CELL BIOLOGY OF VIRUS ENTRY, REPLICATION AND PATHOGENESIS

Organizers: Richard Compans, Ari Helenius and Michael Oldstone

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Plenary Session

Poster Sessions

Cell Biology of Virus Entry, Replication and Pathogenesis

Architecture of Viruses and Viral Antigens

J 001 THREE-DIMENSIONAL STRUCTURE OF A VIRAL ANTIGEN-ANTIBODY COMPLEX

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In the past, many methods have been used in attempts to define antigenic determinants (epitopes) on protein molecules (reviewed in (1)). Although data from some of these experiments have implicated particular amino acid residues as participants in particular epitopes, none have allowed a complete structural description of an epitope.

The first description of the structure of an epitope on a protein molecule was by Amit et al. (2) who determined the three-dimensional structure of a complex between lysozyme and the Fab fragment of a monoclonal antibody. The interaction has been described as conforming to a 'lock-and-key' picture of antibody-antigen interaction, in which, apart from some amino acid side chain movements, no structural changes occur in either the antibody or the antigen.

We have grown crystals of monoclonal antibody Fab fragments NC41 and NC10 complexed with influenza virus neuraminidase of the N9 subtype (3) and in each case, four Fab fragments are bound to the neuraminidase tetramer. The two antibodies recognise overlapping epitopes on the neuraminidase (4).

The structure of the NC41 Fab-N9 neuraminidase complex (5) shows an epitope comprising four surface loops of the neuraminidase. Sequence changes in these loops abolish or diminish antibody binding. The structure also reveals an unusual pairing pattern between the domains of the light and heavy chains in the variable module of the antibody. This implies either that NC41 Fab differs in its three-dimensional structure from other Fab fragments or, more likely we believe, that association of antibody with antigen can induce small changes in the quaternary structure of the Fab, through a sliding of domains at the VL-VH interface.

The NC10 Fab-N9 neuraminidase complex is in progress. Results to date confirm that the epitopes seen by NC41 and NC10 are indeed overlapping.

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J 002 THE STRUCTURE AND ASSEMBLY OF A SIMPLE ENVELOPED ANIMAL VIRUS, Stephen D. Fuller, European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, Federal Republic of Germany.

The three-dimensional structure of Sindbis virus and of isolated Sindbis virus nucleocapsids have been determined by cryo-electron microscopy and image reconstruction. The spikes of the virus form columnar trimers arranged on a T=4 lattice. The lipid bilayer is polyhedral in intact virions but appears rounded when disrupted by freezing and thawing. The structure of the nucleocapsid seen within the virion matches that seen after isolation and has T=3 icosahedral symmetry. The arrangement of spike proteins is complementary to that of the capsid. Two types of capsid-spike interactions are seen: tight ones for the sixty trimers near the five-fold axes and looser ones for the twenty on the three-fold axes. Microscopy of detergent-treated virions supports this difference in interaction strength by showing selective removal of one quarter of the spikes. Sequence alignment with picornaviral vp3's in combination with the structural results suggests that the capsid protein has the same eight-strand β barrel fold as seen for other T=3 RNA viruses but is akin to their expanded forms.

J 003 THE THREE-DIMENSIONAL STRUCTURE OF POLIOVIRUS: IMPLICATIONS FOR CELL ENTRY AND PATHOGENESIS, James M. Hogle, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

We have solved the structure of the Mahoney strain of type 1 and the Sabin (attenuated vaccine) strain of type 3 poliovirus. These structures have provided considerable insight into the architecture, assembly and immune recognition of these viruses. Recent studies of a conformationally altered form of the virus which is produced upon association with susceptible cells have begun to provide insight into the mechanism of cell entry. Using synthetic peptide antibodies and specific proteolytic probes we have shown that this conformational change results in the extrusion of the aminoterminus of the capsid protein VP1 (which is normally inside the viral capsid), and that the exposed aminoterminus confers the ability to attach to lipid vesicles. We have also begun to develop several crystallographic models which we hope will clarify structural factors which contribute to the pathogenesis of poliovirus. These included studies of the structural factors effecting temperature sensitivity in the Sabin strain of type 3 poliovirus (in collaboration with Philip Minor, NIBSC), crystallographic studies of the mouse adapted Lansing strain of type 2 poliovirus (in collaboration with Vincent Racaniello, Columbia University), crystallographic studies of a chimera between the Lansing strain of type 2 and the Mahoney strain of type 1 poliovirus in which the substitution of the Lansing sequence for residues 94-105 into the Mahoney strain confers mouse adaptation on the chimer (in collaboration with Marc Girard, Pasteur Institute), and crystallographic studies of the DA strain of Theiler's virus (in collaboration with Robert Fujinami, UCSD).

J 004 RECOGNITION AT MEMBRANE SURFACES: INFLUENZA VIRUS HA AND HUMAN HLA, Don C. Wiley, Dept. of Biochemistry and Molecular Biology, Howard Hughes Medical Institute, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

The influenza virus haemagglutinin HA and human histocompatibility antigen HLA-A2 are involved in recognition events on the surface of biological membranes. Structure/function studies of these proteins guided by their X-ray crystal structures (Wilson, et al, Nature 289 p. 366-373 (1981), Bjorkman, et al, Nature 329 pp. 506-512 and 512-518 (1987)) will be discussed.

The influenza haemagglutinin attaches virus particles to target cells by binding to N-acetyl neuraminic acid. X-ray Difference Fourier analysis of trisaccharides complexed with wild type and mutant HA's show the details of this interaction and are providing the basis for the design of small molecule inhibitors (Weis, et al, Nature submitted).

The mechanism of the HA's membrane fusion activity by which the virus enters cells is being explored by site-directed mutagenesis (Pfeifer, et al, unpublished). The addition of a disulfide bond covalent linking the three HA_1 polypeptides of the HA trimer, prevents membrane fusion, presumably by blocking a conformational change required to produce the fusion active molecule.

The class I human histocompatibility antigen, HLA-A2, presents foreign antigens in the form of processed peptides to cytotoxic T-lymphocytes. The X-ray structure reveals a large cleft between two long α-helical regions of the molecule. Electron density presumably representing a processed peptide (or peptides) is found in the cleft in two crystal forms of the protein. Evidence indicates that this site is the foreign antigen recognition site and the region of the molecule recognized by T-cell receptors (Bjorkman, et al, Nature 329, p. 512-518 (1987)).

A hypothetical model for the foreign antigen recognition site of class II histocompatibility antigen (involved in T-cell "HELP") will be presented based on analysis of sequence similarities with class I molecules (J. Brown, et al, unpublished).

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Virus Receptors

J 005 EVIDENCE FOR DIRECT INVOLVEMENT OF THE RHINOVIRUS CANYON WITH CELLULAR RECEPTORS, Richard J. Colonno, Jon H. Condra, Satoshi Mizutani, Gordon Abraham, Pia L. Callahan, Joanne E. Tomassini and Mark A. Murcko, Department of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486.
Human Rhinoviruses (HRVs) are members of the picornaviridae family and are the major causative agent of the common cold in man. Competition binding and immunological studies have indicated that the vast number of HRV serotypes can be divided into "major" (78 serotypes) and "minor" (10 serotypes) groups (1). HRV attachment was shown to be a reversible process, since infectious virions could be displaced from cell membranes by an anti-receptor monoclonal antibody. A 90 kDa cellular glycoprotein, present only on membranes of human and chimpanzee cells, has been previously isolated and shown to be involved in "major HRV group" attachment (2). Recent crystallographic studies on HRV-14 have suggested that a deep canyon present on the surface of the viral capsid is involved in viral attachment to cellular receptors (3). To provide direct evidence in support of this concept, a series of mutants was generated by site-directed mutagenesis of an infectious HRV-14 cDNA clone. Amino acids at positions 103, 155, 220, and 223 of VP1 were identified, using the atomic structure, as amino acids residing at the deepest portion of the viral canyon and having side chains protruding upward from the canyon floor. Both conservative and radical amino acid changes were tried at each of the four amino acid locations. Of the 10 site-directed mutants constructed, only the Pro 155 to Tyr failed to yield progeny virus. Binding studies performed on 9 of the resulting mutants have identified 3 different binding phenotypes. One of the VP1 mutants, Pro 155 to Gly, showed an enhanced binding affinity while 6 showed a marked decrease in their dissociation constants. Virion displacement studies support the kinetic data and show that the Pro 155 to Gly mutant was capable of displacing wt HRV-14. None of the mutants were found to be temperature sensitive and most of the mutants demonstrated a small plaque phenotype. These data represent the first experimental evidence that the 25 Å deep canyon is involved in virus-receptor interaction.

(1) Colonno et al., J. Virol. 57:7-12 (1986).
(2) Tomassini and Colonno, J. Virol. 58:290-295 (1986).
(3) Rossmann et al., Nature 317:145-153 (1985).

J 006 CORONAVIRUS RECEPTORS: MOLECULAR BIOLOGY AND ROLE IN SPECIES SPECIFICITY, Kathryn V. Holmes, Charles B. Stephensen, Richard K. Williams, Susan R. Compton, Christine B. Cardellichio and Carl Dieffenbach, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.
Mouse hepatitis virus (MHV) binds specifically to a 110K glycoprotein receptor which is present on the plasma membranes of intestinal brush borders and hepatocytes from genetically susceptible mouse strains (1) and also from several semi-resistant mouse strains such as C3H and A/J which are resistant to killing by MHV although they do permit virus replication. In contrast, no MHV receptor is found on membranes from SJL/J mice which are profoundly resistant to MHV (1). Polyclonal and monoclonal antibodies directed against the MHV receptor have been prepared and used for affinity purification of large amounts of the receptor glycoprotein.

Receptor antibodies are being used to identify receptor-specific cDNA clones from a lambda gt11 library of mouse liver mRNA. The mRNA and genomic DNA from SJL/J and Balb/c liver will be examined to determine the molecular basis for the absence of MHV receptor activity on the membranes of SJL/J mice.

Immunofluorescence and Western blot assays showed that the MHV receptor is only expressed on the plasma membranes of cultured murine cells which are infectable with MHV. Absence of this specific MHV receptor probably explains the resistance of cells from other species to infection with MHV. The intestinal brush border membranes from other species such as pig, dog and cow do, however, express receptors for the coronavirus native to each of these species as shown by solid phase virus binding assays. Thus, it should now be possible to compare the interactions of the peplomeric glycoproteins of different coronaviruses with their specific receptors on membranes from the host species. These studies may elucidate how the receptor binding domain of the viral glycoprotein gene mutated as the coronaviruses adapted to growth in different species during the course of virus evolution.

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J 007 HOST SELECTION OF INFLUENZA VIRUS RECEPTOR VARIANTS.
James C. Paulson, Margaret W. Leigh*, Robert Connor, Jun-Ichiro Murayama, Yoshihiro Kawaoka[+] and Robert G. Webster[+]. Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024; *Department of Pediatrics, University of North Carolina, Chapel Hill, NC 27514; [+]St. Jude Children's Research Hospital, Memphis TN 38101.

A survey of human (6), avian (3) and equine (1) influenza H3 isolates have previously revealed dramatic differences in receptor binding properties which correlate with species of origin [1]. This correlation has now been extended to 10 or more isolates from each of the three species. Without exception, human viruses preferentially bind derivatized erythrocytes containing the SAα2,6Gal linkage, and are sensitive to inhibition of adsorption by equine α_2-macroglobulin, while avian and equine viruses bind cells containing the SAα2,3Gal linkage and are resistant to inhibition by equine α_2-M. The molecular basis of the differences in receptor specificity has been determined to be a single amino acid change at residue 226 with Leu present in human viruses and Gln in avian and equine viruses[2,-5, Kawaoka and Webster, unpublished]. In addition, a similar correlation of receptor specificity with species of origin is seen with human (10 between 1957-68) and avian (12 between 1972-80) isolates with the H2 hemagglutinin. These observations suggest that different host species exert strong selective pressures for emergence of receptor variants best able to grow in that host. Evidence in support of this proposal has been obtained by comparison of the growth of cloned wild type and receptor variant H3 viruses in several hosts. Notably, wild type and receptor variants of the human virus A/Memphis/102/72, exhibit a dramatic difference in virulence in ferrets. [1]Rogers G. N. and Paulson J. C., Virology **127**, 361-373 (1983). [2]Rogers, G. N. *et al.* Nature **304**, 76-78 (1983). [3]Webster, R. G., *et al.* Nature **296**, 115-121 (1982). [4]Daniels, R.S. *et al.* J. gen. Virol. **66**, 457-464. [5]Kida, H. *et al.* Virology **159**, 109-119 (1987).

J 008 RETROVIRUS RECEPTORS ON HUMAN CELLS, Robin A. Weiss, Paul R. Clapham, Jon N. Weber, Myra O. McClure and Maja A. Sommerfelt, Chester Beatty Labs, Institute of Cancer Research, London SW3 6JB, UK.

Retroviruses enter cells via specific cell surface receptors which may determine the cellular tropism and host range of the virus. With a panel of 18 retrovirus strains plating on human cells, we have enumerated 8 distinct receptor types by cross-blocking studies. Using human-rodent somatic cell hybrids, the genes for HTLV-1 & 2, and for D-type primate retroviruses, RD114 & BaEV, have been localized to regions of human chromosomes 17 and 19 respectively.

The CD4 antigen is the only retrovirus receptor that is well characterized biochemically. This molecule serves as the receptor for all HIV-1, HIV-2 and SIV strains tested. CD4 is expressed on T-helper lymphocytes, and some other T-cells, B-cells, macrophages and dendritic cells. Although CD4 is not detectable by immunofluorescence on neural or gut epithelial cells, cell lines of these lineages that express CD4 in RNA are susceptible to HIV infection. Thus the tissue distribution of CD4 can account for HIV pathogenesis including neuropathic and enteropathic AIDS. The binding site for HIV on CD4 is close to that for the leu3a monoclonal antibody. This epitope is highly conserved in primate evolution. Anti-idiotypic sera to leu3a weakly neutralize HIV, and soluble forms of CD4 do so more potently. Following adsorption to CD4 molecules on the surface of human cells, HIV rapidly becomes internalized by a pH-independent mechanism. In contrast, when mouse cells expressing a human CD4 gene are exposed to HIV, the virus binds without effecting entry.

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Viral Persistence

J 009 MOLECULAR MECHANISMS OF PERSISTENCE IN THE SLOW INFECTION CAUSED BY VISNA VIRUS, Ashley T. Haase, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455.
Visna virus is the prototype of a subfamily of retroviruses which cause slow infections in animals and man (AIDS) and persist in their hosts despite an immune and inflammatory response. At one level both the slow evolution of infection and the persistence of virus can be viewed as derivatives of restricted gene expression which limits synthesis of antigen and thus thwarts immune surveillance. This restriction is effected at the level of steady state concentrations of virus specific RNA by mechanisms which will be the subject of this presentation. We will present evidence that a gene in visna virus analogous to the transactivating gene of human immunodeficiency virus plays a critical role in determining the number of copies of virus specific RNA in infected cells.

J 010 REGULATION AND CONSEQUENCES OF PERSISTENT MEASLES VIRUS INFECTIONS, Volker ter Meulen*, Knut Baczko*, Uwe G. Liebert*, Sibylle Schneider-Schaulies*, Roberto Cattaneo+ and Martin Billeter+, *Institut für Virologie und Immunbiologie, Universität Würzburg, FRG, +Institut für Molekularbiologie I der Universität Zürich, Switzerland.
Measles virus is the cause for acute measles in man which normally is an uncomplicated childhood disease. However, occasionally a late central nervous system (CNS) complication may occur years after the onset of acute measles. This disease, referred to a subacute sclerosing panencephalitis (SSPE), is the result of a persistent measles virus infection in brain cells. So far, the pathogenetic mechanisms of this disease are largely unknown and the factors which lead to a progressive brain damage are not understood. Of pathognomonic significance are the findings of a humoral hyperimmune reaction to the majority of measles virus antigens with no or reduced antibodies to measles virus M protein as well as the dissimination of the infection in brain tissue in the presence of a pronounced local antiviral immune response. Biochemical studies on persistently infected cells derived from SSPE brain tissue indicate that the failure to detect antibodies to M protein may be the result of a restriction of measles virus M protein expression. This is either the consequence of a block in translation or the failure of the synthesized protein to accumulate. Similar observations were recently made directly on SSPE brain. Brain material obtained at autopsy and examined for the synthesis of five structural proteins of measles virus in infected brain cells revealed that in none of the material studied all five structural proteins simultaneously occurred. Nucleocapsid (N) and phosphor (P) proteins were found in every diseased area whereas haemagglutinin (H), fusion (F) and matrix (M) protein were either missing or seen only in few infected cells. The characterization of measles virus-specific RNA in SSPE brain revealed plus-strand RNA specific for the viral N, P, M, F and H genes. However, the mRNAs for the envelope proteins were functionally impaired since they could not be translated in vitro. Preliminary evidence indicates that the functional defects of mRNA are a result of accumulating mutations leading to transcriptional alteration. The observation that measles virus envelope proteins are not synthesized in general in SSPE-infected brain cells may explain the absence of infected virus particles and the failure of the immune system to overcome the infectious process.

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Virus Entry

J 011 CELL BIOLOGY OF VIRUS ENTRY: INFLUENZA AND ALPHAVIRUSES.Ari Helenius, Sandra Schmid, Toon Stegmann, Robert Doms, Francois Boulay, Susan Froshauer, Margaret Kielian and Ira Mellman. Department of Cell Biology, Yale School of Medicine, new haven CT 06510.

We are studying viruses which rely on endocytosis and acid-activated membrane fusion for their entry into host cells. Using alpha viruses and influenza virus as our model system, we have determined the key steps in the entry pathway including *attachment, receptor-mediated endocytosis, encounter with acidic pH, acid-induced conformational change in the viral spikes, spike protein-mediated membrane fusion and release of viral genome into the cytoplasm.* With Semliki Forest virus, an alphavirus, quantitative kinetic studies have defined the T1/2 values, the lag times, the asynchrony coefficients and the efficiency of each of these events both in wt cells as well as in mutant cells with endosomal acidification defects. The results show that in CHO cells viruses bound to the surface are rapidly and effciently internalized(T1/2 = 4 min) and the average spike glycoproteins spend 30 minutes in endosomes before delivery to lysosomes. In wild type cells acid-exposure and penetration takes place from the first endosomal pool, but if a mutant virus with a lower pH treshold or mutant cells with endosomal acification defects are used the penetration is delayed.

The most detailed analysis of the *membrane fusion activity* has been performed with Influenza HA. The results using a variety of methods support a model in which the trimeric spike glycoprotein changes its conformation in a cooperative, two step process, where the first step is temperature independent and leads to hydrophobic attachment of the virus to the target membrane via the HA molecules, and the second step is temperature dependent and results in the actual fusion event. Using hybrid trimers obtained in double infected cells we have established that the conformational changes are cooperative between subunits in a HA trimer.

The *early cytoplasmic events* have been studied in the SFV system. We have obtained evidence that the replication of the viral RNA and possibly the assembly of nucleocapsids occurs in complex ribonucleoprotein structures attached to at the cytoplasmic surface of lysosomes.

J 012 STUDIES ON THE SURFACE RECEPTORS FOR INFLUENZA C VIRUS ON A POLARIZED CELL LINE (MDCK CELLS), Georg Herrler and Hans-Dieter Klenk, Philipps-Universität Marburg, D-3550 Marburg, Fed. Rep. Germany

The distribution of surface receptors for influenza C virus on MDCK I cells was analyzed by infecting filter-grown cells from either the apical or basolateral side. While virus budding was detected only at the apical domain of the plasma membrane, influenza C virus was found to be able to infect MDCK I cells from both sides indicating that virus receptors are present on both the apical and basolateral domain of the plasma membrane. A different subline of these cells (MDCK II), which is lacking receptors for influenza C virus, can be infected after incorporation of bovine brain gangliosides into the plasma membrane. The ability of influenza C virus to infect filter-grown MDCK II cells after incorporation of gangliosides into either the apical or the basolateral domain of the plasma membrane was analyzed. Infection was found to be possible from either side irrespective of the site of ganglioside application indicating that the distribution of the receptor gangliosides is not polarized. A mutant of influenza C virus which is able to infect MDCK II cells in the absence of exogenous gangliosides was isolated and analyzed.

J 013 MECHANISMS OF VIRAL ENTRY: INTERACTION OF SENDAI VIRUS WITH BIOLOGICAL AND ARTIFICIAL MEMBRANES, Dick Hoekstra, Steven L. Novick[1], Shlomo Nir[2] and Karin Klappe, Laboratory of Physiological Chemistry, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.

The mechanism of viral membrane fusion was investigated by studying the interaction of Sendai virus with artificial and biological membranes. The kinetics of viral binding and fusion with various biological membranes, such as erythrocytes and murine cells, could be accurately determined using assays, based on the relief of fluorescence selfquenching and resonance energy transfer. Both the rate and extent of fusion of the cell-bound viruses is dependent on the cell-surface particle density, indicating that viral fusion with the cell surface is not an at random process. Rather, in spite of the ability of all virions to display fusion activity only a limited number will actually fuse, the final amount being dependent on the input multiplicity and cell type. Constraints in the mobility of the viral membrane proteins appear to control the fusion of the virus as could be inferred from the dependence of fusion on pH and temperature, in conjunction with the kinetic characteristics of the fusion of reconstituted envelopes. This suggestion is further supported by experiments that rely on cross-linking of the proteins and selfquenching of fluorescently-tagged viral proteins. Hydrophobic interactions between a virus and a target membrane constitute an inherent part of the fusion mechanism of enveloped viruses. Modulation of the state of hydration of the target membrane surface accelerates the kinetics, but does not enhance the extent of fusion. A mass action kinetics analysis reveals that the fusion rate per se increases upon membrane dehydration although such modulations also cause a slight increase in the aggregation rate constant. The nature of the interaction of viral membrane proteins with a target membrane was further investigated using liposomal target membranes. Liposomal membranes affect the fusogenic properties of Sendai virus. The modulating effects of liposomes can be attributed to a lipid composition dependent ability of the viral proteins to penetrate into the target membrane. With the hydrophobic photoaffinity label 3-(trifluoromethyl)-3-(m-[^{125}I]iodophenyl)diazirine (TID), that was incorporated into the hydrophobic core of the liposomal membrane, evidence could be obtained that supports the proposal that hydrophobic penetration of the fusion (F) protein triggers the fusion reaction at neutral pH. However, a direct extrapolation of mechanistic features seen in the fusion between a virus and a model membrane should be done but with extreme caution. Experiments will also be discussed which demonstrate that the fusion between viruses and liposomes can partly be accomplished in a manner that may not bear physiological relevance. 1) California Institute of Technology, Pasadena, CA 91125. 2) University of Jerusalem, Rehovot, Israel.

J 014 HUMAN IMMUNODEFICIENCY VIRUS: ENTRY INTO THE HOST CELL AND PATHOGENESIS Joseph Sodroski, William Haseltine, Mark Kowalski, Joseph Potz, Ladan Basiripour, Bruce Walker, Tatyana Dorfman, Monty Krieger*, Tina Jager-Quinton, Rebecca Hussey, Neil Richardson, Nicholas Brown, Ellis Reinherz. Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA and *Massachusetts Institute of Technology, Cambridge, MA

Human Immunodeficiency Virus (HIV), the etiological agent of acquired immunodeficiency syndrome and other disorders, enters the host cell via a specific interaction between the HIV envelope and the viral receptor, one component of which is the CD4 (T4) molecule. The HIV envelope also induces the fusion of cell membranes, which results in at least part of the cytopathic effects of virus infection on CD4- positive cells. The determinants for receptor binding and membrane fusion of the HIV envelope have been studied using directed mutagenesis. The role of N- and O- linked glycosylation in the processing and function of the envelope proteins have been studied. The ability to inhibit the interaction of the gp120 envelope protein with the CD4 receptor by using recombinant proteins mimicking CD4 will be discussed.

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Intracellular Traffic of Viral Proteins

J 015 STRUCTURE, MODIFICATION, INTRACELLULAR TRANSPORT AND SURFACE EXPRESSION OF INFLUENZA AND PARAMYXOVIRUS SV5 PROTEINS M_2, NB, & SH, AND A HYBRID PROTEIN, ANCHORED PYRUVATE KINASE.

Robert A. Lamb, Scott W. Hiebert, Mark A. Williams and Suzanne L. Zebedee, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL. 60208.

The soluble cytoplasmic protein pyruvate kinase (PK) has been expressed at the cell surface in a membrane anchored form (APK). The hybrid protein contains the N-terminal signal/anchor domain of a class II integral membrane protein (HN of the paramyxovirus SV5) fused to the PK N-terminus. APK contains a cryptic site that is used for N-linked glycosylation but elimination of this site does not affect cell surface localization. Truncated forms of the APK molecule, with up to 80% of the PK region of APK removed, are also expressed at the cell surface. These data indicate that neither native folding nor glycosylation are necessary for intracellular transport of PK to the cell surface, and suggest a lack of specific signals for cell surface localization in the ectodomain.

The small hydrophobic polypeptide (SH) of 44 amino acid residues of the paramyxovirus SV5 has been demonstrated to have properties of an integral membrane protein, is found in the exocytotic pathway and is transported to the infected-cell surface. Biochemical analysis of the orientation of SH in membranes indicates that SH is orientated in membranes with its N-terminal hydrophilic domain exposed on the cytoplasmic face of the plasma membrane and the ~5 amino acid residue C-terminus exposed at the cell surface. These data will be discussed with respect to positive-acting signals being necessary in the ectodomain of SH for cell surface expression.

Both M_2 of influenza A virus and the NB glycoprotein of influenza B virus belong to a small class of integral membrane protein that have a single hydrophobic domain that acts as a signal/anchor and have N-terminal domains exposed extracellularly. NB contains two N-linked carbohydrate chains that are processed from the high mannose form to a heterogeneous form of much higher molecular weight which has the characteristics of polylactosaminoglycan and is expressed at the cell surface. Monoclonal antibodies specific for the extracellular domain of M_2 have been used to resolve the issue of whether M_2 is a component of purified virions and to probe the function of M_2 in infected cells.

J 016 DUAL ROLES FOR THE CYTOPLASMIC DOMAIN OF THE VSV G PROTEIN: PROTEIN TRANSPORT AND VIRUS ASSEMBLY.

M. A. Whitt, R.W. Doms, Aino Ruusala, Jonne Helenius, Ari Helenius and J.K. Rose. Departments of Pathology and Cell Biology, Yale University School of Medicine, 333 Cedar St., New Haven, Ct. 06510.

We have examined a series of 7 mutations deleting amino acid sequences from the cytoplasmic domain of the VSV G protein, or substituting this domain with cytoplasmic domains from other membrane proteins. In general these mutations slowed or blocked exit of the mutant G proteins from the ER without reducing the rate of trimerization of the protein. Using a new assay to measure pH stability of G protein trimers and a series of conformation sensitive monoclonal antibodies, we found that the ectodomains of these mutant proteins were apparently correctly folded whether they were retained in the ER, transported slowly from the ER, or transported normally. Because there was no correlation between folding of the ectodomain and transport for these mutant proteins, we propose that there are important interactions on the cytoplasmic side of the membrane which regulate G protein transport.

In a second series of experiments we devised two assays to analyze the role of the cytoplasmic domain of G protein in VSV assembly. We were able to rescue the tsO45 mutant of VSV (which makes a defective G protein) in cells expressing wild-type G proteins from cloned cDNA and we demonstrated directly that the rescued virus particles contain the protein expressed from cloned DNA. Mutant G proteins with substantial alterations in the sequence of the cytoplasmic domain (but which are expressed on the cell surface), are unable to rescue the ts045 mutant apparently because they are not incorporated into the virus. These experiments suggest that critical interactions between the cytoplasmic domain of G protein, matrix protein, and budding nucleocapsid may direct assembly of this enveloped virus.

Emerging Concepts in Viral Pathogenesis

J 017 B19 PARVOVIRUS: CELLULAR, MOLECULAR, AND CLINICAL STUDIES, Neal S. Young, Keiya Ozawa, Gary Kurtzman, Takashi Shimada, Cell Biology Section, Clinical Hematology Branch, NHLBI, Bethesda MD 20892.
B19 parvovirus, discovered in 1975, has been established by seroepidemiologic studies as the etiologic agent of fifth disease, a common childhood exanthem and a polyarthralgia syndrome in adults. In patients with hemolysis, B19 causes transient aplastic crisis. If neutralizing antibodies fail to form, B19 can persist in marrow and blood and lead to chronic bone marrow failure. In clonal progenitor assays, the virus is a potent inhibitor of erythroid, but not myeloid, hematopoietic colony formation due to cytotoxicity for a progenitor cell at the CFU-E/BFU-E stage. B19 has been propagated in vitro only in primary suspension cultures of human erythroid bone marrow; elimination of erythroid cells and formation of giant pronormoblasts in vitro reproduces two cardinal clinical features of clinical infection. Similar to other Parvoviridae, single stranded DNA viruses, B19 replicates through high molecular weight double stranded intermediate forms, utilizing terminal hairpin structures for self-priming. The genomic organization of B19 also resembles other parvovirues, with the right side encoding two capsid proteins (major VP2, 58, and minor VP1, 83 kd) and the left side a nonstructural (NS) protein of 77 kd. However, the transcription map of B19 is unique among parvoviruses in its use of a single promoter for a large number of differentially spliced, overlapping transcripts, some of which terminate in the middle of the genome at an unusual polyadenylation signal. The quantities of VP1 and VP2 are regulated primarily at the level of translation by the presence of multiple spurious AUG triplets upstream of the VP1 initiation site, which are spliced from the VP2 RNA. NS protein is encoded by the single unspliced RNA, which is relatively rare in permissive cells but a major viral RNA species in nonpermissive cells. The function of NS protein of Parvoviridae species is not known. For B19, transfection of plasmids containing the gene for the NS protein results in transient expression of protein in HeLa cells, but stable transformants fail to form when neo^r is present on the same plasmid or on a cotransfected plasmid, suggesting that the NS protein is lethal in nonpermissive cells. Premature termination of transcription in nonerythroid progenitors, with expression of NS protein without viral propagation, would explain clinical neutropenia and thrombocytopenia.

Cellular Architecture and Virus Assembly

J 018 PATTERNS OF PROTEIN TRAFFIC AND VIRUS RELEASE AS POSSIBLE DETERMINANTS OF VIRAL PATHOGENESIS. Richard W. Compans, R. V. Srinivas, David R. Kilpatrick, Edward B. Stephens, Edward T. Clayson and Lorraine V. Brando, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.
We have been investigating the molecular determinants of protein traffic and viral pathogenesis using murine retroviruses. The Friend murine spleen focus-forming virus (SFFV) encodes a glycoprotein designated gp52, which is responsible for the leukemogenic properties of the virus. Gp52 lacks a cytoplasmic domain and is defective in its transport to the cell surface. We have constructed a chimeric envelope gene which codes for a molecule with an external domain derived from the SFFV envelope gene, and membrane-spanning and cytoplasmic domains derived from the MuLV envelope gene. Like gp52, the chimeric protein was defective in its transport to the cell surface. However, unlike wild type SFFV, the chimeric SFFV genome failed to induce erythroleukemia in adult mice. The results indicate that the altered membrane spanning domain and/or lack of a detectable cytoplasmic tail in gp52 are a prerequisite for the erythroleukemia-inducing properties of SFFV, but are not responsible for the block in intracellular transport of the glycoprotein. The mature form of gp52, designated gp65, is reported to be secreted from SFFV infected cells. To determine the specific changes in the envelope protein which may lead to its lack of stability in associating with membranes, the 3' end of the F-SFFV envelope gene, which encodes the transmembrane domain, was inserted in place of the normal 3' end of the Friend murine leukemia virus genome. This chimeric envelope gene was expressed using the vaccinia virus expression system. The chimeric gp70/p15E glycoprotein molecule lacks the cytoplasmic tail residues and as a consequence is about 3,300 daltons smaller. Immunofluorescence studies demonstrate that the chimeric molecule is efficiently transported to the surface of cells, unlike the SFFV gp52 glycoprotein. However, the chimeric molecule was found to be unstable in its membrane association and is released into the culture medium. These results indicate that the changes in the membrane spanning region and the lack of a cytoplasmic tail do not determine the defective transport of gp52, but may determine the stability of its association with membranes. We have also obtained evidence that the site of surface expression of viral glycoproteins in polarized epithelial cells may be a determinant of viral cytopathology, and have demonstrated that the release of SV40, a non-enveloped virus, is polarized in such cells.

J 019 Interaction between the E2 spike glycoprotein and nucleocapsid in Semliki Forest virus: Immunological reconstitution of intracellular recognition, I. Mellman, D.J.T. Vaux, and A. Helenius, Department of Cell Biology, Yale University School of Medicine, PO Box 3333, New Haven CT 06510

Semliki Forest virus (SFV) is a simple enveloped animal virus consisting of a nucleocapsid, a 42S single stranded genomic RNA, and three spike glycoproteins designated E1, E2, and E3. Like all alphaviruses, SFV buds from the plasma membrane of infected cells in a highly ordered and specific fashion which produces a viral envelope almost completely devoid of host cell proteins. This selectivity is thought to result from an interaction between the nucleocapsid and the 31 amino acid long cytoplasmic tail of E2, which anchors the otherwise laterally mobile spike glycoproteins to the nascent virion. Steric effects are thought to result in host proteins being excluded from this region. Alphavirus biogenesis provides a relatively simple model system for the study of interactions between transmembrane and cytosolic proteins, and thus for membrane sorting. While there is good evidence for the existence of a physical interaction between the nucleocapsid and E2, its specificity has never been established. We have sought to further characterize the spike-capsid interaction and to determine whether it reflects a specific receptor-ligand-like interaction by reconstructing it immunologically, using the structural mimicry of internal image anti-idiotype antibodies. This approach was made possible by a modified *in vitro* immunization procedure, which facilitates the rapid and efficient production of network antibodies. Starting with a synthetic peptide corresponding to the cytoplasmic domain of E2, we made a series of idiotype, anti-idiotype, and anti- anti-idiotype antibodies. The idiotype recognized the cytoplasmic domain of E2, the anti-idiotype recognized the viral nucleocapsid, and the anti-anti-idiotype recognized the E2 cytoplasmic domain once more. Reconstruction of three consecutive steps of an idiotype network proved that there is a specific intermolecular interaction between the nucleocapsid and the cytoplasmic domain of E2 that is likely to be the basis for the selective inclusion of viral proteins, and exclusion of host proteins, during budding. The presumed functional importance of this interaction was supported by the fact that the the determinant recognized by the anti-idiotype was conserved on nucleocapsids of all alphaviruses tested thus far (n=13), and not on flaviviruses. At least a portion of the anti-idiotype-reactive capsids were also associated with characteristic cytoplasmic vacuoles which may represent sites of viral assembly during alphavirus infections. This general approach should be applicable to identifying other specific protein-protein interactions relevant to intracellular membrane transport.

J 020 SORTING IN THE TRANS GOLGI NETWORK USING VIRAL GLYCOPROTEINS AS PROBES, Kai Simons, Mark Bennett and Angela Wandinger Ness, European Molecular Biology Laboratory (EMBL), D-6900 Heidelberg, Fed.Rep. Germany. A useful model for studying epithelial cell polarity is the canine kidney MDCK cell line, which when grown on permeable supports organizes into an epithelial sheet mimicking the organization of epithelial tissues *in vivo*. The envelope glycoproteins of enveloped RNA viruses follow the same biosynthetic pathway as cellular plasma membrane proteins and have frequently been used as probes to study intracellular membrane protein transport. Using this system, it has been demonstrated that viral proteins destined for the apical or basolateral plasma membrane domains are sorted before they reach the cell surface. Our present evidence suggests that the sorting occurs in the trans-Golgi network, the exit compartment in the Golgi complex for outgoing membrane traffic.

We have developed a method for perforating the plasma membrane of MDCK cells while retaining cellular functions (Simons and Virta, EMBO J. **6**:2241-2247, 1987). A nitrocellulose acetate filter is applied and allowed to attach to the apical side of cells, grown on either a glass coverslip or a filter support. Segments of the apical plasma membrane adhere to the filter and are detached from the cell layer by shearing when the filter is peeled off. This allows macromolecules such as antibodies and enzymes to diffuse into the cells. The cells are otherwise intact as judged by light and electronmicroscopy. The perforated cells maintain their capacity to support vesicular transport of proteins and lipids. Our recent results show that the basolateral G protein of vesicular stomatitis virus and the apical hemagglutinin of influenza virus are transported from the trans-Golgi network in perforated cells in the presence of ATP. When Ca^{++} is removed the viral proteins are released from the cell layer into the medium in sealed membrane vesicles. We are presently characterizing these vesicles to determine whether they correspond to basolateral (G protein) and apical (hemagglutinin) carrier vesicles.

Cell Biology of Virus Entry, Replication and Pathogenesis

Intracellular Membranes and Virus Assembly

J 021 TARGETING OF ROTAVIRUS ENDOPLASMIC RETICULUM GLYCOPROTEINS, M.S. Poruchynsky, D. R. Maass, Paul H. Atkinson, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, N.Y. 10461.

It is apparent that specific mechanisms exist to sort membrane glycoproteins from their site of synthesis in the endoplasmic reticulum to their various destinations throughout the cell or to the exterior. Rotavirus gene 9 product, VP7, is a membrane associated glycoprotein which specifically targets to the ER. Similarly, rotavirus gene 10 product, NCVP5, targets to the ER. VP7 has two hydrophobic domains, h1 and h2, near the amino terminus. h1 is not translated in the bulk of VP7 and thus h2 serves as a translocation signal. We previously have shown small deletions in h2 and in the region adjacent to it on the C-terminal side resulted in secretion of VP7. We interpreted this to mean VP7 has a signal in its primary sequence which causes it to be retained in the ER, the absence of which results in the nonspecific secretion.

We have now shown that 2 regions in the amino acid sequence 51 - 111 are responsible for retention in the ER. Whereas we initially though that the h2 domain at the N-terminal side of amino acid no. 51 was involved in this retention it is now apparent this is not the case because this region is a signal sequence and is cleaved from mature VP7. We constructed a gene for a fusion protein with VP7 sequence (amino acids 51-111) on the N-terminus of mature amylase, a normally secreted protein. The fusion protein was retained in the cell. A fusion protein comprising VP7 amino acids 51-63 - amylase was secreted as was wild type VP7 lacking amino acids 51-61. We can conclude therefore that in VP7, the regions 51-61 and 62-111 are necessary for retention in the ER but neither region is sufficient by itself. We have created a series of different sized deletion mutants in VP7 in the region numbers 62-111 in order to narrow down the ER interacting sequences in this part of the molecule, necessary to prevent its secretion.

J 022 THE GOLGI COMPLEX AND BUNYAVIRUS ASSEMBLY. R.F.Pettersson, R.Persson, R.Rönnholm and L.Wikström. Ludwig Institute for Cancer research, Box 60202, S-10401 Stockholm,Sweden

A number of viruses belonging to the Bunyaviridae family have been shown by EM to mature by a budding process at smooth membranes of the Golgi complex (GC). We are studying the underlaying mechanisms that determine this site of assembly of one particular bunyavirus, called Uukuniemi virus. Previous work has shown that the site of maturation is largely determined by the accumulation of the two viral membrane glycoproteins G1 (Mr 70,000) and G2 (Mr 65,000) in the GC. G1 and G2 are slowly transported ($T_{\frac{1}{2}}$ about 45 min) from the endoplasmic reticulum (ER) to the GC, where further transport is arrested. In the presence of cycloheximide, G1 and G2 cannot be chased out of the GC. The accumulation of G1 and G2 in the GC results in a vacuolization of the cisternae. Such an altered GC is still able to transport, terminally glycosylate and sort the Semliki Forest virus glycoproteins E1 and E2, and a number of cellular secretory proteins, suggesting that the structural integrity of the GC is not essential for proper function.

Our aim is currently to determine (i) the role of oligomerization and conformational changes of G1 and G2 during intracellular transport, (ii) the site and mode of virus assembly in the GC, and (iii) the molecular basis for the Golgi-specificity of G1 and G2. In virions, G1 and G2 are present solely as heterodimers. Dimerization occurs shortly after synthesis. Pulse--chase experiments in combination with sucrose gradient centrifugation, subcellular fractionation, immunoprecipitation with conformation-specific monoclonal antibodies followed by SDS-PAGE analyses, indicate that additional conformational changes occur along the transport pathway. We have found that there is a specific interaction between the ribonucleoproteins (RNPs) (i.e. the N protein) and one or both of the glycoproteins both in the virion and in infected cells. We are currently characterizing this interaction. Mapping with antibodies against a synthetic peptide corresponding to the COOH-terminus of G1 suggests that G1 has a 70-80 amino acid-long cytoplasmic extension, whereas that of G2 is only 5 residues long. It will be of interest to find out to which of the glycoproteins the N protein interacts. To study the molecular basis for the Golgi-specificity of G1 and G2, the virion M RNA segment coding for the precursor p110 of G1 and G2 has been cloned and sequenced. A full-length cDNA has been constructed and expression studies are underway. Using this cDNA we hope to study whether both proteins, when expressed individually, are Golgi-specific.

J 023 HERPES SIMPLEX VIRUS: ENTRY INTO AND EGRESS FROM THE CELL, Patricia G. Spear[1], M. Wittels[2], A.O. Fuller[2], D. WuDunn[2] and R. Johnson[2], Northwestern Univ. Medical School[1], Chicago, IL 60611 and Univ. of Chicago[2], Chicago, IL 60637. Herpes simplex virus (HSV) is an enveloped DNA virus that uses both the nucleus and cytoplasm of the infected cell for its replication. Three lines of evidence indicate that infective entry of HSV into human HEp-2 cells can be by fusion of the virion envelope with the plasma membrane and that endocytosis of virus is not required. First, agents known to block endocytosis or to raise the pH of endosomes did not inhibit early events in HSV infection at doses sufficient to block infection by viruses requiring the endocytic pathway. Second, comparisons of the fate of infectious and neutralized virions (equivalent in ability to adsorb to cells) by electron microscopy revealed that fusion of virions with the plasma membrane was associated with infectivity. Third, virus-induced cell fusion is not dependent on exposure of infected cells to low pH, as is the case for some viruses requiring the endocytic pathway.

One implication of the foregoing is that fusogenic activity of HSV must be triggered by events occurring entirely at the cell surface and is not dependent on low pH. Our working hypothesis is that multiple, perhaps sequential, interactions of at least three viral glycoproteins (gB, gD and gH) with the cell surface lead to membrane fusion. One of these glycoproteins mediates attachment of virions to the primary cell surface receptors for HSV. These receptors are heparan sulfate glycosaminoglycans. All three of the glycoproteins mentioned above participate in events occurring between primary adsorption and membrane fusion, as indicated by the phenotypes of viral mutants and the effects of monoclonal antibodies.

Nucleocapsids are assembled in the cell nucleus and acquire an envelope at the inner nuclear membrane. Virions are transported out of the cell in membrane-bounded compartments via the Golgi apparatus. Progeny virions tend to remain attached to the cell surface. A mechanism probably exists to prevent fusion of progeny virions with membranes of infected cells. Recent results show that expression of gD in transformed cells renders the cells resistant to HSV infection. This suggests that gD expression in infected cells might protect progeny virions from eclipse by the infected cell.

Interaction of Cytotoxic T-Cells with Virus-Infected Cells

J 024 VIRAL POLYPEPTIDE PRESENTATION TO AND RECOGNITION BY T LYMPHOCYTES. Thomas J. Braciale, Lynda Morrison, Marianne Sweetser, Lawrence Brown and Vivian L. Braciale, Department of Pathology, Washington University Medical School, St. Louis, MO 63110.

Recent studies strongly suggest that the interaction of viral gene products with the T lymphocyte is by pathways distinctly different from the pathways of viral polypeptide synthesis, transport, and expression leading to virion synthesis and assembly in virally infected cells. We will describe recent evidence indicating that effector T lymphocytes restricted by class I and class II major histocompatibility complex (MHC) products recognize non-native (processed) forms of these viral antigens expressed on cell surfaces in association with MHC locus products. We will summarize results indicating that there are at least two distinguishable pathways of viral polypeptide presentation and that different pathways are preferentially utilized by MHC class I- and class II-restricted T lymphocytes respectively. We will also describe results mapping specific sites on influenza virus polypeptides recognized by T lymphocytes in an MHC restricted fashion.

J 025 CYTOLYTIC LYMPHOCYTE GRANULES: COMPOSITION AND FUNCTION IN CYTOLYSIS, Eckhard R. Podack, Dept. Microbiology and Immunology, University of Miami, School of Medicine, Miami, Florida 33101.
Cytolytic lymphocyte granules contain approximately 6 major and several minor proteins. Of the total granule protein pool a major proportion is comprised of proteinases (granzymes) that are homologous to other cellular proteases of mast cells and polymorphonuclear leucocytes. Although granzymes are predominately associated with cytotoxic T-cells, no direct function in cell mediated cytolysis has been demonstrated. Two granule activities associated directly with cytotoxicity have been demonstrated; one activity (perforin = P1) mediates transmembrane pore formation (perforation) and the other triggers nucleolysis and DNA degradation in the target cell (NTF = nucleolysis triggering factor). We have obtained full-length P1-cDNA clones in the murine system and partial clones in the human system. By Northern blot analysis, P1 expression is absolutely linked to cytotoxicity of lymphocytic killer cells. Both class I MHC specific and class II MHC specific T-cells express P1-mRNA of 2.8 to 3.0 kb size. P1-mRNA levels are significantly increased upon stimulation of CTL with Ca-ionophore and phorbolesters. Non specific mitogens and recombinant IL2, as well as mixed lymphocyte cultures across class I or class II barriers are strong inducers of P1 message. These studies are consistent with a major role of P1 in lymphocyte mediated cytolysis. Supported by: ACS, IM-369A; NIH, RO1 A1-21999; NIH-CA, RO1 CA-39201.

J 026 Characterization of the Cytotoxic T Cell Response to HIV-1 Gene Products, Bruce D. Walker, Timothy J. Paradis, Martin S. Hirsch, Robert T. Schooley, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; Charles Flexner and Bernard Moss, NIAID, NIH, Bethesda, MD 20892.

Characterization of the host immune response to human immunodeficiency virus is critical to the rational design of an effective AIDS vaccine. We have detected circulating HIV-1-specific cytotoxic T cells (CTL) in fresh peripheral blood from twenty of twenty HIV-1 seropositive subjects, but from none of eight HIV-1 seronegative control subjects. CTL targets were prepared by infecting Epstein-Barr Virus (EBV)-immortalized B cells lines (established individually for each subject studied) with recombinant vaccinia viruses expressing HIV-1 genes. CTL directed against autologous B lymphoblasts expressing the HIV-1 envelope gene product were detected in all twenty seropositive subjects. In addition, nine of twelve seropositive subjects tested also had circulating HIV-1 reverse transcriptase (RT)- specific CTL, while only three of twenty had CTL directed against the gag gene product, the major HIV-1 structural protein. HIV-1 gag-specific CTL could, however, be stimulated in vitro. The major cellular effector of the HIV-1-specific CTL response has been identified as a HLA Class I restricted CD3+CD8+ lymphocyte, and we have now been able to expand these cells in vitro. Whereas the magnitude of the observed virus specific CTL response is stable over time in the absence of clinical disease progression, a marked decline in CTL activity has been noted in two subjects progressing to develop AIDS. Longitudinal studies should help to establish whether these HIV-1-specific CTL play a protective role. In addition, this assay provides a means of measuring cellular responses to candidate AIDS vaccines.

J 027 CLASS I MHC RESTRICTED CTL RECOGNITION OF VIRUS ANTIGENS, J. Lindsay Whitton, Hanna Lewicki, Peter J. Southern and Michael B.A. Oldstone, Research Institute of Scripps Clinic, La Jolla CA 92037.

We are attempting to define the nature of virus antigens seen by class I MHC restricted cytotoxic T lymphocytes (CTL), and to determine the viral sequences required both for induction of, and for target cell recognition and lysis by, CTL. To approach these problems we have utilized as a model system lymphocytic choriomeningitis virus (LCMV) infection of its natural murine host. Class I MHC restricted CTL play an important part in controlling LCMV infection, determining whether the outcome will be virus clearance with subsequent immunity, or virus persistence with consequent disease. We have begun to analyze in detail the virus components to which CTL responses are mounted.

LCMV is an arenavirus, with a bisegmented single-stranded RNA genome. Segmental reassortants have been used to show that the short genome segment dictates CTL induction and target cell recognition on the $H2^{dd}$ background. This genome segment encodes two proteins, nucleoprotein (NP) and a glycoprotein precursor, GP-C, which is cleaved to generate the two mature glycoproteins GP-1 and GP-2. We have expressed cDNA clones of these genes in a vaccinia virus vector and used these as target moieties to analyze the anti-LCMV response on $H2^{bb}$, $H2^{dd}$ and $H2^{qq}$ backgrounds. On all three MHC haplotypes LCMV induces a major anti-NP response; in contrast, only on the $H2^{bb}$ haplotype is a response to GP mounted.

We have obtained 18 independent $H2^{bb}$ CTL clones, 17 of which are GP-specific. The precise specificities of these clones have been determined by analyzing their lytic abilities against targets infected with a variety of GP C terminal deletions expressed in vaccinia. By this technique we have identified at least two epitopes in LCMV GP. One of these epitopes has been further analyzed using exogenously applied synthetic peptides to sensitize uninfected $H2^{bb}$ target cells. To compare exogenous application with endogenous synthesis, and to define the minimal sequences necessary for presentation of endogenously-produced molecules by class I MHC, we have expressed in vaccinia a very short (22 amino acid) sequence containing this epitope. This short peptide, when synthesized endogenously, is efficiently seen by appropriate CTL clones. We are currently establishing whether this short protein alone can induce a CTL response or whether accessory sequences, not needed for target cell recognition, are required to allow induction.

Interaction of Viruses and Histocompatibility Antigens

J 028 MHC CLASS I ANTIGENS AND TUMORIGENESIS, Gilbert Jay, Section of Cell Physiology, National Cancer Institute, Bethesda, MD 20892.

Cancer may be thought of as an immunological disorder which arises because certain 'transformed' cells, endowed with the propensity to divide, have learned to evade detection by the immune system. The prospect of intervention by "immunotherapy" depends very much on our ability to either (1) render cancer cells more recognizable by the immune system or (2) potentiate the immune system towards a more effective recognition of cancer cells. There is now direct evidence for the suppression of the major histocompatibility complex class I antigens, a family of cell-surface glycoproteins required for the presentation of cancer cells to the immune system, being directly responsible for the escape of tumor cells from immune surveillance. It has been shown that cancer cells can be made immunogenic either by the expression of an exogenous class I gene introduced by DNA-mediated gene transfer or by the derepression of endogenous class I genes with interferon; these cells are efficiently rejected by the immune system. Even more interesting is the finding that the immune system can be potentiated to reject tumors by immunization with homologous tumor cells that have been manipulated to express normal levels of class I antigens. Since increasing numbers of human tumors have been found to have greatly reduced levels of class I antigens, these findings suggest a direct route to immunotherapy which involves debulking of the tumor mass, raising the level of class I antigens in a small number of explanted tumor cells, and reimmunizing the host.

Virus Structure and Receptors

J 100 INTERACTION OF HUMAN T CELLS WITH HCMV STRUCTURAL PROTEINS, J.D. Adlish, S. St. Jeor, University of Neveda, School of Medicine, Reno, NV 89557.

We previously reported that infection of human T cells with HCMV results in formation of giant cells. These cells fuse together to form multinucleated giant cells containing 2-6 or more nuclei. Human T cells (stimulated with PHA) were infected with HCMV at various m.o.i. ranging from 1-25, the cells were stained with acridine orange and the number of giant cells was observed using UV and phase contrast microscopy. The results indicate that only 2-5% of the T cells form giant cells, indicating that a subset of T cells is susceptible to HCMV induced cell fusion. Giant cell formation appears to be independent of m.o.i., occurs within 30 minutes following infection, and is not blocked by cycloheximide. Southern blot analysis of infected cells showed little or no replication of HCMV DNA at 72 hours post infection. Using SDS PAGE three HCMV proteins, which bind to human T cells have been identified. These proteins were isolated using viable T cells to bind ^{125}I solubilized HCMV protiens. After binding the HCMV proteins were isolated by centrifugation of T cells. These proteins have molecular weights of 10, 25, and 40 KDa. By modified western blotting we have identified at least two T cell proteins to which viral proteins bind. These proteins have molecular weights of 15-25 KDa and 35-50 KDa. These results indicate there is a possible viral receptor specific for T cells.

J 101 RECOMBINANT FRAGMENTS OF THE CD4 MOLECULE CONTAINING THE BINDING SITE FOR HUMAN IMMUNODEFICIENCY VIRUS, Edward A. Berger, Thomas R. Fuerst and Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892.

We have designed vaccinia virus-based mammalian expression systems to produce recombinant fragments of the CD4 glycoprotein, the cellular receptor for human immunodeficiency virus (HIV). One such fragment, representing the amino-terminal half of the extracellular region of CD4, was found primarily in the culture medium. It reacted strongly with several HIV-blocking anti-CD4 monoclonal antibodies, but only weakly or not at all with those anti-CD4 monoclonal antibodies which do not block HIV. This fragment was capable of forming a specific complex with soluble recombinant gp120, the CD4-binding subunit of the HIV envelope glycoprotein. We conclude that the HIV-binding domain is contained within the amino-terminal half of the extracellular region of CD4. This and other recombinant CD4 fragments are under active study as probes for molecular aspects of the CD4/gp120 interaction, as well as for their possible inhibitory effects on HIV infectivity and specific immunological functions involving CD4. The results of such studies will have important implications for the potential therapeutic application of CD4 derivatives in the treatment of acquired immune deficiency syndrome.

J 102 IDENTIFICATION OF A CELLULAR RECEPTOR FOR VISNA VIRUS
Robert G. Dalziel, Neil J. Watt, John Hopkins and Ian McConnell, University of Edinburgh, Scotland.

The ovine lentivirus visna virus causes a variety of slow progressive diseases in infected animals. The virus predominantly infects cells of the monocyte/macrophage lineage with a possible involvement of cells in the CNS. The basis of the cellular and tissue tropism exhibited by visna is not yet understood. Using a western blot/virus overlay assay we have identified a putative cellular receptor for visna virus, present both on tissue culture cells and on cells of the sheep immune system. The receptor has an apparent molecular weight by SDS-PAGE of 30-33 kilodaltons and is present on cells which are permissive for visna virus; the WSCP cell line, primary mammary macrophage and cells derived from afferent and efferent lymph. The receptor cannot be detected on non-permissive cells, e.g. sheep erythrocytes and murine 3T3 cells. We will present data describing further characterisation of this receptor.

J 103 EXPRESSION ISOLATION AND CHARACTERIZATION OF A SECRETED FORM OF THE RECEPTOR, T4: AN INHIBITOR OF THE HIV VIRUSES, Keith C. Deen, J. Steven McDougal*, Richard Inacker, Gail Folena-Wasserman, James Arthos#, Jonathan Rosenberg, Paul J. Maddon+, Richard Axel+ and Raymond Sweet, Smith Kline and French Laboratories, King of Prussia, PA 19406; * Centers for Disease Control, Atlanta, GA 30333; #University of Pennsylvania, Philadelphia, PA 19104; + Columbia University, NY, NY 10032.
T4 is a non-polymorphic protein on the surface of human T-lymphocytes which plays a role in antigen-dependant T-cell activation. T4 is also the receptor for the AIDS viruses. The T4 protein consists of an external domain which displays regions of homology with immunoglobulin variable regions, a transmembrane sequence and a short cytoplasmic region. We have deleted sequences coding for the transmembrane and cytoplasmic regions and overexpressed this molecule, sT4, in mammalian cells by DHFR gene amplification. The protein is secreted into the culture medium in a soluble form, accumulates to a level in excess of 20 mg/l and has been isolated at >95% purity. sT4 retains all surface eptiopes of the native protein tested and thus appears to be an accurate mimic of the T4 surface domain. sT4 specifically recognizes HIV gp120 in viral lysates and completely inhibits HIV binding to sensitive cells at sT4/gp120 molar ratio of about 4 to 1. This blockade of binding results in a dramatic inhibition of HIV infectivity in vitro (4 logs at an estimated sT4/gp120 molar ratio of 10^3). We are pursuing the HIV recognition site on T4 through mutagenesis of the T4 protein.

J 104 ISOLATION OF A SOLUBLE GLYCOPROTEIN OF INFLUENZA C VIRUS. F. Formanowski and H. Meier-Ewert, Inst. fur Med. Virologie, Techn. Universitat, Biedersteiner Str. 29, D-8 Munchen 40, FRG.
The spike glycoprotein of the Johannesburg/1/66 strain of influenza C virus was isolated by incubating MDCK cell grown virions with the protease bromelain. The whole ectodomain of the glycoprotein was recovered. The spike fragment obtained had an apparent molecular weight of 75,000 and consisted of two subunits with molecular weights of 59,000 and 28,000. The molecular weight of the remaining membrane-bound portion of the molecule was calculated to be 3,000-4,500 daltons. The sedimentation coefficient of the isolated spike in sucrose gradients was 10 S, indicating a molecular weight of 206,000 for the spike trimer. The trimeric form required Ca^{2+} ions for stabilization. Studies of the biological activities of the bromelain-released surface spike showed that the solubilized glycoprotein appears to possess both receptor-binding and receptor-destroying enzyme activities. The RDE activity was found to be a property of each monomer of the trimeric spike, and was also demonstrated for the low pH structure of the glycoprotein which was susceptible to trypsin digestion. In contrast, neither hemagglutination nor hemolysis of chicken erythrocytes could be detected for the isolated glycoprotein.

J 105 STUDIES ON THE SURFACE RECEPTORS FOR INFLUENZA C VIRUS ON A POLARIZED CEL LINE (MDCK CELLS), Georg Herrler and Hans-Dieter Klenk, Philipps-Universitat Marburg, Fed. Rep. Germany.
The distribution of surface receptors for influenza C virus on MDCK I cells was analyzed by infecting filter-grown cells from either the apical or basolateral side. While virus budding was detected only at the apical domain of the plasma membrane, influenza C virus was found to be able to infect MDCK I cells from both sides indicating that virus receptors are present on both the apical and basolateral domain of the plasma membrane. A different subline of these cells (MDCK II), which is lacking receptors for influenza C virus, can be infected after incorporation of bovine brain gangliosides into the plasma membrane. The ability of influenza C virus to infect filter-grown MDCK II cells after incorporation of gangliosides into either the apical or the basolateral domain of the plasma membrane was analyzed. Infection was found to be possible from either side irrespective of the site of ganglioside application indicating that the distribution of the receptor gangliosides is not polarized. A mutant of influenza C virus which is able to infect MDCK II cells in the absence of exogenous gangliosides was isolated and analyzed.

J 106 ENTRY OF MURINE LEUKEMIA VIRUSES AND TRAFFICKING THEIR GLYCOPROTEINS, David Kabat and Brian Gliniak, Biochemistry, Oregon Health Sciences Univ., Portland, OR 97201. [^{125}I]Bolton-Hunter reagent was found to label hemoglobin within erythrocytes, indicating that it is membrane-permeable. It also labeled both the envelope glycoprotein and the internal core proteins of intact AKR-strain ecotropic murine leukemia virus (AKR-MuLV) to high specific activities in mild conditions. The [^{125}I]AKR-MuLV bound specifically to the ecotropic MuLV receptors that occur on murine fibroblasts. Shortly after binding the [^{125}I]AKR-MuLV could be efficiently released from the cells by digestion with trypsin. However, a secondary reaction that had a half-time of approximately 15 min at 37°C converted the viral cores into a form that could not be separated from the cells by trypsin, but left the MuLV envelope glycoprotein accessible to the extracellular protease. This secondary reaction required cellular energy metabolism. These results suggest that binding of AKR-MuLV to ecotropic receptors is rapidly followed by fusion of the viral membranes with the cellular plasma membranes. Thereby, viral cores are transferred into the cytosol and viral envelope constituents are implanted into the plasma membranes. The MuLV envelope glycoproteins are later slowly endocytosed and proteolyzed in lysosomes. A related study concerned the inefficient processing of certain MuLV envelope glycoproteins to plasma membranes. Disulfide bonding heterogeneity was the cause for this inefficiency. Moreover, the improperly folded forms were not repaired by disulfide bond isomerization. Efficiency of proper folding was influenced by specific alterations of glutathione metabolism.

J 107 STRUCTURE-FUNCTION STUDIES OF CD4, Nathaniel R. Landau, Maria Warton, Anne Moriarty and Dan R. Littman. Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, California. We have constructed a panel of cDNA's that encode CD4 proteins whose sequences are hybrids between mouse and human CD4. Because the murine protein does not bind HIV, we are using the "chimeric" cDNA's to localize the regions of CD4 that interact with the HIV env protein, gp120. Cell lines stably expressing the chimeric cDNA's have been established and are being used to determine their ability to bind gp120. CD4-gp120 binding is detected in an in vivo binding assay. In this assay, CD4-expressing cells are incubated with recombinant gp120 and bound gp120 is detected by staining with fluoresceinated anti-gp120 antibody. An in vitro assay for CD4-gp120 binding has also been used to localize binding regions of CD4. In this assay, in vitro translated CD4 proteins are studied for ability to bind gp120 by immunoprecipitation with anti-gp120 antibody. We have, in addition established cell lines that express a truncated, soluble form of CD4 that is capable of binding gp120.

J 108 SPECIFIC AND NON-SPECIFIC BINDING-INHIBITION OF RADIOLABELED HUMAN IMMUNODEFICIENCY VIRUS. David Looney[1], Amanda Fisher[2], Hiroaki Mitsuya[3], Samuel Breder[3], Robert Redfield[1], Donald Burke[1], and Flossie Wong-Staal[2]. [1]Walter Reed Army Institute of Research, Washington, D.C. 20307, [2]Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892, [3]Division of Cancer Therapy, National Cancer Institute, Clinical Oncology Program, Bethesda, MD 20892.
We developed a [5 ^{3}U] uridine labeled virus binding assay to investigate binding inhibition (BI) of human immunodeficiency virus (HIV) by sera, monoclonal antibodies, and polyanionic compounds. Binding of labeled HIV to CD4^{+} lymphocyte cell lines (UO Molt3) was specifically inhibited by OKT4 and Leu3^{+}, but not by OKT4^{-} or OKT8^{+}. Scatchard plot analysis revealed 1000-2000 bound virions/cell, suggesting polyvalent binding to cellular CD4. Only modest titers of BI activity were seen in sera from a number of infected patients compared to normal human sera, and binding inhibitory activity was not found in all sera showing HIV neutralizing activity, suggesting other mechanisms of neutralization of virus infectivity. In addition, we examined the binding inhibitory capacity of several anti-HIV agents, including suramin, dextran sulfate, and a phosphorothioate oligomer. Only dextran sulfate appeared to be an extremely potent inhibitor of HIV binding at therapeutically achievable concentrations.

J 109 BINDING OF REOVIRUS TO ENDOTHELIAL CELLS. E. Maratos-Flier, G.L. King, E.M. Verdin. E.P. Joslin Research Lab, Joslin Diabetes Center and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

The uptake of virus from the bloodstream into specific organs is dependent on the interaction of viral particles with the endothelial cells that line the luminal surface of all vessels. To understand this interaction, we have studied binding of reovirus serotypes 1 (T1) and 3 (T3) to primary cultures of rat fat pad capillary endothelial cells. ^{35}S-methionine labeled virus bound rapidly to cells. Binding was specific and saturable (it could be inhibited by an excess of unlabeled virus). Cellular receptors for virus wave of high affinity ($K_d \neq 0.5$ mM) for both T1 and T3. Using Scatchard analysis, endothelial cells express 68,000 binding sites for T1 and 35,000 for T3. Both serotypes apparently bind to the same receptor since the attachment of each radiolabeled serotype is inhibited by both homologous and heterologous ligand. Pretreatment of labeled virus by monoclonal anti-hemagglutinin antibodies inhibited binding indicating that binding is mediated by the viral hemagglutinin. To identify the cellular protein responsible for binding, we subjected solubilized endothelial cell membrane preparations to SDS-PAGE and transferred the proteins to nylon. Nylon filters were probed with ^{35}S-methionine labeled virus. A single band of 54,000 m.w. could be identified by these transfers. No binding could be detected to membranes boiled prior to SDS-PAGE. Binding of virus to this 54,000 m.w. band was inhibited both by an excess of cold virus and by anti-hemagglutinin Ab. We conclude that the interaction of reovirus to endothelial cells is mediated by a population of high affinity receptors of 54,000 m.w. Further studies to characterize this protein are in progress.

J 110 A RECEPTOR DESTROYING ENZYME (ESTERASE) IS ASSOCIATED WITH BOVINE CORONAVIRUS. Willem Luytjes (1), Reinhard Vlasak (2), Peter Palese (2) and Willy J. M. Spaan (1), University of Utrecht, Netherlands (1) and Mount Sinai School of Medicine, New York, N.Y. 10029 (2).

Bovine coronavirus (BCV), a hemagglutinating virus closely related to human coronavirus OC43 and mouse hepatitis virus (MHV), elutes from chicken red blood cells when incubated at 37 C. This suggests the presence of a receptor destroying enzyme activity. Recently, substantial sequence homology was observed between mRNA 2 of MHV strain A59 and the HE gene of influenza C virus, which codes for receptor destroying (esterase) activity. We therefore examined the possibility that coronaviruses possess a receptor destroying enzyme similar to that of influenza C virus. A cell culture adapted BCV strain (Mebus) was purified and tested for esterase activity. The BCV preparation hydrolyzed p-nitrophenylacetate, a synthetic esterase substrate. Incubation of bovine submaxillary mucin, a natural substrate containing O-acetylated sialic acids, with purified BCV resulted in the release of acetic acid. In addition, since erythrocytes pretreated with BCV can no longer be agglutinated by BCV, we suggest that - as in the case of influenza C virus - the virus associated esterase is a receptor destroying enzyme.

J 111 SPECIFIC LOSS OF A GP70 EPITOPE FOLLOWING IN VIVO PASSAGE OF A MURINE LEUKEMIA VIRUS, J.M. Pozsgay *[2] and K.J. Blank[2], *Biomedical Graduate Group, U. of Pennsylvania School of Medicine, and [2] Dept. of Micro and Immunol, Temple Univ. Philadelphia, Pa.

Changes that occur in the viral genome/gene products following serial passage of a murine leukemia virus in adult mice were examined. Inoculation of adult mice with a virus isolated from a tumor cell line induced by Gross murine leukemia virus (B.K-GV1) results in the loss of a specific antigenic determinant associated with the envelope gene product, gp70, as measured by FACS analysis and specific immunoprecipitation. All biological clones derived from B.K-GV1 express the gp70 epitope as defined by mAb 55, suggesting that the loss of this determinant is the result of a specific selective process in vivo. The m55 negative phenotype is stable following both in vivo and in vitro passage of the virus. Preliminary data from Southern blots of unintegrated viral DNA from B.K-GV1 with different in vivo passage histories show random changes throughout the genome. The specific loss of the m55 epitope by the gp70 molecule suggests that this epitope may play a role in host-virus interaction and may influence susceptibility to murine leukemia virus induced leukemogenesis. A similar selective process may occur in other retroviruses (Visna, EIAV, HIV) in addition to the random genomic recombinants observed.

J 112 CELL INTERACTIONS IN THE INITIATION OF INFLUENZA VIRUS INFECTION OF HUMAN MONONUCLEAR LEUKOCYTES (MNL). Norbert Jr. Roberts, Jr., Denise J. Signs, David J. Mock and Steven F. Scheibel, University of Rochester, Rochester, NY 14642.
Influenza virus (INV) infection of leukocytes may alter the defensive capability of the host or, alternately, may play an important role in the generation of an effective immune defense against the virus. We have shown that monocytes-macrophages are required for INV infection of lymphocytes. Direct cell-cell interactions are required; cell-derived soluble factors do not permit lymphocyte infection. Unlike classic antigen processing and presentation, the macrophage-mediated infection of lymphocytes is not HLA-restricted. The infected lymphocytes can then serve as infectious foci for normal macrophages or other cells, although free infectious viral progeny are not released. Furthermore, the manifestations of infection by the lymphocytes (e.g., viral protein synthesis, or susceptibility to cytotoxic lymphocytes) are strongly influenced by HLA class I determinants (e.g., decreased or absent with A2 or Bw6). To investigate cell subtype susceptibility to macrophage-mediated infection, we next obtained purified populations of helper (Th) and suppressor (Ts) lymphocytes, either before or after exposure to macrophages plus INV, using elutriation, rosetting, and/or panning. Th and Ts lymphocytes were equally susceptible to INV infection in the presence of macrophages, and could serve as infectious foci for INV-permissive cells. Such delineation of the processes of INV infection of human MNL may help determine the mechanisms whereby INV infection commonly resolves without serious sequelae and with emergence of effective homotypic immunity.

J 113 ANTIGENIC ANALYSIS OF A RECENT INFLUENZA A (H1N1) VIRUS. James S. Robertson, Phil J. Yates, Janet S. Bootman. National Institute for Biological Standards and Control, South Mimms, Herts, UK, EN6 3QG.
Antigenic and HA structural differences have been described for influenza viruses derived from the same clinical specimen but passaged either exclusively in MDCK cells or adapted to growth in hens' eggs. We have constructed an operational antigenic map of a 1983 A(H1N1) virus isolated and propagated exclusively in MDCK cells. Three distinct but overlapping antigenic sites could be defined corresponding to the previously defined sites Sa, Sb and Ca1. Variants resistant to a fourth group of monoclonal antibodies could not be derived on MDCK cells until an additional substitution occurred in the HA molecule adjacent to the receptor binding site. This could be achieved by passage of the virus in eggs prior to selection of antigenic variants in eggs or in MDCK cells. These and other analyses indicate the importance of HA1 residue 225 in defining receptor binding site specificity in A(H1N1) viruses.

J 114 RECEPTOR BINDING PROPERTIES OF INFLUENZA A VIRUSES BEARING THE H1 HEMAGGLUTININ, Gary N. Rogers and Bruce L. D'Souza, Department of Pharmacology, University of Iowa, College of Medicine, Iowa City, IA 52242.

The influenza virus hemagglutinin mediates viral adsorption to cells by binding to sialic acid (SA) containing oligosaccharides of cell surface glycoproteins and glycolipids. Examination of the ability of viruses to agglutinate human erythrocytes, enzymatically modified to contain either SAα2,3Galβ1,3GalNAc or SAα2,6Galβ1,4GlcNAc sequences, has revealed that viruses can differ dramatically in their specificity for these sequences. Early observations with human influenza A (H1N1) strains isolated prior to 1956 suggested that, in contrast to human H3 isolates which are quite specific for SAα2,6Gal sequences, these viruses could utilize both SAα2,3Gal and SAα2,6Gal containinig oligosaccharides as receptor determinants. We have recently found, however, that human influenza A (H1N1) strains isolated after 1977 preferentially agglutinate erythrocytes containing SAα2,6Gal sequences. Unlike the human H3 isolates, hemagglutination of native erythrocytes by the recent H1N1 isolates is not inhibited by horse serum. Variants of A/PR/8/34 (H1N1) specific for either SAα2,3Gal or SAα2,6Gal sequences have been isolated and their receptor binding properties will be described. (Supported by USPHS AI-25181.)

J 115 VIRAL DETERMINANTS OF THE CYTOTOXIC T-LYMPHOCYTE RESPONSE, Maria S. Salvato, Elaine Shimomaye, Karl Schweighofer and Michael B.A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA 92037.
Lymphocytic choriomeningitis virus (LCMV) has long been studied for its ability to elicit a cytotoxic T-lymphocyte (CTL) response upon primary infection of a murine host. We have isolated and characterized variants of LCMV which fail to elicit a CTL response and thereby persist. We have compared the genomic sequence of two variants of LCMV Armstrong, one which elicits the CTL response (CTL^+) and one which suppresses it (CTL^-). The genome of LCMV contains two single stranded RNA segments; two glycoproteins and a nucleoprotein are encoded on the small (S) segment, and a large protein with homology to other viral polymerases on the large (L) RNA segment. The CTL^- phenotype maps to the L RNA segment of LCMV within the gene for the putative viral polymerase. We have devised a model for the function of this gene product and speculate on its role in viral persistence.

J 116 THE EFFECTS OF LYSOSOMOTROPIC AGENTS ON THE INTRACELLULAR PROCESSING OF HIV-1 gag (p24) AND ENVELOPE (gp120) PROTEINS IN CD4+ HUMAN T LYMPHOBLASTIC CELLS, Barry S. Stein and Edgar G. Engleman, Stanford University School of Medicine, Blood Center, Palo Alto, CA 94304.
Human lymphoblastic CD4+ T cells (VB) acutely infected with HIV-1 were treated with various lysosomotropic agents including 500 uM chloroquine, 20 mM ammonium chloride, and 20 uM monensin, doses sufficient to neutralize acidic compartments of the cell, and concomitantly metabolically radiolabeled. Immunoprecipitation of both envelope (gp160 and gp120) and gag (p24) determinants were performed followed by SDS-PAGE/fluorography. gp160, gp120, and p24 were all clearly demonstrable in cells treated with chloroquine and ammonium chloride; although a significant loss of signal was observed with chloroquine treated cells (compared to the control) secondary to the inhibitory effects of this agent on protein synthesis. However, monensin did dramatically alter viral envelope processing resulting in a reduction of the molecular weight of gp120 to gp100; gp160 and p24 remained unchanged. These data indicate that acidic compartments of the cell do not play a major role in the proteolytic cleavage of gp160 to gp120 and gp41 or in the cleavage of gag precursors (p55) to p24. The disruptive effects of monensin on gp120 processing can be explained on the basis that this agent is a carboxylic acid ionophore which in addition to being a potent lysosomotrope, profoundly disrupts glycosylation and transport functions of the Golgi apparatus. Preliminary evidence suggests that monensin inhibits trans Golgi processing of the cleaved precursor of gp120 derived from gp160, resulting in a gp100 species with deficient sialylation.

J 117 EXPRESSION OF THE MHV RECEPTOR IS REQUIRED FOR SUSCEPTIBILITY TO MHV INFECTION, Charles B. Stephensen, Susan R. Compton, Christine B. Cardellichio, Richard K. Williams and Kathryn V. Holmes, Pathology Dept., Uniformed Services University of the Health Sciences, Bethesda, MD 20814
The receptor for mouse hepatitis virus, a murine coronavirus, is a 110K glycoprotein. In a virus overlay protein blot assay (VOPBA), MHV bound to a single 110K protein of intestinal brush border membranes from susceptible Balb/c mice (1). The MHV receptor can also be detected in Western blots using polyclonal or monoclonal anti-receptor antibody. This receptor is the only mechanism for entry of MHV into susceptible murine fibroblasts, since a monoclonal antibody, CC1, completely inhibits virus infection. To date, all mouse strains tested express the MHV receptor on their enterocyte and hepatocyte membranes, except SJL/J mice which are profoundly resistant to MHV infection. Cell lines of other species do not express the MHV receptor and are not infectable with MHV. In addition, intestinal brush border membranes from many other species of animals fail to express the MHV receptor as detected by VOPBA or Western blot with polyclonal or monoclonal antibody. Thus, the species specificity of MHV binding is determined by expression of the MHV receptor glycoprotein.
(1) Boyle, J. F., Weismiller, D. G., and Holmes, K. V., J. Virol. 61: 185-189 (1987).

J 118 HIV gp 120/160 INDUCES INTERFERENCE AND SUPERINFECTION RESISTANCE IN CYTOLYSIS SENSITIVE $CD4^+$ CELLS, Mario Stevenson, Craig Meier, Angela Mann, Nora Chapman, Andrzej Wasiak, University of Nebraska Medical Center, Omaha, NE 68105. Masking of host cell receptors following retroviral infection is the basis for the phenomenon of virus interference. Interference involves an interaction of the viral envelope glycoprotein with its corresponding host cell receptor and typically leads to the establishment of superinfection resistance in a number of retroviruses. A similar property also appears to be possessed by the human immunodeficiency virus (HIV). In certain cell lines, resistance to HIV mediated cytolytic effects and the initiation of a persistent viral infection appears to correlate with the loss of expression of the host T4 receptor. We investigated the ability of the HIV envelope gene to induce interference and superinfection resistance in a cytolysis sensitive T cell line. Expression of the HIV envelope gene in CD4 positive CEM Cells is accompanied by a 60% reduction in expression of surface T4 receptor and correlates with the presence of intracellular HIV/envelope-T4 receptor complexes. CEM cells expressing the HIV envelope gene acquire a cytolysis resistant phenotype such that infection of these cells with HIV leads to a persistent infection and a reduced release of progeny virus rather than a cytolytic infection which appears in control cultures. TPA mediated induction of virus replication in persistently infected cells results in renewed cytolytic effects which are independent of syncytium formation. We propose that envelope mediated interference and acquisition of superinfection resistance in $CD4^+$ lymphocytes provides the basis for viral persistence in the course of AIDS.

J 119 THE EPITOPE MAPPING OF THE FUSION PROTEIN OF THE MEASLES VIRUS. José P. Versteeg-van Oosten*, Simon A. Langeveld*, Petra de Vries#. Ad D. Osterhaus#, Peter J. Weisbeek*. *State University of Utrecht, Department of Molecular Cell Biology, #National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.
The aim of our research is the production of a subunit vaccin against the measles virus. The fusion protein is one of the two glycoproteins of the measles virus and appears to be important for the induction of the immune respons. It is therefore important to know the immunologically active sites (epitopes) of the fusion protein. We localized the epitopes with the aid of a cDNA clone containing the complete F-gene and mouse monoclonal antibodies raised against the fusion protein.
We have inserted well-defined 3′ and 5′ deletions of the F-gene into the transcription vector pSPT18, which contains the promotors for T7 and SP6 polymerase. With T7 polymerase we produced in vitro RNA, which was translated in the wheatgerm in vitro translation system into radioactive protein fragments. These fragments were immune-precipitated with different monoclonal antibodies and made vissible on a polyacrylamide gel. By comparing the precipitation of the different deletions it is possible to map continous epitopes on the protein. We localized three epitopes this way.

J 120 *AFRICAN SWINE FEVER VIRUS RECEPTOR AND ATTACHMENT PROTEIN*, E. Vinuela, A. Alcami and A. L. Carrascosa, Centro de Biologia Molecular (CSIC-UAM), Madrid, Spain. African swine fever (ASF) virus is an enveloped deoxyvirus which does not induce the synthesis of neutralizing antibodies in the infected animal. The virus shows a strict host range and cell tropism, since natural infections are confined to porcine species and virus replication takes place mainly in mononuclear phagocytes.

The morphological data obtained by electron microscopy and the sensitivity of the infection to lysosomotropic drugs suggest that ASF virus adapted to VERO cells enter into virus-producing cells (VERO cells and swine macrophages) by a receptor-mediated endocytosis mechanism. Binding studies of ^{3}H-ASF virus and competition experiments with UV-inactivated virus have shown that the virus entry that leads to a productive infection is mediated by saturable binding sites on the plasma membrane of susceptible cells. In nonproducing cells (L cells and rabbit macrophages) virus-cell interaction was mediated by nonsaturable binding sites. As a result of the nonsaturable interaction, the virus was not able to enter into L cells and led to an abortive infection in rabbit macrophages, suggesting that the presence of specific receptors is a factor that determines the cellular susceptibility to ASF virus.

The presence of cellular receptors that mediate ASF virus binding to the cell involves the existence of a virus attachment protein in the virus particle and suggests that ASF virus could be neutralized by blocking the interaction of the virion with the virus receptors.

When ^{35}S-labeled ASF virus surface proteins were incubated with ASF virus-sensitive VERO or nonsensitive L or IBRS cells, we found a specific binding to the susceptible cell monolayers similar to that obtained with the whole ASF virus. Moreover, the electrophoretic analysis of the material associated to the cells either at 37°C or 4°C, showed that protein p12 was bound to VERO but not to nonsensitive cells. The binding of p12 or intacct virions to sensitive cells was specifically competed by unlabeled ASF virus. These results suggest that protein p12 is the ASF virus attachment protein.

J 121 CHARACTERIZATION OF THE CELL MEMBRANE RECEPTOR FOR CORONAVIRUS MHV, Richard K. Williams, John F. Boyle, Stuart Snyder, Christine B. Cardellichio, Mark F. Frana and Kathryn V. Holmes, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814
A 110K protein identified as the cellular receptor for mouse hepatitis virus (MHV) is found on membranes of enterocytes and hepatocytes from susceptible Balb/c mice (1). The MHV receptor contains N-linked oligosaccharides and has a pI of about 3.5. Studies with detergents show that the virus binds to a linear domain of the receptor. Removal of oligosaccharides from the receptor by endoglycosidase F does not destroy virus binding activity. Thus, a linear amino acid sequence of the receptor may be the site of virus attachment. In contrast, SDS denaturation of the E2 viral glycoprotein abolished its ability to bind to the MHV receptor. The MHV receptor is a minor component of the cell membrane, consisting of <0.1% of total membrane protein. A monoclonal antibody directed against the receptor has been used for affinity purification of the receptor from detergent solubilized membranes of mouse liver. This affinity purified receptor protein will be useful in identifying the specific residues of the receptor which interact with the E2 glycoprotein of MHV.
(1) Boyle, J. F., Weismiller, D. G., and Holmes, K. V., J. Virol. 61: 185-189 (1987).

J 122 CELL SURFACE RECEPTOR FOR HERPES SIMPLEX VIRUS IS HEPARAN SULFATE, Darrell WuDunn[1] and Patricia G. Spear[2], Univ. of Chicago[1], Chicago, IL 60637 and Northwestern Univ. Medical School[2], Chicago, IL 60611. Heparin, a highly sulfated glycosaminoglycan, has long been known to inhibit HSV infection. This led us to investigate the possibility that cell surface heparan sulfate moieties are primary receptors for HSV. We found that heparin blocks infection by blocking the adsorption of virions to cells (human HEp-2 cells) and that virions bind to heparin-Sepharose, indicating that heparin interacts with the virus anti-receptor. Agents known to bind to cell surface heparan sulfate, such as poly-L-lysine and platelet factor 4, were shown to modify the cells so as to prevent virion adsorption, also preventing infection. Finally, enzymatic digestion of cell surface heparan sulfate, but not of dermatan sulfate or chondroitin sulfate, rendered the cells resistant to infection and reduced the number of viral receptors.
Previous studies indicate that the cell receptors for HSV-1 and HSV-2 are different. Our results show that the receptors for both serotypes are heparan sulfate glycosaminoglycans but that different structural features of heparan sulfate may be recognized by HSV-1 and HSV-2. Consistent with their broad host ranges, HSV-1 and HSV-2 use as their primary receptors ubiquitous cell surface components known to participate in interactions with the extracellular matrix and with other cell surfaces.

J 123 NUCLEOTIDE SEQUENCE OF VACCINE STRAIN OF RUBELLA VIRUS (HPV77) 24S RNA AND COMPARISON WITH WILD TYPE STRAIN (M33), Dexian Zheng, Lillian Dickens, Teh-Yung Liu and Hira L. Nakhasi, Food and Drug Administration, Bethesda, MD 20892.
To understand the mechanism of attenuation in vaccine strain of rubella virus we have undertaken studies to elucidate the structure of vaccine strain of rubella virus, compare with the parent wild type strain and to find out what modifications have occured which may be responsible for the attenuation process. Vaccine strain (HPV77) of rubella virus replicates slowly in Vero 76 cells as compared to wild type strain which manifests in delayed appearance of cytopathic effect. Comparison of the structural proteins synthesized by two strains of rubella virus in the cell shows that the E2 envelope protein of vaccine strain is about 11KDa smaller than E2 protein of wild type strain. To analyze this difference and other differences, a full length cDNA clone for 24s RNA of vaccine strain was isolated from cDNA library constructed from infected cell RNA and nucleotide sequence was determined. Comparison of the sequence with that of wild type progenitor indicates that attenuation has brought about 13 point mutations in the coding region which are scattered throughout the 24s RNA. Five out of 13 mutations result in change in amino acids, of which 2 are in the capsid protein, 3 in the E2 protein, and none in E1 protein. In addition there are 4 point mutations in the 3'-noncoding region. Whether the point mutations observed in 24s RNA are responsible for attenuated phenotype is not yet known, but it is likely that one or more of these 5 coding changes are involved.

Cell Biology of Virus Entry, Replication and Pathogenesis

Virus Entry and Persistence

J 200 MECHANISM OF INHIBITION OF HVJ ENTRY BY PEPTIDE DERIVATIVES, Akira Asano and Kimiko Asano, Sapporo Medical College, Sapporo 060 & College of Medical Technology, Kyoto Univ., Kyoto 606, Japan.

Several peptide derivatives having some homology with N-terminal portion of F_1 of paramyxovridae, i.e. putative fusogenic segment, is reported to be inhibitory to infection of some strains of paramyxoviruses (such as measles and canine distemper). We recently found that prolonged incubation upto 2 h with inhibitory peptides at 30-37° C is required for full expression of their inhibitory activity on HVJ-induced hemolysis. Furthermore, preincubation of these peptide derivatives with virions but not with cells was found effective for the inhibition. Although some carbobenzoxy-derivatives of N-terminal homologues, such as Z-D-Phe-Phe-Gly, Z-Phe-Tyr and Z-Phe, were inhibitory, no appreciable inhibition was detected with peptides having different spacing between benzene rings, such as Z-Gly-Phe and Z-Glu-Phe. Thus, aromatic-aromatic interaction between inhibitory peptide derivatives and N-terminal fusogenic sequence containing Phe-Phe-Gly was suspected.

To check this hypothesis, we prepared ^{125}I-labeled Z-Phe-Tyr and measured binding of the label to purified F- and HN-proteins. We also used thermolysin-truncated F-protein which was also used for cholesterol-binding studies as a inactive control. Labeled peptide derivatives were found to bind to intact F-protein but not to HN- and thermolysin-truncated F-proteins. Furthermore, cholesterol binding of F-protein was inhibited by the addition of Z-Phe-Tyr and Z-D-Phe-Phe-Gly. Therefore, the inhibitory peptide derivatives seems to inhibit the fusion reaction by binding to the fusogenic portion of F-protein and thus inhibiting cholesterol binding.

J 201 SPECIFIC BINDING OF CHOLESTEROL TO F-PROTEIN OF HVJ (SENDAI VIRUS): IMPLICATION IN THE VIRUS ENTRY, Kimiko Asano and A. Asano, College of Medical Technology, Kyoto Univ., Kyoto 606 & Sapporo Medical College, Sapporo 060, JAPAN

Active form of F-protein is required for fusion of the viral envelope with cell membranes, and by this process the virus genome enters target cells. A notion that N-terminal segment of F_1 subunit is the membrane penatrating portion is well accepted from its hydrophobic nature. But direct demonstration of this hypothesis is missing.

We have previously shown with limited proteolysis and iodination studies that this putative fusogenic sequence of F_1 is exposed on the surface of the protein despite of its hydrophobic nature. Furthermore, close contact of F-protein with target cell membranes at agglutination stage (ice-cold condition) was shown by use of a lipid-soluble photoaffinity label, TID. Next and the most important question is how the fusogenic sequence induces membrane perturbation which may be the prerequisite for membrane fusion. We found recently that F-protein binds cholesterol rather specifically in substantial amounts. Furthermore, site of cholesterol binding was shown to be the fusogenic N-terminal segemnt of F_1 by using thermolysin-truncated F-protein (which lost about 2,500 dalton segment from N-terminal of F_1). Specificity of sterol binding to F-protein was studied by binding-competition with several sterol and other lipidic compounds. Cold cholesterol, ergosterol and A/B-trans sterols tested were active for competition with ^{3}H-cholesterol, whereas a A/B-cis sterol, squalene and phosphatidylethanolamine were not competitive. Rate of cholesterol binding was found to be very rapid being completed within 2 min. Thus, cholesterol binding of F-protein seems to be a important step of the viral entry to target cells.

J 202 MECHANISMS OF MEMBRANE FUSION MEDIATED BY VIRAL SPIKE GLYCOPROTEINS, Robert Blumenthal, Debi Prasad Sarkar and Anu Puri, NIH, Bethesda, Md. 20892

We studied fusion between membranes of VSV and Vero cells using an assay for lipid mixing based on the relief of self-quenching of octadecylrhodamine (R18) fluorescence. We could identify the two pathways of entry by the kinetics of R18 dequenching, effects of inhibitors, temperature dependence, and dependence on osmotic pressure. The pH profile of Vero-VSV fusion at the plasma membrane as measured by the dequenching method, paralleled that observed for VSV-induced cell-cell fusion. A simple model for membrane fusion mediated by viral spike glycoproteins is presented. The viral proteins are considered to be allosteric proteins which undergo concerted conformational transitions when they bind the ligand. The effect of the conformational transition is to bring membranes together and induce their fusion. An equation is derived for the dependence of fusion rates on ligand concentration, for a given dissociation constant (K_d), equilibrium constant for the conformational change (L), and number of cooperating subunits (n). Curves generated by this equation provide a reasonable fit to data on the rates of fusion of Vesicular Stomatitis virus with cells for a pK_d of 6.3, L=1000 and n=6. To examine the role of the target membrane we studied fusion between membranes of Sendai virus and human erythrocytes ghosts (HEG), or liposomes, containing the appropriate receptors. The same receptor, Gd1a, inserted into asialo-HEG induced fusion at a 30-fold faster rate then when inserted into liposomes composed of phosphatidylcholine and cholesterol. Why the biological membranes are much better targets for viral fusion than the liposomes containing the appropriate receptors still remains to be explored.

J 203 PRONOUNCED TRANSCRIPTION ATTENUATION AND MUTATIONS CAN CAUSE DEFECTIVE MEASLES VIRUS GENE EXPRESSION IN DISEASED HUMAN BRAINS

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In rare cases measles virus (MV) induces subacute sclerosing panencephalitis (SSPE) or measles inclusion body encephalitis (MIBE), two lethal diseases of the human central nervous system. In SSPE and MIBE brain autopsies MV transcripts were analysed by quantitative Northern blots. Transcripts from the first MV gene were surprisingly abundant, amounting to about one-tenth of that in lytically infected cells. However, the quantity of transcripts decreased sharply for each subsequent MV gene. This resulted in gradients of transcript levels much steeper than in lytically or persistently infected cultured cells. These altered ratios of mRNAs most likely lead to reduced expression of the viral envelope proteins, encoded by distal MV genes, at the surface of brain cells (Cattaneo et al. (1987) *Virology* **160**, 523-526). Furthermore full length copies of MV genes from these brain autopsies were cloned. From these cDNAs synthetic mRNAs were produced and translated *in vitro*. Whereas for the nucleocapsid proteins only minor differences in electrophoretic mobility were observed, for one matrix protein an important size alteration was monitored. The sequence of the corresponding gene disclosed mutations in the translation initiation and termination codons, as well as many other U to C transitions. Thus, in addition to the generally occuring pronounced transcription attenuation, multiple mutations can also cause defective MV gene expression in SSPE and MIBE. Defective MV gene expression can account for the lack of viral budding observed in infected brains, and could allow persistent MV infections to elude immune surveillance by reducing or supressing exposure of viral envelope proteins at the cell surface.

J 204 ENTRY OF SV40 IS RESTRICTED TO APICAL SURFACES OF POLARIZED EPITHELIAL CELLS.

Edward T. Clayson and Richard W. Compans, University of Alabama at Birmingham, Birmingham, Alabama 35294

The entry of SV40 virions was investigated in polarized Vero C1008 and primary AGMK cells. Cells were grown on nitrocellulose filters and inoculated either on the apical or basolateral surface to determine if adsorption and penetration could occur on either or both surfaces. Electron micrographs of polarized Vero C1008 cells infected at high multiplicities revealed SV40 virions lining the surfaces of apically infected cells at 15 minutes post infection. In contrast, the surfaces of basolaterally infected cells were devoid of virus particles, suggesting that virus binding is restricted to apical surfaces. Indirect immunofluorescence studies revealed that productive infection occurs in polarized Vero C1008 cells only when virions have access to the apical surface. Virus yields from cells infected apically or basolaterally were titered by plaque assays. Yields from apically infected polarized cells were 10^2-10^3 times greater than yields obtained from basolaterally infected polarized cells. When similar experiments were performed on nonpolarized Vero or CV-1 cell lines, productive infection occurred irrespective of the direction of virus input. These results indicate that a specific receptor for SV40 is expressed exclusively on the apical surfaces of polarized epitheial cell lines.

J 205 **EARLY EVENTS IN THE ESTABLISHMENT OF PRODUCTIVE AUTONOMOUS PARVOVIRAL INFECTION**

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Fibrotropic and lymphotropic strains of minute virus of mice (MVM) are reciprocally restricted for the initiation of viral transcription in each other's normal host cell. The region responsible for the ability of the fibrotropic strain to turn on its genes in fibroblasts, maps to a small hypervariable region of the capsid gene. Extended host range mutations also map to this small region, and involve changes in the primary sequence of the coat protein. Comparison natural infection with plasmid-mediated transfection shows that the establishment of productive transcription patterns in differentiated cells is particle-mediated. The first gene product to be expressed is the 83kd major non-structural protein NS-1, a nuclear phosphoprotein which is expressed early in infection, and which is intimately involved in viral DNA replication. Although, over time, a minor fraction is processed to a 65kd polypeptide, presumably by proteolytic cleavage, NS-1 is a relatively stable polypeptide. A small subset of NS-1 molecules can be detected covalently bound to each 5' end of both viral monomer and dimer RF DNA, and also to pulse-labeled single-stranded progeny DNA. Soon after infection of permissive cells trans-activation of expression from the P38 promoter occurs. Directed mutagenesis of sequences coding for NS-1, and for NS-2, a 24kd, transiently-expressed cytoplasmic protein, shows that both of these products of P4 transcription are required for P38 trans-activation. Trans-activation is not detected during restrictive infection, and expression from either promoter of a virion-delivered, but restricted, genome cannot be rescued in trans by expression from a co-infecting, non-restricted virus. Initiation of productive infection, therefore, appears to require an interaction between the incoming virion and a differentiation-specific intra-nuclear "receptor", for the correct activation of P4- and P38-directed viral transcription.

J 206 FUSION AND NEUTRALIZATION SITES ON VISNA VIRUS ARE SEPARATE EPITOPES, Sharon Crane and Janice Clements, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Lentiviruses are non-oncogenic retroviruses which cause slowly evolving multi-organ diseases. Visna virus, a lentivirus of sheep, causes extensive fusion in cell cultures both without virus replication (fusion from without) as well as during the replication of the virus (fusion from within). Visna virus has been studied to determine the location of the fusion epitope in the viral envelope and to determine if the fusion and neutralization epitopes were distinct. Using a combination of strain specific polyclonal antibodies and antigenically distinct strains of visna virus, the viral neutralization and fusion epitopes can be distinguished. Further, the data suggest that while the neutralization epitope mutates rapidly during natural infection, the fusion epitope appears to be conserved.

J 207 THE SUBCELLULAR ROUTING OF RHINOVIRUS IN HELA CELLS INVESTIGATED WITH NEW VIDEO MICROSCOPIC TECHNIQUES AND WITH NEW INHIBITORS OF UNCOATING, F. Aerts, M. De Brabander, G. Geuens, R. Nuydens, B. Dewindt, K. Andries, Div. Cellular Biology & Chemotherapy, Janssen Research Foundation, B-2340 Beerse, Belgium.
The route by which RV enter cells and the way in which the RNA is released into the cytoplasm are still enigmatic. We are using a combination of several novel approaches to gain insight.
We prepared neutralizing and non-neutralizing Mabs against RV 9. Hela cells were incubated with RV 9 and fixed at various times. The location of the RV 9 antigen was localised at high resolution in whole cells using 1 nm gold particles followed by silver enhancement and detection with video contrast enhancement. In addition labelling was performed on ultrathin frozen sections. Our first data show that RV 9 entered cells by endocytosis into small vesicles that fused within ~ 15 minutes into larger endosomes which eventually became concentrated at the cytocentre. R 61 837 a new compound that binds to RV 9 and inhibits infection did not inhibit binding or endocytosis of RV 9. However, the fusion of small endosomes into larger ones was clearly arrested. The antigen remained distributed in small vesicles and eventually disappeared totally after ± 8 hr. Previous data suggest that R 61 837 inhibits uncoating of RV 9 by binding to a hydrophobic site. Our observations suggest that exposure of the hydrophobic site which is induced by a fall in pH inside endosomes and which is blocked by R 61 837, induces fusion of early into late endosomes and further that the viral RNA may be released from these late endosomes.

J 208 EXPRESSION OF RETROVIRAL ENVELOPE GENES AND THEIR POSSIBLE EFFECT ON VIRAL INFECTION. Mark J. Federspiel, Stephen H. Hughes* and Lyman B. Crittenden, USDA-ARS Regional Poultry Research Lab, East Lansing, MI 48823;* NCI-Frederick Cancer Research Facility, P.O.Box B, Frederick, MD 21701.

Experiments have been initiated to examine the in vitro expression of retroviral envelope genes and the interactions between envelope proteins and virus infection. Earlier work has shown that resistance to avian leukosis virus (ALV) infection was conferred by a defective inserted ALV that expressed only the envelope gene presumably because the envelope glycoproteins interfere with viral attachment and penetration. Envelope genes from bovine leukemia virus (BLV) and two isolates of reticuloendotheliosis virus (REV), spleen necrosis virus (SNV) and REV-A, are currently under study. These envelope genes have been cloned into the plasmid expression vector 779-NEO which consists of a cloning site between two Rous sarcoma virus LTR's and neo with a beta-actin promotor. D17 cells, a canine cell line derived from an osteosarcoma, will be transfected with the BLV and REV envelope constructs and G418-resistant cell lines established. Data will be presented on the expression and cellular location of envelope proteins in the cell lines generated as analyzed by flourescent antibody staining and gel electrophoresis. Envelope protein producing cell lines will be challenged with virus to assay possible envelope protein and virus interactions.

J 209 SPECIFIC INFECTION OF CENTRAL NERVIOUS SYSTEM WHITE MATTER BY A MURINE RETROVIRUS, Glen N. Gaulton*, Louis A. Rosenthal+, Willaim F. Hickey*, Kenneth J. Blank+ and Nancy A. Simonian*, *University of Pennsylvania and +Temple University, Philadelphia, PA 19104. The retroviruses human immunodeficiency virus and human T-lymphotrophic virus type I have been linked to neurological disease, however, the tropism and pathogenetic mechanisms of this class of virus in the central nervous system is poorly understood. In this study we describe a murine model of retroviral CNS infection using a variant of Gross Passage A murine leukemia virus. Intracerebral inoculation of neonatal mice resulted in a selective infection of CNS white matter in the cerebral hemispheres. Replication of virus in constituent glial cells in the CNS was verified in in vitro cultures. Virally treated glial cells were positive for reverse transcriptase activity, and expression of the retrovirus envelope glycoprotein gp70 as determined by microfluorometric analysis. Gp70 expression was most apparent in the oligodendrocyte component, however, expression in astrocytes or microglia cells could not be ruled out. These results establish a novel system for analysis of retrovirus disease of the CNS.

J 210 LOW pH INDUCED CELL FUSION BY ENVELOPE GLYCOPROTEINS OF HSV, H.P. Ghosh, M. Butcher, N. Ghosh-Chowdhury, L. Rasile and K. Raviprakas, Dept. Biochem., McMaster University, Hamilton, Ont., Canada, L8N 3Z5. Enveloped animal viruses enter host cells by either direct fusion with the plasma membrane at neutral pH or by internalization via enocytosis into acidic endosomes where fusion between the viral envelope and the endosomal membranes are induced by the acidic pH. Herpes simplex virus (HSV-1) is believed to enter cells by direct fusion at the cell surface. The glycoproteins gB, gC and gD of HSV-1 have been implicated in the viral entry and fusion process. We have previously shown that the glycoprotein gB expressed from cloned gene can induce cell fusion under acidic condition in the absence of other viral gene products (PNAS, 84, 5675 (1987)). Cells expressing gC-1 and gD-1 proteins were also observed to undergo fusion to form polykaryons by exposure to acidic pH. Low pH induced fusion was also observed in permanent cell lines expressing gB or gC. The cell fusion was specifically inhibited by monoclonal antibodies. Infection of cells expressing gB glycoproteins with HSV-1 inhibited the low pH induced cell fusion suggesting that the fusion process is regulated by expression of other viral gene products. Studies involving site directed mutagenesis of the glycoprotein genes are in progress to determine the roles of the various domains of the glycoprotein in the fusion process. Supported by Medical Research Council and National Cancer Institute of Canada.

J 211 IDENTIFICATION OF THE MEMBRANE-INTERACTING POLYPEPTIDE OF THE BROMELAIN-SOLUBILIZED ECTODOMAIN OF INFLUENZA VIRUS HEMAGGLUTININ BY HYDROPHOBIC PHOTOLABELING.
C. Harter, T. Bächi, G. Semenza and J. Brunner, Laboratorium für Biochemie, ETH Zürich and Institut für Virologie und Immunologie, Universität Zürich, Switzerland.
The bromelain-solubilized ectodomain of influenza virus hemagglutinin (BHA) has been shown previously to bind to membranes at low pH. To investigate the molecular basis of this interaction, which possibly represents a fundamental step in a variety of biological fusion reactions, we applied the technique of hydrophobic photolabeling using two different photoactivatable probes. Photolysis of BHA in the presence of liposomes containing [^{125}I]TID, a small, lipid-soluble, photosensitive molecule, resulted in label incorporation predominantly in the BHA2 subunit under both neutral and acidic conditions. Although labeling at pH 5 was about two times stronger than at pH 7, the clear labeling of BHA2 at neutral pH may reflect the presence of a hydrophobic pocket in that subunit. In contrast to the results found with [^{125}I]TID, photolabeling with the new phospholipid [^{3}H]PTPC/11, whose distribution is confined to the lipid core of a membrane, resulted in label incorporation in the BHA2 subunit in a strictly pH-dependent manner, thus providing direct evidence that the low pH-induced interaction of BHA with membranes is mediated by this subunit. Protein chemical degradation of BHA2 is now being used to identify this (those) polypeptide segment(s) which is (are) involved in the membrane interaction.

J 212 FUNCTIONAL ANALYSIS AND GENE MAPPING OF EBV GP85, Lindsey Hutt-Fletcher, Nancimae Miller and Douglas Oba, University of Florida, Gainesville FL 32610. Epstein-Barr virus (EBV) codes for three major glycoproteins exposed on the surface of the virion. The two largest, gp350 and gp220, are encoded by the same differentially spliced gene and are thought to be the virus attachment proteins. Less is known about the third molecule, gp85, which has been characterized biochemically by use of the neutralizing antibody F-2-1. As part of a long term goal to understand the role of gp85 in virus infectivity, we set out to identify the gene that encodes it and determine at what point the antibody F-2-1 interferes with virus replication. Analysis of the EBV DNA sequence identified the BXLF2 open reading frame as potentially coding for a molecule similar to gp85. Antibody was therefore made to a seventeen residue peptide derived from the BXLF2 sequence and its reactivity compared with that of F-2-1. Both antibodies immunoprecipitated glycosylated molecules with identical electrophoretic mobilities, digestion of the two immunoprecipitated proteins with V8 protease generated comparable peptides and the anti-peptide antibody reacted in Western blots with the molecule immunoprecipitated by F-2-1. In addition, a monospecific antibody made against native gp85 reacted with the peptide used for immunization; these results suggested that the BXLF2 reading frame codes for gp85. Antibody F-2-1 had no effect on the ability of EBV to bind to B cells. We therefore adapted the fusion assay of Hoekstra et al. (Biochemistry 23:5675, 1984) to measure entry of EBV into either lymphoblastoid cell lines or normal peripheral B cells. Antibody F-2-1, but not a non-neutralizing antibody to gp85, interfered with virus penetration, indicating that gp85 may play an active role in this process.

J 213 ISOLATION OF AN HTLV-1 LIKE RETROVIRUS FROM PERIPHERAL BLOOD AND CEREBROSPINAL FLUID OF PATIENTS WITH TROPICAL SPASTIC PARAPARESIS. [1]Steven Jacobson, [2]Cedric S. Raine, [1]Elizabeth S. Mingioli, and [1]Dale E. McFarlin, [1]Neuroimmunology Branch, NINCDS, NIH, Bethesda, MD 20892, [2]Neuropathology Division, Albert Einstein College of Medicine, The Bronx, NY 10461. Tropical spastic paraparesis (TSP) is a slowly progressive myelopathy associated with increased serum and cerebrospinal fluid (CSF) antibody to the human T-lymphotropic retrovirus type I (HTLV-1). Complete virus has been consistently difficult to isolate from patients with TSP using techniques that have successfully cultured and isolated HTLV-1 from patients with adult T cell leukemia. Here we present the isolation of an HTLV-1 like virus from 12 T cell lines derived from the peripheral blood and CSF of 6 TSP patients. Infectious virus was demonstrated by co-culturing techniques and complete, replicating intact virions were visualized ultrastructurally. Non-transformed T cell lines that express HTLV-1 antigens were generated. Additional biological differences between the TSP viral isolates and conventional HTLV-1 have been identified. Infection of a $CD4^+$ HLA class II restricted cytotoxic T cell line specific for influenza virus by co-cultivation with a conventional HTLV-1 infected T cell line (Hut 102) inhibited the cytotoxic function. In contrast, no inhibition was observed when the same influenza T cell line was co-cultured with the PBL T cell line of the TSP patient, even though comparably infected.

J 214 HUMAN AND MOUSE CELLS EXPRESSING HERPES SIMPLEX VIRUS (HSV) TYPE 1 GLYCOPROTEIN D ARE RESISTANT TO INFECTION BY HSV, Ray Johnson[1] and Patricia G. Spear[2], Univ. of Chicago[1], Chicago, IL 60637 and Northwestern Univ. Medical School[2], Chicago, IL 60611. Human HEp-2 cells and mouse LMTK- cells were transfected with plasmids containing a selectable marker and the coding region for HSV-1 glycoprotein D (gD-1) under control of the human metallothionein promoter. Stable transformants expressing variable levels of gD-1 were isolated. For most cell lines the amount of gD-1 detected by Western blotting or FACS analysis could be increased by Cd++ or Zn++ induction. Human or mouse cells expressing sufficiently high levels of gD-1 were resistant to HSV infection, probably due to a block in viral penetration. No quantitative differences were observed in the adsorption of radiolabeled virus to resistant and control cells. Exposure of resistant cells to virus did not result in synthesis of viral proteins or in inhibition of cell protein synthesis. Because this inhibition is known to be caused by a virion component, early inhibition of cell protein synthesis can be an indicator of viral penetration. Resistance of the cells to virus infection was not absolute and could be overcome by exposure of the cells to high multiplicities of virus. The cells appeared to be somewhat less resistant to HSV-2 than to HSV-1, suggesting some serotype specificity in the phenomenon.

Investigations of the molecular basis of this phenomenon, reminiscent of retrovirus interference, should shed light on the process of HSV penetration.

J 215 EFFECT OF LYSOSOMOTROPIC AGENTS ON THE ENTRY OF VACCINIA VIRUS INTO CV-1 CELLS,
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Vaccinia virus (VV), a member of the genus Orthopoxvirus, is a large DNA virus enveloped by lipoprotein. Although the host range of VV is very wide, the mechanism of virus entry has not been elucidated. The observation that infection with some, though not all, strains of VV can lead to the spontaneous fusion of the plasma membranes of the host cells, led us to investigate if membrane fusion plays any role in the infectious entry of this virus. Different strains of VV display varying phenotypes with respect to membrane fusion activity. The IHD-W strain causes spontaneous cell-to-cell fusion during infection under normal, neutral pH medium. The WR strain does not lead to spontaneous polykaryon formation, but brief acidic treatment (<pH 6.4) of infected cells induces extensive syncitia formation. Finally, polykaryon formation of cells infected with the IHD-J strain has not been observed even after low pH (pH 5.0) treatment. Lysosomotropic agents (NH_4Cl, methylamine and chloroquine) inhibited plaque formation by all 3 VV strains in a dose-dependent and reversible manner. Brief treatment at low pH (pH 5.0, 5 min) after viral adsorption accelerated the uncoating of viral DNA, and could reverse the inhibitory effect of NH_4Cl on virus entry. These and other results to be presented suggest that the entry of VV into host cells may involve a pH-dependent step.

J 216 IDENTIFICATION AND CHARACTERIZATION OF A PUTATIVE FUSION REGION OF THE RHESUS ROTAVIRUS VP3 PROTEIN, Erich R. Mackow, Robert D. Shaw, Suzanne M. Matsui, and Harry B. Greenberg. Department of Medicine, Stanford University, Stanford, CA.

Three VP3 specific monoclonal antibodies that neutralize a serotypically diverse group of rotaviruses were used to select rhesus rotavirus variants which escaped neutralization. Each variant gene 4 was sequenced and the position of single base changes encoding single amino acid (AA) substitutions were determined. Variant mutations were identified in a unique region of VP3 at amino acids 388 and 393. This neutralization region is contained within a central hydrophobic region of 20 amino acids which is surrounded by 2 additional hydrophobic AA stretches of 14 and 11 residues. Monoclonal antibodies selecting for mutations in this region were able to neutralize virus prebound to Ma104 cells and appeared to inhibit viral penetration. Interestingly, residues 384 to 401 of the rotavirus VP3 share a region of specific homology with the putative fusion protein sequences (AAs 75-93) of Semliki Forest virus and Sindbis virus.

J 217 DETERMINATION OF PATHOGENICITY OF H7 AVIAN INFLUENZA VIRUSES

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A laboratory-adapted strain of avian influenza (Fowl plague) virus has been used to study determinants of pathogenicity of fowl plague virus, other than the HA. The maximum temperature of replication in vitro has been shown to correlate with the ability to kill 6 week-old chicks. By use of recombinant viruses, the "critical cut-off" temperature for virus to kill chicks was shown to be 41.5°. A mutation in A/FPV/Rostock/34, strain S_3 which accounts for virus attenuation by reduction of the maximum temperature has been shown to result in a change from leucine to isoleucine in PB2 at amino acid position 512. To investigate whether there is a correlation between growth in different cells in vitro and pathogenicity, a virus was isolated that failed to replicate in MDCK cells but could replicate in chick embryo fibroblasts. Initially the virus failed to kill 6 week-old chicks, but virus was isolated from the brains of infected birds, which upon bird to bird passage increased in pathogenicity. After attainment of a fully pathogenic phenotype, the virus still failed to replicate in MDCK cells. We conclude that growth in MDCK cells does not always correlate with pathogenicity in 6 week-old chicks.

J 218 HIV REPLICATION IN MONOBLASTOID CELLS IS REGULATED STRINGENTLY AND AFFECTS DIRECTLY CELLULAR GENE EXPRESSION, C. David Pauza, The Salk Institute, P.O. Box 85800, San Diego, CA 92138. HIV infection of monocytes is likely to be a crucial step in the development of AIDS. In particular, establishment of a persistent infection within this cell population may constitute the reservoir for virus and participate directly in the pathognomonic destruction of helper T-cells. Accordingly, we have characterized the replication of HIV in the human monoblastoid cell line U937 as a model for infection of monocytes. Infection of these cells with the LAV1 strain of HIV resulted initially in a productive infection as determined by the accumulation of either reverse transcriptase activity of viral p24 in the culture medium. Subsequently, virus replication was "restricted" and no further release of particles was observed. The change in virus replication appears to be regulated at the level of viral RNA accumulation; there were no observable cytopathic effects on these cells. When infected U937 cells were induced to differentiate by the addition of phorbol ester a complete and irreversible cessation in virus production was observed, despite the continued intracellular accumulation of high levels of both viral RNA and protein. Infection also altered the pattern of cellular oncogene expression. Accumulation of stable protooncogene RNA was observed to be correlated to viral RNA expression instead of the state of differentiation as it is in uninfected cells. These data suggest one possible mechanism for the establishment, and maintenance of a persistent HIV infection in monocytes and demonstrate, for the first time, a direct link between viral replication and cellular gene expression.

J 219 EVENTS IN THE INFECTION OF MICE WITH THE LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV). P.G.W. Plagemann, J.T. Harty, S.P.K. Chan, R. Davis, X. Li, and C. Onyekaba. Department of Microbiology, University of Minnesota, Minneapolis, MN 55455. Infection of mice (regardless of age or strain) with LDV results in rapid production of virus yielding plasma LDV titers of 10^{10} infectious units/ml 1 to 2 days p.i. This acute phase of infection is followed by a progressive 3-4 log decrease in plasma LDV titers during the next two weeks invariably leading into a lifelong persistent infection. Immunosuppression of BALB/c mice either before LDV infection or during the persistent phase by treatment with cyclophosphamide, cyclosporine, mAbs to various T lymphocyte surface antigens (Lyt2, Thy1.2 or L3T4), or by lethal X-irradiation had no effect on plasma LDV levels. The results support our previous conclusion that the decrease in plasma LDV beginning about 2 days p.i. is not mediated by a host immune response to LDV, despite coinciding with the development of antibodies to the glycoprotein of LDV(VP-3), but reflects the depletion of the mice of the subpopulation of macrophages that supports LDV replication. Persistence, according to this model, is maintained by the infection of new permissive macrophages that seem to be slowly but continuously generated in the animal. Evidence is presented supporting the view that LDV replication in macrophages is cytocidal. Infection by LDV also leads to a polyclonal activation of B cells. This may be an indirect effect, since it is accompanied by an about 50% decrease in the responsiveness of spleen lymphocytes from infected mice to concanavalin A, whereas their responsiveness to LPS is about normal.

J 220 *IN VITRO* AND *IN VIVO* MODELS OF VARICELLA-ZOSTER VIRUS PERSISTENCE IN THE NERVOUS SYSTEM, C. Sadzot-Delvaux, M.P. Merville-Louis, C. Bourdon-Wouters, P. Delrée, J. Piette, G. Moonen and B. Rentier, Liège University, B23, B4000 Liège, Belgium.

We have developed a persistent VZV infection in primary dissociated adult rat peripheral nerve cells (dorsal root ganglia, DRG), by coculture with infected MRC5 (human embryonic lung) cells. Infection was neuron-specific and non productive. Very few neurons displayed virus-specific antigens during the first days after infection Antigens were not expressed at later times. However, viral genome was detected by *in situ* hybridization in up to 40% of neurons for the duration of the experiments (i.e. 10 days).

A complementary model has been set up in a mouse neuroblastoma (Neuro-2A) cell line. This model, which appears to have the same characteristics, allows us to perform quantitative biochemical studies on this type of infection, such as identification of mRNA and early proteins.

The 3rd model is an *in vivo* persistent infection of adult rats with VZV. The virus was inoculated subcutaneously in ±20 injections along the spine. Its genome was found in DRG six months later and, at the same time, virus was rescued from DRG by cocultivation with MRC5 cells.

These models open new possibilities in the study of VZV persistence and reactivation, as well as in monitoring of antiviral approaches.

We thank the FNRS, FRSM, IRSIA and National Lottery of Belgium for financial support.

J 221 ALLELOCHEMICALS: INDICATIONS OF CELL DISTURBANCE SIMILAR TO VIRUS INFECTIONS, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

The tropane alkaloids, scopolamine and hyoscyamine, washed from thornapple seeds, have been identified as allelochemicals. Effects of these compounds include reduced germination and interference with seedling development of some crop species.
An experiment with 0.05% total thornapple alkaloids in aqueous solution in which the effects on root tip cells of sunflower has been described: (i) the abundance of amyloplasts and microbodies in the cells, indicate slowing down of the metabolism of food reserves (a process which takes place rapidly during the early phases of germination); (ii) increase in size and number of microbodies.
The latter interaction could be direct or indirect, followed by intense activity of the Golgi vesicles which may play, in turn, an important role in the formation of the primary and secondary cell wall.
Evidence for malformation of the cell wall possibly indicates a response to allelochemicals analogous to that reported following viral infections (Kim & Fulton, 1973. J. Ultrastruct. Res. 45:328.

J 222 IMMEDIATE-EARLY GENES INVOLVED IN HSV-1 LATENCY: A MUTATIONAL APPROACH D. Leib[1,2], D. Coen[2], D. Knipe[1], K. Tyler[1], N. DeLuca[1,2], K. Yager[1], K. Hicks[1], J. Jacobson[1], C. Bogard[2], and P. Schaffer[1,2]. Harvard Medical School[1] and the Dana-Farber Cancer Institute[2], Boston, MA 02115.
We have studied the roles of immediate-early proteins in the establishment and reactivation of latent herpes simplex virus (HSV) infection following corneal inoculation of adult CD-1 mice. HSV-1 strain KOS (the wild-type virus for our mutants) was able to establish reactivatable latency in trigeminal ganglia of 50% of animals at dosages 1000-fold lower than its LD_{50}. Viral DNA was readily detected in latently infected ganglia by slot-blot hybridization. Mutants in three of the five HSV immediate-early genes have been tested for their ability a) to replicate in the cornea during acute infection b) to reach trigeminal ganglia as judged by the presence of viral DNA, and c) to reactivate from latently infected ganglia by cocultivation with permissive cells. One deletion mutant in the gene for ICP27 (5d1 2.1) failed both to replicate in corneas and to reactivate from ganglion explant cultures. Similarly, two nonsense mutants in the gene for ICP4 (n12 and n18) which specify peptides lacking the carboxy terminal three-quarters and two-thirds of the ICP4 peptide, respectively, failed to replicate in corneas and to establish latency. By contrast, the replication-competent nonsense mutant n6, which specifies a peptide lacking the amino terminal 90 amino acids of ICP4, and ΔAT, a mutant lacking the nucleotides AT from the transcriptional start site of ICP4 (ATCGTC) established latency and were readily recovered from ganglion explants. A deletion mutant in the gene for ICP0 (d1X 3.1) was found to replicate in corneas during acute infection and viral DNA was readily detected in latently infected ganglia. This mutant, however, could not be reactivated from ganglion explant cultures. These findings suggest that ICPs 4 and 27 function in the establishment of latency by virtue of the requirement for these two proteins for virus replication at the site of inoculation, and that ICP0 is required not for establishment, but for reactivation of virus from latency in this model. Current efforts are designed to identify the stage(s) at which ICP0 mutants are blocked in the reactivation process.

Intracellular Trafficking and Pathogenesis

J 300 DIFFERENTIAL SYNCYTIUM-INDUCING CAPACITY OF LONGITUDINAL AND TRANSSECTIONAL HIV ISOLATES, M. Tersmette, B.J.M. Al, R.A. Gruters, R.E.Y. de Goede, I.N. Winkel, J. Goudsmit*, H.T. Cuypers, H.G. Huisman, F. Miedema, Central Lab.Neth.Red Cross Blood Transfusion Service and Lab. Exp. and Clin. Immunol. of the Univ.of Amsterdam, *Dept.of Virol. Univ. of Amsterdam, Amsterdam, The Netherlands

Longitudinally and transsectionally obtained HIV isolates were studied with respect to syncytium-inducing capacity, replicative properties and host range. In the transsectional panel 50% of the isolates from patients with ARC or AIDS, but only 17% of the isolates from asymptomatic individuals were able to induce syncytia in peripheral blood mononuclear cell (MNC) culture. Longitudinal sets of HIV isolates were obtained from 6 individuals, two of whom went on to develop AIDS. Sequential isolates exhibited similar biological characteristics despite minor differences at the molecular level. Syncytium-inducing capacity was consistently absent in isolates from 5 individuals but present in all isolates from one individual obtained over a 16-month period before development of AIDS. Syncytium-inducing isolates were reproducibly obtained from the same MNC sample in over 90% of the cases. Syncytium-inducing capacity was shown to be a stable property of an isolate. In primary culture, isolation of syncytium-inducing isolates was associated with early and high reverse transcriptase activity. Evidence was obtained that this may be due to higher infectivity of these isolates. All syncytium-inducing, but none of the non-syncytium-inducing isolates could be transmitted to the H9 cell line. The frequent isolation of syncytium-inducing isolates from individuals with AIDS or progressing to AIDS and their apparent higher in-vitro infectivity suggests that these isolates may unfavorably influence the course of HIV infection.

J 301 INTERACTIONS OF EPSTEIN-BARR VIRUS AND GLUCOCORTICOIDS.
Graham P. Allaway, Edward L. Oates and Bellur S. Prabhakar. National Institutes of Health, Bethesda, MD. 20892.

Pro-opiomelanocortin (POMC) gene-related transcripts were found in human B and T lymphocytes using an RNA probe. The highest transcript levels were found in Epstein-Barr virus (EBV)-infected B cell lines. Unlike the situation in the pituitary, expression of POMC in an EBV-infected lymphocyte cell line was not depressed by the synthetic glucocorticoid dexamethasone. POMC is the precursor for several hormones, including ACTH which stimulates glucocorticoid production by the adrenal gland. Conversely, it is known that glucocorticoids can stimulate EBV antigen expression and virus particle production in vitro. The effect of dexamethasone on the expression of EBV in lymphocytes was examined by northern blot analysis. The levels of EBV transcripts were much higher in cells treated with dexamethasone for 48 hrs than in untreated cells. This enhancement may result from the presence in the EBV genome of sequences which are similar to known glucocorticoid receptor binding sites.

It is hypothesized that enhanced POMC expression by EBV-infected lymphocytes might lead to increased levels of ACTH and glucocorticoids in vivo. Stimulation of EBV replication by these glucocorticoids could result in more EBV-infected cells. This mechanism would lead to altered regulation of both EBV and glucocorticoids.

J 302 THE AMINO TERMINAL SIGNAL PEPTIDE ON THE MATRIX PROTEIN OF THE PORCINE TRANSMISSIBLE GASTROENTERITIS CORONAVIRUS IS NOT REQUIRED FOR MEMBRANE TRANSLOCATION AND GLYCOSYLATION, David A. Brian[1], Brenda G. Hogue[1,3], Paul A. Kapke[2], and Frank Y. T. Tung[1], University of Tennessee, Knoxville, TN 37996-0845[1], and U.S. National Animal Disease Center, Ames, IA 50010[2]. The deduced amino acid sequence for the matrix protein of the porcine transmissible gastroenteritis coronavirus (TGEV) revealed that this protein differs significantly from the matrix protein of the prototypic mouse hepatitis coronavirus. The matrix protein of the mouse hepatitis coronavirus is 228 amino acids long, has no N-terminal signal peptide, and resides in the endoplasmic reticulum after becoming translocated by means of one or more of three "internal" hydrophobic signal domains that map between amino acids 25 and 114 (Rottier et al., 1986. Biochemistry 25:1335). The TGEV matrix protein, in contrast, is 262 amino acids long and has an N-terminal hydrophobic signal sequence of 16 amino acids in addition to three internal hydrophobic domains. The internal hydrophobic domains map between amino acids 53 and 135 and may also function as internal signals for translocation. When the TGEV matrix protein without its amino terminal signal peptide was translated in vitro in the presence of microsomes it became translocated and glycosylated, but not as well as the protein that still has its N-terminal signal peptide. The N-terminal signal peptide on the TGEV matrix protein, therefore, may function to enhance membrane translocation of an especially long (34 amino acid) extravirion, hydrophilic amino terminus.
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J 303 CHARACTERIZATION OF FELINE HERPESVIRUS GLYCOPROTEINS, Teresa Compton, Cetus Corporation, Department of Microbial Genetics, Emeryville, CA 94608.

Glycoproteins synthesized by the herpesviruses are important determinants of viral pathogenicity. Feline herpesvirus (FHV), a member of the Alpha herpesviridae, is associated with a variety of clinical syndromes, including respiratory tract infections, and it causes a high mortality rate in kittens. The biochemical and biological properties of FHV-encoded glycoproteins have not been described. High titers of FHV were obtained from propagation in Crandell-Rees feline kidney cells (CRFK). To examine FHV glycoproteins, infected CRFK monolayers were metabolically labelled with 35S-methionine or 3H-glucosamine. Analysis of purified virions, infected-cell extracts and culture supernatants by SDS-PAGE have revealed the presence of at least five glycoprotein species. In cell extracts, a group of three, closely-migrating polypeptides of 107K, 103K and 102K molecular weight are prominently labelled with 3H-glucosamine; however, only the 107K and 103K species were present in mature virions. The 102K protein was sensitive to endoglycosidase H digestion, suggesting it may be the high-mannose precursor to either the 107K or 103K proteins. In addition to this major complex, other glycoproteins of 85K (a broad, diffusely-migrating band), 68K and 59K were detected in both cell extracts and virions. The 68K glycoprotein, while present in both cells and mature virus, is completely sensitive to endoglycosidase H digestion. FHV glycoproteins were also detected in culture supernatants. As has been observed for other herpesviruses, FHV releases glycoproteins into the culture medium. The 107K species is detectable in infected cell culture supernatants and may represent a "shed" form of the cell-associated glycoprotein. Further, a prominently-labelled, novel species of 75K was also observed in medium. Studies are currently in progress to further define the biological properties of FHV glycoproteins.

J 304 THE DETECTION OF COXSACKIEVIRUS RNA IN HUMAN BIOPSY MATERIAL BY IN SITU HYBRIDIZATION, A. J. Easton and R. P. Eglin, University of Warwick and John Radcliffe Hospital, Oxford, U.K.

Coxsackieviruses produce common enteric infections in man and have been shown to be the causitive agents of a number of clinically important diseases. In man, serological studies have indicated a strong link between infection with Coxsackie B virus and the onset of acute pencarditis and myocarditis. A cloned cDNA probe derived from Coxsackie B4 infected cell RNA was used to detect virus-specific RNA sequences in biopsy material obtained from patients diagnosed as having a coxsackievirus infection. Virus RNA was detected using the technique of in situ hybridization in 45% (6/13) of cardiac samples, with no signal detected in normal, control cardiac samples. The probe was also used to show the presence of virus in human brain tissue. The hybridization data will be presented and the patterns of hybridization and the possible significances of these discussed.

J 305 THE LYSOSOMAL MEMBRANE IS THE SITE OF ALPHAVIRUS REPLICATION, Susan Froshauer, Jurgen Kartenbeck, Hans Stukenbrok, Eva Bolzau, and Ari Helenius, Department of Cell Biology, Yale University School of Medicine, New Haven CT 06510.

Semliki Forest virus and Sindbis, both alphaviruses, are enveloped and have positive-stranded RNA genomes. The early interactions of these viruses with the cell have been well-characterized. After uptake by endocytosis, virus particles enter prelysosomal, acidic organelles, endosomes. The low pH of endosomes triggers a conformational change of viral spike proteins, resulting in fusion of the membranes of the virus and the endosome, and viral penetration. There is little information about the subsequent early cytoplasmic events in the virus life cycle-- uncoating, initiation of translation, assembly of membrane-associated replication complexes. Our studies have aimed at characterizing these early cytoplasmic events, both to gain a greater understanding of the organelles of the endocytic pathway and of viral pathogenesis in general.

Previous investigators suggest that alphavirus infection-specific organelles, cytopathic vacuoles I (CPVIs) are the site of viral replication. Recently, we have obtained additional evidence identifying CPVIs as viral replication factories. We also have demonstrated that CPVIs are components of the endocytic pathway, and they are lysosomal in nature. With antibodies highly specific for the lysosomal membrane proteins, lgp110, lgp120 and lgp96, and antibodies specific for two viral RNA polymerase subunits, nsp3 and nsp4, we have localized these antigens to CPVIs by several immunocytochemical strategies. We also detect fluid-phase markers in CPVIs. In addition, CPVIs fractionate with lysosomal enzyme activity during free-flow electrophoresis. Further studies will focus on learning how the replication complex is directed to the lysosomal membrane and on determining what functions of lysosomes are altered.

J 306 MANNOSE 6-PHOSPHATE (MAN 6-P) IN AN ENVELOPE GLYCOPROTEIN (GP) OF VARICELLA-ZOSTER VIRUS (VZV): POSSIBLE ROLE IN INFECTIVITY. C. Gabel, L. Dubey, S. Steinberg, M. Gershon,& A. Gershon. Depts. of Anatomy & Cell Biology and Pediatrics, Columbia University College of P & S., New York, NY 10032.

Cultured human embryonic lung fibroblasts (HELF) infected with VZV do not release infectious virus particles into the medium. Intracellular virions are found in acid phosphatase-containing vacuoles and extracellular virions have irregular, ragged envelopes, suggesting that lysosomal degradation of VZV may occur prior to exocytosis. Since attachment of Man 6-P to high mannose-type oligosaccharides of acid hydrolases directs these enzymes to the lysosomal compartment, we analyzed intracellular VZV for the presence of this specific recognition marker. HELF, infected with VZV for 18 hr, were pulse-labeled for 30 min with (2-^{3}H) mannose and chased for 1-3 hr. Cell extracts were subsequently prepared, the viral gps were recovered by immunoprecipitation, and were analyzed by SDS-PAGE and radioautography. The individual radiolabeled VZV gps were excised from the dried gels and solubilized with pronase. Resulting glycopeptides were fractionated by concanavalin A-Sepharose chromatography into triantennary-, biantennary-, and high mannose-type asparagine-linked units. After acid hydrolysis of the glycopeptides, the resulting monosaccharides were characterized by paper chromatography. The high mannose-type glycopeptides isolated from the 3 major VZV gps did not contain Man 6-P; however, proteins corresponding to precursor and mature forms of gp3 contained Man 6-P residues in association with the triantennary-type glycopeptide fraction. Thus, although the recognition marker is not associated with high mannose oligosaccharides as is the case with acid hydrolases, cell-associated VZV contains at least one gp that possesses Man 6-P residues. Since Man 6-P receptors on plasma membranes are known to mediate endocytosis of acid hyrolases, we sought evidence that this unusual phosphorylated oligosaccharide promotes VZV entry into HELF. Exogenous Man 6-P was found to protect HELF from infection with cell-free VZV. The effect of Man 6-P was concentration dependent (between 5- 20 mM) and was not mimicked by glucose 6-phosphate, a poor ligand for the Man 6-P receptor. HELF that support growth of VZV were found to efficiently endocytose Man 6-P-bearing lysosomal enzymes, indicating that the cells contain the 215 kDa Man 6-P receptor at their surface. These data support the hypothesis that the Man 6-P residues of VZV gp3 mediate the attachment of infectious virions to Man 6-P receptors on the surface of target cells and play a role in viral entry. Supported by NIH grants AI 24021 , GM 33342, and NS 12969.

J 307 GLIAL CELL PLASTICITY AND REMYELINATION FOLLOWING A59 MOUSE HEPATITIS VIRUS INDUCED DEMYELINATION, C. Godfraind, V. L. Friedrich Jr., C. A. Jordan, K. V. Holmes, and M. Dubois-Dalcq, Laboratory of Molecular Genetics, NIH, Bethesda, MD 20892 and Department of Pathology, USUHS, Bethesda, MD 20854.

The A59 strain of mouse hepatitis virus, injected intracerebrally in C57 black mice, causes a transient paresis and ataxia associated with glial cell infection and demyelinating lesions in the spinal cord, followed by functional recovery and remyelination. Here, we analyze how the spread and clearing of virus correlates with the disease and identify glial cell precursors which might be involved in myelin repair. In situ hybridization with A59 RNA probes revealed abundant virus specific transcripts in both gray and white matter at the end of the first week post-infection (PI). A progressive decrease in viral mRNA occurred during the second and third week PI with transcripts being cleared from the gray matter and persisting in clusters in the white matter. At that time, astrocytes identified on 1μ frozen sections by their reactivity to glial fibrillary acidic protein (GFAP), increased in number in both gray and white matter. At three weeks, the first signs of remyelination were identified by electron microscopy while demyelination was still progressing. Prior to this, very large stellate cells expressing markers (such as 0_4 antigen) specific for precursors of oligodendrocytes emerged in and around the lesions. Several of these cells also expressed GFAP. Using enzymatic dissociation of the spinal cord, hundreds of 0_4 positive cells could be cultured from remyelinating animals while only a few grew from control animals. After a few days of culture, many 0_4 positive cells expressed either GFAP or galactocerebroside, a marker for differentiated oligodendrocytes. A few of the 0_4 and 0_4-GFAP cells had incorporated thymidine injected into the animals two hours before sacrifice. Our results suggest that cells active in remyelination are recruited from gray and white matter at the time of virus clearing and may derive from a slowly dividing precursor which is 0_4 positive and can also express GFAP.

J 308 THE BASIS OF SINDBIS VIRUS NEUROVIRULENCE, Diane E. Griffin[1], Shlomo Lustig[2], Alan C. Jackson[1], Chang S. Hahn[2], Ellen G. Strauss[2] and James H. Strauss[2], [1]Johns Hopkins University, School of Medicine, Baltimore, MD and [2]California Institute of Technology, Pasadena, CA

Wild-type Sindbis virus (strain AR339) (SV) causes fatal encephalitis in suckling mice and non-fatal encephalitis in weanling mice. A neuroadapted strain of virus (NSV) which causes fatal encephalitis associated with kyphoscoliosis and hind limb paralysis in weanling mice has been isolated after intracerebral passage of SV. Both NSV and SV infect neurons and ependymal cells in the brain but NSV replicates more rapidly, is more likely to cause neuronal death, and causes more extensive infection than SV. The HRSP laboratory strain of SV is avirulent for weanling mice and has reduced virulence for suckling mice. Nucleotide sequence analysis of the glycoprotein regions shows 8 nucleotide changes which result in 4 amino acid differences between NSV and SV. Construction of recombinant viruses using an avirulent infectious clone (toto1101) demonstrates that most of the neurovirulence of NSV is accounted for by the surface glycoproteins. Changes in either E2 (his55 in NSV to glu in SV) or in E1 (asp 313 in NSV to gly in SV) abolishes virulence for weanling mice. A number of changes in E2 (e.g. gly 172 to arg) and E1 (e.g. ala 237 to ser) lead to decreased virulence in suckling mice effectively forming a gradient of virulence for various strains of SV.

J 309 EXPRESSION OF HIV ENVELOPE PROTEINS AND THE CD4 RECEPTOR USING A SV40 LATE REPLACEMENT VECTOR. Marie-Louise Hammarskjold, Frank Ferro, Alan J. Smith and David Rekosh, Departments of Microbiology and Biochemistry, Suny at Buffalo, Buffalo, N.Y. 14214.

A fragment of HIV proviral DNA containing the genes for the envelope proteins as well as the regulatory genes art/trs and tat was inserted into a SV40 late replacement vector. Upon transfection of CV1 or COS cells this construction expresses the envelope protein precursor (gp160) which is cleaved into gp120 and gp41. Pulse-chase experiments demonstrate mobility shifts of gp160 on SDS gels consistent with sequential changes in glycosylation. These experiments also show that in this system cleaved gp120 appears slowly and that some uncleaved gp160 remains even after a 24 hour chase. In addition to the envelope proteins the vector expresses tat as determined by activation of a LTR-CAT construction in cotransfections. Experiments using deletion mutants suggest that the vector also produces art/trs and that this is important for efficient envelope gene expression. Primer extension analysis has been carried out to determine the structure of the different mRNAs produced by the vector. We have also used the SV40 late replacement vector to express the HIV receptor, CD4. Cotransfection of COS cells with this construction and the envelope expression vector results in the formation of large syncytia. Results of experiments using this system to study gp120/CD4 interactions will be presented.

J 310 EXPRESSION OF M13 PHAGE PROCOAT-COAT IN MAMMALIAN CELLS, James F. Hare and David Triebwasser, Biochemistry Dept., Oregon Health Sciences Univ., Portland, OR 97201
Previous studies of the expression of mutant animal virus proteins in transfected cells have shown that proper folding and quartenary structure are a requirement for targeting to appropriate membranes. Moreover, establishment of native structure in the endoplasmic reticulum may be rate limiting for expression at the cell surface. To test whether a viral protein having minimal structural complexity can target to the cell surface by a default pathway, we have expressed the M13 phage procoat gene in stable cell lines of tsCOS cells. The expressed protein is present as both procoat and proteolytically processed coat. Antibody binding and surface labeling approaches will determine if and how fast coat appears at the cell surface.

J 311 CHARACTERISTICS OF THE SOLUBLE FORM OF THE G GLYCOPROTEIN OF RESPIRATORY SYNCYTIAL VIRUS, David A. Hendricks, Max Niebert, Kenneth McIntosh, and Jean L. Patterson, Gene Trak Systems, Framingham, MA and Harvard Medical School, Boston, MA. A soluble (non-virion-associated) form, G_s protein, of the attachment (G) protein of respiratory syncytial (RS) virus was recovered from the fluids of infected HEp-2 cells. The G_s proteins of the Long and 18537 strains have molecular weights of 82 kd and 79 kd respectively, 6-9 kd smaller than the virion-associated (G_v) forms. The G_s protein of the Long strain was further characterized. Approximately one in six of all of the ^{3}H-glucosamine-labeled G proteins in cultures at 24 hours post infection was present in the culture fluids as the G_s protein. The G_s protein was clearly evident in culture fluids at six hours post infection but the G_v protein could not be discerned until 12 hours after infection, an observation that is consistent with the 12 hour ecllipse period for RS virus. Therefore, the G_s protein was shed, in part at least, from infected, intact cells and before the appearance of viral progeny. The appearance of a smaller G_s protein (74 kd) in fluids of infected cells which were incubated with tunicamycin shows that addition of N-linked oligosaccharides is not required for the genesis and shedding of the G_s protein. Sequencing of the amino terminus of purified G_s protein revealed two different termini whose generation is consistent with cleavage of the full length G protein between amino acids #65 and #66 and between amino acids #74 and #75. We present an hypothesis which suggests that the G_s proteins of vesicular stomatitis, rabies, and RS viruses are generated from their full length forms by a signal peptidase-like protease.

J 312 ANALYSIS OF THE SIGNAL/ANCHOR DOMAIN OF INFLUENZA NEURAMINIDASE, Brenda G. Hogue, Donald J. Brown, and Debi P. Nayak, Department of Microbiology and Immunology, UCLA School of Medicine and Jonsson Comprehensive Cancer Center, Los Angeles, CA 90024
The influenza virus neuraminidase is a typical class II membrane glycoprotein. A single hydrophobic domain (29aa) at the amino terminus functions both as an uncleaved signal sequence and as a transmembrane anchor. We have examined this signal/anchor domain by creating a series of deletion mutants within this region. Analysis of these mutants using an *in vitro* transcription/translation system indicates that the entire stretch of 29 amino uncharged amino acids is not required for anchoring. The anchor function appears to be provided by 20 amino acids between residues 7 and 26. The data also suggest that within the anchoring domain a smaller subdomain (residues 13-26) exists which provides the signal for translocation. Mutants which were positive for translocation *in vitro* have been expressed by generating vaccinia virus recombinants to further characterize the mutants for the ability to be transported to the cell surface. The structural characteristics of this multifunctional domain which define the signal/anchor regions will be presented.

J 313 EXPRESSION OF THE SPLEEN FOCUS-FORMING VIRUS ENVELOPE GENE IN A POLARIZED EPITHELIAL CELL LINE. David R. Kilpatrick, Ranga V. Srinivas and Richard W. Compans. University of Alabama at Birmingham, Birmingham, Alabama

Friend spleen focus forming virus (F-SFFV) encodes a glycoprotein designated gp52, which is defective in its intracellular transport and accumulates in the rough endoplasmic reticulum. Only 3-5% of the mature form of gp52 eventually reaches the cell surface. Compared to transport-competent murine leukemia virus (MuLV) glycoproteins, the gp52 molecule exhibits several structural differences which may have resulted in the possible loss of signals required for transport to the cell surface. To determine the effect of these alterations on the specific sites of surface expression of the molecule, the SFFV env gene was expressed from a vaccinia virus recombinant in a polarized epithelial cell line in which retrovirus glycoproteins are expressed exclusively on basolateral surfaces. We also expressed a chimeric env gene which contains the external domain of SFFV gp52 and the cytoplasmic tail residues of Friend MuLV. The wild type and chimeric env gene products were found to exhibit similar processing rates in CV-1 cells using pulse-chase analysis. They were both defective in transport, as evidenced by low levels of expression on surfaces of CV-1 cells at 6 hpi. However, both glycoproteins were expressed at detectable levels on the basolateral surfaces of MDCK cells, a line of polarized epithelial cells. These results indicate that the presence or absence of a cytoplasmic tail as well as a 585 base deletion in the external domain have no affect on the polarized expression of a murine retrovirus glycoprotein.

J 314 TRANSPORT TO THE CELL SURFACE OF NON-NATIVE AND NATIVE, GLYCOSYLATED AND UNGLYCOSYLATED FORMS OF THE HN PROTEIN OF THE PARAMYXOVIRUS SV5. Davis Ng, Scott W. Hiebert and Robert A. Lamb. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

The HN (Hemagglutinin-Neuraminidase) of SV5 is a type II integral membrane protein with a N-terminal cytoplasmic domain and a C-terminal ectodomain. A single N-terminal hydrophobic domain acts as a combined signal-anchor. HN contains six potential sites for N-linked glycosylation in the ectodomain. Oligonucleotide-directed mutagenesis was done on a cDNA clone of HN changing the coding sequence for asparagine to serine at each of the six sites. Expression of these six mutant proteins in vivo showed that four of the six sites are used. An additional seven mutants were constructed altering the four sites in various combinations including a non-glycosylated form of the protein. The proper folding of the mutant proteins in vivo were analyzed using conformation-specific monoclonal antibody recognition and disulfide bonded oligomer formation. Transport of the mutant proteins to the plasma membrane was studied by cell surface proteolysis and indirect surface immunofluorescence.

J 315 STUDIES ON THE STRUCTURE AND FUNCTION OF THE SV5 FUSION PROTEIN, Reay G. Paterson and Robert A. Lamb, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

Deletions involving the basic amino-acid residues of the connecting peptide of the SV5 fusion (F) protein have been constructed by site directed mutagenesis. The effect of the deletions on both cleavage of F_0 to F_1 and F_2 and fusion activity will be discussed. The N-terminus of the F_1 subunit is implicated as being directly involved in membrane fusion and to examine the properties of this region hybrid proteins have been constructed. We have previously shown that the F_1 N-terminus is sufficiently hydrophobic to function as a membrane anchorage domain (Paterson and Lamb, 1987, Cell, 48, 441-452). Here we describe a series of experiments in which the extended signal/anchor of the influenza virus neuraminidase is replaced with the F_1 N-terminus. The neuraminidase "reporter molecule" is expressed as a biologically active soluble protein and the implications concerning the interaction of hydrophobic domains with the cellular transport machinery will be discussed.

J 316 VIRUS SPECIFIC CTL RESPONSE IN A NEUROTROPIC MURINE LEUKEMIA VIRUS INFECTION, Deanna S. Robbins and Paul M. Hoffman, University of Maryland at Baltimore, Baltimore, MD 21201 and Veterans Administration Medical Center, Baltimore, MD 21218.
Previous studies demonstrated that inbred NFS/N mice developed an age acquired T-cell mediated resistance to Cas-Br-M murine leukemia virus (MuLV) induced neurodegenerative disease. To determine the functional T-cell subsets involved in resistance, an in vitro ^{51}Cr release assay measuring MuLV specific cytotoxicity against a ^{51}Cr labeled, syngeneic target cell line (NS467) was developed. Using this assay we determined that NFS/N mice infected with Cas-Br-M MuLV at 10-21 days of age developed a brisk cytotoxic response. This MuLV specific cytotoxic response had the kinetics of a cytotoxic T lymphocyte (CTL) response, was MHC restricted, and could be inhibited by the addition of unlabeled NS467 target cells but not by unlabeled allogeneic conA splenic blast cells. High levels of MuLV specific cytotoxicity were present by 1 week post-infection and were maintained in spleens of immune mice longer than 20 weeks post-infection. In contrast NFS/N mice infected with Cas-Br-M MuLV at 2 days of age failed to develop an MuLV specific CTL response prior to or during the development of neurologic disease. However, spleen cells from these same mice maintained the ability to mount an in vitro allogeneic cytotoxic response throughout the course of neurologic disease. These studies indicate that Cas-Br-M MuLV specific CTL may play an important role in the resistance to the paralytic effects of Cas-Br-M MuLV infection possibly by preventing virus dissemination to the central nervous system.

J 317 MEMBRANE INSERTION AND INTRACELLULAR TRANSPORT OF THE CORONAVIRUS E1 PROTEIN, P.J.M. Rottier and J. Rose*. Institute of Virology, Utrecht, The Netherlands
*Yale University, New Haven
Coronaviruses, a group of positive-stranded, enveloped RNA viruses, have the interesting property of budding intracellularly, either into ER or into Golgi membranes depending on the type of host cell. One of the two virus envelope proteins, E1 localizes this particular site of virus assembly. The O-glycosylated E1-protein of mouse hepatitis virus strain A59 has previously been shown to be cotranslationally inserted into the ER membrane in an SRP-dependent manner using an internal signal sequence. The 24K integrated protein is tightly associated with the lipid bilayer: it has 3 membrane-spanning domains and only small portions of the N- and C-terminus are susceptible to proteolysis at the lumenal and cytoplasmic face, respectively. When expressed from a SV40 based vector in COS cells, the protein is glycosylated and accumulates in the Golgi region. By in vitro mutagenenesis of the cloned E1 gene, a series of mutations have been introduced. These mutant genes are now being expressed both in vitro, to study the role of the various protein domains in membrane integration and topology, and in vivo, to elucidate the determinants for its localization in Golgi. In addition, we have started to analyze the state of the wild-type protein as it moves from ER to Golgi to find out if some quarternary organization is required for this transport. The results of these studies will be presented.

J 318 ISOLATION OF A NOVEL PROTEIN TYROSINE KINASE GENE, Martin Ruta, Richard Howk, George Ricca, Joseph Schlessinger, William Drohan, and David Givol, Rorer Biotechnology Center, Rockville MD.
We have isolated a novel human gene from a cDNA library using a tyrosine kinase gene as a probe. This novel gene designated FLK contains a open reading frame encoding a putative protein tyrosine kinase. The FLK gene contains unusual structural features suggesting that it represents a novel class of protein tyrosine kinase. Flk expression has been analyzed in both normal and tumor cell lines and in various differentiation systems. These results as well as protein data will be presented.

J 319 THE FUNCTION OF THE CORONAVIRUS SPIKE PROTEIN IN VIRAL PATHOGENESIS, W. Spaan, W. Luytjes, D. Geerts, M. Koolen. Institute of Virology, University of Utrecht, The Netherlands.

Mouse hepatitis virus (MHV), a coronavirus is an enveloped virus containing a nucleocapsid protein N and two membrane glycoproteins E1 and E2. The E2 glycoprotein forms the peplomers (or spike) on the surface of the virion. It is involved in the attachment of virus to host cells, induction of cell fusion and elicitation of virus-neutralizing antibodies. Three major neutralizing epitopes A, B and C have been identified. Studies on antigenic variants (escape mutants) have suggested that mutations in the E2 protein can drastically change the neuropathogenic properties of the virus. Recently we have cloned and sequenced the E2 gene and fragments of the gene have been used to produce beta-galactosidase fusion proteins. This allowed us to identify the monoclonal antibody binding site corresponding to epitope A. To define the epitope more precisely we have synthesized a series of peptides which were tested in an ELISA. Using this approach we were able to identify a continuous strech of 9 amino acids that binds efficiently to the antibody. Currently we are examining the effect of immunization with synthetic peptides or B-gal fusion proteins containing the aminoacid sequence of epitope A on the neuropathogenicity of the virus. To study the relation between cleavage of the E2 protein and induction of cell fusion we have introduced the E2 gene into the genome of vacciniavirus. This recombinant virus will be used to examine the cleavage and cell fusion in different host cells.

J 320 THE ROLE OF ASPARAGINE-LINKED OLIGOSACCHARIDE PROCESSING IN THE ASSEMBLY AND TRANSPORT OF PROTEOGLYCANS, Robert C. Spiro, Ralph A. Reisfeld and John R. Harper, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The covalent linkage of glycosaminoglycan (GAG) side chains to core protein structures defines the proteoglycan class of molecules. Although well-characterized as major components of the extracellular matrix and cartilage tissue, more recent studies have demonstrated the post-translational addition of GAG chains to glycoproteins such as the transferrin receptor, the class II invariant (gamma) chain and the melanoma-associated antigen (MPG). In these systems, both glycoprotein and proteoglycan forms are synthesized and expressed. The melanoma model system has been utilized to examine the mechanisms that regulate the expression of proteoglycan forms, particularly the role that processing of asparagine-linked oligosaccharides (N-linked sugars) might play in this event. Using agents which block discrete steps in the trimming and processing of N-linked sugars (castanospermine, 1-deoxynojirimycin, 1-deoxymannojirimycin and swainsonine) it has been demonstrated that removal of the three glucose residues from the precursor N-linked sugar $(Glu)_3(Man)_9(GlcNac)_2$-ASN is required for the addition of GAG side chains to the melanoma MPG and class II invariant chain. Conversion of the N-linked sugars to the "complex" form is not, however, required for this modification or for the cell surface expression. The known effects of these inhibitors on the transport and secretion of other glycoproteins supports the hypothesis that the processing of N-linked sugars is involved in the regulation of transport of core proteins to the site of GAG addition.

J 321 TRANSPORT AND LEUKEMOGENICITY OF TRUNCATED SFFV GP52 MOLECULES THAT LACK A MEMBRANE ANCHOR. Ranga V. Srinivas, David R. Kilpatrick and Richard W. Compans, University of Albamam at Birmingham, Birmingham, AL 35294

The gp52 glycoprotein encoded by the replication defective Friend spleen focus-forming virus (F-SFFV) is a leukemogenic, transport defective protein that accumulates in the rough endoplasmic reticulum of the infected cells. A small proportion of gp52 is expressed on the cell surface in a processed form and eventually shed into the culture medium. The cell-surface or the extracellular forms are thought to mediate the leukemogenic effects of SFFV gp52. Previous studies involving substitution of the gp52 transmembrane domain with the transmembrane and cytoplasmic domains of transport competent murine leukemia virus (MuLV) envelope proteins resulted in chimeric proteins which exhibited a transport defect similar to SFFV gp52. Recombinant viruses expressing these chimeric glycoproteins were found to be nonleukemogenic to mice. To further investigate the role of different domains of SFFV gp52 in its transport and leukemogenicity, we have constructed mutant SFFV envelope genes that code for gp52 molecules without a membrane anchor. The anchor-minus gp52 molecules were also found to be transport defective, and were not secreted from the cells suggesting that the transport defect of SFFV gp52 can be attributed to its external domain. Recombinant SFFV (MuLV) pseudotypes expressing the truncated gp52 were found to be nonleukemogenic in mice, indicating that the SFFV transmembrane domain is required for its full biological activity.

J 322 NOVEL GLYCOSYLATION PATHWAYS OF RETROVIRAL ENVELOPE PROTEINS, Wen-Po Tsai, and Stephen Oroszlan, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

Two mature glycoproteins, gp90 and gp20 of avian reticuloendotheliosis virus (REV-A) envelope were previously identified. An intracellular polyprotein, $gPr77^{env}$ antigenically related to both gp90 or gp20, was also found, but the precise metabolic relationship of $gPr77^{env}$ to the viral envelope proteins was not determined (Tsai *et. al.*, 1986). We have now identified an intracellular higher-molecular weight *env* polyprotein designated $gPr115^{env}$. Kinetic studies using ^{35}S-Cys for metabolic labeling of the glycoprotein suggested that $gPr77^{env}$ is the immediate precursor to $gPr115^{env}$. Prior to virion assembly $gpr115^{env}$ but not $gPr77^{env}$ is intracellularly cleaved into gp90 and gPr22. In the virion, the latter is further processed into gp20 and p2. REV-A infected cells treated with tumicamycin yield only a single *env* protein of 58 KDa indicating that the 38 KDa size difference between $gp115^{env}$ and $gPr77^{env}$ may be due entirely to sugar moieties. Digestion of proteins from virus and cell lysates with a panel of glycosidases showed that $gPr77^{env}$, gPr22, and gp20 are high mannose-type glycoproteins whereas $gPr115^{env}$ and gp90 are at the complex-type rich in terminal sialic acids. Taken together, the results suggest that $gPr77^{env}$, initially modified with oligomannosides at both gp90 and gp20 domains, is converted to $gPr115^{env}$ by further glycosylation (sialylation) only at sites in the gp90 domain. This metabolic pathway together with the presence of large size of three N-linked complex-type carbohydrate moieties (~ 15KDa/site) appears to be unique among retroviruses. (Sponsored by the NCI, under contract No. NO1-CO-74101 with Bionetics Research, Inc.

J 323 **DEVELOPMENT OF A PRIMATE MODEL FOR PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY USING A CHIMERIC JC VIRUS CONTAINING A SV40 ENHANCER**, Dominick A. Vacante, Gregory A. Elder, Sidney A. Houff, Eugene O. Major. Infectious Diseases Branch, DIR, NINCDS, National Institutes of Health, Bethesda, MD 20892.

A chimeric polyomavirus was constructed by inserting the 72-base-pair (bp) and 21-bp repeats of simian virus 40 (SV40) into the JC virus (JCV) regulatory region on the late side of the JCV 98-bp repeats. Although this chimeric polyomavirus was able to propagate well in human fetal glial cells, deletions were found in the chimeric regulatory region. Sequence analysis and mapping of the transcriptional start sites of a selected clone indicated a regulatory region with a JCV replication origin, an early viral promoter that consisted of JCV sequences, and a late viral promoter that consisted of SV40 sequences. This genome had an extended host range in tissue culture, producing infectious virus in human fetal brain and embryonic kidney as well as in rhesus fetal and adult glial cells. Thus the clone was not restricted by tissue type or species. The extended species host range suggested a potential primate model for progressive multifocal leukoencephalopathy, the acute human demyelinating disease caused by JCV. Therefore, infectious virus was inoculated intracranially into cyclosporin-treated juvenile rhesus monkeys. After 6 weeks, evidence derived from brain scans using magnetic resonance imaging, indicated white matter brain lesions that are consistent with demyelinated foci. Virological, immunological, and neurological evidence will be presented for the involvement of this virus in the development of the central nervous system disease.

J 324 DISASSEMBLY OF THE CYTOSKELETON IN BHK CELLS INFECTED WITH VESICULAR STOMATITIS VIRUS, Keiko Ozaki, Patricia A. Whitaker-Dowling, Julius S. Youngner and Christopher C. Widnell, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have shown earlier that endocytosis is inhibited in BHK cells infected with vesicular stomatitis virus (VSV). To determine whether changes in the organization of the cytoskeleton could contribute to the effect, we have studied, by fluorescence microscopy of VSV-infected cells, the distribution of: F-actin (with phalloidin); microtubules (with anti-tubulin); intermediate filaments (with anti-vimentin). The distribution of actin changed at 1h post-infection and at 3h no stress fibers were evident. The organization of microtubules began to change at 3h post-infection and at 4-6h, when the cells changed from a spread to a rounded morphology, only a few microtubules remained as recognizable structures. The last change to be detected was in the organization of intermediate filaments, which correlated with the rounding of the cells. Studies with ts mutants of the virus indicated that the viral proteins G, M and N were not required for these effects. In contrast, cells infected with a ts mutant in L (at 39.5°) and UV-irradiated virus (at 37°) exhibited the same morphology as control, uninfected cells even after 6h. The results suggest that these changes in the organization of the cytoskeleton represent a coordinated response to infection with VSV, which requires viral gene expression, and which is mediated by the viral L and/or NS proteins. The changes in cytoskeletal organization seem to mediate, at least in part, the cytopathic effect of the virus on BHK cells.

J 325 THE RATE OF BULK FLOW FROM THE ENDOPLASMIC RETICULUM TO THE CELL SURFACE, Felix T. Wieland, Michael L. Gleason, Tito A. Serafini and James E. Rothman, Stanford University, Stanford, CA 94305.
Tripeptides containing the acceptor sequence for Asn-linked glycosylation (Asn-X-Ser/Thr) were added to Cho and HepG2 cells. The tripeptides were glycosylated in the ER and then secreted into the medium, via the Golgi complex in which the oligosaccharide chains were processed. The half-time secretion, ~10 min, was faster than that of known proteins transported through the same pathway. Since much evidence suggests that oligosaccharide chains are not signals for transport, it appears that no signal is necessary for rapid and efficient transport from the ER to the Golgi, or from the Golgi to the cell surface. Rather, it appears that proteins retained as permanent residents en route through the ER-Golgi transport pathway must contain specific retention signals.

J 326 THE ROLE OF GLYCOSYLATION IN FORMATION OF NEUTRALIZING EPITOPES OF LCMV, Kathryn E. Wright and Michael J. Buchmeier, Research Institute of Scripps Clinic, La Jolla, CA 92037.
The arenavirus LCMV contains three major structural proteins, two of which are glycoproteins with molecular weights estimated to be 44,000 (GP-1) and 35,000 (GP-2). These glycoproteins are cleavage products of a nonstructural glycopeptide termed GPC. We have observed that monoclonal antibodies against neutralizing epitopes on GP-1 of the Armstrong strain of LCMV used in our laboratory recognize predominantly conformational epitopes and one of these conformational epitopes appears to require glycosylation for expression. Using such monoclonals as probes of conformation we have undertaken experiments examining the effects of glycosylation on folding. Using inhibitors of glycosylation we have demonstrated a role for sugar moieties in promoting GP-1 folding into the appropriate native conformation. Further, these experiments have revealed a requirement for glycosylation for proper proteolytic cleavage of GPC. We are using conformation dependent and independent monoclonal antibodies to further probe the processing and intracellular transport of GPC and its cleavage to form GP-1 and GP-2.

Virus Assembly

J 400 THE ROLE OF THE ROTAVIRAL NONSTRUCTURAL GLYCOPROTEIN NS28 IN VIRUS MORPHOGENESIS. K.S. Au, W.K. Chan and Mary K. Estes, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030. Rotavirus morphogenesis involves the budding of subviral particles through the rough endoplasmic reticulum (RER) membrane of infected cells. During this process, particles acquire the outer capsid proteins and a transient envelope. Previous immunocytochemical and biochemical studies suggested that a viral nonstructural glycoprotein, NS28 encoded by gene 10 (G10), is a transmembrane RER protein and about 10K of its carboxy-terminus is exposed on the cytoplasmic side of the RER. We have used in vitro binding experiments to examine whether NS28 serves as a receptor that binds subviral particles and mediates the budding process. Specific binding activity was observed between purified SA11 single-shelled (ss) particles and RER membranes from (1) SA11-infected monkey kidney cells, (2) SA11 G10 baculovirus recombinant-infected insect cells, and (3) rabbit reticulocyte lysates (RRL) programmed with SA11 G10 mRNA. Comparison of the binding of ss-particles to microsomes from infected monkey kidney or insect cells indicated membrane associated component(s) from SA11-infected monkey kidney cells interfered with binding. A monoclonal antibody previously shown to interact with the C-terminus of NS28 also blocked the binding of radiolabeled ss-particles effectively. Protease treatment of the RER membrane (known to cleave the C-terminus of NS28) significantly reduced the amount of ss-particle binding. Evidence for a direct interaction of NS28 expressed in RRL and the ss-particles was also obtained by co-pelleting the preformed receptor-ligand complex through sucrose gradients. These experimental data provide support for the hypothesized receptor role of NS28 in the budding process of rotavirus morphogenesis.

J 401 VIRION BASIC PHOSPHOPROTEIN OF HUMAN CYTOMEGALOVIRUS CONTAINS O-LINKED N-ACETYLGLUCOSAMINE. Donna Benko,[1] Robert Haltiwanger,[2] Gerald Hart,[2] and Wade Gibson.[1] Virology Laboratories, Depts. of Pharmacology and Molecular Sciences,[1] and Biological Chemistry,[2] The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205.

A 149kDa virion protein of human strains of cytomegalovirus (HCMV) is the principal acceptor for galactose added *in vitro* by galactosyltransferase (GT, EC 2.4.1.22). Peptide comparisons of the galactosylated protein, taken together with other biochemical characteristics, identified it as the virus encoded basic phosphoprotein. This protein is thought to be a tegument, rather than an envelope, constituent and has not been recognized as a glycoprotein in earlier studies based on biosynthetic radiolabeling, lectin binding assays, and sensitivity to glycosidic enzymes. The galactosylated carbohydrate was resistent to endo-ß-N-acetylglucosaminidase F, but sensitive to removal by ß-elimination, indicating an O-linkage to the protein. Chromatographic size determinations showed that most of the galactosylated, ß-eliminated carbohydrate was present as a disaccharide, and high voltage paper electrophoresis identified that molecule as Galß1-4GlcNAcitol. These results establish that the HCMV virion basic phosphoprotein contains O-linked N-acetylglucosamine, and constitute the first example of a virus protein with this recently discovered modification.

J 402 **INSERTION OF THE ROTAVIRUS NON-STRUCTURAL GLYCOPROTEIN NCVP5 IN THE RER MEMBRANE INVESTIGATED BY SITE-DIRECTED MUTAGENESIS, Cornelia C. Bergmann, K.F.H. Powell and A.R. Bellamy, University of Auckland, New Zealand.**

Rotavirus particles mature by budding through the membrane of the rough endoplasmic reticulum (RER) and become transiently enveloped in the process. The temporary envelope is later removed, leaving mature viruses within the lumen of the ER. NCVP5 is a 175 amino acid non-structural glycoprotein thought to be involved in the budding process. This protein is directed to the ER of the cell and retained as an integral membrane protein with the COOH-terminal region exposed on the cytoplasmic side. The inferred amino acid sequence of the cloned gene predicts three hydrophobic domains at the NH2-terminus with two carbohydrate attachment sites in position 9 and 19. To determine the potential function of these regions as leader sequences or membrane spanning domains we have constructed a series of mutants deleted in specific regions at the NH2-terminus of the protein. These altered proteins have been analysed for membrane insertion and sequestration using a cell free transcription/translation system and dog pancreatic microsomes. Our results suggest the following: (i) each truncated protein is capable of membrane insertion. (ii) Glycosylation only occurs when the second hydrophobic domain is present. (iii) None of the mutants are sequestered into the lumen. (iv) The third hydrophobic sequence is the membrane- spanning domain in both the wild-type NCVP5 and mutants Δ(1-16) and Δ(40-48). These results lead to a model in which the glycosylated NH2-terminal region of NCVP5 is located on the luminal side of the membrane, whilst the third hydrophobic domain acts as the membrane spanning domain anchor. The function of the second hydrophobic domain remains to be assigned.

J 403 MEMBRANE-ASSOCIATED ASSEMBLY OF A PHAGE T4 DNA ENTRANCE VERTEX STRUCTURE STUDIED WITH EXPRESSION VECTORS, G. Michaud, A. Zachary, V.B. Rao and L. Black, Biochemistry Dept, Univ of Maryland Med Sch, Baltimore, MD 21201.

Assembly of the phage T4 prohead occurs on the cytoplasmic membrane through a specific attachment located near the gp20 DNA entrance vertex or connector. The gp20 DNA entrance vertex is a dodecameric ring required to initiate prohead assembly. In the absence of this protein in a T4 infection, open ended polyheads rather than proheads are assembled with a delay. Mutants of gene 40, an early gene unlinked to the head genes, whose protein product is not structural, exhibit a morphological phenotype similar to gp20 mutants. It is hypothesized that gp40 and gp20 form a membrane-associated "stalk" from which the prohead is assembled and later detached for DNA packaging. However, the postulated 40-20 interaction could be mediated through numerous other head proteins. Low levels of gp40 and gp20 synthesized in a T4 infection have made direct study difficult.

T4 head subassembly has been studied through use of expression vectors containing lambda pL promoters. Vectors were constructed with inserts containing T4 genes 20(pr20.34), 40(ps40.12) and 20+40(pr20.40.6). Gp20(67kd) and gp40(13kd) could be dectected as several percent of total cell protein following heat induction.

When the clones were heat induced and cytoplasmic membranes were purified, gp20 was strongly associated with purified membranes only in the gp20+g40 containing construction. In these gp20+gp40 preparations gp20 was the major membrane associated protein. Our experiments show that specific gp20 proteolytic fragments are protected by association with the membrane when gp40 is present. Both proteins are membrane associated, and gp20 is a membrane spanning protein requiring a membrane potential when gp40 is present. Our experiments establish a direct role of gp40 in assembling and attaching the DNA entrance vertex structure to the membrane, and raise the question of whether specific host attachment sites and membrane insertion systems are implicated.

J 404 HIV PROTEASE EXPRESSED IN ESCHERICHIA COLI EXHIBITS AUTOPROCESSING AND SPECIFIC MATURATION OF THE GAG PRECURSOR, Christine Debouck, Joselina G. Gorniak, James E. Strickler, Thomas D. Meek, Michael L. Moore, Brian W. Metcalf and Martin Rosenberg, Smith Kline & French Laboratories, King of Prussia, PA 19406.
The mature gag and pol proteins of HIV and all retroviruses derive from large gag and gag/pol polyprotein precursors by post-translational cleavage. A highly specific, virally encoded protease is required for this essential proteolytic processing. We have expressed the HIV protease gene product in E. coli and shown that it autocatalyzes its maturation from a larger precursor. In addition, this bacterially produced HIV protease specifically and accurately processed an HIV p55 gag polyprotein precursor when co-expressed in E. coli. This system will allow detailed structure-function analysis of the HIV protease and provides a simple assay for the development of potential therapeutic agents directed against this critical viral enzyme.

J 405 RETROVIRAL INFECTION INTERFERENCE FROM A VIRAL ENVELOPE GLYCOPROTEIN AND A TRANSLOCATION DEFECTIVE VARIANT, Eric L. Delwart and Antonito T. Panganiban, McArdle Laboratory, University of Wisconsin, Madison, WI 53706.
Cells transfected with reticuloendotheliosis (REV) env expression plasmids will translocate the viral glycoprotein to their membranes. Such cells exhibit membrane enclosed cytoplasmic projections on their surface as do virus infected cells. Envelope expression also leads to a transient formation of syncytia. Cell lines with env as the only viral component are a hundred fold resistant to REV infection.
A mutant envelope glycoprotein with the transmembrane and cytoplasmic tail domains replaced by 20 random amino acids is defective in translocation.
This mutant induces no syncytia, no surface distortions and by FITC immunostaining appears blocked in the RER. It does not label with golgi added fucose and does not proteolytically process what remains of its transmembrane region. This glycoprotein still induces a hundred fold resistance to REV infection presumably by interacting with its receptor while both are translocating through the RER.

J 406 **ROLE OF CYTOPLASMIC VACUOLES IN VARICELLA-ZOSTER VIRUS GLYCOPROTEIN TRAFFICKING AND VIRION ENVELOPMENT,** Frank Jones and Charles Grose, University of Iowa, Iowa City, IA 52242.

Varicella-zoster virus (VZV) encodes several glycoproteins which are present on both mature viral envelopes and the surface of infected cell membranes. Mechanisms of VZV glycoprotein transport and virion envelopment were investigated utilizing both continuous radiolabeling and pulse-chase analyses with tritiated fucose in VZV-infected cells. Fucose was selected because it is a terminal sugar that is not converted into other sugars. When the specific activity in subcellular compartments was defined by quantitative electron microscopic autoradiography utilizing crossfire analysis, we documented a progression of activity originating in the Golgi and travelling through the post-Golgi region into cytoplasmic vacuoles and finally to areas of the cellular membrane associated with the egress of viral particles. Significant label was not observed in the cytoplasm or nucleus and only low levels of label were associated with the cellular membrane not involved with the egress of viral particles. These observations suggested that cytoplasmic vacuoles harbored VZV-specified glycoproteins and were also the predominant site of varicella-zoster virion envelopment. Viral particles were not observed within the Golgi apparatus itself.

J 407 LOCALIZATION OF M2 PROTEIN IN INFLUENZA VIRUS-INFECTED MDCK CELLS. Patsy G. Hughey,[1] Richard W. Compans,[1] Suzanne L. Zebedee,[2] and Robert A. Lamb[2]
[1]University of Alabama at Birmingham, Birmingham, AL 35294, [2]Northwestern University, Evanston, IL 60201.

The M2 protein of influenza virus is a small, non-glycosylated membrane protein of undetermined function in virus replication. Monoclonal antibodies specific for the external domain of M2 or hemagglutinin were used to analyze and compare the time course of surface appearance of the proteins in infected MDCK cells. Immune fluorescence and ^{125}I-protein A binding studies indicate that M2 is expressed on the apical surfaces with kinetics similar to those of HA. Treatment of infected cell monolayers with EGTA did not significantly increase the amount of label bound, indicating that M2 is predominantly localized on the apical surface. The ultrastructural localization of M2 in infected cells was investigated by electron microscopy using the monoclonal antibody specific for M2 followed by a gold conjugate. At 6-7 hpi, a time when extensive virus budding was occurring, the apical cell membrane was labeled with gold particles evenly distributed between microvilli and the surrounding membrane. In addition, 35-45% of the gold particles were apparently associated with virions. The HA specific monoclonal antibody exhibited a similar pattern of labeling of virions and microvilli. These results suggest that M2 may be involved in virus maturation.

J 408 ANALYSIS OF THE COMBINED SIGNAL-ANCHOR DOMAIN OF A SMALL INFLUENZA VIRUS PROTEIN M_2, WHICH HAS AN N-TERMINAL ECTODOMAIN

J. David Hull[1], Reid Gilmore[2] and Robert A. Lamb[1],
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The M_2 protein of influenza A virus is a small (97 amino acids) integral membrane protein that is expressed on the surface of infected cells. Examination of the requirements for integration of M_2 into the endoplasmic reticulum (E.R.) demonstrated that this process is co-translational and dependent upon the presence of signal recognition particle (SRP). This finding raises interesting questions regarding the orientation of M_2 in the E.R. as despite having an internal signal-anchor, M_2 is orientated in membranes with its C-terminus on the cytoplasmic side. Deletion of 6 or more residues from the 19 residue signal-anchor of M_2 prevents its integration into the E.R. and deletion of as few as 2 residues decreases the efficiency of integration substantially. None of the deletion mutants has a functional signal sequence and non-functional anchor, suggesting that the two activities cannot be separated out of a combined signal-anchor region.

J 409 OLIGOMERIZATION OF ROUS SARCOMA VIRUS GLYCOPROTEIN COMPLEX. Eric Hunter and David Einfeld. University of Alabama at Birmingham, Birmingham, AL 35294.

The oligomeric nature of the Rous sarcoma virus envelope protein was investigated in an SV-40 expression vector system. Lysates from vector-infected cells, labeled with [^{35}S]-methionine in pulse-chase experiments, were analyzed by sucrose gradient fractionation and immuno-precipitation. The 95kd high-mannose glycoprotein precursor, present in cells pulse-labeled for 5 min., sedimented as a monomer but was observed to associate into a discrete oligomer during 30-60 min. chase periods. The oligomeric form of the envelope glycoprotein appears to be a trimer since it has a sedimentation rate equivalent to that of the influenza virus NA tetramer (approx 300kD). The precursor remains in a stable, trimeric form as it is converted to the mature disulfide-linked glycoproteins (gp85 and gp37) in the Golgi and is transported to the cell surface. Mutant proteins lacking the 22 amino acid cytoplasmic domain or both the cytoplasmic domain and the 27 amino acid transmembrane region also form trimers, although the rate of oligomerization appears to be altered. A mutant envelope protein with a C-terminal 95 amino acid deletion, that encompasses 46 amino acids of the extracellular domain along with the transmembrane region and the cytoplasmic domain, does not form oligomers. This mutant has previously been shown to be defective in intracellular transport and accumulates in the endoplasmic reticulum. These data suggest that oligomerization is critical to normal intracellular transport of the viral glycoproteins and may play an important role in virus assembly.

J 410 SEMLIKI FOREST VIRUS AS MODEL SYSTEM FOR PRESENTATION OF HETEROLOGOUS EPITOPES: PRELIMINARY RESULTS, Danny Huylebroeck, Peter Liljeström[1] and Henrik Garoff[1], EMBL, Heidelberg, Fed. Rep. Germany, [1]Karolinska Institute, Center for Biotechnology, Huddinge, Sweden.
We are interested in developing different viral delivery systems for heterologous antigens and epitopes. This requires a detailed understanding of the biosynthesis of the envelope glycoproteins and the assembly of the viral particle used as a carrier. We use SFV as a model system. One approach towards a new vaccine generation of enveloped RNA viruses aims at producing a defective virus preparation suitable to be used as a vaccine. The basic strategy is to package a replication-incompetent genome into virus particles in a helper cell line that produces the missing gene products needed for viral replication. We will report on the progress in constructing an infectious cDNA clone of SFV. We plan to use this for the generation of chimeric particles containing epitopes from other Toga- and Flaviviruses. We have also localized the translocation signal of the p62 glycoprotein from SFV by inserting the coding regions of two reporter molecules in frame at a site 40 codons away from the 5'-end of the p62 coding region. Both fusion proteins translocate in an *in vitro* transcription-translation-translocation assay, remain uncleaved and are glycosylated at the only potential site present in either hybrid, i.e., at Asn (13) of the translocation-active p62 part. Alkaline treatment of the lysates demonstrates secretion of the fusion proteins into the lumen of microsomes. However, a small but significant fraction of the translocated molecules remain membrane-attached when glycosylation is inhibited.

J 411 STRUCTURE, MODIFICATION, INTRACELLULAR TRANSPORT AND SURFACE EXPRESSION OF INFLUENZA AND PARAMYXOVIRUS SV5 PROTEINS M_2, NB, & SH, AND A HYBRID PROTEIN, ANCHORED PYRUVATE KINASE.
Robert A. Lamb, Scott W. Hiebert, Mark A. Williams and Suzanne L. Zebedee,
Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL. 60208.

The soluble cytoplasmic protein pyruvate kinase (PK) has been expressed at the cell surface in a membrane anchored form (APK). The hybrid protein contains the N-terminal signal/anchor domain of a class II integral membrane protein (HN of the paramyxovirus SV5) fused to the PK N-terminus. APK contains a cryptic site that is used for N-linked glycosylation but elimination of this site does not affect cell surface localization. Truncated forms of the APK molecule, with up to 80% of the PK region of APK removed, are also expressed at the cell surface. These data indicate that neither native folding nor glycosylation are necessary for intracellular transport of PK to the cell surface, and suggest a lack of specific signals for cell surface localization in the ectodomain.

The small hydrophobic polypeptide (SH) of 44 amino acid residues of the paramyxovirus SV5 has been demonstrated to have properties of an integral membrane protein, is found in the exocytotic pathway and is transported to the infected-cell surface. Biochemical analysis of the orientation of SH in membranes indicates that SH is orientated in membranes with its N-terminal hydrophilic domain exposed on the cytoplasmic face of the plasma membrane and the ~5 amino acid residue C-terminus exposed at the cell surface. These data will be discussed with respect to positive-acting signals being necessary in the ectodomain of SH for cell surface expression.

Both M_2 of influenza A virus and the NB glycoprotein of influenza B virus belong to a small class of integral membrane protein that have a single hydrophobic domain that acts as a signal/anchor and have N-terminal domains exposed extracellularly. NB contains two N-linked carbohydrate chains that are processed from the high mannose form to a heterogeneous form of much higher molecular weight which has the characteristics of polylactosaminoglycan and is expressed at the cell surface. Monoclonal antibodies specific for the extracellular domain of M_2 have been used to resolve the issue of whether M_2 is a component of purified virions and to probe the function of M_2 in infected cells.

J 412 SEPARATION OF VESICULAR STOMATITIS VIRUS L PROTEIN FROM NS PROTEIN PHOSPHORYLATING ACTIVITY. Douglas M. Massey, Gail M. Clinton and John Lenard. UMDNJ-Robt. Wood Johnson Med. Sch. and Ore. Health Sci. Ctr. U.

Isolated vesicular stomatitis virus (VSV) L protein was subjected to gel filtration on Sephadex G-200 to test whether it can be separated from its associated ability to phosphorylate the viral NS protein. L protein was isolated from gradient purified 35S-methionine labeled VSV in 3 steps, as previously described by others: (i) Nucleocapsid preparation from detergent disrupted virions by centrifugation on discontinuous glycerol gradients; (ii) Dissociation of L+NS from N-RNA by high salt disruption of nucleocapsids, followed by separation on glycerol gradients; (iii) Separation of L from NS protein by phosphocellulose chromatography. >90% of the radioactivity of the L fraction thus prepared was eluted in the void volume of a G-200 column, while >90% of the NS phosphorylating activity was retarded, eluting with an apparent m.w. of ca. 75 kDa. The NS phosphorylating fractions were also active against casein, but small differences in the activity profiles against the two substrates suggested the presence of more than one kinase. The kinase containing fractions were not significantly labeled with 35S-methionine, suggesting that they were of cellular rather than viral origin. Since phosphorylation of NS at specific sites is required for polymerase activity (Chattopadhyay and Banerjee, Cell, 49, 407, 1987), it appears that one or more cellular kinase(s) may be involved in regulation of VSV transcription.

J 413 PATHWAYS OF VIRAL ANTIGEN PRESENTATION TO CLASS II-RESTRICTED CYTOTOXIC T CELLS ARE DEFINED BY THE MODE OF VIRUS ENTRY, Eric O. Long[1], Rafick P. Sekaly[1] and Steve Jacobson[2], [1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, and [2]Neuroimmunology Branch, National Institute of Neurology, Communicative Disorders and Stroke, NIH, Bethesda, MD 20892.
Class II MHC-restricted CD4-positive cytotoxic T lymphocytes (CTL) specific for virus-infected cells are an important component in the immune response to certain viruses. The requirements for antigen presentation to influenza virus-specific or to measles virus (MV)-specific class II-restricted CTL are different. Influenza virus-specific class II-restricted CTL recognize antigens processed through the chloroquine-sensitive endocytic pathway but do not recognize antigens synthesized intracellularly (1). In contrast, MV-specific class II-restricted CTL recognize matrix and nucleocapsid proteins synthesized in the target cell. Unlike influenza viruses which fuse only with endosomal membranes, many other viruses, such as MV and HIV, fuse with the cell membrane at neutral pH. Whereas influenza virus antigens can be rapidly processed and presented via the endocytic pathway, the primary source of MV antigen for processing and presentation may rather be newly synthesized material. The identification of the antigen presentation pathway for specific viral pathogens will be important for the design of strategies to enhance the host's cellular immune response.
(1) Morrison et al. (1986) J. Exp. Med 163:903-921.

J 414 THE LATERAL MOBILITY OF VIRAL GLYCOPROTEINS IS DETERMINED BY THE GLYCOPROTEIN STRUCTURE AND THE HOST CELL. Shari L. Lydy, Sukla Basak, and Richard W. Compans, Univeristy of Alabama at Birmingham, Birmingham, Alabama 35294
The lateral mobility of viral glycoproteins has been found to be host cell dependent. In the presence of specific antibody, both measles and parainfluenza type 3 (PI3) viral glycoproteins are mobile on the surfaces of infected HeLa cells, forming unipolar caps. In contrast, no such redistribution is observed with influenza virus HA or vesicular stomatitis virus (VSV) G glycoproteins on the surfaces of infected HeLa cells. However, both influenza virus and VSV glycoproteins are mobile in the plasma membrane of CV-1 cells. When comparing the above cell lines, no significant difference in the synthesis of influenza or VSV viral proteins was found, thus indicating that the defect in lateral mobility in HeLa cells is not due to differences in the relative amounts of viral proteins. In HeLa cells doubly infected with vaccinia and PI3 virus, the PI3-HN and F glycoproteins retain their lateral mobility, whereas vaccinia superinfection of influenza-infected HeLa cells does not alter the restricted mobility of influenza HA. Influenza HA and VSV-G glycoproteins expressed from genes cloned into vaccinia virus were found to be immobile in HeLa cells and mobile in CV-1 cells, thus indicating that the restricted mobility in HeLa cells is an intrinsic property of the glycoprotein molecule itself and not the result of interaction with other viral components. It is possible that differences in the association of viral glycoproteins with either the cytoskeleton or the membrane proteins of cells may be related to the observed differences in lateral mobility.

J 415 DNA-PROTEIN INTERACTIONS IN VIRUS ASSEMBLY, Richard N. Perham, Gary J. Hunter and David H. Rowitch, University of Cambridge, Cambridge, CB2 1QW, U.K.
The filamentous bacteriophages occupy a central place in molecular biology as models for many important processes, among them DNA-protein interactions and DNA packaging, DNA replication, protein synthesis and secretion across membranes, and self-assembly. The 50-residue major coat protein (encoded by gene VIII) of the filamentous bacteriophage fd (M13) forms a singled tube of overlapping α-helices surrounding the DNA, with the positively-charged region at the C-terminal end of each protein abutting the DNA and the negatively-charged region at the N-terminal end forming the hydrophilic surface. Site-directed mutagenesis of gene VIII has shown that positive charge on the cluster of four lysine residues near the C-terminus of the coat protein directly governs virus assembly; mutation of lysine-48 to a neutral side chain such as alanine, threonine or glutamine causes a change in the nucleotide:protein subunit packing ratio and with it the length of the assembled viral particle. On the other hand, some other mutations do not affect the essential prior insertion of the processed coat protein into the bacterial membrane but prevent subsequent elongation step(s) in the extrusion of the viral particle. Fibre diffraction analysis of the mutated filaments is capable of providing answers at atomic resolution to investigations of the role of membrane protein processing and DNA-protein interactions in virus assembly.

J 416 PRIMARY SEQUENCE DOMAINS REQUIRED FOR THE RETENTION OF ROTAVIRUS VP7 IN THE ENDOPLASMIC RETICULUM, Marianne S. Poruchynsky and Paul H. Atkinson, A. Einstein College of Medicine, Bronx, N.Y. 10461.

Rotavirus VP7 is a membrane-associated glycoprotein of the endoplasmic reticulum (ER), characterized by two NH_2-terminal hydrophobic domains, h1 and h2, each preceded by an initiation codon. Previous studies showed that deletions of h2 plus adjacent distal coding sequences for amino acids (aa) #51-61, resulted in secretion of the altered VP7, suggesting that information for retention was present in h2. In current studies, comparison in the presence or absence of membranes, of in vitro translated messages of wild type (wt) VP7 or deletions (d.) eliminating the first MET and h1 (dh1), and of VP7-amylase fusions, indicated that initiation occurred at the second MET and h2 was cleaved, as did the comparison of secreted products from cells transfected with deletion mutants, confirming studies by others (Stirzaker C. et al, 1987). Wt VP7 translated product was the same size as the major VP7 product in infected cells. The VP7 region aa #51-61, absent from the secreted mutant 47-61, was tested for its ability to retain salivary α-amylase, a secretory protein, in the ER. The coding region extending from aa #1-63 in wt VP7, in d.1-14 or in d.51-61/dhl, was fused with the mature amylase coding region and the chimeric proteins were seen to be secreted from transfected cells. Since d.47-61 and the $VP7_{63}$-amylase chimeric proteins are secreted, wt VP7 is not, and h2 is cleaved for each, we conclude that the region spanning aa # 51-61 is necessary but not sufficient for ER retention. Extending the VP7 contribution to the chimera to aa # 111, resulted in retention of d.1-14_{111}/Am product and secretion of d.47-61_{111}/dhl/Am. A pulse chase study showed that d.47-61/dhl, wt amylase, or 47-61_{111}/dhl/Am products were each secreted efficiently. Therefore, two regions of VP7 mediate its retention in the ER. The first, is within the region aa #51-61 and the second is within aa # 62-111, which contains the glycosylation site for VP7.

J 417 INTERACTION OF THE AVIAN RETROVIRAL GAG PRECURSOR WITH CELLULAR MEMBRANES. Daniel M. Putterman and Volker M. Vogt, Cornell University, Ithaca, NY 14853.

The gag proteins of Avian Sarcoma and Leukemia Viruses (ASLV) are synthesized on free polysomes as a polyprotein precursor denoted Pr76gag. It is the precursor which assembles at the plasma membrane and buds from it to form nascent virions. Little is known about this gag-membrane interaction other than that it probably occurs near the N-terminus of the molecule[1], and that interactions with the envelope glycoproteins also synthesized by the virus are not required for particle formation[2]. In addition, while N-terminal myristylation of the murine gag precursor Pr65gag has been shown to be necessary for membrane binding[3], no such N-terminal modification has been discovered for ASLV gag (however, the molecule is acetylated). Recent evidence from our lab implies that Pr76gag binds to membranes peripherally. Attempts to label Pr76gag with the hydrophobic carbene-generating reagent [^{125}I]TID (trifluoromethyl-meta-[^{125}I]iodophenyl diazirine) have not been successful, while extraction of ALV infected cells with Triton X-114 has led to recovery of Pr76gag in the aqueous phase. Recently, we have cloned a gag gene construct denoted pPrgag into a T7 transcription vector to allow coupled in vitro transcription/translation of the gag precursor. Efforts to reconstitute binding of Pr76gag so derived with chick embryo fibroblast membranes will be described, as well as our attempts to dissect the mechanism of this binding.

1 Pepinsky and Vogt, J. Mol. Biol. 131, 819, 1979.
2 RNA Tumor Viruses, Weiss, et al. eds., chapter 6 (Cold Spring Harbor Laboratory, 1982).
3 Rein, et al., PNAS 83, 7246, 1986.

J 418 MECHANISM OF SYNTHESIS OF THE BOVINE LEUKEMIA VIRUS PROTEASE, Nancy R. Rice, Terry D. Copeland, and Stephen Oroszlan, Basic Research Program, Bionetics Research, Inc., NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

In bovine leukemia virus (as in HTLV-I and -II) the sequence encoding the viral protease lies downstream of the *gag* gene but in a different reading frame. Using antisera to synthetic peptides and to purified viral proteins we have tested whether translation of the protease occurs by a frameshift mechanism. Our results are perfectly consistent with such a mechanism: we found a 15 kD protein in the virus containing both p12 and downstream determinants, and an intracellular protein of about 66 kD which is the likely *gag*-protease precursor. These findings are consistent with our prediction based on the BLV sequence [Rice *et al.*, *Virology* 142: 357, 1985] and agree with the results from Rous sarcoma virus [Jacks and Varmus, *Science* 230: 1237, 1985] and mouse mammary tumor virus [Hizi *et al.*, *PNAS* 84: 7041, 1987] where frameshifting has been definitively demonstrated.

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J 419 MEMBRANE PERTURBATIONS RESULTING FROM HERPES SIMPLEX VIRUS TYPE 1 PENETRATION, Ken S. Rosenthal, Deborah Roess, and B. George Barisas, N.E. Ohio Universities Coll. of Med., Rootstown, OH 44272 and Colorado State Univ., Fort Collins, CO

Both viral and cellular structures and functions are involved in the uptake of herpes simplex virus (HSV) into the cell. Upon interaction of HSV-1 KOS with target cell, global changes in cell surface protein mobility can be observed indicative of cytoskeletal involvement. While the virus was bound to HEp-2 cells, a restriction in cell surface protein (CSP) mobility (Rosenthal et al. 1984. J. Virol. 49:980) occurred, which has been termed anchorage modulation (Gall and Edelman. 1981. Science 213:903). Concurrent with virus penetration, CSP mobility increased to 3-4 times that of control cells for 20-30 minutes and then returned to normal levels upon completion of penetration for HEp-2 and VERO cells. Inhibition of HSV penetration at mild acidic pH prevented the increase in CSP mobility. The antiviral drug tromantadine also inhibits penetration and inhibited the increase in CSP mobility. Cytochalasin D, an inhibitor of microfilament function, inhibited both penetration and the increase in CSP mobility while colchicine, a microtubule effector, did not inhibit HSV penetration, but inhibited the return of CSP mobility to normal levels following the penetration period. These studies indicate that both microfilaments and microtubules play a role in HSV penetration, that virus binding and penetration have significant effects on the cell membrane and that penetration and the related changes in membrane structure can be specifically blocked.

J 420 A DELETION OF THE SIGNAL PEPTIDE PREVENTS THE INCORPORATION OF A HERPESVIRUS GLYCOPROTEIN INTO THE VIRUS ENVELOPE, Patrick Ryan, Calvin Keeler, Jr., Mary Whealy, Alan Robbins, and Lynn Enquist, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898. The envelope of the swine herpesvirus, Pseudorabies virus (PRV) contains at least five glycoprotein species. Our laboratory has cloned, mapped, and sequenced a nonessential yet major constituent, glycoprotein gIII. Analysis of the DNA sequence indicates the presence of a twenty-two amino acid signal peptide at the amino-terminus, and a single transmembrane spanning domain near the carboxy-terminus. Previous work has demonstrated a rapid and efficient conversion of a 74 kDa pre-Golgi form of gIII, containing primary N-linked glycosylation, to a 92 kDa complex glycosylated mature species that is found both on the infected cell surface and in the virus envelope. To demonstrate that this process was dependent upon the signal peptide predicted by DNA sequencing, codons 2-22 of the wild-type sequence were deleted from a plasmid clone of a portion of the gIII gene. The mutant allele was subsequently recombined into an otherwise wild-type genome, giving rise to a strain, PRV56, whose plaques did not react with gIII-specific monoclonal antibody. Analysis of PRV56 infected-cells revealed the presence of a single polypeptide that specifically reacted to polyvalent serum raised against purified gIII. This stable, nonglycosylated 57 kDa protein migrated similarly in SDS-polyacrylamide gels to the in vitro translated and endoglycosidase H-treated forms of the wild-type gIII polypeptide. In no instance was the mutant gIII detected in viral envelopes. Relative to wild-type gIII, the mutant allele unexpectedly was found to be expressed at reduced levels, as judged by slot blot and Northern analysis of infected-cell RNA. This is the first demonstration of a signal sequence deletion mutant of a herpesvirus envelope glycoprotein, and the isolation of PRV56 will greatly facilitate the recombination into the gIII gene of more discrete signal sequence alterations that may result in a "leaky" phenotype.

J 421 SPECIFIC INTERACTION BETWEEN THE CORONAVIRUS NUCLEOCAPSID PROTEIN AND THE MHV LEADER RNA SEQUENCES. Stephen A. Stohlman, Robert Deans, Ralph Baric, Gary Nelson and Michael M.C. Lai. University of Southern California School of Medicine, Los Angeles, CA 90033.

Mouse hepatitis virus (MHV) is the prototype of the RNA plus-stranded enveloped coronavirus group of animal viruses. The virion is composed of two envelope glycoproteins and the nucleocapsid (N) protein which associates with the 20 Kb RNA to form a helical nucleocapsid. Using monoclonal antibody (mAb) specific for the N protein we found that N was not only associated with virion genomic RNA but also with intracellular genomic RNA and each of the other six MHV-specific mRNA. The common feature of all these RNA species is a leader sequence of approximately 72 bases found at the 5' end of each mRNA. Anti-N mAb also immunoprecipitated the small intracellular leader containing intracellular RNAs of 65 nucleotides or longer. These RNAs are functional intermediates released during pausing of the transcriptional complex at areas of secondary structure on the negative strand template. In addition, the anti-N mAb immunoprecipitated the RI complex from infected cells. Specificity for (+) or (-) strand RNA was demonstrated by an RNA-protein overlay blot assay (ROBA). Specific binding of PT 7 derived RNA transcripts to N in the presence of competitor RNA was found for (+) but not (-) sensed RNA. In addition, the binding site on the RNA for N was mapped between leader RNA nucleotides 56 and 72 by ROBA. Internal sequences lacking leader RNA did not bind to N.

J 422 DEVELOPMENT OF A NOVEL MoMLV-BASED VECTOR SYSTEM WITH THE HOST RANGE OF A TYPE C PRIMATE RETROVIRUS, Carolyn Wilson, Marvin S. Reitz, Hiroto Okayama and Maribeth Eiden. Laboratory of Tumor Cell Biology, NCI and Laborator of Cell Biology, NIMH, Bethesda, Maryland.

A transient assay was performed to determine if the envelopes of the gibbon ape leukemia viruses, GaLV SEATO and/or GaLV SF could be assembled with Moloney murine leukemia virus, MoMLV, gag and pol gene products to generate infectious viral particles. Three plasmids were cotransfected into NIH-3T3 cells :1) a MoMLV env-minus plasmid containing the MoMLV 5' LTR, the coding regions of the MoMLV gag and pol and an SV40 polyadenylation signal 2) an env plasmid containing a MoMLV 5' LTR and the gene encoding either the GaLV SEATO or GaLV SF envelope 3) the evx plasmid containing the MoMLV 5' and 3' LTRs, the MoLV packaging site, and the gene conferring resistance to the antibiotic G418. Filtered supernatant from the transfected cells containing between 10^3 and 10^4 infectious viral particles/ml allowed for the infection of cell lines suseptible to infection with these gibbon ape retroviruses but not NIH 3T3 cells which are refractory to infection by GaLV retroviruses. The components of this system will be useful for the expression cloning of the GaLV receptor, and for characterizing envelope regions that determine primate retrovirus host range. Furthermore by generating retroviruses carrying GaLV-MoMLV hybrid envelopes one can identify regions involved in murine versus primate virus receptor interactions. We are currently using this approach to identify other retroviral envelopes that complement MoMLV gag and pol genes to generate recombinant retroviruses.

Interaction of Viruses and the Immune System

J 500 **B-CELL KILLING OF MOUSE HEPATITIS VIRUS-INFECTED CELLS: FUSION OF TARGETS AND EFFECTORS,** **Maria Wysocka[1], Robert Korngold[2], Robert Knobler[2], Jonathan Yewdell[3], and Jack Bennink[3], [1]The Wistar Institute, Philadelphia, PA 19104, [2]Thomas Jefferson University, Philadelphia, PA 19107, [3]National Institutes of Health, Bethesda, MD 20892.**

Effector cells (phenotypically IgM^+, IgG^+, $J11d^+$, Ia^+, Fc^+, $Thy1^-$, $MAC\text{-}1^-$ and asialo-$GM1^-$) capable of lysing mouse hepatitis virus (MHV)-infected target cells have been found in the spleen and lymph node of non-immunized mice, confirming previous reports. Immunoglobulin does not appear to be required for the B-cell cytolysis since the B-cell hybridoma, Sp2/0, which has no surface or secreted immunoglobulin, lyses MHV-infected targets. Furthermore, using Sp2/0 ^{51}Cr labeled cells, both the effector and MHV-infected target cells were shown to undergo cytolysis. Results from studies labeling target and effector cells with different fluorescent dyes suggest that lysis is due to fusion of B-cells and MHV-infected cells. In addition, using SJL x BALB/c recombinant-inbred mice differing in susceptibility to MHV-induced encephalomyelitis, a direct correlation was observed between disease and the presence of cytolytic B-cells. This suggests a roll for B-cells in MHV-induced demyelination.

J 501 T-LYMPHOCYTE INVOLVEMENT IN SYSTEMIC COXSACKIEVIRUS INFECTION OF BALB/C AND DBA/2 MICE, Roy A. Blay and Sally A. Huber, University of Vermont, Burlington, VT 05405.

Histological evidence suggests that mature, male Balb/c and DBA/2 mice exhibit differential systemic effects after coxsackievirus (CVB3) infection. One week after intraperitoneal infection with CVB3, mice of both strains were sacrificed and hearts, pancreases, and livers removed. These organs were examined for the presence of inflammation and virus-induced cellular damage. Hearts from both strains were myocarditic. The acinar tissue of pancreases from both strains exhibited profound cellular damage while the islets were spared. The livers of Balb/c mice appeared relatively normal in contrast to DBA/2 livers which exhibited a greater number of inflammatory cells and enlarged hepatocytes with "frothy" cytoplasms. Monoclonal antibodies GK 1.5 (anti-L3T4) and 2.43 (anti-Lyt 2.2) were administered to infected mice to deplete them of T helper and T cytolytic cells, respectively. Neither antibody protected the acinar tissue of Balb/c mice nor did GK 1.5 protect the acinar tissue of DBA/2 mice suggesting that the cellular damage was not immunologically mediated. However, GK 1.5 protected both the livers and hearts of DBA/2 mice demonstrating that L3T4-positive T helper cells mediate cell damage in infected DBA/2 mice, and the same immunopathogenic mechanism is responsible for myocarditis and hepatitis in this strain. Adoptive transfers of myocyte-immunoadsorbed lymphocytes into thymectomized, bone-marrow reconstituted animals will be performed to determine if L3T4-positive T helper cells arising during infection recognize a common antigen on myocytes and hepatocytes, or if distinct T cell subpopulations arise that recognize antigens specific for each organ.

J 502 NUCLEOSOMAL FRAGMENTS IN SERUM:MARKERS FOR CELL-MEDIATED IMMUNE ATTACKS IN VIVO?

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It has been shown by in vitro experiments that cell-mediated immune attacks result in an almost instantaneous fragmentation of the target cellular DNA(1). These fragments can be visualized by gel electrophoresis as forming a ladderlike pattern with spacings of about 200 base pairs, the length of the nucleosomal fragment. A spectrum of patterns can be detected by nick-translation and electrophoresis of DNA isolated from the serum of acute hepatitis B patients, ranging from a single DNA band representing the HBV genome till a long ladderlike pattern with fragments differing about 200 base pairs in length and having a maximal length of 10-15 kb. These same long ladderlike patterns can also be found in sera of hepatitis A and PBC(primary biliary cirrhosis)-patients. In the pathology of these diseases cell-mediated cytotoxicity has been implied. In many of these sera also mitochondrial DNA can be detected, the relevance of which is presently unclear. In reconvalescent sera the same pattern is found as in the sera of healthy humans, i.e. a small ladder consisting of fragments with a maximal length of 1.2 kb, presumably representing a background caused by either cell-mediated immunity or by physiological cell death (apoptosis). By processing many samples simultaneously it is possible to follow the course of a disease, giving possible insight into the relative importance of cell-mediated cytotoxicity in its pathology. Examples of this will be presented.

1):Russell et al.,J.Immunol.124:1100 (1980).

J 503 TOPOGRAPHY OF SENDAI-VIRUS GLYCOPROTEINS IN MURINE CELL MEMBRANES AFTER FUSION WITH RECONSTITUTED SENDAI-VIRUS ENVELOPES

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Sendai virus (Sv) infects a cell after fusion with the plasma membrane. In this process, viral envelope components, including hemagglutinin/neuraminidase (HN) and fusion (F)glycoproteins (GP),are left behind in the cell membrane. To study whether these membrane-inserted GPs provide recognizable antigenic structures for cytotoxic T-lymphocytes (CTL),detailed knowledge of their level and mode of expression is required. Reconstituted Sv envelopes(RSVE) were prepared containing only HN and F. These RSVE were compared with intact Sv for their capacity to introduce envelope material in murine cell membranes by immuno EM. EL4 cells or ConA-stimulated spleen cells were incubated for 1h at 37°C with either RSVE or Sv. Bound and fused particles were recognized by an anti-hemagglutinin MoAb or a mouse anti-Sendai serum, followed by incubation with gold-labeled anti-mouse IgG. Fusion resulted in isolated HN and F molecules spread over the cell membrane. For a reliable comparison of Sv and RSVE-derived GPs inserted in the plasma membrane, the number of gold particles bound to the cell surface of 40 cells was counted. In both cases HN and F molecules were present in similar amounts. The RSVE-treated cells were consistently killed to a lower extent than target cells treated with whole virus, although we demonstrated with the ultrastructural studies that the same amount of GPs are introduced on the cell surface. These suggest that HN and F may not provide the dominant antigens for CTL recognition.

J 504 ISOTYPIC MODULATION OF MURINE ANTIBODIES PRODUCED AFTER VIRAL INFECTIONS.

Jean-Paul Coutelier (1,2), Jos van der Logt (3), Frans Heessen (3) and Jacques Van Snick (2,4); (1) LOM, National Institutes of Health, Bethesda, MD 20892, (2) University of Louvain, Bruxelles, Belgium, (3) University of Nijmegen, Nijmegen, The Netherlands and (4) Ludwig Institute for Cancer Research, Bruxelles, Belgium.

The isotypic distribution of murine IgG antibody responses was examined after infection with a panel of DNA and RNA viruses. The results indicated that: (i) IgG2a is the predominant subclass in antiviral IgG antibodies, which contrasts with a preponderance of IgG1 in anti-protein IgG antibodies produced after immunization. (ii) Infection with some viruses is followed by an hypergammaglobulinemia restricted to the IgG2a and, to a lesser extent, IgG2b subclasses. Most of these immunoglobulins are not antiviral antibodies. (iii) When mice are infected with some viruses concomitantly with the immunization with soluble protein antigens, a modification in the isotypic distribution of anti-protein antibodies is observed, with a preferential production of IgG2a.

These observations strongly suggest that viral infections can influence the immunoglobulin switch and selectively stimulate the production of the IgG2a subclass.

J 505 MODULATION OF IMMUNE RESPONSES BY DEFECTIVE INFLUENZA VIRUS, Nigel Dimmock and Lesley McLain, University of Warwick, Coventry, U.K.
Influenza in the mouse has an immune pathology mediated by delayed hypersensitivity T cells. Treatment with homologous defective virus (A/WSN) gives 80% protection against death. Since there is no detectable alteration in virus multiplication, we infer that defective virus is modulating the immune response. This requires active defective virus since betaproprolactone-inactivated defective virus does not protect. So far we have found alterations in both B cell and T cell responses. Only mice treated with defective virus make a local anti-HA IgG response and unusually this is non-neutralizing. Both make anti-neuraminidase. Further, mice treated with defective virus have a reduced lung CTL response. However killing by CTLs is doubled when local antibody is added either to CTLs themselves or target cells. Preliminary data indicate that this ADCC is MHC class I restricted.

J 506 THE ACID-INDUCED CHANGE IN THE INFLUENZA HEMAGGLUTININ MOLECULE IS A SIGNIFICANT STEP IN ANTIGEN PROCESSING FOR HELPER T (T_H) CELLS, Laurence C Eisenlohr, Walter Gerhard and Charles J. Hackett, Wistar Institute, Philadelphia, PA 19104

Three linear T_H cell determinants (sites 1, 2 and 3) of known location on the heavy chain of hemagglutinin molecule of the A/Puerto Rico/8/34 influenza virus each exhibited distinct requirements for generation and stability of expression on surfaces of antigen presenting cells (APC). Exposure of whole virus to acid pH, a step which occurs in the endosome during viral infection, and which causes a well-characterized conformational change in the HA structure, was sufficient processing to render sites 2 and 3 presentable by pre-fixed APC. The protease inhibitor leupeptin was shown to inhibit efficient generation of site 2, as well as the appearance of site 1 when active APC were pulsed with normal virus. In contrast, the same leupeptin-treated APC showed an enhanced presentation of site 3. Therefore, on a single protein with a programmed response to acidification, T_H show distinctly different antigen processing requirements. Site 1 requires protease activity for its expression. Sites 2 and 3 appear to require no more processing than acidification within the endosome for their expression, although expression of an efficient site 2 does require protease activity.

J 507 MOLECULAR MIMICRY OF HLA CLASS II ANTIGENS BY HIV-I ENVELOPE LEADS TO GENERATION OF AUTOANTIBODIES IN AIDS, Hana Golding, Frank A. Robey, Phil Lucas, Michael Phelan, Thomas Hoffman,and Basil Golding. Div.of Virology and Lab of Cell Biology DBBP, FDA. Bethesda MD 20892
Homologous regions of five amino acids were identified in the C-terminus of HIV-I envelope (gp41) and the N-terminal domain of all human class II beta chains. The regions are highly conserved among HIV-I isolates and class II haplotypes. The possibility that this "molecular mimicry" could lead to the generation of antibodies, in HIV-I-infected individuals, which would crossreact with self-class II molecules was tested. It was found that 36% of 78 patients with AIDS (but not from normal volunteers), contained antibodies which bound to plates coated with the class II-derived peptide or with intact class II molecules from cellular extracts, as determined by ELISA. Binding to either plate could be blocked by the soluble class II-derived peptide. Furthermore, murine monoclonal antibodies specific for the HIV I-derived peptide also crossreact with "native" human class II antigens expressed on EBV-transformed B cells and DR-transfected mouse L cells. These mAb could efficiently block activation of an allospecific CD4+ human T cell line by class II-bearing accessory cells. Therefore, the anti-class II antibodies found in AIDS patients, with specificity against the homologous regions of class II antigen and HIV-I gp41, could contribute to the immunological abnormalities present in AIDS.

J 508 PRESENTATION OF ENDOGENOUSLY-SYNTHESIZED VIRAL PROTEINS BY TRANSFECTED- OR BYSTANDER ANTIGEN-PRESENTING CELLS (APC) TO MURINE T-HELPER CELLS IN VITRO. C.J. Hackett, J.W. Yewdell[1], L.C. Eisenlohr, K. Eager [2], W. Gerhard, and R.P. Ricciardi. Wistar Institute,Philadelphia PA. 19104; [1] NIH, NIAID, Rockville, MD 20852;[2] Squibb and Sons, Inc., NJ.

To study the presentation of endogenously-synthesized viral proteins to class II MHC (Ia)-restricted T cells, cell lines of various mouse MHC haplotypes were transfected with genes encoding individual influenza virus proteins known to be recognized by $I\alpha^d$-restricted T cell hybridoma clones. Ia-compatible A20 cells ($h\text{-}2^d$ APC line), transfected with the hemagglutinin (HA) gene of A/Puerto Rico/8/34 virus, constitutively presented antigen to HA-specific T cells. These T cells were also specifically stimulated in response to Ia-incompatible ($H\text{-}2^k$) HA-transfected cells if these were co-cultured with normal A20 APC. Further experiments showed that compatible APC could present both the membrane glycoprotein HA and influenza virus nucleoprotein aquired from transfected cells in co-culture. This suggests that viral proteins endogenously synthesized in cells lacking the appropriate class II molecules may be presented to T cells by activity of bystander APC.

J 509 NEUTRALIZING EPITOPES ON THE ENVELOPE GLYCOPROTEIN OF THE LACTATE DEHYDROGENASE-ELEVATING VIRUS AND THEIR ROLE IN PERSISTENT INFECTION. John T. Harty and Peter G.W. Plagemann, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455.
The lactate dehydrogenase-elevating virus (LDV), an unclassified togavirus which causes a persistent infection in mice, evokes only a poor neutralizing antibody response in persistently infected animals. In order to address the reason for the poor neutralizing antibody response, hybridomas secreting anti-LDV mAbs have been isolated from BALB/c mice primed with inactivated virus. Four hybridomas secreting mAbs which react with the envelope glycoprotein VP-3 and neutralize LDV infectivity hgave been isolated from BALB/c mice primed with formalin inactivated virus. These hybridomas secreted antibodies of the IgG2a and IgG2b isotypic classes, while 6 non-neutralizing anti VP-3 secreting hybridomas isolated from the same fusion were of the IgG1 isotype. ELISA competition analysis indicated that each of the neutralizing mAbs recognized spatially contiguous viral epitopes. Polyclonal antiserum obtained from persistently infected mice did not compete for binding with any of the neutralizing mAbs indicating that formalin inactivation may result in alteration of the neutralizing epitope recognized by these mAbs, thus permitting its recognition by the immune system. Inability to recognize this neutralizing epitope in a natural infection may contribute to the poor neutralizing antibody response in persistently infected mice. Purified neutralizing mAbs were coupled to hemocyanin and used to elicit polyclonal anti-idiotype antibodies in BALB/c mice.

J 510 L3T4-POSITIVE LYMPHOCYTES MEDIATE DIABETES IN EMC VIRUS-INFECTED BALB/C BYJ MICE, Mark K. Haynes, Sally A. Huber and John E. Craighead, University of Vermont, Burlington, Vermont, 05405.
Diabetes mellitus development in adult male Balb/c ByJ mice infected with encephalomyocarditis (EMC) virus is dependent upon T cell immunity. Mice were treated with either an anti-L3T4 monoclonal antibody (GK 1.5), an anti-Lyt2.2 monoclonal antibody (2.43), or a combination of both reagents to investigate whether a particular T cell subset controls this immunopathogenic response. These monoclonal antibodies have been shown to inhibit helper T cell function and cytotoxic T cell function, respectively. The incidence of diabetes, as measured by non-fasting blood glucose concentration, was 79% in both the control-infected and anti-Lyt 2.2-treated mice 7 days after virus inoculation. In contrast, anti-L3T4-treated mice had a greater than 50% reduction in the incidence of diabetes (37%) as did mice given a combination of both reagents (42%). Virus titration of the pancreas on days 3, 6 and 9 post-inoculation demonstrated that all groups sustained equivalent pancreatic infection. Histologic examination of pancreatic tissue revealed that islet lesions with concomitant beta cell degranulation only occurred in control-infected and anti-Lyt2.2-treated mice. Complement dependent islet damage was investigated by treating virus infected mice with daily injections of cobra venom factor (CVF). This treatment was effective in eliminating serum complement activity as assessed by a chromium release assay, however CVF treatment had no effect on the incidence or severity of diabetes. The production of anti-islet cell antibodies by EMC-infected diabetic mice is currently being investigated using immunohistochemical techniques and isolated islet tissue.

J 511 ANTIVIRAL ACTIVITY OF HUMAN RECOMBINANT IFN ALPHA BDDD IN MICE, H.K. Hochkeppel, B. Poncioni, A. Matter and D.S. Gangemi, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland

A human recombinant hybrid IFNPBDDD which is active on mouse cells has been tested for antiviral activity in several murine models of experimental viral disease (Rauscher and Friend Leukemia as well as Herpes Simplex Type 1 Pneumonitis). Administration of 10^6 IU of IFNαBDDD per mouse beginning on the day of virus challenge and given every other day through day 20 post infection was highly effective in protecting (>80 % survival) mice against infection with Rauscher or Friend Leukemia Viruses. In addition, treated mice which eventually died had prolonged mean survival times. Moreover, mice treated with IFNαBDDD had at least a 5 fold reduction in spleen weights and white blood cell elevation as compared to control mice. While a single dose of IFNαBDDD (6×10^4 U/mouse) on the day of virus challenge had little effect, when used together with MTP-PE which by itself exerted only marginal activity the combination of both resulted in a synergistic effect (80 % protection). Continuing studies are now evaluating the synergistic activity of IFNαBDDD and MTP-PE in both in vitro models of macrophage antiviral activity and in other in vivo models of viral diseases (e.g. murine AIDS).

J 512 **THE PATHOGENESIS OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY (PML) INVOLVES JC VIRUS (JCV) INFECTION OF MONONUCLEAR CELLS IN BONE MARROW AND SPLEEN, Sidney A. Houff, Eugene O. Major, David L. Katz, Conrad V. Kufta, Kei Amemiya, Gregory Elder, Dominick A. Vacante and Renée Traub, Infectious Diseases Branch, DIR, NINCDS, National Institutes of Health, Bethesda, MD 20892.**

PML, a subacute demyelinating disease occurring most often in immunocompromised patients, results from JCV infection of oligodendrocytes and astrocytes. JCV has been isolated from urine in PML patients and other immunosuppressed individuals. However, the route by which virus enters the brain has remained unknown. We have demonstrated JCV infection of mononuclear cells in the bone marrow of three PML patients and the bone marrow and spleen of an additional one. AIDS was responsible for immunosuppression in three patients. The fourth patient had no detectable underlying disease and an extensive search for co-infection with other lymphotropic viruses was unsuccessful. The infected lymphocytes in this patient were shown to be B lymphocytes using double labeling techniques to detect viral DNA or capsid antigens and lymphocyte markers in the same cell. Our findings offer a new site for JCV latency. They suggest a means by which immunosuppression can activate JCV replication through failure of immune regulation by T suppressor cells. The presence of JCV infected lymphocytes in Virchow-Robins spaces found at autopsy in one patient suggest that JCV infected mononuclear cells reach the extravascular space in the CNS and offer a means by which virus can enter the brain. Finally, these findings expand the host cell range for JCV which previously was restricted to glial cells. The study of the molecular biology of JCV infection of lymphocytes should offer new insights into the regulation of virus expression in the brain.

J 513 AUTOIMMUNITY TO THE COXSACKIEVIRUS B3 RECEPTOR ON MYOCYTES CAUSES MURINE MYOCARDITIS, Sally A. Huber, Nicholas Heintz and Russell Tracy, University of Vermont, Burlington, VT, 05405.

Balb/c mice infected with a cardiotropic variant of coxsackievirus, group B, type 3 (CVB3M) develop severe cardiac inflammation. Although the disease is clearly initiated by viral infection, tissue injury results exclusively from immune rather than viral mechanisms since T cell deficient mice lack significant cardiac pathology despite equivalent virus titers in the hearts of infected immunodeficient and immunocompetent animals. Selective depletion of CD4+ (T helper) and CD8+ (T cytolytic) cell subsets in vivo by administering monoclonal GK 1.5 (anti-L3T4) and 2.43 (anti-Lyt 2.2) antibodies indicates that only CD8+ cells participate in disease pathogenesis. Distinct cytolytic T cell populations have been identified by immunoadsorption of CVB3M-immune lymphocytes to uninfected and infected myocyte monolayers. Autoreactive CTL (ACTL) specifically adsorb to and lyse uninfected myocytes but do not react to either infected cardiocytes or non-heart cells. Both the ACTL-specific antigen and the CVB3M receptor on myocytes disappear within 1 hour of infection and a monoclonal antibody presumably directed to the viral receptor inhibits ACTL mediated killing of myocyte targets suggesting that ACTL recognize the viral receptor on the myocyte. Virus specific CTL (VSCTL) lyse both heart and non-heart derived targets infected with CVB3M but do not lyse uninfected targets. Both ACTL and VSCTL populations can induce cardiac damage when adoptively transferred into infected T cell deficient mice although ACTL are approximately 4 time more pathogenic than VSCTL. However, only ACTL can induce myocarditis in uninfected animals. Thus ACTL are presumably the predominant effector of cardiac injury in this disease.

J 514 HERPES SIMPLEX VIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTES RECOGNIZE CELLS EXPRESSING GLYCOPROTEIN gC BUT NOT gB, gD or gE, David C. Johnson, James R. Smiley, Frank L. Graham and Kenneth L. Rosenthal, Molecular Virology and Immunology Program, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Herpes simplex viruses (HSV) induce the synthesis of over 60 polypeptides in infected cells. In order to determine which viral polypeptides are recognized by HSV-specific cytotoxic T lymphocytes (CTL) we constructed a series of murine cell lines which express HSV glycoproteins gB, gC, gD and gE. Mouse cells able to express the HSV-1 ICP4 gene, which acts as a transactivator of viral genes, were transfected with viral glycoprotein genes and the neomycin resistance gene (pSV2neo). Stable transformants were tested for susceptibility to anti-HSV-specific CTL. Cells expressing gB, gD or gE both internally and on the cell surface were not lysed. However, after infection of these cells with HSV the cells were lysed by anti-HSV CTL. In contrast, target cells expressing barely detectable levels of gC were lysed by CTL as efficiently as infected target cells. These results suggest that murine class I MHC-restricted anti-HSV CTL recognize HSV-1 gC but do not recognize gB, gD or gE. Preliminary results from cold target inhibition experiments suggest that a majority of murine anti-HSV CTL recognize gC. We are attempting to confirm the finding that murine CTL primarily recognize HSV gC and extend our studies to humans using adenovirus vectors which express gB, gC, and a variety of internal HSV polypeptides including ICP4, ICP0, thymidine kinase, and ribonucleotide reductase.

J 515 HUMORAL IMMUNE RESPONSE AND VIRUS-INFECTED CELLS IN THE BLOOD OF SHEEP EARLY AFTER INFECTION WITH BOVINE LEUKEMIA VIRUS. Lynn C. Kidd, Donna Lagarias, Deborah Grossman, and Kathryn Radke. Department of Avian Sciences, University of California, Davis, California, 95616.

We have experimentally infected sheep with bovine leukemia virus (BLV). The humoral immune response to viral infection was assessed in parallel with the presence of infected cells in the blood. The humoral response to viral surface glycoproteins was studied by monitoring the production of neutralizing and precipitating antibodies. The antibody response to internal viral structural proteins was examined by immunoblot analysis. BLV-infected mononuclear cells from the blood of experimentally infected sheep were quantified by in situ hybridization to detect cells capable of expressing viral RNA, and by an infectious center assay to measure cells capable of producing BLV virions. The use of closely spaced samples and combined methods to monitor the progress of BLV infection has revealed new information about early infection. The production of neutralizing and precipitating antibodies at rapidly increasing titers did not prevent the spread of the virus or eliminate the persistent infection. This suggests that BLV infects new cells at sites that are inaccessible to concentrations of antibodies sufficient to block interaction of the virus with cellular receptors. Analysis by immunoblotting has shown that sheep respond very early after infection to the major core protein of the virus. Lysis of infected cells during immune surveillance is one route by which this normally masked protein could be made available as an antigen.

J 516 ALTERED MHC CLASS I EXPRESSION IN RETROVIRUS-INDUCED TUMOR CELLS. Karen K. Klyczek and Kenneth J. Blank, Dept. Microbioloby and Immunology, Temple Univ. Sch. of Med., Philadealphia, PA.

Thymic lymphoma cells induced by Gross murine leukemia virus (GV) display alterations in expression of major histocompatibility complex (H-2) class I molecules. Some tumor cell lines express no H-2K or H-2D class I molecules by FACS analysis, specific immunoprecipitation, and Northern analysis. However, two observations suggest the expression of some class I-like determinants on these cells. 1) These tumor cells are rejected by immunocompetent, allogeneic mice. Furthermore, cytotoxic T lymphocytes (CTL) can be generated in primary in vitro cultures of allogeneic splenocytes plus irradiated tumor cells. The resulting CTL lyse only the inducing tumor cell line. 2) These tumor cells express beta$_2$ microglobulin (beta$_2$m) and unique beta$_2$m-associated molecules not found on normal lymphoid cells.

Other GV-induced tumor cell lines which do express H-2K and H-2D proteins are apparently resistant to lysis by allospecific CTL. This suggests some alterations in class I expression on these cells which abrogates CTL recognition and lysis. Experiments are in progress to further examine these alterations in class I expression and to characterize the unique beta$_2$m-associated molecules on GV-transformed cells. These studies may provide insight into the mechanism of H-2-mediated resistance to MuLV-induced disease.

J 517 CYTOMEGALOVIRUS INDUCED IMMUNOSUPPRESSION BY VIRAL INHIBITION OF IL-1, Debra MacKenzie-Thompson, Leroy C. McLaren, Arthur D. Bankhurst, University of New Mexico, School of Medicine, Albuquerque, N.M. 87131.

Cytomegalovirus (CMV) is known to suppress normal human immune responses both in vivo and in vitro. CMV-induced suppression of Phytohemagglutinin (PHA)-stimulated proliferation of peripheral blood lymphocytes (PBL's) appears to be mediated, at least in part, by interference with Interleukin 1 (IL-1) activity. Supernatant fluids derived from infected PBL's have reduced IL-1 activity as measured by the murine thymocyte co-stimulation assay with Concanavalin A (Con-A) both in our studies and as reported by others. Additionally, supernatant fluids from CMV-infected fibroblasts significantly suppress murine thymocyte proliferation in the IL-1 activity bioassay. This suppression does not appear to be due to direct infection of the thymocytes as no CMV immediate-early antigens could be detected by immunofluorescent studies with monoclonal antibodies and neutralizing antibody to CMV did not abrogate the suppression. Addition of exogenous IL-1 to the supernatant fluid did not relieve the observed suppressive effect suggesting that CMV-infected fibroblasts release an inhibitor of IL-1 activity. Current research is directed toward investigating the effect of CMV on IL-1 production and activity both at the level of the infected fibroblast and the infected peripheral blood lymphocyte.

J 518 INTERNALIZATION AND DE NOVO EXPRESSION OF MHC ENCODED CLASS I MOLECULES BY DIFFERENT CELL TYPES, P. Machy (1), A Truneh (2), D. Gennaro (2) and S. Hoffstein (2). (1) Centre d'Immunologie de Marseille-Luminy, case 906, 13288 Marseille cedex 9, France. (2) Smith Kline and French Laboratories, 1500 Spring Garden St, Philadelphia, PA 19101, USA.

T lymphocytes are able to lyse virus infected cells upon recognition of viral antigens in association with the self encoded MHC class I molecules. So far, the mechanism of association of MHC molecules with foreign antigens has not been elucidated. In order to have a better understanding of the function of these molecules we have studied their expression and endocytosis in different cell types. Fibroblasts internalize all their class I molecules via a smooth tubular/vesicular pathway only when cross-linked by multivalent ligands. T lymphocytes internalize spontaneously part of their class I molecules (20-40%) via coated pits/coated vesicles. B lymphocytes never internalize class I molecules. In fibroblasts and T lymphocytes the majority of internalized class I molecules do not cycle back to the surface but are degraded with their ligands in lysosomal compartments. Only fragments of cleaved class I molecules are detectable both in the cytoplasm and at the cell surface. To replace their surface class I molecules the cells express those which are stored within intracellular pools. These observations are in agreement with differential regulation of class I molecules depending on the cell type. Knowledge of the molecular mechanisms for the processing of class I molecules should provide a better understanding of their function in the immune system with regard to MHC restriction.

J 519 VACCINATION OF MICE WITH ADENOVIRUS VECTOR EXPRESSION HSV-1 gB ELICITS HSV-SPECIFIC IMMUNITY, Mark R. McDermott, David C. Johnson and Frank L. Graham, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Replicating viral vaccines best engender the expression of protective immunity, particularly at mucosal epithelia interfaced with the external environment. We have studied the potential of Adenovirus type 5 (Ad5), containing replacement of the non-essential E3 region with the Herpes simplex virus type 1 (HSV-2) glycoprotein gB gene (Ad5gB) to protect female mice against lethal infection by HSV-1 and its serotypic couterpart, HSV-2. Three weekly inoculations via the intraperitoneal (I.P.) route with $5x10^7$ PFU of highly-purified Ad5gB protected mice from lethal challenge with virulent HSV. A small degree of such protection was noted after a single I.P. inoculation with live or irradiation-inactivated Ad5gB. In contrast, inoculation with parent Ad5 or medium alone did not elicit any protection against HSV. Mice inoculated with Ad5gB were clinically healthy and did not show histological signs of Ad5gB or Ad5 mediated disease. Interestingly, Ad5gB-inoculated mice produced serum antibodies to HSV gB but the presence of these did not correlate with resistance to challenge with HSV. These results indicate that Adenovirus, which has been used to vaccinate humans against respiratory disease, may be valuable as a vaccination vector against a variety of systemic and mucosal pathogens. Supported by the NCI, MRC and NSERC.

J 520 IN VIVO AND IN VITRO ANALYSIS OF CYTOTOXIC T CELLS (CTL) TO RESPIRATORY SYNCYTIAL VIRUS, Jose L. Munoz, Carol A. McCarthy, Mary Clark, U Rochester, Rochester, N.Y. Respiratory Syncytial virus (RSV) is the major cause of lower respiratory tract infection in children under one year of age. There is evidence that the T lymphocyte is important in protection from infection and that it contributed to the pathogenesis of enhanced pulmonary disease observed during a trial with a formalin killed RSV vaccine in the 1960's. We have begun to study the cytotoxic T cell (CTL) response to RSV in the murine model.

RSV specific, MHC restricted CTL are readily detected in both C57Bl/6 and Balb/c mice. The CTL's lyse RSV infected peritoneal exudate cells (PEC's). The Balb/c CTL's lyse the BCH_4 cell line, a fibroblast line chronically infected with RSV. RSV infected PEC's and the best stimulator for CTL's in our system. BCH_4 can stimulate CTL but only with an exogenous source of IL-2.

CTL lines and clones have been derived by stimulating in vivo primed spleen cells with RSV infected PEC's in vitro and cloning by limiting dilution in the presence of IL-2. 2D9 a Balb/c CTL clone, is H-$2K^d$ restricted and can lyse targets infected with both A and B strain RSV. 7-11E1 is one of nine C57Bl/6 clones developed. It is H-$2K^b$ restricted, sees targets infected with both A and B strain RSV, requires both IL-2 and antigen for optimal growth, and does not see RSV infected targets that carry the H-$2K^{bm1}$ mutation. 10^7 7-11E1 cells can completely clear RSV from infected mice with or without exogenous IL-2. Work is in progress to define the RSV protein specificity of these clones and to further define the protective and possibly pathological role of these CTL lines and clones.

J 521 INTERLEUKIN-2 AS AN ADJUVANT TO INACTIVATED RABIES VIRUS VACCINE, Jack H. Nunberg, Michael V. Doyle, Arthur D. Newell, and Charles J. York[1]. Cetus Corporation, Emeryville, CA 94608; [1]BioTrends International Inc., Winters, CA 95694.

Interleukin-2 (IL-2) occupies a central position in the cascade of cellular events involved in the immune response. IL-2 supports the proliferation of antigen-activated T cells and the generation of effector T cells, including helper, suppressor, and cytotoxic T cells. Proliferating T cells also produce other lymphokines that affect cells of B cell and macrophage lineages. Taken together, the data suggest that IL-2 may function as a potent adjuvant to vaccination, to increase the specific and durable response to vaccine immunogens.

We were interested to examine the ability of recombinant human IL-2 to enhance the potency of inactivated Rabies virus vaccine in mice. These studies were performed using the NIH Test for Rabies Vaccine Potency. Outbred mice were vaccinated with serial dilutions of Rabies vaccine in conjunction with continued systemic IL-2 treatment and were subsequently challenged with multiple LD_{50}s of virulent CVS Rabies virus.

In an initial study, IL-2 administration was shown to markedly increase the potency of inactivated Rabies virus vaccine. Under conditions where challenge is fatal in 100% of mice receiving vaccine alone, there are no deaths in the group receiving vaccine plus IL-2. This effect is vaccine dose-dependent and is not attributable to the IL-2 treatment per se. Extrapolating from survival curves, we estimate that IL-2 is able to increase the potency of this inactivated Rabies vaccine at least 25-fold. Work is underway to further characterize this potent adjuvant effect and to dissect the mechanisms of IL-2 action and disease protection.

J 522 MECHANISM OF INFLUENZA VIRUS NEUTRALIZATION ON CILIATED CELLS OF THE TRACHEA. M C Outlaw, N J Dimmock. Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England.

Mechanisms of influenza virus neutralization have, until now, been studied using tissue culture cell systems. Such cells are dedifferentiated and often unlike the cells of the natural target tissue. The dependence of neutralization on the host cell has recently been demonstrated with picorna and bunya viruses, and stresses the need to take the type of cell into account in such studies.

Here we describe an organ culture system derived from mouse trachea, which has been used to study the mechanism of neutralization by mouse IgG antibodies. We find that attachment of radiolabelled influenza virus to ciliated epithelial cells is affected to a varying degree depending on the proportion of virus and antibody in the neutralization mixture.

J 523 VIRUS BINDING AND MODULATION OF MHC COMPONENTS IN THYROID FOLLICULAR EPITHELIAL CELLS BY REOVIRUS TYPE 1, Joanne C. Pratt*, Miguel Stadecker+ and Glen N. Gaulton*, *University of Pennsylvania, Philadelphia, PA 19104 and Tufts University, Boston, MA 02111. The reasons and mechanisms by which immunological tolerance to self antigens is broken in autoimmune thyroid and other diseases remains unsolved. We have investigated the potential role of virus in the modulation of autoimmune reactivity to cultured thyroid follicular epithelial cells (TFEC) through effects on the expression of components of the murine major histocompatibility complex. Isolation of a clone of reovirus type 1 with tropism for TFEC was acheived by serial passage and cloning of virus on the murine TFEC line M.5. The binding of this virus to M.5 was shown to induce the expression of class II antigens on cells in a fashion similar to that seen following treatment with interferon-gamma. Class II protein expression was, however, independent of the production or presence of interferon suggesting the direct induction of MHC components by this virus.

J 524 PASSIVE IMMUNITY MODULATES GENETIC REASSORTMENT BETWEEN ROTAVIRUSES IN MIXEDLY-INFECTED MICE. Robert F. Ramig and James L. Gombold, Baylor College of Medicine, Houston, TX 77030. Mixed infection of nonimmune suckling mice with rotaviruses SA11 and RRV resulted in high frequencies of reassortant progeny virus (Gombold, J.L. and Ramig, R.F., J. Virol. 57: 110-116, 1986). We have now examined the effects of passively-acquired homotypic or heterotypic immunity on reassortment *in vivo*. Passively-immune suckling mice, obtained from dams immunized with either a serotype 3 (SA11) or a serotype 6 (NCDV) rotavirus, were infected orally with either SA11, RRV, or a mixture of SA11 and RRV (both serotype 3 viruses). At various times postinfection, disease symptoms were noted and the intestines of individual mice were removed and homogenized for determination of viral growth and isolation of progeny plaques. Viral growth and severity of disease in mice passively immune to serotype 3 (homotypic immunity) was minimal following infection with either virus alone or the virus mixture. Immunity to rotavirus serotype 6 (heterotypic immunity) afforded less protection from disease and allowed more virus replication when compared to mice with homotypic immunity. The severity of disease and level of replication was significantly reduced relative to nonimmune mice. Gel electrophoresis of genomic RNA was used to identify reassortants among the viral progeny. No reassortants (<0.5%) were detected among 224 clones examined from mixedly-infected homotypically-immune mice. Twenty-nine of 272 progeny clones (10.7%) from mixed-infected heterotypically-immune mice were reassortant. Thus, reassortment was reduced more than 50-fold by homotypic immunity and approximately 3-fold by heterotypic immunity when compared to mixed infections of nonimmune mice. Control mixed infections of nonimmune mice revealed significantly reduced frequencies of reassortant progeny when the dose of virus was lowered. Taken together, these results suggest immune responses modulate the frequency of reassortment, probably by reducing the effective multiplicity of infection in the case of homotypic immunity. The basis of reduced reassortment in heterotypically immune mice is not understood, but this reduction may be a desirable result of immunization with heterotypic viruses.

J 525 THE CYTOTOXIC T CELL RESPONSE TO ADENOVIRUS IN MICE. Frances C. Rawle, William S.M. Wold* and Linda R. Gooding. Emory University School of Medicine, Atlanta, GA 30322 and *St. Louis University School of Medicine, St. Louis, MO 63110

The E3 region of adenovirus is believed to encode proteins which provide protection against the host immune system. The E3 encoded Gp19K protein has been shown to bind class I MHC antigens and reduce their expression on the cell surface. We wished to investigate whether this effect was sufficient to interfere with the generation of a cytotoxic T lymphocyte (CTL) response to adenovirus. We were able to generate a CTL response to the human Group C adenoviruses Ad2 and Ad5 in mice of strains C3H/HeJ (H-2k), C57BL/10 (H-2b), BALB/cJ (H-2d) and A/J (H-2k/d). Using wild type and E3 deletion mutants we investigated the effect of the E3 region on priming, secondary in vitro stimulation and target cell lysis. The presence of the E3 region inhibited CTL lysis in some strain/virus combinations (e.g. BALB/c:Ad5 and A/J:Ad2) but not others (e.g. C3H/HeJ:Ad2). In general, the weaker the CTL response, the greater the effect of E3 observed. The effect of E3 was greatest on target cell lysis, and deletion of E3 had no effect on in vivo priming under our expermental condition (10^7 pfu per mouse, i.p.). Using a panel of deletion mutants, we have mapped the inhibitory effect of E3 on BALB/c target cell lysis to the gp19K gene.

J 526 IMMUNOSELECTION OF A NEUTRALIZATION-RESISTANT VARIANT OF HIV-1 (HXB2D), Marvin Reitz, Marjorie Robert-Guroff, Carolyn Wilson, Christine Naugle, and Robert Gallo, National Institutes of Health, Bethesda, MD 20892.

HIV-1 virus produced from H9 cells containing HXB2D, a biologically active molecular clone of viral DNA, was transmitted to H9 target cells and grown in the continuous presence of natural neutralizing antiserum. After a lag of 4-5 weeks, the target cells began to produce virus particles. This virus was resistant to the selecting antiserum, although it maintained sensitivity to other neutralizing sera. The resistant variant was molecularly cloned and a major portion of its envelope gene was substituted into the HXB2D clone. Transfection of this clone yielded virus which, unlike the parental virus, was specifically resistant to neutralization by the selecting serum. The two clones differed by a single amino acid substitution in the gp41 transmembrane protein. The data show that selection of resistant variants of HIV-1 can occur in the presence of neutralizing antibodies, that minimal differences in the primary amino acid sequence can result in substantial changes in neutralizability, and that these changes can be in the transmembrane protein.

J 527 HUMAN ADENOVIRUS LATENCY: THE ROLE OF DEFECTIVE NK CELL ELIMINATION OF VIRUS-INFECTED CELL, John M. Routes and James L. Cook, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

Infection of hamster or rat cells with human adenovirus (Ad) types 2 or 5 induces susceptibility of target cells to lysis by either rodent or human natural killer (NK) cells. This viral induction of cytolytic susceptibility in infected rodent cells is associated with expression of the products of the Ad E1A gene. In contrast to the results of studies using rodent target cells, infection of established (A549, KB) or primary (tonsillar fibroblasts) human target cells does not change their inherent resistance to lysis by human or rodent NK cells. Furthermore, NK susceptible human cell lines (293, Jurkat) that are permissive for Ad infection and E1A gene expression exhibit no reduction in susceptibility to NK cell-mediated lysis. While Ad E3 gene expression is known to induce alterations in infected cell surface expression of class I major histocompatibility antigen (MHC), no correlation was found between levels of MHC expression and the cytolytic phenotypes of Ad-infected human cells. These data suggest that the failure of Ad infection to induce cytolytic susceptibility in human cells is due to a difference in the function of Ad E1A gene products in human, compared to rodent, cells that results in the lack of indiction of cytolytic susceptibility in infected human targets. This postulated resistance of Ad-infected human cells to elimination by NK cells due to species-specific differences in Ad E1A gene function provides one explanation for Ad latency in man.

J 528 PRESENTATION OF NEWLY SYNTHESIZED CYTOPLASMIC VIRAL ANTIGENS TO HLA CLASS II RESTRICTED CYTOTOXIC T CELLS. R.P. Sekaly, S. Jacobson, & E. O. Long. Laboratory of Molecular Immunology, Clinical Research Institute of Montreal, Neuroimmunology Branch, NINCDS and Laboratory of Immunogenetics, NIAID, NIH Bethesda.

T cells restricted by class II antigens, of the major histocompatability complex (MHC) usually are not cytotoxic and recognize soluble Ags which have been endocytosed and processed by Ag presenting cells. MHC class I restricted cytotoxic T cells (CTL) recognize peptides derived from processed viral proteins which are either intracellular or cell surface Ags. CTL specific for measles virus (MV) are predominantly class II restricted. To show if these class II restricted CTL recognized newly synthetised cytoplasmic viral Ags murine fibroblasts transfected with the HLA-DR 1 or HLADR 4 alpha and beta chains were supertransfected with matrix (MVM) or nucleocapsid (MVN) cDNAs. The level of MVM or MVN RNA expressed in the tranfectants was only 10^{-4} to 10^{-3} of that found in a measles virus infected human cell line. Viral proteins were undetectable in the tranfectants by immunofluorescence or by immunoprecipitation. Nevertheless the DR_1 MVN or DR_4 MVN murine L cell tranfectants were lysed efficiently by bulk populations of T cells previously stimulated in vitro with MV. Lysis occured in a class II retricted manner. L cells expressing DR_4 and MVM were also killed efficiently by a bulk MV specific T cell line. Therefore both viral proteins are recognized by MV specific CTL. These results demonstrate that there is no requirement for cell surface viral Ags in this class II restricted CTL response and that endogenously synthetized proteins can be presented by MHC class II Ags.

J 529 CLASS I MHC RESTRICTED T LYMPHOCYTE RECOGNITION OF INFLUENZA A HEMAGGLUTININ, Marianne T. Sweetser, Lynda A. Morrision, David Kittlesen, Vivian L. Braciale and Thomas J. Braciale, Washington University School of Medicine, St. Louis, MO 63110.
The class I MHC restricted cytotoxic T lymphocyte (CTL) response directed against the influenza A hemagglutinin (HA) glycoprotein in the BALB/c mouse ($H\text{-}2^d$ haplotype) is limited to a few immunodominant sites. This can be demonstrated by showing killing of target cells sensitized with synthetic oligopeptides representing portions of the HA molecule by both bulk populations and a panel of $H\text{-}2^d$ restricted CTL clones. This panel of clones can also appropriately distinguish target cells infected with recombinant vaccinia virus constructs containing either an 168 or 394 amino acid deletion of the A/JAP/305/57 influenza hemagglutinin gene. Using these recombinant vaccinia, it has been possible to show that the class I CTL response in CBA mice ($H\text{-}2^k$ haplotype) is not directed against the same immunodominant sites. The importance of these immunodominant sites in <u>in vivo</u> stimulation will be presented.

J 530 A 14.7K PROTEIN CODED BY THE E3 TRANSCRIPTION UNIT OF HUMAN ADENOVIRUS PROTECTS VIRUS INFECTED CELLS AGAINST LYSIS BY TUMOR NECROSIS FACTOR. A. Tollefson[1], L. Gooding[2], A.L. Elemore[2], W. Wold[1]. [1]St. Louis University School of Medicine, St. Louis, MO 63110 and [2]Emory University School of Medicine, Atlanta, GA 30322.
Tumor necrosis factor (TNF) is secreted by monocytes/macrophages in response to inflammatory stimuli. It has diverse functions which include inhibition of virus replication. We have found that TNF lyses cells infected by adenoviruses with deletions in region E3. Uninfected cells and cells infected by wild type adenovirus are not lysed. These results indicate that adenovirus renders cells susceptible to lysis by TNF, and that a product of E3 protects against lysis. The E3 product also protects against lysis by TNF in cells in which susceptibility to TNF was induced by cycloheximide. Using a variety of E3 deletion mutants, we have shown that protection against TNF is conferred by a 14.7K protein encoded by E3. The 14.7K protein is distantly related to IL-6. Other workers have reported that the presence of IL-6 in cells correlates with resistance to lysis by TNF. We hypothesize that the sequence similarity between 14.7K and IL-6 reflects their common ability to protect against TNF lysis. Thus, two separate E3 products have been linked to host anti-viral defense mechanisms: the 14.7K to TNF and gp19K to cytotoxic T-cells (see Abstract by Rawle et al.).

J 531 SUCCESSFUL TREATMENT OF MURINE EMC INDUCED DEMYELINATION WITH ANTI-CD4 and ANTI-CD8 ANTIBODIES; David J. Topham, Subramaniam Sriram, University of Vermont, Department of Neurology, Burlington, VT 05405
The M variant of the encephalomyocarditis virus (EMC-M) causes an acute demyelinating *paralytic syndrome in BALB/c mice beginning 9-12 days following viral innoculation* and is characterized by lymphocytic perivascular cuffing and demyelination. We studied the effects of in vivo therapy with monoclonal antibodies to CD4 (helper-inducer) and CD8 (cytotoxic-suppressor) subsets of T cells on this disorder. Twelve week old female BALB/c mice were innoculated with 60 pfu of virus intraperitoneally (ip.) and observed for the development of neurological disorder. One day prior to recieving virus, control mice recieved 1mg purified rat IgG ip. while the other two groups recieved an equivalent amount of either anti-CD8 or anti-CD4 monoclonal antibody. Ninety-three percent (93%) of control animals (25/27) developed paralysis and had a histological score of 12.2. In contrast, only 21% of the anti-CD4 treated animals (6/28) and 18% of the anti-CD8 treated animals (5/28) developed neurological signs. Histological scores for the latter two groups were 3.1 and 5.2 respectively. These experiments show that both subsets of T cells acting either independently or in concert are involved in the development of demyelinating disease caused by EMC-M virus infection.

J 532 INTERACTION OF COXSACKIEVIRUS B3 WITH LYMPHOID CELLS, Raija Vainionpaa, Department of Virology, University of Turku, SF-20520 Turku, Finland

Coxsackie B-viruses are associated with various clinical symptoms including aseptic meningitis, encephalitis, pancreatitis, carditis and myositis. After the initiation of infection in mucosal epithelial cells, the replication of the virus takes place in lymphoid tissues. The infection of lymphoid cells is supposed to be responsible for viremia and it may mediate the infection to the central nervous system. Virus latency and immunosuppression may also occasionally be observed in coxsackie virus infections. Very few reports have been published concerning coxsackievirus B3 infection in lymphoid cells.

We have analyzed the susceptibility of human peripheral blood mononuclear cells (PBMC) and lymphoblastoid cell lines to coxsackievirus B3 infection. The infection was characterized by determining the amount of viral RNA by spot hybridization and in situ hybridization, intracellular virus-specific proteins by immunofluorescence and Western blotting tests, and the release of infectious virus by end-point titration.The virus-infection in non-stimulated and mitogen-stimulated PBMC was highly restricted. No clear-cut release of infectious virus could be detected. Both spot hybridization and in situ hybridization could demonstrate viral RNA in PBMC, but no increase in the amount of RNA during the observation period could be seen. Using indirect immunofluorescence test virus-antigen positive cells could be detected in PBMC . These results suggest that coxsackievirus B3 could infect human PBMC, but the virus replication was very restricted. On the other hand , the lymphoblastoid cell lines with B- and T-cell characteristics supported the virus replication to high titers.
We are currently analyzing these phenomenons in in vitro-infected mouse immune cells and by using immunomodulation of experimental animals.

J 533 DETECTION OF VISNA VIRUS IN LYMPH OF PERSISTENTLY INFECTED SHEEP
Neil J. Watt, Douglas J. Roy, Robert G. Dalziel, Yvonne Ligertwood, David R. Sargan and Ian McConnell, University of Edinburgh, Scotland.

In sheep lentiviruses cause slowly progressive diseases of the lungs, joints central nervous system and mammary gland. Cells of the monocyte/macrophage series are target cells for the virus. The direct interaction of visna virus with these cells in lymphoid tissue can be studied uniquely in sheep using the isolated lymph node model. Afferent and efferent lymph was collected from adult sheep which were persistently infected with the K184 (Weybridge) isolate of visna virus. Isolation of virus was attempted from lymph plasma and from fractionated lymph cells by co-cultivation with susceptible choroid plexus cells. Virus nucleic acids were detected by in situ and slot-blot hybridisation techniques.

In resting lymph (non-antigen stimulated) virus was associated with the antigen presenting cell fraction of afferent lymph but could not be detected in efferent lymph. Following antigenic stimulation of the lymph node, virus could be detected in efferent cells. The kinetics of this response, the cell types infected and the possible consequences for the pathogenesis of lentiviral infections will be discussed.

J 534 POLYCLONAL GAMMA GLOBULIN THERAPY PREVENTS COXSACKIEVIRUS B3-INDUCED MYOCARDITIS IN BALB/C MICE, Ann H. Weller, Mitchell Hall, Mario Estrin, and Sally A. Huber, University of Vermont, Burlington, VT 05405.

Coxsackievirus B3 (CVB3) induces severe myocarditis in susceptible mouse strains, and we have shown the disease to be T-cell mediated. Susceptibility is dependent largely on MHC antigen expression, and within susceptible strains different T cell subsets are responsible for the immunopathology. In DBA/2 ($H\text{-}2^d$) mice, cardiac damage is mediated by CD4+ (T-helper) cells and in Balb/c ($H\text{-}2^d$) mice by CD8+ (T-cytolytic) cells. The genetically-determined immunopathologic mechanism can determine the success of immunosuppressive treatment in murine myocarditis: cyclosporin A or prednisone completely abrogates CD4+ cell mediated disease in DBA/2 but has no protective effect in Balb/c mice. However, both CD4+ and CD8+ cell mediated autoimmunity in mice has been successfully treated with polyclonal gamma globulins. This therapy is thought to act by increasing the balance of anti-idiotypic antibodies to self-damaging idiotypic immunity. Male Balb/c mice were given two courses of either pooled healthy-donor mouse IgG, $F(ab)_2$ fragments of the former, anti-IA^K monoclonal (as an irrelevant antibody control), BSA (as a protein control), or PBS and then infected with 6×10^4 PFU CVB3. We found significant reduction of myocardial lesions in both the IgG and $F(ab)_2$-treated groups but not in anti-IA^K or BSA treated groups. Protection by $F(ab)_2$ fragments was equivalent to that of whole IgG, supporting the hypothesis that protection is acheived through an idiotype-anti-idiotype interaction on autoreactive cytolytic T cells.

J 535 LOSS OF CYTOTOXIC T LYMPHOCYTE RESPONSES IN A MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY SYNDROME, Robert A. Yetter, Gene M. Shearer, Matthew W. Miller, and Herbert C. Morse, III, Veterans Administration Medical Center, Baltimore, MD, 21218, Department of Pathology, University of Maryland at Baltimore, Baltimore, MD 21201, and Immunology Branch, NCI and Laboratory of Immunopathology, NIAID, Bethesda, MD 20892.

Adult C57BL/6 mice infected with the LP-BM5 murine leukemia viruses (MuLV) develop a syndrome of lymphoproliferation and profound immunodeficiency. Spleen cells from these mice were tested in vitro for the ability to generate cytotoxic T lymphocyte (CTL) responses to TNP-modified syngeneic cells (self + x) and to alloantigens. Depression of CTL responses to self + x were apparent by two weeks following infection, while responses to alloantigens did not become depressed until seven to nine weeks post-infection. Interleukin-2 (IL-2) production by spleen cells from LP-BM5 MuLV-infected mice was impaired by 3 weeks after infection. Addition of recombinant IL-2 to CTL induction cultures from spleen cells of mice 2-8 weeks post-infection restored both self + x and alloantigen responses to near normal levels. These studies suggest that functional loss of a T helper ($Ly\text{-}4^+$) cell population participating in IL-2 induction may be responsible for the failure of both these CTL responses.

J 536 INACTIVATION OF NEURAMINIDASE ACTIVITY ALLOWS NON-INFECTIOUS INFLUENZA VIRUS TO SENSITIZE CELLS FOR LYSIS BY CYTOTOXIC T LYMPHOCYTES, Jonathan W. Yewdell, Jack R. Bennink and Yasuhiro Hosaka[1], Laboratory for Viral Diseases, NIAID, NIH, Bethesda, MD 20892, and [1]Osaka University, Osaka, JAPAN. Past studies have shown that non-infectious influenza virus fails to sensitize histocompatible cells for lysis by class I restricted cytotoxic T lymphocytes [CTL]. We have found that this finding, while strictly correct, is misleading: sensitization can be achieved if the neuraminidase [NA] of non-infectious virions is inactivated thermally or by addition of a competitive inhibitor. Both internal proteins [nucleoprotein and polymerase] and external proteins [hemagglutinin] derived from non-infectious, NA inactivated virions are processed into CTL recognition structures by target cells. Evidence strongly suggests that sensitization depends on fusion of viral and cellular membranes. These findings indicate that processing of intact proteins for CTL recognition is independent of biosynthetic pathways. Further, we have found that non-infectious, NA inactivated virions are able to induce CTL responses in vivo. Such preparations represent a novel vaccine which could optimally induce cellular and humoral immunity to influenza virus.

J 537 *EFFECT OF HUMAN CYTOMEGALOVIRUS INFECTION ON EXPRESSION OF CLASS 1 HLA.* *S.A. Young*,[1,2] and R.M. Stenberg[2]. Department of Otolaryngology[1] and Department of Microbiology[2], West Virginia University, Morgantown, West Virginia.*
Human Cytomegalovirus (HCMV) infection of human foreskin fibroblasts (HFF) resulted in a decrease in detectable class 1 HLA molecules. Murine antibody W6/32, which recognizes monomorphic determinates on the HLA A,B and C molecule, was used for immune precipitation or immunofluorescent staining. HCMV infection of HFF at a MOI of ten resulted in a decrease in immune precipitable class 1 HLA. Class 1 HLA decreased gradually over the first twenty-four hours post-infection but resulted in a significant decrease in immune precipitable class 1 HLA by 24 hours post-infection. Fluorescent activated flow cytometry was used to quantitate HCMV immunofluorescent staining of class 1 HLA molecules on HCMV infected verses mock-infected HFF. Twenty-four hours post HCMV infection FACS analysis profiles demonstrated a decrease in mean intensity channels of infected verses mock-infected HFF. To determine if the observed loss in detection of class 1 HLA by immune precipitation or immunofluorescent staining was due to a decrease in class 1 HLA mRNA, a DNA fragrent representing class HLA mRNA was hydrizided to northern blots of HCMV infected and mock-infected HFF whole cell RNA. The primary band detected in mock-infected HFF cells was 1.6kb, the size RNA anticipated for class 1 HLA mRNA. The levels of detectable 1.6kb RNA hydridizing to the class 1 HLA probe decreased as time post-infection increased in infected verses mock-infect HFF. The pattern of decrease in this 1.6kb RNA was similar to that seen for the decreased expression of the class 1 HLA molecule.