Virus

**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 Retiviral 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 peptides 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 reactive (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.

**J2-237** BONE MARROW IS A MAJOR SITE OF LONG-TERM ANTIBODY PRODUCTION AFTER ACUTE VIRAL INFECTION, Rafi Ahmed, Mark K. Slika and Mehmed 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 bone marrow 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 antiviral 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=0.82, P<0.001). The percentage of cells staining CD4+CD29+ (bright plus dim) was 43.3 (95% CI 37.4-49.4) in group 1, 28.9 (27.5-30.4) in group 2, and 10.2 (6.6-11.9) in group 3. The respective values for these groups were CD4+CD29+ (bright plus dim) 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 9.8 (8.5-11.3). In single factor discriminant function tests, the %CD4+CD29+ cells best predicted subject group (67% correct), proving to be a better discriminator than %CD4+CD29- (77% correct), %CD4+CD29- (51%), %CD4+CD45RO+ (75%) and %CD4+CD45RO-CD45RA- (83%).

Overall, no advantage was seen in 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+ and CD45RO+ 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 INFECTIOUS VIRUS-SPECIFIC CD8+ CTLS AND CORRELATION WITH IFN-γ PRODUCTION, Innocent N. Mba-Wiakulé,1 Simonetta Di Fabio2, Kohtarou Fujishashi3, Hiroshi Kiyono3, Jerry R. McGhee1 and Robert B. Couch1, The Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030 and The Departments of Microbiology and Virology, 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 CD8+ and CD9+ T cells by their expression on the surface of distinct D45R isoforms. CD45RA is expressed on naïve 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 51Cr release assay while IFN-γ production and expression was assessed by ELISPOT and quantitative RT-PCR, respectively. CD8+CD45RO+ (memory) 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-CELL LYPHOCYTE ACTIVITY BY HIV-GP160 RECOMBINANT CANARYPOXVIRUS: CTL MEMORY WITHOUT ANTIGEN, Yves Riviére1, Geneviève Janvier1, Béatrice Reusly1, Jean Louis Excler2, Stanley Plotkin2, Gilles Plaouzès3 and James Targataglia4, 1 UVic, Institut Pasteur,75724 Paris cedex 15, 2 FMSV,Marcy l'Etoile, France, 3 Hop.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 alun or incomplete freund adjuvant (IFA).

HIV-envelope specific cytotoxic activities were detected from CTL lines derived from PBMC stimulated by specific simulation 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 CD8+CD8+, MHC class I restricted cytotoxic activity, and for at least two volunteers, this activity was still present two years after the first canary-pox/IFA injection.

Because avian poxviruses are incapable of complete replication under 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. Nahil, K. Vergiliis 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 reduction of secondary immune responses to certain viruses over time if there is no re-exposure to the original infectious agent.
Molecular Aspects of Viral Immunity

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 (1/364), and CD49d-high (VLA-4-high) (V102). This is useful in 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 (1/364), and CD49d-high (VLA-4-high) (V102). 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 C57BL6 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 Bil and Carol S. Reiss1,2, 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 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-acetylpenicillamine (SNAP), while 10μM of the control compound N-acetylpenicillamine (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.
Molecular Aspects of Viral Immunity

**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 P. 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 the demyelinating encephalomyelitis in susceptible rodents. 40-90% of disease caused by MHV-JHM.

In vivo with respect to the acute encephalitis and chronic demyelinating disease caused by MHV-JHM infected C57BV6 mice. Three epitopes were identified that are recognized by a bulk population of CTLs from the CNS of the chronic demyelinating encephalomyelitis nor the clinical symptoms. Recently, it was shown that lymphocytes isolated from the central nervous system (CNS) of C57BV6 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 C57BV6 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 (CIZKNFHFL, Db), 598-605 (RCQIFANI, Kb), and 1143-1151 (NEFGQNGNI, Db). Thus, the results indicate that the T lymphocytes responsive to the S protein of MHV-JHM in C57BV6 mice recognize both Kb and Db-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 SURFACE (S) GLYCOPROTEIN OF A MOUSE NEUROTROPIC CORONAVIRUS AND THEIR POSSIBLE ROLE IN VIRAL DEMYELINATING DISEASE. Raymond K. Cheung,1 Hansi J. Dea,1 Janice M. Miller2 and Mark R. Ackermann,1 Virology Swine,1 Virology Cattle,2 and Avian Diseases2 Research Units, National Animal Disease Center, USDA. Agricultural Research Service, Ames, IA 50010

A recombinant pseudorabies virus (PRV) (LLTBA2) was constructed which contains a 3.0 kb deletion of 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 (LTL) and truncated one copy of the diploid immediate early IIE0 gene. Replication and viral gene expression of LLTBA2 in Medin-Darby bovine kidney cells was similar to that of the parental virus and a virus rescued for the deleted sequences (LLTBA4). When inoculated intranasally in 4-week-old or 4-day-old pigs, LLTBA2 replicated efficiently at the site of inoculation yet caused markedly reduced mortality, neuroanatomical distribution and spread of infection in mice 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 immune response, CD8+ T-cells recognise and destroy infected glial cells leading to demyelination.

**J2-247** AGE-RELATED RESTRICTED REPLICATION AND SPREAD OF SEMLIKI FOREST VIRUS IS RELATED TO SYNAPTOGENESIS AND MATURITY OF NEURONAL SYSTEMS John K. Fazakerley, Kevin Oliver, Martina F. Scallan and J. Pedro Simas, Department of Pathology, University of Cambridge, Cambridge, CB2 IQP, UK.

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 neurons undergoing synaptogenesis, absent in mature neurons but inducible by administration of gold compounds. Infection of neurons undergoing synaptogenesis is productive and virus is transmitted along neural pathways, infection spreads rapidly around the brain, destroys cells and anidias die of a fulminant encephalitis. In mice infected after 14 days of age replication in mature neurons 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 immune 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.

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MACROPHAGE-DERIVED NITRIC OXIDE-MEDIATED INHIBITION OF VIRUS REPLICATION, Gunaenarru Varnavilli 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 (IFN) 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 antigen dependent, correlated with NO production in cultures and was reversible by the NOS inhibitor Nω-nitro-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-acetylpenicillamine. 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.

CNS PERSISTENCE, TROPISM AND GENETIC VARIABILITY OF THEILER'S VIRUS
J. Pedro Simas, Anthony A. Nash and John K. Fazakerley, Department of Pathology, University of Cambridge, CB2 1QP, UK. Theiler's murine encephalomyelitis virus, a natural occurring 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 infection. 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 preceding 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 VPI capid 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.

THE ROLE OF CD4+ T CELLS IN THE ENTRY OF CD8+ CTL INTO THE CENTRAL NERVOUS SYSTEM DURING ACUTE INFECTION WITH THE NEUROTOPIRIC 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 I 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 unactivated 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, W. A. Attenuent, T. C. VanCott, J. R. Mascola, J. K. Carr, P. A. Hegerich, J. Gaywee, E. Sanders-Buell, M. L. Robb, D. E. Dayhoff, S. Thiitrichanlert, S. Nityavatham, 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 serotyping 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 |
|---------|----------|-------------|---------------------|----|----|
| Case 1  | B +E     | <50         | 200                 | 3  | 97 |
| Case 2  | B + E    | 1600        | 3200                | 86 | 50 |
| Subtype E | E       | <50         | 3200                | 88 | 0  |
| Subtype B | B       | 1600        | <50                 | 105| 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, Kay Townsend, Joanne O'Dea, David Millich, Jan Hughes, Stephen Chang, Douglas Jolly, and William T.L. Lee, Viagen, 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, Viagen has demonstrated that recombinant retroviral-mediated expression of HBV 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 C57Bl/6 (H-2k) mice induced both humoral and cellular immune responses. Specifically, IgG antibodies to the HBV core were induced as well as CDS 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 BY FORMULATED IN OIL-IN-WATER EMULSIONS AND ADJUVANTED WITH MPL AND QS21, Carine Baain, Daniel Gilles, Omer Van Opsai, Patricia Monnot, Nathalie Garwn, Myriam Fraconnet, Michel Hendrickx, Martin Cumberbach, Moncef Slaoui, and Pietro Paaso, RAD Department, SmithKline Beecham Biologics, Rixensart, Belgium; *Service de Génétique Appliquée, CRULIB, 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. 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 intralymph node injections of formulations containing 5 μg of NS1-ICP27. Responder cells obtained from draining lymph nodes were re-stimulated in vitro with P815 cells transfectected 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 gpl60 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.
Molecular Aspects of Viral Immunity

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

Human immunodeficiency virus type 1 (HIV-1) Env-, Gag-, Pol-, Nef-, and Tax-specific cytotoxic T-lymphocytes (CTL) activities were quantitated temporally in five patients with symptomatic 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 characterize 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. Morris, 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 gua. 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 EPITOPES. SPECIFIC CTL PRECURSORS BY LIMITING DILUTION. Andreas Cerny*, 5 Bonita Grabcheidt*, Mary A. Brothers*, Werner J. Pichler* and Francis V. Chisari*. The Scripps Research Institute, La Jolla CA 92037, 6Department of Medicine and 7Institute 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 vivo 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. HBV or HCV and r-hu IL-2 added to peripheral blood mononuclear 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 effectors cells specific for influenza matrix M1-68 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 140-1415) 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 HLA-A2 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 Coe, Cheryl Cook, Catherine McCaill, Christie Ohnemus, James Blodgett, Lisa Ailen and Vidal de la Cruz, Cortec 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. 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, (DPPPPNPMP), 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 10 ug 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.

1. Dintzis, H.M., R. Dintzis and B. Vogelstein (1982) Proc. Natl. Acad. Sci. USA 79: 396.
Molecular Aspects of Viral Immunity

J2-308 PROTECTION AGAINST INFLUENZA IN MICE BY VACCINATION WITH A VENEZUELAN EQUINE ENCEPHALITIS VIRUS VECTOR EXPRESSING THE HA PROTEIN, Nancy L. Davis, Kevin W. Brown, Ian J. Calvi, Ronald I. Swanson and Robert E. Johnston. Departments of Microbiology & Immunology and Biochemistry, Univ. of North Carolina, Chapel Hill, NC 27599

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 x 10^6 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 organism of tropism of VEE. Mice were challenged three weeks after immunization by intranasal administration of 10^6 EID50 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 2,46, 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 x 10^6 pfu/ml, respectively. This vector also has been expressed in the form of a protein 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 MODULATION 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 an 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 induced 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 HPV16E7 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 tumoural 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-2b mice with HPV16E7 protein with Quil A as adjuvant could induce cytotoxic T cells able to kill HPV16E7 expressing tumour cells in vitro. We then used similar immunisation with E7/Quil A to induce E7 specific immunity in FVB (H-2b) mice. H-2b skin grafts expressing E7 were not rejected by E7 immunised H-2b mice, though immunisation induced antibody to E7, and similar grafts were rejected, as expected, across an allotumour mismatch in H-2b mice. We conclude either that HPV16E7 lacks a Tc epitope in the context of H-2b, or that expression of E7 in the skin from the E7 transgenic line is insufficient for recognition by primed effector cells, and further experiments will address this distinction.

1. Lambert, P.F., Pan, H., Pilot, H.C., Lien, A., Jackson, M., and Griep, A.E. Epidermal cancer associated with expression of human papillomavirus type 16 E7 oncogenes in the skin of transgenic mouse. Proc Natl Acad Sci USA 80:5583-5587, 1983.
2. H Frazer, H. Leippe, D.M. Dunn, L.A. Lien, A. Tindle, R.W. Fernando, G.J. Phillips, W.C. and Lambert, P.F. An HPV16 E7-E7 transgenic mouse as a model for the immunology of HPV16 associated cervical cancer. J Biol Chem (Suppl) 1994.
Molecular Aspects of Viral Immunity

J2-312  INDUCTION OF CTL RESPONSES USING HYBRID TYPE-1 VIRUS-LIKE PARTICLES, Tim French, Steve Harris, Daniel West, Jeremy Mylon, Nichole Meyers, Michele Hill-Pettis, Sarah Woodrow and Guy Layton. British Biotech Pharmaceuticals Ltd, Watlington Rd, Oxford, OX4 5LX, 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 alam abut inhibited CTL induction but augmented T helper cell priming.

We have now demonstrated 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 virus 16 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 (HIN1) or the mouse adapted A/HK/68 (HIN2) 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 injected 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/Beijing/89 and A/Georgia/93, and were similar to mice that had been previously infected with A/Beijing/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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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 responses 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 virus 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 induce CTL responses and also in destroying tumour cells. The virus has been shown to be able to synthesize 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-2Dd restricted epitope, E7 residues 49-57.
52-318 CONJUGATION OF A PEPTIDE FROM THE V3 LOOP OF HIV TO THE VACCINE CARRIER BRUCELLA ABORTUS INDUCES A SPECIFIC, MHC-RESTRICTED CYTOTOXIC RESPONSE AGAINST HIV gp120. Cheryl K. Lapham1, Hans Golding2-John Immerv, Robert Blackburn3, and Basil Golding2-3.

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 gpl60 polypeptide. 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 D5. We found that two in vivo immunizations with 10⁵ 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 D5 but not V3 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-CTLA4 prior to immunization caused a severe depletion of CD4+ lymphocytes from 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.

52-319 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 cytotoxic 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 utilize 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 CD8+ and CD8+-cytotoxic T cell responses, (CTL). Antigens used have been bovine 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+ respsonse, and even whole fixed viruses, makes them attractive candidates for vaccines where clearance of infected cells is important in protection and recovery.

52-319 LONG-TERM PROTECTION FOLLOWING VACCINATION WITH A gH-DELETED HERPES SIMPLEX VIRUS TYPE 1 . C.S. McLean1, I.A. Duncaen1, S.E. Chishol1, S.L. Boll1, M.E.G. Boursnell1, S.C. Ingulis1, A.C. Minson2 and J.G. Shields1.

1Cantab Pharmaceuticals Research, 184 Cambridge Science Park, Cambridge CB4 4GN, U.K. 2Division 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.
Molecular Aspects of Viral Immunity

J2-320 COMPARISON OF FORMULATIONS FOR INDUCTION OF CTL RESPONSES USING SYNTHETIC PEPTIDE, Douglas F. Nixon, Pel-de Chen, Peter J. Kuebler, Catarine Hoo, Tim Zamb, J. Paul McGee, Derek T. O'Hagan, Howard Tsu, 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) lipopeptide - peptide conjugated to tricaprylin-6-glycerol-2-cysteine (ParPeptide) and dissolved in a 1% DMSC/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 blank) was administered to groups of 3 mice intra-peritoneally or sub-cutaneously at 1,10 and 20 days. 7 days following the last immunization spleenocytes were cultured in vitro in the presence of appropriate peptide or control with Con A, a supernatant as a source of growth factors. CTL activity was measured in a standard 4 hour chromium release assays 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 peptides in emulsions. The lipopeptide P3-5C2-252-260 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, L. Pirjola, M. Roivainen, L. Lienemann, J. Eskola, A.C. Bevers* and T. Hovi

National Public Health Institute, Helsinki, Finland

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A pilot enhanced-potency inactivated poliovirus vaccine (EI-IPV) with asummarily improved immunogenicity containing trypsin-treated type 3 poliovirus (strain Sabin) 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 EI-IPV (TrIPV) 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 immunization mice and rabbits with 10-fold concentrated type 3 component of TrIPV. 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 TrIPV. Already one month 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 30 children who were due to receive the third dose of the regular immunization schedule at about 2 years. In both groups, 25 individuals received TrIPV 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 TrIPV was at least as immunogenic as the regular EI-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, Andrea Robinson, Kathe Robinson, John Crowley and Carolyn Weeks-Lévy, Viral Vaccine Research, Lederle-Praxis Biologica, 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 have 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 polypeptide 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. Santoro1, E.M. Fynan2, and H.L. Robinson1, 1Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655; 2Department 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 μg 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.
**Molecular Aspects of Viral Immunity**

### J2-324

**A TRANSGENIC MOUSE MODEL FOR IDENTIFYING HTLV-I T-CELL EPITOPES: GENERATION OF HLA-B*3501-RESTRICTED CTL DIRECTED AGAINST SYNTHETIC PEPTIDES AND NATURALLY PROCESSSED VIRAL ANTIGENS, Christian Schleinbach, Ai Kariyone*, Kyoshi Nikohara**, Karl-Heinz Wasmullner and Masafumi Takiguchi**, Departments of Tumor Biology*, Immunogenetics*, Institute of Medical Science, University of Tokyo, Tokyo (Enzymes and Genetic Engineering, Japan)**

The majority of human T-cell leukemia virus type-I (HTLV-I), HLA class I-restricted T-cell epitopes have been identified by cloning HTLV-I patient-derived T cells. Here we describe for the first time a rapid method (reverse immunogenetics) for identifying T-cell epitopes, together with a transgenic mouse model as a guide for testing the cellular immune response to a mixture of the lipopeptide immunoadjuvant PamgCys-Ser-(Lys)4 and synthetic HTLV-I peptides which seem suitable for vaccine design. HTLV-I amino acid sequences were searched for eight to 10 inner patterns carrying the anchor residues of the HLA-B*3501 peptide motif at positions two and eight to 10. 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 Pam3Cys-Ser-(Lys)4 and used to immunize C3H/He (H-2K) mice. One week after 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 peptide loaded RMA-S-B*3501 cells only. Bulk CTL generated by six peptides derived from env-Gp46 (PVMVSSSTTPLLY, SPSTTPLLY, VFFSPSTPTLY, VPPSSTTPLLY, VPSPSTTPLLY, POL) and gag-p19 (CPINTYSSLASL) protein sequences of four HTLV-I strains killed peptide pulsed RMA-S-B*3501 and P815-B*3501 target cells infected with the corresponding vaccinia virus-HTLV-I 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 gp1160 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 LAI ≥ 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). Curiously missing is reactivity to the V3 loop peptide (LAI #200). Although the highest 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 INFECTIOUS IN MHC CLASS I DEFICIENT MICE, Patricia R. Slev, Bradley S. Bender, Wayne K. Ports, Department of Pathology, Pathway 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 control (normal 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 (gC4). None of these types of inoculations have 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 intermediate 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 RECOMBINTN LISTERIA MONOCYTOGENES AS A LIVE VACCINE VEHICLE. Silvia M.K., Shen, H., Mathebath, 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 precursors 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.

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inclusion in candidate EBV vaccines. Demonstrated concurrent with initial detection of virus in the during EBV seroconversion. EBV-specific CTL activity was from whom cryopreserved PBMC samples were available before. and Inhibited by antibody against MHC class-I but not antibody against chromium release assays. We were fortunate in identifying one child detected against the EBV latent proteins EBNA 1, 2, LP or LMP 1. EBNA 3C was recognized most frequently. No CTL responses were infected with recombinant vaccinia constructs expressing single latent genes (Epstein-Barr nuclear antigen [EBNA] 1, 2, 3A, 3B, leader protein [LP], latent membrane protein [LMP] 1 and 2A) in vitro. Recognition of individual EBV gene products by bulk CTL lines was measured by combining CTL lines with B cells 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 products. 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 HLA 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 of initial CTL responses against B-LCL pulsed with overlapping 20-mer peptides derived from p24. Eleven children (908 and 617) had CTL against p24.14 (a(mino) acids 263-282, KRWIIGLINKKVRMYSP17), two others (938 and 157) recognized p24.17 (aa293-312, FRDYVDRFYKTLRAEQAS0). Similarly we found two SI infected rhesus macaques reacting against the analogous aa sequences of SIV; i.e. p26.14 (8653) and p26.17 (19V). 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. Fine mapping of the p24.17 epitope 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 41, 1612, 1991, Buseyne JV 67,694,1993) and to p26.17 in cynomolgus macaques (Gottch JMPrmt 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.
Molecular Aspects 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 immune cells. 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 viral gene product that is found in spleen and lymph node follicles.

Experimental Designs: We have further examined virus-immune cell interactions after infection of immune cells derived from adolescent C3H/HeJ 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 10^7 plaque forming units of CVB3, at day 0 and with 10^5 SRBC's at days 2, 3 and 4 post-CVB3 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, was associated with 12.5% 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 virus replication. A 24% and 73% increase in virus titers 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 positive 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 during the acute phase of enterovirus-induced disease was increased at day 0, and decreased at day 2, 3 and 4 post-CVB3 infection. Splenocytes were harvested 4 days post-SRBC infection, mixed with target SRBC's and guinea pig complement and incubated. Plaques were then quantitated.

Conclusions: These data indicate that CVB3, associates with and replicates in murine splenic immune cells, and that infection of susceptible mice with CVB3, modifies the immune response to a third party antigen during the acute stages of disease. An understanding of such CVB3-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 Y6 T CELL-MEDIATED RECOGNITION OF NONVIRAL CELL SURFACE ANTIGENS INDUCED BY ACUTE VIRUS INFECTION. Jack F. Bukowski, Richard M. Stenberg, and Ann E. Campbell, Department of Medical Genetics, University of British Columbia, Vancouver, B.C. V6Z 1Y6

We have isolated a CHO mutant clone, VV5-4 that is resistant to VSV killing. Since this mutant clone was isolated by retroviral infection of a nonvirally-resistant CHO cell clone, VV5-4 may play a role in the immune resistance to VSV killing.

J2-333 PATHOGENICITY OF HUMAN 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 virus 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 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. Wen Chang, Che-Sheng Chung and Chi-Hong Bali, Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, ROC

We have isolated a CHO mutant clone, VVS-4 that is resistant to vaccinia virus (VSV) cytolysis. Vaccinia virus can infect VVS-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 VVS-4 cells. However, viral intermediate and late gene products are not detected in infected VVS-4 cells. It therefore suggests that in VVS-4 progression of VSV life cycle is blocked at the uncoating or replication stage. Furthermore, host protein synthesis in VVS-4 cells is not shut-off after VSV infection suggesting uncoating/replication is important for determining cell fate.

Since this mutant clone VVS-4 was isolated by retroviral insertion mutagenesis. A cellular gene that was integrated by single retrovirus was isolate and codes for a 5Kb transcript in Northern blot analyses. 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 VSV infection. In addition, a two-hybrid system will be employed to identify any viral gene product interacting with this cellular target.
**Molecular Aspects of Viral Immunity**

**J3-335** **VIRAL INFECTION 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.*

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*The Beineke Carter Center for Immunology Research, University of Virginia, Charlottesville, VA 22908, fand program in Molecular and Cellular Biology, Washington University, St. Louis, MO 63110.

Using our laboratory’s standard strain of A/Japan/305/57 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 hemagglutinin (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 HAUs) 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.

**J3-336** **INTERLEUKIN-12 PROMOTES RESISTANCE TO EXPERIMENTAL HERPES VIRUS INFECTION**, Michael J. Mulqueen, Jackie Carr, Jane Rogerson, Alan Lanom*, Vivien Gibson and Robert F. Booth, Departments of Virology and Anti-Inflammation, Roche Products, Welwyn Garden City, Herts, U.K., A/73 AY

The therapeutic and prophylactic antiviral efficacy of interleukin-12 (IL-12) was studied using mice 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. IL-12 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 supernatants at various time points for assay. The preliminary data showed that: (a) adherent cells were infected with DEN2 and DFV virus strains replicated 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 (DFP and DF strains peaked at 6.3x10^4 PFU/ml and 1.5x10^4 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 the patients had better replication capability in human bone marrow cells. Future studies on cytokines and immunologic evaluation are in progress.
Molecular Aspects of Viral Immunity

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 remains in the acinar cells of the salivary glands. Finally a third stage of infection exists which is characterized by a lack of detectable viral infection. It is not known whether MCMV remains persistent at some low level in the salivary glands. Finally a third stage of infection exists.

To test this, spleens, kidneys and salivary glands from mice 2-8 months post infection were evaluated by sensitive assays. Sensitivity was tested using virus co-cultured on mouse 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 for 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 infection with 2x10^5 kidney cells from latently infected mice. Following identical transfers of latently infected cells, MCMV was not detected in SCID mice although explants from the same organs reactivated in vitro. Using FACS sorting, magnetic bead separation, and reactivation assays, individual cell types have been analyzed for the presence of MCMV genome and the ability to reactivate virus.

VACCINIA VIRUS REPLICATION IS INDEPENDENT OF THE HSP70 RESPONSE TO VIRUS INFECTION. Lisa Sedge, Ian Ramshaw, and Janet Ruby.

Division of Cell Biology, John Curtin School of Medical Research, Australian National University, PO Box 534, Canberra, 2601 Australia.

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 titers as control viruses, both in vitro and in vivo. In particular, kinesthetic 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.

CONSTRUCTION OF A SIMPLIFIED FORM OF RABIES VIRUS GLYCOPROTEIN. Boguslaw Wojcik, 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, it 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 1 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 and purified from 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 Asn19 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 recombinant glycoprotein, which did not affect secretion, antigenicity, of assembly of the recombinant glycoprotein, but did allow purification using Ni-2-garose 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.
Molecular Aspects of Viral Immunity

Emerging Viral Infections

J2-343 WIDESPREAD ZENZOTIC OF A NOVEL HANTAVIRUS OF THE HARVEST MOUSE IN NORTH AMERICA. Bruce Anderson, Terry Yates, Norah Torrez-Martinez, Wannin 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 Comers 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 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, Wannin 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 "SGGRQKRFVC" as opposed to the consensus sequence "SGGRQQRF" 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.
Molecular Aspects of Viral Immunity

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

William E. Biddison, Kenneth C. Parker, John E. Coligan, Steven Jacobson, and Ursula Utz. Neuroimmunology Branch, NINDS and Laboratory of Molecular Structure, NAID, NIH, Bethesda, MD 20892

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-A2+ patients, HTLV I-specific CD8+ CTL restricted by HLA-A2 recognize a peptide derived from the HTLV I Tax protein (Tax 11-19 LLFGYPVW). In the present study, we have presented by HLA-A2. These observations suggest that the demyelination process in HAM/TSP may be, 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 OVER-LAPPING K4-RESTRICTED CD6+ CTL INFLUENZA HEMAGGLUTININ EPITOPES: IMPLICATIONS OF LIMITED T CELL REPERTOIRE FOR A SELF-MIMIC EPITOPE, Wonjuung Cao, Brenda A. Myers-Powell, and Thomas J. Braciale, The Beineke 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-2K*)-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 NH2-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 subunits 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/Japan/57 in short term bulk cultures, and the FACS analysis of TCR Vγ 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 E Azami El Idriissi, Charles J. Piao, 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 anemia 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 OKT4.1 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 anemia in these mice. Our results strengthened the central role of the immune system in the anemia 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 anemia 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, Howard Nolding, Robert A. Kaslow, Robert Giacchino, 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 of 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 hyperglybolaminemia 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.
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 ev loci and, compared to ev negative birds, these lines exhibit a reduced CTL response in birds expressing the ev-21 locus is reduced 85% compared to ev negative birds having nearly identical (eighth backcross) genetic backgrounds. Other ev loci (18, 10 and 11) also reduce the CTL response to ALV in MHC matched chickens with different genetic backgrounds.

**Late Abstracts**

**SV40 LARGE T ANTIGEN AND P53 RELATIONSHIP IN PLEURAL MESOTHELIOMA**

M. Carbone, P. Rizzo, A. Procopio, D.J.Y. Mew, M.T. Giulianos, S.M. Steinberg, A.S. Levine, P. Grimly, and H.I. Pass.

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The role of p53 alterations in human malignant pleural mesotheliomas (MPM) is unclear. Immunostaining (IPOX) of 52 specimens revealed p53 expression in 31 of 52 (60%). p53 detection by SSCP for exons 5-9 revealed p53 alterations in 2 of 25 specimens, and LOH at exon 11 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. Not only did these findings suggest co-precipitation of p53 and Tag, but our 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.
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+ cells. 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 60% and 41% respectively. FACs analysis determined that this CTL population was 92.8% CD3+CD8+ and 73.3% CD3+CD8-. 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.

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

Britta Köppe, Paul Kiermer, Ute-Christian Meter, David Lallos, Steve McAdam, Sarah Rowland-Jones, Andrew McMichael and Rodney Phillips

**Molecular Aspects of Viral Immunity**

**HLA-A2 RESTRICTED CTL RESPONSE TO THE HPV E7 PROTEIN IN THE PERIPHERAL BLOOD OF CIN PATIENTS:** Carys Wyn Evans, Thomas Grubert, Stefan Bauer, Hermann Wagner and Grayson B. Lipford, Institute for Medical Microbiology, Technical University of Munich, Munich #1675, 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+ cells. 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 60% and 41% respectively. FACs analysis determined that this CTL population was 92.8% CD3+CD8+ and 73.3% CD3+CD8-. 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**

Michel R. Klein; C.A. van Baalen, A.M. Holwerda, S.R. Kerkhof-Gladz"I.P.M. Keet"**,** J.K.M. Eefinck Schattenkerk**,** and F. Miedema**

"Dept. of Clinical (Viro) Immunology of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. "Dept. Virology, Erasmus Un., Rotterdam "Academic Medical Centre, Amsterdam. "Municipal Health Service, Amsterdam, The Netherlands.

**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 3C 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.

**GENOTYPIC AND PHENOTYPIC VARIATION AMONGST ENTEROVIRUS ISOLATES,**

Tom A. McKen, Jasper D David & Shao 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 Italy. 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 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 for these findings is that this anomalous isolate is a natural recombinant between the parental 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. Lacker et al. have recently described a dominant gene, PyVv, carried by the C3H/Bi mouse strain, which confers susceptibility to Py virus-induced tumors.

Mapping and immunological analyses indicate that PyVv is the mouse mammary tumor virus 7 superantigen (MTV 7 sag) gene, which deletes T cells required for Py tumor immunosurveillance in H-2a 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 I1 and Peru/attack (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 I1, 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 Czeck II genomic DNA. These results indicate that, like the C3HiBi, the Peru/attack and Czech II contain gene(s) which override 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.

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

G.I.Troen (1), H.Y.Dai (1) and A.Dalen (2), (1) UNIGEN Center for Molecular Biology, (2) Department of Microbiology, University of Trondheim, N-7005 Trondheim, NORWAY.

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 immunodeficiency syndrome induced by HIV. Restriction mapping and nucleotide sequence analysis of FIS-2 show a high degree 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.

IMMUNOLOGICAL RECOGNITION OF FMDV AND ITS PROTEINS IN THE BOVINE R. Michael B.Parkhouse, Jana Baran, Ananda Childerstone, Trevor C. Collee, Tim Doel, Michael Flint, Mildred Foakter-Guevas, Mercedes Garcia-Valcarcel, Martin Ryan and Haru Takamatsu, Department of Virology, Institute for Animal Health, Pirbright Lab., Wokingham, GU24 6TD, 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 persistently infected). The principal findings were:

1. RECOGNITION OF IMMUNE ANTIBODY VIRUS
   a) Humoral: Unlike classical neutralizing antibodies, total anti-FMDV antibodies, as measured in an ELISA 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 immunization 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 pBR-322, and purified by SDS-PAGE.
   b) Humoral: Structural and non-structural proteins were recognized by infected animals. A good monospecific 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 recognized and some were cross reactive. Interestingly, VP1 was strain specific, and the polymavirus (3D) was the most immunogenic and cross reactive.
   d) A construct comprising 3D and the immunodominant VP1 epitopes was prepared and tested.

IMMUNE RESPONSES TO BACULOVIRUS-EXPRESSED EQUINE HERPESVIRUS 1 GLYCOPROTEINS IN A MURINE MODEL, J.Millar Whalley1, Deepanker Tewar1, Daria N. Love3 and Hugh J.Field2, 1School of Biological Sciences, Macquarie University, Sydney, Australia 2109; 2Department of Clinical Veterinary Medicine, Massey University, Cambridge, CS3 OES, UK; 3Department of Veterinary Pathology, University of Sydney, Sydney, Australia 2006.

In common with other herpesviruses, the enveloype 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 abortifacient 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 accelerated 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. Hagewans', 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.