CELL BIOLOGY OF VIRUS ENTRY, REPLICATION AND PATHOGENESIS

Organizers: Michael Oldstone, Ari Helenius and Richard Compans

February 28- March 5, 1992

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Keynote Address

N 001 NUCLEAR TRANSLOCATION OF THE JUN ONCOPROTEIN. PETER K. VOGT AND KAZUHIRO CHIDA DEPARTMENT OF MICROBIOLOGY AND NORRIS CANCER CENTER, USC SCHOOL OF MEDICINE, 2011 ZONAL AVENUE, HMR 401, LOS ANGELES, CA 90033-1054.

The Jun protein is a transcription factor of the AP-1 complex, it is concentrated in the cell nucleus. While the cellular Jun protein is transported into the nucleus in a cell-cycle independent fashion, the oncogenic viral version of the protein translocates into the nucleus most rapidly during G2 and only slowly during G1/S. This cell-cycle dependence of nuclear transport has been mapped to the C to S mutation in the carboxy terminal portion of viral Jun. We have identified a complex nuclear translocation signal located in the basic region of viral Jun. This signal has the sequence ASKSRKRKL. A peptide of this sequence synthesized *in vitro* and conjugated to IgG can mediate cell-cycle dependent translocation of the microinjected conjugate from the cytoplasm into the nucleus. The nuclear translocation signal has two functional domains. The pentapeptide RKRKL is sufficient as a cell-cycle independent nuclear address. The entire signal is needed for cell-cycle dependent nuclear translocation. The amino terminal tetrapeptide contains the C to S substitution responsible for cell-cycle dependence. Deletion analysis of the Jun protein suggests that the nuclear translocation signal identified in the basic region is the only such signal in the molecule. This work was supported by U.S. Public Health Service Research Grant No. CA 42564 from the National Cancer Institute and Grant No. 1951 from the Council of Tobacco Research

Virus Structure

N 002 STRUCTURE AND FUNCTIONS OF SINDBIS CORE PROTEIN: A CAPSID PROTEIN THAT IS A SERINE PROTEASE. Michael G. Rossmann[1], Hok-Kin Choi[1], Liang Tong[1], Wladek Minor[1], Philippe Dumas[1*], Ulrike Boege[2], and Gerd Wengler[3], [1]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, [2]Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada, [3]Institut für Virologie, Justus-Liebig-Universität Giessen, Frankfurter Strasse 107, D-6300 Giessen, Germany and *Present address: Laboratoire de Cristallographie Biologique, Institut de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, 15 rue René Descartes, 67084 Strasbourg Cedex, France.

Sindbis virus is the type member of alphaviruses, a group of insect-transmitted enveloped ("toga") viruses. They consist of a nucleocapsid core surrounded by a lipid membrane through which penetrate 80 glycoprotein trimers in a $T = 4$ (1) surface lattice arrangement. The structure of the core protein that comprises the coat surrounding the genomic RNA has been determined. It forms the same stable dimer in at least two quite different crystal forms. The polypeptide fold of Sindbis core protein from residue **114** to residue **264** is homologous to that of chymotrypsin-like serine proteinases. Although serine-like proteinases are common to RNA viruses, this is the first time that their use as a structural protein has been clearly established. The previously identified catalytic residues (2) His **141**, Asp **163** and Ser **215** are positioned as in other serine proteinases. The carboxy-terminal tryptophan remains in the P_1 substrate site subsequent to the autocatalytic *cis* cleavage of the capsid protein from its own polyprotein, thus rendering the proteinase inactive. Model building of the Sindbis core protein dimer into $T = 3$ and $T = 4$ surface lattices of appropriate radius shows that the nucleocapsid is more likely to have $T = 4$ quasi-symmetry. This model identifies those residues likely to act as anchor to the carboxy-terminal residues of the E2 glycoprotein spike. This interaction is important for the budding process.

1. Caspar, D. L. D. & Klug, A., *Cold Spring Harbor Symp. Quant. Biol.* **27**, 1-24, 1962

2. Hahn, C. S. & Strauss, J. H., *J. Virol.*, **64**, 3069-3073, 1990

Viral Receptors and Entry

N 003 CORONAVIRUS RECEPTORS, Kathryn V. Holmes, Gabriela S. Dveksler, Richard K. Williams, Michael N. Pensiero, Christine B. Cardellichio, Curtis L. Yeager, Sara E. Gagenten and Carl W. Dieffenbach, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799

Coronaviruses are enveloped RNA viruses which cause infections of respiratory and enteric epithelium of many species of domestic animals and man. Although these viruses share a common replication strategy, they have evolved to form three serologically distinct groups. At least three different types of molecules on the surfaces of epithelial cells can serve as coronavirus receptors. For mouse coronavirus MHV, a murine member of the carcino-embryonic antigen family in the immunoglobulin superfamily serves as a specific receptor. This receptor determines the species specificity and mouse strain specificity of MHV infection, and affects the tissue tropism of virus infection. Human coronaviruses and rat coronaviruses do not bind to the respective human or rat-specific CEA-related homologs of the MHV receptor, but instead identify other glycoproteins as receptors. Some coronaviruses express a hemagglutinin esterase (HE) glycoprotein which binds specifically to a carbohydrate moiety on epithelial cells. Some of the HE expressing coronaviruses do not appear to utilize the carbohydrate moiety as a receptor leading to infection, but other coronaviruses may use it as a receptor in specific cell types. Thus, closely related viruses may recognize strikingly different types of receptors on the same type of tissue, and a single virus may utilize more than one mechanism for binding to and infecting the same type of host cell.

N 004 THE CELL SURFACE RECEPTOR FOR ECOTROPIC MURINE RETROVIRUSES IS A BASIC AMINO ACID TRANSPORTER, Hao Wang[1], Michael Kavanaugh[2], R. Alan North[2], and David Kabat[1], Department of Biochemistry and Molecular Biology, and Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon 97201-3098.

We recently discovered that the cell surface receptor (ecoR) that mediates attachment and infection of ecotropic host-range murine retroviruses is the major transporter for basic amino acids in mammalian cells (i.e., the y^+ permease). We expressed ecoR in Xenopus laevis oocytes by injecting a synthetic messenger RNA and we detected the transport function by sensitive electrophysiological voltage clamp methods as a Na^+-independent cationic amino acid influx. Consistent with earlier studies of y^+, we found that this permease could also transport certain neutral amino acids (e.g., homoserine and cysteine) by a low affinity Na^+-dependent mechanism. Continuing studies are in progress to analyze the molecularly cloned ecoR transporter by electrophysiological, biochemical, and virological methods. The uptake of cationic amino acids is markedly accelerated by hyperpolarization of the membrane without significant effects on Km. Our results suggest that the transporter changes conformation during its transport cycle, and that electrostatic interactions are critical determinants in this conformational transition. In addition, we have begun to analyze the interactions between the viral glycoprotein and ecoR in mammalian cells at 37°C. Preliminary evidence suggests that infection of murine cells with ecotropic retroviruses causes a substantial inhibition (decrease of V_{max}) of the cell's y^+ permease activity. However, the percentage of inhibition may depend upon the level of y^+ expression by the cells. These results raise the possibility that diminution of essential amino acid transport in some cells might contribute to pathogenesis by this leukemia virus. The possibility that cell surface receptors for other retroviruses might also be permeases is suggested by structural features of the Gibbon ape leukemia virus receptor.

N 005 STUDIES ON THE MECHANISM OF HIV AND SIV ENTRY, Dan R. Littman[1,2], Kathleen Page[2], and Kurt Zingler[2], [1]Howard Hughes Medical Institute and [2]Department of Microbiology and Immunology, University of California, San Francisco, CA 94143-0414.

Entry of HIV into target cells involves high affinity binding of the viral envelope glycoprotein to CD4, fusion of the viral and cellular membranes, and uncoating of the viral core. From a number of studies with HIV's as well as SIV, it is clear that binding of virus to CD4 is not sufficient to insure entry. The structure of the envelope glycoprotein, as well as poorly defined cellular factors, determine whether a particular virus can gain entry subsequent to binding to the receptor. To study this problem, we have developed means of packaging *env*- HIV genomes encoding selectable markers in transiently-transfected COS cells. Viral particles produced in these cells can incorporate various envelope glycoproteins encoded by co-transfected plasmids. Cells infected by these particles are detected as drug-resistant colonies. Using this system, we have shown that numerous cell lines are resistant to either HIV or SIV infection, but can be infected by HIV pseudotyped with appropriate envelope glycoproteins. For example, no HIV-1 or SIV envelope glycoproteins could mediate entry into murine cells expressing human CD4, but incorporation of murine ecotropic and amphotropic envelope glycoproteins permitted HIV to infect these cells. Likewise, HeLa-CD4 cells were resistant to HIV(SIV) pseudotypes, but several human T cell lines were readily infected by these particles. Taken together, these studies strongly suggest that a productive match between tissue-specific target cell cofactors and envelope glycoproteins must occur for infection to proceed subsequent to envelope-CD4 interaction.

The V3 region of the HIV-1 envelope glycoprotein appears to have a critical function in specifying the appropriate match of viral strain and target cell. This region contains the principal neutralizing determinant and also has a key role in target cell tropism. It has been suggested that interaction of this region of gp120 with host factors, such as proteases, is required for viral infection to proceed. To determine whether the V3 region has a critical role in viral entry, we have analyzed the ability of envelope glycoproteins bearing a number of mutations in this region to mediate infection and syncytium formation. HIV particles containing selectable genomes and mutant envelope glycoproteins were produced in COS cells and tested for infectivity of $CD4^+$ cells. None of the mutations affected envelope biosynthesis or binding to CD4. However, several mutations in V3 abrogated viral entry and the ability of gp160, expressed in COS cells, to mediate syncytium formation with HeLa-CD4 cells. The V3 loop therefore appears to have a critical role in mediating fusion of the viral and cellular membranes.

Interaction of Viral Glycoproteins with Cells

N 006 UPTAKE AND INTRACELLULAR SORTING OF CELL SURFACE RECEPTORS, Colin R. Hopkins, MRC Laboratory for Molecular Cell Biology, University College London WC1E 6BT, Membrane proteins internalised by receptor mediated endocytosis concentrate within clathrin coated domains prior to uptake. We have studied this process by electron microscopy using an expression system devised by I S Trowbridge (JING et al. J. Cell Biol. 110 289-301, 1990) in which human transferrin receptor cDNA is transfected into primary chick fibroblasts. We find (Miller et al, Cell65. 621-632,1991) that the level of expression has no detectable influence on membrane invagination but that it can at the highest levels increase clathrin lattice formation. Our most recent studies suggest that transferrin receptors appear on the surface, they bind adaptor complexes and it is the receptor-adaptor complexes which interact with growing lattices.

In a related study we have used low light video microscopy to follow the intracellular processing of recycling transferrin receptors (HOPKINS et al. Nature, 346 335-339. 1990). We have found that the intracellular compartments involved are extensively interconnected by branching tubular cisternae. Further work in which epidermal growth factor receptors travelling to the lysosome were followed through the transferrin labelled system, showed that multivesicular bodies were an integral part of this tubular network and that as they accumulated EGFR within their internal vesicles, they moved along the tubules. Correlative electron microscopy demonstrates that during this process, EGFR destined for the lysosomes are removed into the internal vesicles of the multivesicular body and thus separated from the transferrin receptors which remain on the perimeter membrane. This segregation of EGFR requires their endogenous kinase to remain active and is accompanied by phosphorylation of clearly defined endosome associated substrate protein (Felder et. al. Cell 61.623-634, 1990).

N 007 THE ION CHANNELS OF INFLUENZA VIRUSES, Robert A. Lamb[1,2], Leslie J. Holsinger[2], and Lawrence H. Pinto[3], [1]Howard Hughes Medical Institute, [2]Department of Biochemistry, Molecular Biology and Cell Biology, and [3]Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois, 60208-3500.

The influenza A virus M2 protein has been shown to have an associated ion channel activity by microinjecting M2 mRNA into *Xenopus laevis* oocytes and measuring whole cell currents using a two electrode voltage clamp. The M2 associated channel activity was markedly activated by changing the pH from pH 7.6 to pH 5.4. The anti-influenza virus drug, amantadine hydrochloride, significantly attenuated the inward current induced by hyperpolarization of oocyte membranes. The wild-type M2 protein associated channel permits the flow of both Na^+ and K^+ ions, and it would be surprising if this did not extend to protons. Various mutations in the M2 membrane spanning domain, that when found in influenza virus confer resistance to amantadine, exhibited channel currents that were resistant to the effect of the drug. The ion selectivity of the channel activity of several mutant M2 proteins was different from each other and from wild type M2, strongly suggesting that the M2 protein is a channel *per se* and not a regulator of an endogeneous but normally inactive oocyte channel. The pivotal role of the M2 ion channel in permitting the flow of protons from endosomes into the virion interior to facilitate virion uncoating will be discussed.

The influenza B virus NB glycoprotein shows a remarkable degree of similarity of structure to the influenza A virus M2 protein. As the known biological activities of influenza B virus are very similar to influenza A virus, we thought it likely that NB would also function as an ion channel. It should be stressed that this could be the case despite the specific effect of amantadine on influenza A virus M2 and its lack of effect on influenza B virus replication: the effect of amantadine is thought to be because of a specific macromolecular interaction with M2 that does not occur with influenza B virus. When oocytes were injected with NB mRNA and the membrane currents were measured, they were found to evoke a slowly-activated inward membrane current that was modulated by $[Ca^{2+}]_{out}$. Investigation of the ion selectivity of the NB associated channel activity indicates that both Na^+ and Cl^- contribute to the observed inward current.

Virus-Induced Cell Fusion

N 008 MECHANISMS OF ENVELOPE MEDIATED CELL FUSION IN HIV-2, Beatrice H. Hahn[1], Prasanna Kumar[1], Feng Gao[1], Lawrence Brass[2], John C. Kappes[1], Mark J. Mulligan[1], Raymond W. Sweet[3], George M. Shaw[1], James A. Hoxie[2], [1]University of Alabama at Birmingham, Birmingham, AL, [2]Hospital of the University of Pennsylvania, Philadelphia, PA, [3] SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

Naturally-occurring isolates of human immunodeficiency virus (HIV) are known to vary in their ability to fuse with and kill CD4 positive target cells, and such biological heterogeneity may reflect differences in their *in vivo* pathogenic potential. In an attempt to better understand envelope mediated cell fusion and its relation to HIV pathogenicity, we molecularly characterized an attenuated strain of HIV-2 (HIV-2/ST) previously shown to infect CD4 positive target cells without inducing fusion and cell killing and two fusogenic and cytopathic variants derived from this virus by repeated cell free passage. Using an assay in which the affinity of cell-associated envelope for recombinant soluble CD4 was measured, we found that the binding affinity of attenuated HIV-2/ST for CD4 was two orders of magnitude reduced, while the two cytopathic variants exhibited a high CD4 binding affinity comparable to that of cytopathic HIV-1 and HIV-2 isolates. To analyze these differences on a molecular level, we molecularly cloned both the attenuated parental strain and the two cytopathic variants. Five infectious proviral clones (one from the attenuated isolate and four from the two cytopathic variant cultures) were obtained and confirmed to exhibit the biological phenotype of their parental viruses. Construction of chimeric viruses and vaccinia expression studies mapped the fusogenic and cytopathic differences to the viral envelope genes. Direct and competition binding assays, however, using vaccinia virus expressed soluble gp120 and recombinant soluble CD4 revealed a low binding affinity for both attenuated and cytopathic HIV-2/ST gp120 envelope glycoproteins. Subsequent sequence analysis of cytopathic and noncytopathic envelope genes identified a total of eight potentially causative amino acid sequence changes (3 within gp120 and 5 within gp41), none of which was located in the putative CD4 binding region of gp120. From these results we conclude that the fusogenic and cytopathic potential of HIV depends, at least in part, on its receptor binding affinity. The increased CD4 binding affinity of the native envelope glycoprotein complex but not the soluble gp120 indicates that cooperative interactions between SU and TM and/or oligomers of these molecules can act to positively regulate receptor binding affinity. Moreover, the finding that this affinity is subject to *in vitro* selection pressures suggests that similar selection pressures may also occur *in vivo* and may contribute to the emergence of more virulent strains in infected individuals over time.

N 009 REQUIREMENTS FOR MEMBRANE FUSION DIRECTED BY THE NEWCASTLE DISEASE VIRUS FUSION GLYCOPROTEIN, Trudy Morrison, Catherine McQuain, Theresa Sergel, and Lori McGinnes, University of Massachusetts Medical School, Worcester, MA 01655

Paramyxoviruses such as Newcastle disease virus (NDV) encode two glycoproteins, the attachment protein (HN) and the fusion (F) protein. To explore the role of these proteins in membrane fusion, the cDNAs encoding these proteins have been expressed in chick embryo cells using a retrovirus vector. The fusion protein expressed in this system is transported to the cell surface and efficiently cleaved into the disulfide linked F_1-F_2 form found in infectious virions. Cells expressing this protein show no evidence of fusion. However, these cells will fuse if infected with an avirulent strain of NDV (without a cleaved fusion protein) or if transfected with the HN gene, suggesting that both the HN and the F proteins are required for fusion. The HN and F proteins must be in the same membrane since mixtures of cells expressing the F protein and cells expressing the HN protein do not fuse. Neither the retrovirus env protein nor the influenza HA protein will substitute for the HN protein, suggesting that the HN protein is providing more than just an attachment function. Furthermore, the HN protein of the closely related paramyxovirus, Sendai virus, will not substitute for the NDV HN protein indicating that there are very specific HN sequence requirements for fusion. All these observations suggest that a specific interaction between the fusion glycoprotein and the HN glycoprotein is necessary for membrane fusion. Indeed, using polyclonal antisera raised against a portion of the HN protein expressed in bacteria, precipitation of the HN protein also precipitates the fusion protein. The HN-F protein interaction detected by co-precipitation occurs posttranslationally but prior to the cleavage of the fusion protein. The structural requirements for this interaction will be discussed.

The amino terminus of the F_1 has long been implicated in membrane fusion. Computer analysis of this sequence predicts an extensive amphipathic alpha helix. The effect of mutations in this predicted structure on membrane fusion will be discussed.

N 010 FUSION MECHANISM OF THE INFLUENZA HEMAGGLUTININ: INHIBITOR DESIGN, *Judith M. White*, Dale L. Bodian, George W. Kemble, and Irwin D. Kuntz, University of California, San Francisco.

The influenza hemagglutinin is responsible for both binding and fusion between the viral and host plasma membranes. We and others have studied the fusion activity of this well characterized glycoprotein in detail. We present recent findings on (i) the fusion mechanism of HA, (ii) the conformational changes required to elicit fusion, and (iii) attempts to design an inhibitor of the fusion-inducing conformational change.

(i) Models have recently been proposed in which the fusion junction involves an aggregate of several HA trimers and fusion begins in the interior of the aggregate. The proposed structure has been likened to a pore. If this is the case, then the transmembrane region of HA might be critical for the stability of the pore and/or to aid in promoting the lipid rearrangements required for fusion. As a first test of this hypothesis, we analyzed the structure, conformational changes, and fusion activity of glycophosphatidylinositol (PI) anchored HA, HA-PI. HA-PI was found to be a trimer expressed at the surface of transfected cells. The ectodomain of HA-PI was released as a trimer with PI-specific phospholipase C (PI-PLC). Both membrane anchored HA-PI and its PI-PLC-released ectodomain changed conformation (became protease sensitive) at low pH like wt-HA. Like the ectodomain of wt-HA (BHA), the PI-PLC released ectodomain of HA-PI became hydrophobic after exposure to low pH (partitioned into TX-114). Nevertheless HA-PI was greatly impaired in its membrane fusion capacity. The rate of fusion of RBCs with HA-PI expressing cells (using a fluorescence dequenching assay) was ≤15% of the corresponding rate for cells expressing wt-HA. Thus although HA-PI appears to undergo the necessary low pH-induced conformational changes preparatory for fusion, it is greatly impaired in its ability to promote membrane fusion. These findings suggest that the transmembrane region is critical for fusion.

(ii) We previously showed that the conformational change in HA (in response to low pH) occurs in two major stages with changes in the stem of the molecule, notably release of the fusion peptides, preceding major dissociation of the globular head domains. We recently found, however, that a change in the globular head domain interface consistent with partial dissociation does occur during the first stage of the conformational change. When HA was treated at low pH, the kinetics of fusion peptide exposure (monitored with an anti-fusion peptide antibody) and loss of two trimer specific epitopes located in the globular head domain interface (N1 and N2) were found to be identical. This result suggests that partial dissociation of the head domains may be required for fusion peptide exposure and hence for fusion activity.

(iii) Since fusion peptide exposure is the key event in eliciting HA-mediated fusion, we wish to design a low molecular weight inhibitor of this step in the fusion reaction. The HA crystal structure was examined and a potential binding site in the vicinity of the fusion peptide was identified. The computer program DOCK was used to select molecules with shape complementarity to the site. 92 compounds thus identified were screened for their ability to prevent fusion peptide exposure. One was inhibitory at mM concentrations. 30 derivatives of this compound have now been screened. Several are inhibitory at a concentration of ~10 μM.

Virus Infection of the Nervous System

N 011 **MUTATIONS OF MEASLES VIRUSES CAUSING LETHAL HUMAN BRAIN DISEASES,** M. A. Billeter*, R. Cattaneo*, A. Schmid*, P. Spielhofer, K. Kaelin*, M. Huber*, K. Baczko+, and V. ter Meulen+, **Institute for Molecular Biology I, University of Zürich, Switzerland and +Institut für Virologie und Immunbiologie, Universität Würzburg, Federal Republic of Germany.*

Both subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (MIBE) are lethal diseases, occurring occasionally subsequent to measles virus (MV) infections after a long latant period, spreading in the brain apparently without virion formation. SSPE develops very rarely, in presence of intact humoral and cell-mediated immune defenses, whereas MIBE results at higher incidence, in immunocompromized patients.

Although environmental and host factors play a role in disease development, mutations of the MV genomes appear of prime importance. By cloning and sequencing of entire MV genes, derived either directly from brain autopsy material or from persistently infected cell lines obtained by cocultivation techniques from SSPE brains, we and others have identified many mutations affecting primarily the envelope proteins matrix (M), fusion (F) and hemagglutinin (H), whereas the proteins required for transcription and replication seem less compromized. Particularly, the V protein produced from edited transcripts of the phosphoprotein (P) gene is well conserved. Not only errors made by the viral polymerase, resulting in single site replacements, contribute to mutagenesis. Hypermutations, converting many U residues to C (usually in the plus strand sense) in entire genes or gene segments, occur frequently, presumably due to multiple A to I conversions caused by ds RNA unwinding activity present in various amounts in essentially all cell types. Replacement of wt by mutated genomes, endowed with a selective advantage in the diseased tissue environment, can be clearly followed; in one case, several subsequent hypermutations in one brain have been documented.

The M gene is most heavily affected; in many cases no M protein can be produced. The F gene is generally highly conserved, but significantly, in all ten SSPE cases investigated, important changes occur in the region specifying the small cytoplasmic anchor domain, leading to premature termination, nonconservative aminoacid replacements or elongation beyond the natural termination. This suggests that the fusion function is maintained, but the expression of the F protein at the cell surface is modified. The H gene is in general well conserved, although one case of limited hypermutation has been noted and in some cases possible glycosylation sites are changed.

To evaluate the biological meaning of these mutations, artificially expressed mutated proteins are analyzed in cultured cells, revealing in particular altered transport. To fully understand the effect of MV genes mutated in SSPE cases, introduction of their cloned copies in otherwise normal MV genomic cDNA and rescue of reconstructed virus is attempted.

Transgenic Models of Viral Diseases

N 012 HEPATITIS B VIRUS CYTOTOXIC T CELL RESPONSE: CHARACTERISTICS IN MAN AND CONSEQUENCES IN TRANSGENIC MICE.

Francis V. Chisari[1], Kazuki Ando[1], Susanne Wirth[1], Takashi Moriyama[1], Stephane Guilhot[1], Shao-nan Huang[2], Gabriele Missale[1,3], Antonio Bertoletti[1,3], Amalia Penna[3] and Carlo Ferrari[3]. [1]The Scripps Research Institute, LaJolla, CA 92037, [2]Sunnybrook Health Science Center, Toronto, Canada, [3]University of Parma, Parma, Italy.

It has long been assumed that viral clearance and liver cell injury in hepatitis B virus (HBV) infection are mediated by a cytotoxic T cell (CTL) response to one or more HBV-encoded antigens. This assumption has not been testable due to the limited host range of HBV and to the absence of expression systems suitable for such studies. We have recently developed the reagents and experimental systems necessary to test this hypothesis in HBV-infected patients and in HBV transgenic mice.

Using a strategy involving stimulation of PBMC with synthetic HBV peptides we have determined that HBV nucleocapsid (HBcAg) specific CTL are readily detectable in the peripheral blood of patients with acute HBV infection, whereas HBV envelope specific T cells are not. In contrast, we have not been able to detect HBcAg-specfic CTL in patients with chronic HBV infection, suggesting that such CTL may play a role in viral clearance and liver cell injury during acute infection. Specifically, we have demonstrated that: 1) HBcAg peptide-specific CTL are capable of recognizing endogenously synthesized nucleocapsid antigen. 2) the CTL response is mediated by CD8 positive T cells. 3) the response is focussed on a limited number of epitopes within the nucleocapsid antigen, each of which is restricted by a different HLA class I allele. 4) an HLA-A2 restricted CTL epitope between residues 18-27 (FLPSDFFPSV) of HBcAg is recognized by all HLA-A2 positive patients with acute hepatitis studied thus far. Current studies are designed to characterize the CTL response to the remaining HBV gene products in order to elucidate its potential role in HBV pathogenesis and to explore the possibility of developing a peptide-based, epitope-specific immunotherapy to terminate persistent HBV infection.

Although the foregoing studies demonstrate the existence of HBV specific CTL during acute viral hepatitis, they do not establish the pathogenetic postential of this response in vivo. To this end we have examined the consequences of adoptive transfer of cloned HBV specific CTL into transgenic mice whose hepatocytes express the corresponding HBV-encoded antigen (in this instance HBsAg). We have shown that an H-2^d restricted, CD8-positive CTL clone, specific for residues 21-40 (LLTRILTIPQSLDSWWTSLN) of HBsAg can recognize and destroy HBsAg positive hepatocytes in vivo. We now report that the entire disease spectrum of acute viral hepatitis, including lethal fulminant hepatic failure, can be reproduced by this and related CTL clones, thereby providing the first definitive evidence that an analogous response to HBV encoded antigens should be sufficient to cause all of the acute hepatic manifestations of this disease in man. We have also demonstrated that the adoptively transferred CTL represent only a minor component of the inflammatory infiltrate within the liver in this lesion. Furthermore, blocking studies demonstrate that gamma interferon, produced by these CTL, is an essential mediator of this response and that the activated macrophage plays a dominant role as the final effector of hepatocellular injury in this model. Thus, antigen nonspecific amplification mechanisms increase the efficiency of the antigen specific CTL response to an extent that it can be lethal for the host. Current studies focus on the establishment of chronic immunologically mediated liver disease in this transgenic mouse model of viral hepatitis.

N 013 VIRUS INFECTION TRIGGERS INSULIN-DEPENDENT DIABETES MELLITUS IN A TRANSGENIC MODEL: ROLE OF ANTI-SELF (VIRUS) IMMUNE RESPONSE, Michael B. A. Oldstone, The Scripps Research Institute, La Jolla, CA.

The potential association between viruses and insulin-dependent (type 1) diabetes (IDDM) by developing a transgenic mouse model. By inserting into these mice a unique viral protein that was then expressed as a self-antigen in the pancreatic islets of Langerhans, we could study the effect on the expressed antigen alone, or in concert with an induced antiviral (i.e., autoimmune) response manifested later in life in causing IDDM. Our results indicate that a viral gene introduced as early as an animal's egg stage, incorporated into the germline, and expressed in islet cells does not produce tolerance when the host is exposed to the same virus later in life. We observed that the induced anti-self (viral) CTL response leads to selective and progressive damage of β cells, resulting in IDDM. The role of viral protein alone or specific viral peptide in causing anti-self response was evaluated.

N 014 TRANSGENIC MICE AND PRION DISEASES, Stanley B. Prusiner, University of California, San Francisco, California.

Advances in our knowledge of the infectious pathogens causing scrapie and other transmissible neurodegenerative diseases over the past decade support the hypothesis that these pathogens are novel and different from both viroids and viruses. Besides scrapie of sheep and goats, prion diseases include bovine spongiform encephalopathy of cattle, as well as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS) of humans. Enriching fractions from Syrian hamster (SHa) brain for scrapie prion infectivity led to the discovery of the prion protein (PrP). Determination of the *N*-terminal sequence of the protease resistance core of PrP permitted retrieval of molecular clones encoding PrP from cDNA libraries. The finding of PrP mRNA in uninfected tissues led to discovery of the normal PrP isoform denoted PrP^C. Transgenic (Tg) mice expressing both SHa and mouse (Mo) PrP genes were used to probe the molecular basis of the species barrier and the mechanism of scrapie prion replication. Our results argue that the species barrier for scrapie prions resides in the primary structure of PrP and formation of infectious prions is initiated by a species-specific interaction between PrP^{Sc} in the inoculum and homologous PrP^C. Ataxic GSS in families shows genetic linkage to a mutation in the PrP gene leading to the substitution of Leu for Pro at codon 102. Discovery of point mutations in the PrP gene of humans with GSS or familial CJD established that prion diseases are unique among human illnesses — they are both genetic and infectious. Tg mice expressing MoPrP with the GSS point mutation spontaneously develop neurologic dysfunction, spongiform degeneration and astrocytic gliosis. Inoculation of brain extracts prepared from these Tg(GSSMoPrP) mice into Syrian hamsters and Tg mice expressing wild-type PrP transgenes has produced neurodegeneration in recipient animals after prolonged incubation times. If convincing data on serial passage of prions from the inoculated recipients can be obtained, then these results will argue that prions are devoid of foreign nucleic acid. Studies of inherited prion diseases have revised thinking about sporadic CJD suggesting it may arise from a somatic mutation. Pulse-chase radiolabeling experiments of scrapie-infected cultures of mouse neuroblastoma cells indicate that protease-resistant PrP^{Sc} is synthesized during the chase period with $t_{1/2}$ ~1-3 h from a protease-sensitive precursor, consistent with the conclusion that PrP^C and PrP^{Sc} differ due to a post-translational event. The acquisition of PrP protease resistance in scrapie-infected cultured cells was found to be independent of Asn-linked glycosylation. PrP^C is bound to external surface of cells by a glycoinositol phospholipid anchor. In contrast, PrP^{Sc} accumulates within cytoplasmic vesicles of cultured cells. Attempts to demonstrate a scrapie-specific nucleic acid within highly purified preparations of prions have been unrewarding to date. Although it seems likely that transmissible prions are composed of PrP^{Sc} molecules alone, a hypothetical second component such as a small polynucleotide remains a formal possibility. Studies on the structure of PrP^{Sc} and PrP^C have been unsuccessful in defining a posttranslational chemical modification that distinguishes one PrP isoform from the other. These findings suggest that the difference between PrP^{Sc} and PrP^C may be conformational. Whether distinct prion isolates or "strains" with different properties result from multiple conformers of PrP^{Sc} remains to be established. The study of prion diseases seems to be emerging as a unique area of investigation at the interface of such disciplines as genetics, cell biology and virology.

Protein Trafficking in Infected Cells

N 015 INTRACELLULAR TRANSPORT AND TARGETING OF RETROVIRAL *gag* AND *env* GENE PRODUCTS, Eric Hunter, David Bedwell, Brian Brody, Jianjun Dong, David Einfeld, Sung Rhee, Tomas Ruml, Maja Sommerfelt and Caterina Strambio, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

We have investigated the process of retrovirus capsid protein transport and assembly using Mason-Pfizer monkey virus (M-PMV) since in this Type D virus the processes of capsid assembly, intracellular transport and membrane extrusion (budding) are unlinked. We showed previously that in M-PMV infected cells capsids were preassembled in the cytoplasm from three Gag-containing precursor proteins. Mutagenesis of the matrix protein coding region indicated that intracytoplasmic transport of preassembled capsids and the initiation of membrane extrusion were active processes that involved specific interactions between viral capsid and cellular components. These mutagenesis studies also provided clues to why Type-D precursor proteins assembled in the cytoplasm while those of the Type-C viruses were assembled at the plasma membrane. A non-infectious mutant virus, with a single codon change (an arginine to tryptophan change at position 55 in the approximately 750 amino acid long Pr78 precursor) within the MA region of the *gag* gene provided the key to this problem. Although mutant virus producing cells were found to release virus particles with a normal complement of capsid proteins, a biochemical assay for preassembled intracytoplasmic capsids in these cells was negative. Thin section electron microscopy of the cells revealed budding structures at the plasma membrane identical to those of a Type-C virus. Therefore, a single amino acid change within the MA protein was sufficient to change the intracellular site of capsid assembly and type of morphogenesis for M-PMV. Thus it is likely that the intracellular site to which capsid protein precursors are targeted defines the morphogenesis of the virus and that M-PMV capsid proteins contain a dominant targeting sequence that directs them to an intracytoplasmic assembly site. By comparing the MA proteins of MPMV and MMTV we have identified a sequence that is present in Type-D and Type-B capsid precursors but absent in Type C precursors. We have engineered this 18aa sequence into the Gag protein of MuLV and showed that the chimeric capsids now assemble in the cytoplasm. We hypothesize therefore that there is a novel transport pathway in mammalian cells that targets proteins to a specific intracytoplasmic compartment. Type-D/B retroviruses utilize this pathway to concentrate capsid precursors so that they can self-assemble in the cytoplasm. The self-assembly process appears to be optimized by additional regions of the Gag precursor such as the p12 and p27 domains. In addition, host cell proteins such as the chaperonins may be involved. We have demonstrated that expression of the M-PMV capsid precursor protein in yeast results in capsid assembly, therefore, it should be possible to address the role of host proteins in this process.

In order for an infectious virus to be assembled, retroviral envelope glycoproteins must be transported to the site of virus budding and must be incorporated into virions. We have shown previously that shortly after synthesis, the *env* gene product associates into a stable oligomer and that the kinetics of oligomer formation and transport out of the ER were similar. We have investigated which regions of the retroviral *env* gene product are involved in the oligomerization process. When the mature oligomer is disrupted by pH (M-PMV) or reducing agents (Rous sarcoma virus, RSV), the SU domain is released as a monomer while the TM protein remains oligomeric. Expression of the TM protein domain in the absence of the SU domain shows that TM is capable of efficiently forming an oligomer which can be transported intracellularly. In contrast when the SU domain is expressed independently it does not form stable, detectable oligomers even though it is efficiently secreted from the cell. Thus the TM protein appears to play the critical role in initiating and stabilizing retroviral glycoprotein structures. For some retroviruses, it may also play an important role in targeting glycoproteins for incorporation into the virus particle. Recent experiments that address this problem will be discussed.

N 016 PROTEIN SORTING IN VIRUS-INFECTED EPITHELIAL AND NEURONAL CELLS, Lukas Huber, Carlos Dotti, Marino Zerial and Kai Simons, European Molecular Biology Laboratory, Heidelberg, Germany.

Recent experiments in our laboratory suggest that neurons and epithelial cells sort membrane glycoproteins in a similar manner. Upon infection of polarized neurons in culture with vesicular stomatitis virus, the basolateral G glycoprotein was exclusively delivered to the dendrites, whereas in neurons infected with fowl plague virus, the apical hemagglutinin (HA) glycoprotein was preferentially delivered to the axon. We also investigated whether endogenous proteins which are anchored to the membrane via a glycosyl phosphatidylinositol (GPI)-anchor undergo axonal delivery in neurons. These GPI-anchored proteins are known to be exclusively sorted to the apical domain of epithelial cells. We observed by light and electron microscopy techniques that Thy-1, a neuronal GPI-anchored protein, was exclusively localized to the axonal membrane (Nature 349: 158-161, 1991). Since polarized delivery of membrane proteins only occurred in neurons which have developed an extensive synaptic network, it is possible that the machinery responsible for molecular sorting is assembled at the time of synaptogenesis. Searching for components involved in the specific targetting of apical and basolateral proteins in MDCK cells, we have identified a GTP-binding protein, rab 8, in the basolateral vesicles using 2D gel electrophoresis and GTP ligand blots. Immunofluorescence studies using anti rab 8 antibodies revealed a vesicular staining in MDCK cells. In neurons, rab 8 was demonstrated in the cell body and in the dendrites. It was absent from the axon. Further studies are in progress to study the function of the GTP binding protein.

Presentation of Viral Antigens

N 017 PEPTIDES NATURALLY PRESENTED BY MHC CLASS I MOLECULES, Hans-Georg Rammensee, Kirsten Falk, Olaf Rötzschke, Max-Planck-Institut für Biologie, Abteilung Immungenetik, W-7400 Tübingen, Germany.

The peptides naturally presented by MHC class I molecules can be isolated from whole cells or from purified MHC molecules by acid extraction followed by HPLC separation. Analysis of such peptides representing normal self peptides, viral peptides, minor histocompatibility peptides, and peptides recognized by alloreactive T cells indicated the following: i) Each normal class I-expressing cell simultaneously presents hundreds or thousands of peptides derived from cellular proteins. ii) The peptide content of cells is dependent on the expression of MHC class I genes. For example, the H-2K^b-restricted minor H peptide H-4^b is not detected in H-4^b cells not expressing K^b. iii) Cells may contain peptides that are not dependent on coexpression of MHC molecules. For example, H-4^b cells (irrespective of MHC expression) contain an additional peptide recognized by H-4^b-specific, H-2K^b restricted CTL with low efficiency. Such MHC-independent peptides may be precursors for the peptides finally presented by MHC molecules. iv) Several peptides naturally presented by MHC class I molecules have been identified, for example, ASNENMETM (D^b-restricted) and TYQRTRALV (K^d-restricted) are naturally presented by influenza-infected cells. v) the peptides presented by class I molecules adhere to allele-specific rules or motifs, which require allele-specific lengths (8 residues for K^b, 9 for K^d, D^b, and HLA-A2.1) and correct occupancy of two anchor residues, one of which is always C-terminal and is aliphatic for the alleles mentioned. Based on these data, we propose the following model for peptide processing in the MHC class I pathway. Proteases in various cellular compartments (e.g., cytosol) are degraded by an endopeptidase cutting C-terminal of aliphatic residues. The resulting peptides that share the C-terminus but not the N-terminus with the final product are then translocated to the compartment of MHC class I assembly and bind there to MHC molecules. Binding requires accomodation of side chains of the allele-specific anchor residues into the allele-specific pockets of MHC molecules. Finally, the N-terminal residues of the precursors are trimmed by an hypothetical protease activity. Thus, class I molecules have an instructive as well as a selective role in processing.

N 018 ANTIVIRAL CYTOTOXIC T LYMPHOCYTES: THE USE OF MINIGENES TO DISSECT THEIR BIOLOGICAL SIGNIFICANCE, AND TO ANALYSE PEPTIDE/MHC INTERACTIONS. J Lindsay Whitton, Tom McKee, & Michael BA Oldstone, Dept of Neuropharmacology, The Scripps Research Institute, 10666 N Torrey Pines Rd, La Jolla, CA 92037.

Cytotoxic T lymphocytes (CTL) are critical to the control of most virus infections as indicated by several human immunodeficiency syndromes (primary, secondary, and iatrogenic); patients lacking antibody responses cope well with most viruses, but are plagued by bacterial infections, while patients lacking T cell responses are highly susceptible to viral diseases. While the role of CTL in controlling primary virus infection has been well established in several animal model systems, it is frequently assumed that they play no role in secondary immunity (eg post-immunisation), which is characterised by an overwhelming 'memory' antibody response. Using the lymphocytic choriomeningitis (LCMV) model system, we have investigated the ability of CTL to protect against subsequent virus challenge, and have found that they can, in the absence of a secondary antibody response, efficiently protect against lethal-dose virus challenge.

The protective cells, CTL, recognise antigen presented to the immune system as peptides bound in the 'groove' of class I MHC glycoproteins. The mechanism of peptide generation within the presenting cell remains uncertain, but may involve degradation of intact proteins by the 'proteasome', followed by transport of the resulting peptides into the endoplasmic reticulum. Some time ago we reasoned that, if such a two-step system was present, it might be possible to directly manufacture short peptides in the cytoplasm and have them presented by class I MHC. Consequently, we designed "minigenes", open reading frames encoding CTL epitope peptides as short as 10 amino-acids, expressed from vaccinia virus. These short peptides are recognised by CTL, indicating that they are successfully presented by the MHC molecules; we have not yet determined whether the minigene peptides are processed prior to their interaction with class I MHC. The minigene peptides can induce biologically relevant responses, since a single administration of recombinant vaccinia containing a 10 amino acid LCMV sequence can confer complete protection upon mice of the appropriate haplotype; and a 'string of beads' vaccine, in which minigenes are fused to produce a polypeptide containing two CTL epitopes, each for a different MHC haplotype, is protective.

The factors which control peptide selection by class I molecules are not known. Initial schemes to predict epitopes from entire protein sequences met with limited success, but increasing databases of MHC allele-specific epitopes, and recent techniques to isolate endogenously-processed peptides, have allowed the identification of possible structural similarities among peptides presented by individual class I alleles. We have found that a single CTL epitope is capable of protecting several mouse strains, suggesting that this motif is able to bind efficiently to several class I alleles. We have performed mutational analysis of this epitope when expressed as a minigene, to define the critical amino acid residues required for presentation by the class I L^d molecule, as well as on the other haplotypes which present this epitope region. We have carried out similar minigene-based analyses of epitopes presented by the D^b molecule, to determine the effect of mutating the N (Asn) residue, thought to be critical for D^b presentation; is this residue important when the epitope is made in 'processed' form?

Viral Structure, Viral Receptors and Entry

N 100 MYRISTYLATION OF POLIOVIRUS CAPSID PRECURSOR PROTEIN P1 IS REQUIRED FOR ASSEMBLY OF SUB-VIRAL PARTICLES. David C. Ansardi, Donna C. Porter, and Casey D. Morrow, Dept. of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294. The poliovirus capsid proteins are initially synthesized as part of a large polyprotein, P1, which is proteolytically processed by the virus protease 3CD into the individual capsid proteins found in subviral particles and virions. The P1 polyprotein is myristylated at its amino terminal glycine residue, and the presence of myristate on P1 and the subsequent products of its processing is required for viral infectivity. In order to investigate the importance of P1 myristylation in the assembly pathway of poliovirus, we have generated two recombinant vaccinia viruses which express either myristylated (VVP1) or non-myristylated (VVP1myr-) forms of P1. When cells are coinfected with VVP1 or VVP1myr- and another recombinant vaccinia virus (VVP3), which expresses the protease 3CD, the P1 molecules are correctly cleaved into the single capsid proteins VP0, VP1, and VP3; thus, myristylation of P1 does not appear to be necessary for proteolytic processing by 3CD. In cells coinfected with VVP1 and VVP3, the processed capsid proteins assemble into empty capsid-like structures. In contrast, in cells coinfected with VVP1myr- and VVP3, no assembly of the processed capsid proteins can be detected. Analysis of the subcellular localization of the myristylated and non-myristylated forms of P1 by separation of intracellular membranes from cytosolic components revealed no major differences in association with intracellular membranes. Additionally, we found that both myristylated and non-myristylated forms of P1 expressed from the recombinant vaccinias are correctly proteolytically processed and are incorporated into virions in a mixed infection with wild-type poliovirus. These results suggest that the assembly defect detected for the capsid proteins derived from non-myristylated P1 is not likely the result of improper localization within the cell, nor are non-myristylated versions of P1 excluded from sites of virion assembly. Evidence will also be presented demonstrating that myristylated and nonmyristylated P1 proteins interact with the cytosolic heat shock proteins hsp 72/73, indicating a possible role for cellular chaperones in poliovirus assembly. We conclude, then, that myristylation of P1 is required for the efficient interaction of poliovirus capsid proteins at the initial stages of the assembly process. Finally, assembly of poliovirus capsid proteins may be required for their stability. We have observed that processed capsid proteins derived from non-myristylated P1 are more rapidly degraded than assembly competent capsid proteins derived from myristylated P1.

N 101 TRANSFERRIN RECEPTOR MEDIATES UPTAKE AND PRESENTATION OF HEPATITIS B ENVELOPE ANTIGEN BY T LYMPHOCYTES. Alessandra Franco*#, Marino Paroli*#, Ugo Testa§, Cesare Peschle§, Francesco Balsano#, & Vincenzo Barnaba*#. *Lab. Immunologia, #Fondazione "Andrea Cesalpino", I Clinica Medica, Università "La Sapienza"; §Lab. Ematologia ed Oncologia, Istituto Superiore di Sanità, 00161 Roma, Italy.

Human activated T lymphocytes expressing class II molecules overcome their inability of antigen presentation only for those complex antigens that bind to their own surface receptors, and thus can be captured, internalized, and processed through class II major histocompatibility complex (MHC) processing pathway. In the light of this, here we use for the first time the antigen-presenting T cell system as a tool to identify a viral receptor, as that used by hepatitis B virus (HBV) to enter cells. In deed, we demonstrate that both CD4+ and CD8+ T clones can process and present H8 envelope antigen (HBenvAg) to class II-restricted cytotoxic T lymphocytes (class II-CTLs) and that CD71 transferrin receptor (TfR) is involved in the efficient HBenvAg uptake by T cells. Since TfR is also expressed on hepatocytes, it might represent a portal of cellular entry for HBV infection. Furthermore, we suggest that the system of antigen presentation by T cells should serve as a model to study lymphocyte receptors used by viruses.

N 102 CELLULAR TROPISM OF ROTAVIRUS IN TISSUE CULTURE IS DETERMINED BY VIRAL PENETRATION OF THE CELL PLASMA MEMBRANE, Dorsey Bass, Michael Baylor and Harry B. Greenberg, Departments of Pediatrics and Medicine, Stanford University, Stanford, CA 94305.

Rotaviruses are an important cause of gastroenteritis in young mammals including human infants. In vivo, rotavirus displays striking cell, tissue and host tropism with viral replication generally restricted to the villus tip enterocytes of the small intestine of a specific species. We studied a panel of four cell lines which vary in their permissivity to rotavirus infection. Murine L cell fibroblasts and Hep 2 human laryngeal epithelial cells were approximately one thousand fold less susceptible to rotavirus infection compared to permissive simian renal MA 104 cells and human colonic carcinoma line HT29 cells, both in terms of infectious progeny and antigen synthesis measured by immunohistochemical staining. RNA transcription among the cell lines was proportional to antigen synthesis making a translational block an unlikely source of observed differences in susceptibility. All of the cell lines bound and internalized radiolabelled virus equally well as measured by escape from cell surface protease treatment. A time course analysis of the escape of infectious cell-bound virus from neutralizing monoclonal antibody revealed that in L cells and Hep 2 cells internalized rotavirus did not enter an eclipse phase but was internalized in an infectious form, possibly within endocytic vacuoles. L cells and Hep 2 cells were as permissive as MA 104 and HT29 cells when rotavirus infection was mediated by transfection of single- or double-shelled rotavirus particles using cationic liposomes (Lipofectin™). We conclude that rotavirus cell tropism in permissive tissue culture cells is determined by the ability of infecting virions to traverse the plasma membrane of the cells into the cytoplasmic compartment. In non-permissive cells, infectious virus binds to the plasma membrane and is internalized but does not traffic to a cytoplasmic compartment where encoating and transcription can occur.

N 103 ANTIBODY-COMPLEXED FOOT-AND-MOUTH DISEASE VIRUS, BUT NOT POLIOVIRUS, CAN INFECT CELLS VIA THE Fc RECEPTOR, B. Baxt[1], P. Mason[1], F. Brown[1], J. Harber[2], A. Murden[2], and E. Wimmer[2]. [1]USDA, ARS, Plum Island Animal Disease Center, Greenport, NY 11944; [2]Dept. of Microbiology, State University of New York at Stony Brook, Stony Brook, NY 11794

It is known that poliovirus (PV) and foot-and-mouth disease virus (FMDV) initiate infection by binding to specific cell surface receptors. For both viruses, binding is followed by a poorly understood disassembly process. To probe these early steps of infection, the ability of PV and FMDV to infect cells following binding through an alternative receptor was examined. For these studies, a Chinese hamster ovary cell line (CHO) expressing the B2 isoform of the murine Fc receptor (FcR) was used. Both viruses, when complexed with the appropriate antibodies, were able to bind to this cell line in an antibody dependent manner. Only FMDV, however, was able to productively infect these cells following binding through the FcR. We were able to demonstrate that FMDV was able to infect several other FcR-expressing cell lines, however PV was unable to infect any of these cells. These results suggest that the poliovirus receptor may serve dual roles of binding and destabilizing the virus particle, while the FMDV receptor may only serve in virion binding. These findings are also consistent with differences in virion architecture which predict a more intimate virion-receptor association in PV than in FMDV. The ability of FMDV to infect cells via this pathway may have important implications in FMD pathogenesis and in the establishment of latent (carrier) infections.

N 104 THE INTEGRIN VLA-2 IS AN ECHOVIRUS RECEPTOR. Jeffrey M. Bergelson, Michael P. Shepley, Bosco M. C. Chan, Martin E. Hemler, and Robert W. Finberg, Dana-Farber Cancer Institute, Boston, MA.

To identify cell surface receptors for echovirus, a common human pathogen, we generated two monoclonal antibodies that protected susceptible cells from infection by preventing virus attachment to specific receptor sites. These antibodies recognized the α and β subunits of the integrin VLA-2 ($\alpha^2\beta_1$), a receptor for collagen and laminin. RD rhabdomyosarcoma cells expressed minimal levels of VLA-2, and bound minimal amounts of radiolabelled virus. When transfected with cDNA encoding the α^2 subunit these cells expressed cell-surface VLA-2, gained ability to bind virus, and showed increased susceptibility to infection. Integrins, adhesion receptors important in cell-cell interactions and interactions with extracellular matrix, are capable of mediating virus attachment and infection.

N 105 SHEDDING OF A RHINOVIRUS MINOR GROUP BINDING PROTEIN: EVIDENCE FOR A Ca^{++}- DEPENDENT PROCESS, Dieter Blaas, Franz Hofer, Berthold Berger, Martin Gruenberger, Herwig Machat, Rudolf Dernick* and Ernst Kuechler, Institute of Biochemistry, University of Vienna, Vienna, Austria. *University of Hamburg, Heinrich Pette Institute of Experimental Virology and Immunology, Hamburg, Germany. Soluble rhinovirus minor group binding activity was found to be shed into the medium upon incubation of HeLa cells at 37°C. Although substantial amounts of this protein were released, no decrease of virus binding to the cell surface was seen. When the membrane associated receptor was stripped from the cells with trypsin, virus binding was rapidly restored from an intracellular receptor pool even in the absence of *de novo* protein synthesis. The release of the 85 kDa virus binding activity was inhibited by metal chelators such as EDTA, EGTA or 1,10-phenanthroline. The inhibition by EDTA was overcome by excess Ca^{2+} but not by Mg^{2+} or Zn^{2+} - ions. Sedimentation analysis showed that the soluble binding activity is not associated with membranous material. The virus binding protein was also released from cells grown under serum free conditions excluding its presence in the cell culture medium as a serum component. The potential involvement of a Ca^{2+}-dependent protease and/or a phospholipase in the release process is discussed.

N 106 CHARACTERIZATION OF THE CELLULAR RECEPTOR FOR LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV). Persephone Borrow and Michael B.A. Oldstone, Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, CA 92037

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus which produces a widespread infection in its natural host, the mouse, and also infects many other species, including man. The nature of the cellular receptor(s) for this virus were investigated. Two techniques were used to detect virus binding to membrane receptors: 1) cells were incubated with biotinylated LCMV followed by streptavidin FITC, and the extent of virus binding analyzed by flourescence activated cell sorter and 2) membrane proteins were separated by SDS-PAGE, and the receptor component detected using a virus overlay protein blot assay (VOPBA). With both techniques, LCMV was shown to bind specifically to cell types it is known to infect (e.g. MC57, Hela), but not to lymphoid cell lines that it does not readily infect (e.g., RMA, WIL-2). Protease treatment of cells/membranes abolished binding, whereas phospholipases, including phosphatidyl inositol-specific phospholipase C did not, indicating that the receptor is a transmembrane protein, not a membrane lipid or a protein with a glycosyl phosphatidyl inositol anchor. The protein is glycosylated, and has a molecular mass of approximately 120-150 kD, as demonstrated by VOPBA. The murine receptor protein is currently being purified, and cDNA libraries are being screened to isolate the gene which encodes it.

N 107 PRELIMINARY CHARACTERIZATION OF PUTATIVE CELLULAR RECEPTOR FOR ROTAVIRUS, M. Bremont, R. l'Haridon, J. Cohen and Harry B. Greenberg, Stanford University, Stanford, CA 94305.
In attempt to identify and characterize the putative cellular receptor for rotaviruses, rhesus monkey kidney cells (MA104) were used to immunize mice in order to generate a panel of hybridomas against MA104 cells. Positive monoclonal antibodies (Mabs) were selected for their capacity to protect MA104 cells from infection by rotaviruses. Two monoclonals (32Y21 and 94Y26) efficiently inhibited rotavirus replication and were further studied: a)-Both Mabs strongly blocked replication of three different rotavirus serotypes but did not affect replication of another virus (VSV). b)-Infection of other cells susceptible to rotavirus replication, such as COS cells and HT29 cells, was also inhibited by these two Mabs. c)-Mab 32Y21 and 94Y26 are able to stain the plasma membrane of non-fixed cells in an immunofluorescence test. d)-Mab 32Y21 immunoprecipitates a 50 Kd protein, while Mab 94Y26 immunoprecipitates an 80 and 63 Kd protein from MA104, COS and HT29 cells. These Mabs do not precipitate proteins from L cells which are refractory to rotavirus replication. Determination of the NH2 terminal sequences of these proteins is being undertaken and this sequence is likely to provide more information on the nature of these proteins and their potential role as rotavirus receptors.

N 108 INFECTIOUS ENTRY PATHWAY FOR CANINE PARVOVIRUS, Sukla Basak, Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

We have investigated whether canine parvovirus (CPV) infection involves a low pH-dependent entry pathway. The effects of two lysosomotropic bases, NH_4Cl and chloroquine, on CPV entry was studied by immunofluorescence and ultrastructural and biochemical methods. In the presence of these reagents, input virions appear to accumulate in large vacuoles. Ultrastructural studies indicated that uptake of virions takes place predominantly in small uncoated vesicles that appear to fuse with larger vesicles. In the presence of NH_4Cl, virions accumulate in the latter structures and their uncoating appears to be prevented. Viral DNA as well as antigen synthesis were found to be significantly inhibited in the presence of these reagents. In addition, inhibition of viral DNA and antigen synthesis appeared to be most extensive when NH_4Cl was present from 30 min preinfection, whereas no significant inhibition was observed when the cells were treated after 2 hr post infection. Thus, the results indicate that CPV requires exposure to low pH in an endosomal compartment to initiate a productive infection.

N 109 INTERACTION OF VESICULAR STOMATITIS VIRUS MATRIX PROTEIN WITH CELLULAR MEMBRANES AND RIBONUCLEOCAPSID CORES

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The matrix (M) protein of vesicular stomatitis virus (VSV) is a major structural component of the virion and may interact with both the ribonucleocapsid (RNP) core and the viral envelope. To investigate the interaction of M protein with cellular membranes, we examined its distribution in transfected Hela cells by subcellular fractionation. M protein, expressed without other viral proteins, was found both free in the cytoplasm as well as associated with membranes. This distribution remained unaltered when the VSV glycoprotein (G) or nucleocapsid (N) protein was co-expressed with M protein. Conditions known to release peripherally associated membrane proteins were ineffective in detaching M protein from isolated membrane vesicles. Membrane associated M protein was also capable of binding RNP cores *in vitro*. We further determined that the amino terminal 15 amino acids of M protein were essential for RNP binding and were also necessary for stable membrane association.

N 110 CAPSID RESIDUES THAT CONTROL STRUCTURAL TRANSITIONS IN POLIOVIRUS DURING CELL ENTRY

Elizabeth M. Colston and Vincent R. Racaniello, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032

Poliovirus entry into cells can be experimentally divided into two steps. At 4°C poliovirus binds reversibly to its immunoglobulin-like receptor on the surface of a susceptible cell. If these virus-receptor complexes are shifted to 37°C, the virus undergoes a dramatic, irreversible receptor-mediated conformational transition. The resulting altered particles differ from native virus in that they are not infectious, have lost the internal capsid protein VP4, have externalized the N-terminus of VP1, are hydrophobic, possess altered antigenic and sedimentation properties and can be found intracellularly as well as extracellularly.

Our approach to understanding the structural transitions associated with binding and alteration is to use soluble poliovirus receptor (PVR) to generate viral mutants defective in these steps. PVR was overexpressed in insect cells using the baculovirus expression system. Insect cells expressing PVR bind poliovirus and cytoplasmic extracts of insect cells expressing PVR neutralize infectivity by converting native virus to altered particles at 37°C.

We have isolated mutants that escape neutralization by the soluble receptor. Sequence analysis of 21 mutants has identified 10 amino acid changes, all in capsid protein VP1, that each confer resistance to neutralization by soluble receptor. The three-dimensional structure of poliovirus is known and the mutations fall into two general structural classes - surface residues, in the GH loop of VP1, and internal residues, near the drug-binding pocket of VP1. We hypothesize that mutations in surface residues may map receptor contact sites and that internal mutations may affect structural transitions associated with cell entry.

Because the soluble receptor can both bind and alter virus we also predicted two functional classes of mutants - those that do not bind well and those that bind normally but do not alter. Binding assays suggest that mutants with surface changes bind poorly, whereas mutants with internal changes bind normally. As one test of the mutants' ability to undergo transitions we assayed sensitvity to WIN 51711, an antiviral drug that binds in the drug-binding pocket of VP1 and prevents alteration. Two mutations greatly increase drug sensitivity and eight mutations have no effect on drug sensitivity. Experiments are currently under way to test the alteration ability and thermal stability of these mutants. It is our goal to understand the structural and functional basis of the virus-receptor interaction by studying mutants.

N 111 BASIS FOR THE RECEPTOR SPECIFICITY OF H2 INFLUENZA VIRUSES

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Influenza binding to a host cell is dependent upon the structure of the sialyloligosaccharide receptors which are recognized by the viral hemagglutinin. Our lab has demonstrated two major species specific binding types: H2 and H3 isolates from humans preferentially bind the SAα2,6 Gal linkage whereas avian and equine isolates preferentially bind the SAα2,3 Gal linkage. Sequencing of the H3 isolates revealed that a single amino acid change in the receptor binding pocket was responsible for the difference in receptor specificity. In order to determine the molecular basis for the receptor specificity of the H2 isolates, we are sequencing the A/RI/5+/57 and A/RI/5-/57 hemagglutinins. Sequencing of these antigenically similar substrains which exhibit SAα2,6 Gal and SAα2,3 Gal receptor specificity should provide insight into the molecular basis of this binding.

N 112 DISTRIBUTION AND CHARACTERIZATION OF FELINE LEUKEMIA VIRUS RECEPTORS, Gregg A. Dean and Edward A. Hoover, Department of Pathology, Colorado State University, Fort Collins, Colorado 80523

Feline leukemia virus is a retrovirus in the family Oncovirinae associated with the induction of anemia, leukemia, and immunodeficiency. FeLV subgroups A, B, and C are defined by viral interference and host cell range *in vitro* . The pathogenic and host range determinants of FeLV are located in the envelope gene which codes for the surface glycoprotein (gp70) that interacts with a cellular receptor to gain entry into the host cell. Although the cellular receptor used by FeLV has not been identified, there is evidence that receptors may play a role in the pathogenesis of FeLV. Studies reported here describe the distribution of FeLV receptors and character of the gp70/cellular receptor interaction using flow cytometry and partially purified gp70 from FeLV isolates 61E-A (F6A), Rickard B (FRB), and Sarma-C (FSC), representing FeLV subgroups A, B, and C, respectively. Partially purified gp70 specifically bound the FeLV receptor on a feline T-lymphocyte cell line (3201) as demonstrated by saturability of receptors, competition of binding by homologous gp70 and inhibition of binding by an anti-FeLV monoclonal antibody as well as immune cat serum. Cell receptor binding correlated with ability to infect with FRB and FSC but not F6A. Glycoprotein from each of the three virus subgroup bound less than 5% of bone marrow lymphocytes, 6-17% of erythroid progenitors and 6-9% of myeloid progenitors, thus demonstrating the presence of FeLV receptors on hemopoietic cells. 3201 cells infected with any of the three subgroups of FeLV retained the ability to bind both homologous and heterologous gp70. These studies suggest superinfection interference occurs at a post-binding step of infection. Finally, although the receptor used by subgroup C viruses has been proposed to be the transferrin receptor, transferrin did not block binding of gp70 of any of the three subgroups.

N 113 Isolation and Characterization of a Null Mutation in the Major Capsid Gene of Herpes Simplex Virus Type 1 (HSV-1). Prashant Desai, Joseph C. Glorioso and Stanley Person. Department of Molecular Genetics and Biochemistry. University of Pittsburgh Medical Center, Pittsburgh, Pa. 15261

The goal of our research is to identify the steps in the capsid assembly pathway of HSV-1. To achieve this goal deletion mutations will be introduced into each capsid gene. The seven capsid proteins and their approximate molecular weights in kilodaltons(kD) are: VP5(150), VP19C(52), VP21(44), VP22a(40), VP23(33), VP24(25), and VP26(12). VP5 is the major capsid protein and comprises approximately 60% of the capsid mass. It is the gene product of the unique long, open reading frame, UL19, of HSV-1 and is encoded by the Bg1 II N restriction fragment. A Vero cell line, designated G5, transformed with the EcoR1 restriction fragment of HSV-1, specifying genes UL16 through UL21 has been isolated. This cell line complements a temperature-sensitive mutation in VP5 at the non-permissive temperature. The mutant used is ts1178, a member of the G complementation group isolated and characterized by Dr. P.A. Schaffer and colleagues. A VP5 null mutation was introduced in the Bg1 II N fragment by the insertion a *lacZ* (B-galactosidase) reporter gene cassette such that expression of LacZ utlizes the VP5 promoter and initiation codon. The VP5 null mutation was transferred into the KOS genome by co-transfection of G5 cells with plasmid and viral DNA. Plaques that stained blue were isolated and plaque purified. The absence of VP5 in this virus was confirmed by SDS-PAGE analysis of labelled infected-cell extracts prepared from VP5$^-$ infected Vero cells. Currently EM studies are being carried out to determine what structure, if any, is formed by this virus under non-permissive conditions.

N 114 CHARACTERIZATION OF THE VIRUS BINDING DOMAINS OF THE MOUSE HEPATITIS VIRUS RECEPTOR, Gabriela S. Dveksler, Christine B. Cardellichio, Michael N. Pensiero, Kathryn V. Holmes and Carl W. Dieffenbach, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814. The cellular receptor for murine coronavirus MHV-A59 is a member of the carcinoembryonic antigen (CEA) family of glycoproteins. The MHV receptor (MHVR) has 4 immunoglobulin-like domains including an N-terminal domain stabilized by a salt bridge, followed by 3 C2-like domains, a hydrophobic transmembrane region and an intracytoplasmic tail. A series of deletion mutants was engineered by polymerase chain reaction to localize the virus-binding domain in the MHVR. Receptor mutants maintained the domain structure through the elimination of entire loops and conservation of the inter-loop spacing. The constructs were tested two ways. First, we used a transient expression system to determine whether the deletion constructs could render MHV-resistant hamster cells susceptible to MHV. The transfected cells were challenged with MHV-A59 and infection was monitored by detection of viral antigens by immunofluorescence. Second, the constructs were expressed in BHK cells using the vaccina virus T-7 RNA polymerase system. This high level expression allowed for the analysis by SDS-PAGE electrophoresis of the size of the modified glycoproteins. Additionally, we were able to determine the in vitro virus binding and monoclonal antibody binding of the altered receptor proteins. The combination of the two approaches distinguishes between simple binding and binding leading to productive infection. Our results indicate that specific loops are dispensable for virus infection and virus-induced cell fusion.

N 115 INTRODUCTION OF HUMAN GENOMIC SEQUENCES CONFERRING AMPHOTROPIC-SPECIFIC RETROVIRAL INFECTION ON CHO-K1 CELLS, Martin A. Eglitis, Michael Kadan, Lydia Gould, Edmund Wonilowicz, and Paul Tolstoshev, Genetic Therapy, Inc., Gaithersburg, MD 20878

Chinese hamster ovary (CHO-K1) cells are normally resistant to infection by amphotropically packaged mouse leukemia viruses. As part of an effort to isolate and characterize the human protein responsible for infection by amphotropic retroviruses, we introduced total human genomic DNA into CHO-K1 cells and used a retroviral vector conferring resistance to G418 to isolate transfected clones which had acquired the ability to be infected by amphotropically packaged retroviruses. One clone, CHO-18, had more than 50% of cells positive for β-galactosidase activity after transduction with a second vector carrying the bacterial lacZ gene. This clone's DNA was analyzed by Southern blot, and was shown to contain both alu and LINE human repetitive sequences. Furthermore, when an antibody-based retrovirus binding assay was used to evaluate the nature of virus-cell interaction, amphotropic but not ecotropic viruses were found to specifically bind to the CHO-18 clone. Neither type of virus can bind to wild type CHO-K1 cells. A genomic library was constructed from CHO-18 DNA, and screened with a human-specific alu probe. A clone was isolated which contained human unique and repetitive sequences. One of the unique human sequence probes detected transcripts in infectable human cells as well as in CHO-18 cells, but not in uninfectable CHO-K1 cells. Thus, CHO-18 cells provide a useful model for evaluation of the acquisition of retroviral infectability, and sequences obtained from the genomic library should provide probes useful for the isolation and analysis of the authentic human gene conferring this infectability.

N 116 DIFFERENT MECHANISMS ACCOUNT FOR HAMSTER CELL RESISTANCE TO INFECTION BY MURINE AND GIBBON APE LEUKEMIA VIRUSES.

Maribeth V. Eiden and Carolyn A. Wilson, Unit on Molecular Virology, Laboratory of Cell Biology, NIMH, Bethesda MD 20892.

Hamster cells are resistant to infection by most retroviruses including Moloney murine leukemia virus (MoMLV), the murine amphotropic virus, 4070A, and gibbon ape leukemia viruses (GaLVs). We have constructed hybrid virions to resolve the contributions of different viral components to the block of efficient replication of these viruses in hamster cell lines. The substitution of MoMLV core components for GaLV core components circumvents the resistance of hamster cells to infection by GaLV, demonstrating that hamster cells have receptors for GaLV but are not efficiently infected by this primate retrovirus due to a post-penetration block. Conversely, the resistance of hamster cells to infection by MoMLV and 4070A is attributable to envelope-mediated restrictions. Substituting a GaLV envelope for a MoMLV envelope facilitates infection of hamster cells by these hybrid virions. CHO K1 or BHK 21 hamster cells bear a receptor for MoMLV that can be rendered functional after treatment with tunicamycin, an inhibitor of N-linked glycosylation. Pretreatment of CHO K1 and BHK 21 target cells with tunicamycin does not render these cells susceptible to infection by 4070A virus. Different mechanisms account for the resistance of hamster cells to infection by GaLV, MoMLV and 4070A.

N 117 THE β-ADRENERGIC RECEPTOR ON HL60 CELLS DOES NOT FUNCTION AS A RECEPTOR FOR REOVIRUS,

Ali A. El-Ghorr and Eleftheria Maratos-Flier, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215

Controversy exists over the nature of the reovirus receptor on mammalian cells. Some reports have suggested that the β-adrenergic receptor is used by reovirus type 3 to facilitate its entry and initiate an infection in some mammalian cells, the mouse thymoma (R1.1) cell line among them.

We have used the human promyelocytic leukemia cell line (HL60), R1.1 cells and mouse L929 fibroblasts (L-cells) to study the nature of the reovirus receptor for serotypes T1 and T3. In our hands, the R1.1 cell line did not bind T1 or T3 reovirus and was not susceptible to infection. One million L-cells efficiently bound 70-90% of input virus (2-6μg) as indicated by a virus binding assay using radiolabelled reovirus. However, L-cells have already been shown not to bear β-adrenergic receptors.

The HL60 cell line was able to bind 55-85% of input T1 or T3 reovirus and was shown to bear β-adrenergic receptors using the tritiated β-adrenergic antagonist (-)3H-dihydroalprenolol (DHA). 15nM non-labelled ligand blocked 45% of the specific binding of 15nM radiolabelled DHA. The T3 reovirus was not capable of blocking the DHA binding in concentrations ranging between 0.6 and 60μg in 100μl. These competitive binding assays indicated that the β-adrenergic receptor did not act as the reovirus receptor on this cell line.

Additionally, the HL60 cells could be differentiated to granulocytes or monocytes using dimethyl sulfoxide or phorbol ester. Upon differentiation, the granulocytes bound 6-26% more reovirus while the monocytes bound 60-93% less reovirus than untreated HL60 cells. The β-adrenergic antagonist binding, however, did not mirror this pattern. The granulocytes bound equal amount of DHA while the monocytes bound twice the amount of DHA as untreated HL60 cells. These data argue against the theory that the β-adrenergic receptor acts as the universal reovirus receptor on mammalian cells.

N 118 X-RAY STRUCTURAL ANALYSIS OF NODAVIRUSES

Andrew J. Fisher, Bonnie R. McKinney, Jean-Pierre Wery and John E. Johnson. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Nodaviruses are the simplest group of RNA animal viruses. Their genome consists of three genes encoded on two messenger sense RNA molecules (RNA1-3,100 and RNA2-1,400 nucleotides). Nodaviruses infect insects but the type member, Nodamura virus, infects mammals as well. The gene product of RNA2, 407 amino acids, is protein α. 180 copies of protein α assemble into a capsid with T = 3 icosahedral symmetry. Approximately 80% of the α protein undergoes a post-assembly maturation cleavage that stabilizes the virion. This cleavage is auto-catalytic and necessary for infectivity.

Two nodaviruses, black beetle virus (BBV) and Flock House virus (FHV), have been analyzed at 3.0Å resolution by x-ray crystallography in our laboratory. BBV and FHV are 87% identical in protein α sequence, but exhibit significant biological differences. Most amino acid substitutions map to the exterior surface of the virus, yet there is evidence the viruses share a common receptor. The more conserved regions are the β-barrel domain and the inner-most region where the cleavage site is located. A conserved aspartic acid, near the cleavage site (ASN363/ALA364), may play a crucial role in catalysis.

The structures have lead to hypotheses concerning the mechanism for maturation cleavage, assembly, and cell receptor binding. We are testing these hypotheses by expressing FHV protein α in baculovirus. The expressed protein spontaneously assembles into virus-like particles that sustain the maturation cleavage (A. Schneemann & R. Rueckert, personal communication). We have grown crystals of these virus-like particles that are similar to the native virus crystals. Schneemann and Rueckert have also mutated the cleavage site resulting in assembly of provirions but inhibited maturation cleavage. Structural studies are currently underway of these provirions.

N 119 LOW pH-DEPENDENT CONFORMATIONAL CHANGE OF HUMAN RHINOVIRUS 2 IN ISOLATED ENDOSOMES,

R. Fuchs, L. Prchla and D. Blaas*. Dept. Gen. Exp. Pathology and *Inst. Biochem, Univ. Vienna, Austria.

Receptor-mediated endocytosis followed by low pH-induced conformational change of the viral capsid to C-antigenic particles are early events in rhinovirus 2 (HRV 2) infection of HeLa cells. Previous data suggest that uncoating and/or penetration of viral RNA into the cytoplasm most likely occurs from endosomes. It was the purpose of this study: 1. to determine the kinetics of virus entry and conversion, 2. to demonstrate the role of the endosomal proton pump in virus conversion and uncoating, and 3. to induce virus conversion in isolated endosomes *in vitro*. HeLa cells were incubated with ^{35}S-labeled HRV 2 at 34°C for various time periods. C-antigenic particles and native virus were monitored by immuno precipitation with monoclonal antibody 2G2 and polyclonal antiserum, respectively. 2G2 has been shown to immunoprecipitate exclusively C-antigenic particles, generated either *in vivo* or *in vitro* by pH<5.5 treatment of isolated HRV 2. Continous incubation of HeLa cells with HRV 2 showed a linear increase of total cell-associated virus with incubation time up to 25 minutes. In contrast, the conversion of native virions to C-antigenic particles occurred after a 2-3 minutes lag. During that time period 60% of total virus had been converted to C-antigenic particles. Thereafter, C-antigenic particles and to a lesser extent native virons were rapidly degraded in lysosomes. When HeLa cells were infected with HRV 2 in the presence of NH_4Cl or Bafilomycin A, a specific inhibitor of vacuolar-type proton pumps, the conformational change as well as virus replication was almost completely inhibited. In addition, internalization of HRV 2 in the presence of NH_4Cl and preparation of an endosome-enriched Golgi fraction demonstrated accumulation of native virions, but not C-antigenic particles in this fraction. *In vitro* incubation of this fraction in NH_4Cl-free medium, that leads to passive endosome acidification, did not result in a conformational change. However, addition of ATP to the incubation medium- to stimulate the vacuolar proton pump - resulted in 15% conversion of native virions. Maximal conversion (55%) was obtained by incubation in pH 5.5 buffer. In conclusion: 1. Intracellular conversion of native virus to C-antigenic particles occurs after a 2-3 minutes lag period. 2. C-antigenic particles are more susceptible to lysosomal degradation. 3. Virus is enriched in isolated endosomes. 4. Endosome acidification is necessary to induce the formation of C-antigenic particles *in vivo* as well as *in vitro*.

Supported by Hochschuljubiläumsstiftung der Stadt Wien.

N 120 LOW INFECTIOUS YIELDS OF PSEUDORABIES VIRUS FROM HUMAN CELLS ARE DUE TO A DEFECT SUBSEQUENT TO ENTRY THAT MAY INVOLVE BOTH VIRUS AND CELL–SPECIFIC FACTORS. A. Oveta Fuller and G. Subramanian, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620.

Pseudorabies virus (PRV) is an alphaherpesvirus closely related to herpes simplex (HSV). These viruses have a similar genome size and arrangement, location and number of viral envelope glycoproteins, infectious cycle in their native hosts, and entry process into cultured cells. Unlike some other mammalian herpesviruses like monkey herpesvirus B, PRV does not seem to infect humans. From studies to understand the molecular events in entry of HSV and PRV, we find that PRV produces poor infectious yields from cultured human cells (HEp-2 and HEL). Increased input virus can increase PRV yields, but not to levels equal to that of PRV from native swine cells or HSV from human cells. As with HSV, PRV binds well to HEL and HEp-2 cells and that binding can be reduced by wash with heparin. Early PRV proteins are expressed in human cells indicative of successful penetration. Transfection of viral DNA produces good infectious yields of PRV on susceptible swine and Vero cells, but no infectious progeny from human cells. Thus, bypassing the normal entry process does not correct the block to PRV infection of these cells. Transfection of PRV DNA produces especially high infectious yields in native swine cells (as high by transfection as by infection). We conclude that the defect to PRV infection of human cells occurs subsequent to virus entry at gene expression, genome replication, or viral assembly or egress. Experiments are in progress to further define the specific location of the defect and the nature of critical cellular or virus factor(s). These studies will contribute to understanding at a molecular level the viral and cellular factors that determine host tropisms of alphaherpesviruses.

N 121 PROTEASE INDUCED ENTRY OF HEPATITIS B VIRUS, Wolfram H. Gerlich, Lu Xuanyong and Klaus-Hinrich Heermann, Institute of Medical Virology, University of Giessen, and Department of Medical Microbiology University of Göttingen, Germany.

Hepatitis B Virus (HBV) is highly infectious in vivo, but it cannot infect efficiently human hepatoma cells in vitro, although such cells are able to replicate and secrete HBV after transfection. The human hepatoma cell HepG2 has two not-yet identified receptors for the preS1 domain and for the pre2-glycan of HBV, but virus entry does not occur after attachment. Pretreatment of HBV or hepatitis B surface antigen (HBsAg) particles with trypsin or V8-protease cleaves the middle sized HBs protein at a proteolysis-hypersensitive site in the preS2 domain. Such protease-treated particles do no longer attach specifically to Hep G2 cells but bind irreversibly to all types of cells. In contrast to the specific binding the nonspecific binding is optimal at low pH. The nonspecific binding depends on a sequence at the aminoend of the S domain which is homologous to fusion sites of other viral surface proteins. It becomes exposed by trypsin or V8-treatment. Chymotrypsin removes that sequence and abolishes also the nonspecific binding. V8-treated, but not untreated or chymotrypsin-treated HBV enters HepG2 cells. We postulate that the low infectivity of HBV for hepatoma cell cultures is caused by a lack of suitable protease and/or insufficient endosomal uptake.

N 122 ROLE OF HIV-1 PROTEASE IN EARLY STEPS OF INFECTION. Helmut Jacobsen, Ludwina Ahlborn-Laake, Roland Gugel, Jan Mous, F.Hoffmann-La Roche Ltd., CH-4002 Basel, Switzerland

The *pol*-reading frame of HIV-1 encodes an aspartate protease which cleaves *gag* and *gag-pol* precursor proteins to yield mature progeny virus. Specific inhibitors of the protease have been described recently. We have used the inhibitor Ro 31-8959 to study a possible role of the HIV-1 protease in early steps of replication, i.e., during the establishment of a primary infection in the T-cell line MT-4. Towards this end, we have analyzed the synthesis of cDNA, its integration into the host genome and its subsequent transcription in one-step growth experiments. Protease inhibitor at concentrations known to be inhibitory in antiviral assays proved to be without effect on these early events of HIV-1 growth. However, no mature progeny virus was produced and thus infection was restricted to the primary infected cells in the continued presence of Ro 31-8959 . Virus growth and spread of infection resumed when the inhibitor was removed from the infected culture. These results argue against an essential function of HIV-1 protease during early events of infection up to transcription of the infecting viral genome.

N 123 SATURABLE ATTACHMENT SITES FOR THE POLYHEDRA-DERIVED VIRUS OF *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS ON *LYMANTRIA DISPAR* CELLS

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Among baculoviruses, the nuclear polyhedrosis viruses (NPV) consist of two distinct phenotypes, the polyhedra-derived virus (PDV) and the budded virus (BV). PDV initiates the primary infection in the insect midgut, while BV is responsible for establishment of secondary infections of other tissues. While previous studies suggest that BV exhibits specific receptor binding to insect cells, the present research represents the first evidence for specific binding of PDV. Fluorescence-activated cell sorting (FACS) analysis revealed saturable binding of FITC-labeled PDV (PDV/FITC) from *Lymantria dispar* NPV to the gypsy moth cell line, IPLB-EIta. Competition for limited receptors was found when PDV/FITC was incubated with excess levels of unlabeled PDV. In addition, excess levels of unlabeled BV effectively competed with PDV/FITC suggesting that both viral phenotypes may be able to use the same receptor. While it is known that BV enters host cells by the pathway of adsorptive endocytosis, PDV does not use this pathway and the exact mechanism of entry of this phenotype is unclear. The present work will provide initial information necessary for an understanding of the mechanism by which PDV establishes the primary infection in the insect midgut.

N 124 IDENTIFICATION OF A RECEPTOR FOR HERPES SIMPLEX VIRUS GLYCOPROTEIN D, A PUTATIVE RECEPTOR FOR HSV, D.C.

Johnson[1], T. Gregory[2], and R.-L. Burke[3]; [1]McMaster University, Hamilton, Canada L8N3Z5; [2]Genentech, South San Francisco, CA, 94080; [3]Chiron Corp., Emeryville, CA 94608

Entry of HSV into cells is a complex process, initially involving adsorption of virions onto very numerous, charged molecules on the cell surface which include heparan sulfate proteoglycans. However, subsequent interactions are required for virus entry into cells which involves fusion of the virion envelope with the plasma membrane of cells. Glycoprotein D (gD) plays an essential role in entry of HSV into cells and cell-cell fusion but is not required for the initial adsorption step. Virus mutants lacking gD cannot interact with a limited set of cellular receptors required for virus entry and these receptors can be blocked by soluble forms of gD, so that viruses adsorb onto the cell surface but do not enter. Furthermore, soluble forms of gD bind to saturable, protease-sensitive sites on the surfaces of cells. These results support the hypothesis that gD interacts with cell surface proteins, which are much more limited in number than the heparan sulfate molecules or other charged molecules to which the virus can initially adsorb, and which are essential for virus entry into cells. Soluble forms of gD binds to a cellular protein which has an apparent molecular weight of 180 - 200 KDa on semi-native SDS gels and which is a component of the plasma membrane. A soluble form of HSV glycoprotein B (gB) does not bind to this protein. This putative HSV receptor is found in most mammalian cell lines and in several human tissues and is unrelated to two well characterized FGF receptors, flg and bek. Attempts are underway to purify this protein and clone the gene which encodes the protein.

N 125 DETECTION OF RECEPTOR-SPECIFIC MURINE LEUKEMIA VIRUS BINDING TO CELLS BY IMMUNOFLUORESCENCE ANALYSIS, Michael J. Kadan[1], Sabine Sturm[2], W. French Anderson[2], Martin A. Eglitis[1], [1]Genetic Therapy, Inc., Gaithersburg, MD 20878 and [2]Lab of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892

Four classes of murine leukemia virus (MuLV) have been described which display distinct cellular tropisms and bind to different retroviral receptors to initiate virus infection. Retroviral vectors derived from MuLV have proven to be valuable tools for gene transfer in mammalian cells. In the present study, we describe a rapid, sensitive immunofluorescence flow cytometry assay useful for characterizing the initial binding of MuLV to cells. Using the rat monoclonal antibody 83A25 (L.H. Evans et al., 1990, J.Virol. 64:6176), which recognizes an epitope of the envelope gp70 molecule common among the different classes of MuLV, it is possible to analyze binding of ecotropic, amphotropic, or xenotropic MuLV using only a single combination of primary and secondary antibodies. The MuLV binding detected by this assay is envelope-receptor specific and correlates with the susceptibility to infection determined for a variety of human and rodent cells. That is, human cells infectable by only amphotropic retrovirus show amphotropic but not ecotropic virus binding. To further define the nature of the virus binding, amphotropic MuLV binding to NIH-3T3 cells was characterized in detail. The binding to NIH-3T3 cells is rapid, saturable and temperature dependent; at 37°C, maximal amphotropic virus binding is observed within 15 min of virus exposure. This binding is dramatically reduced at 4°C. Chinese hamster ovary (CHO-K1) cells normally lack ecotropic virus binding and are not infectable by ecotropic vectors. Expression of the cloned ecotropic retroviral receptor gene (Rec) in CHO-K1 cells confers high levels of ecotropic-specific binding and ecotropic infectability. Characterization of MuLV binding to primary cells may provide insight into the infectability of cells by retrovirus and aid in the selection of appropriate vectors for gene transfer experiments.

N 126 INHIBITION IN THE FUNCTION OF COATED PITS AND COATED VESICLES BY A TRANSDOMINANT MUTATION OF CLATHRIN LIGHT CHAIN LCB3, T. Kirchhausen, T. Toyoda and S. Schlesinger, Dept. of Anatomy & Cell. Biology, Harvard Medical School, Boston, MA 02115 and Dept. of Microbiology, Washington University, St. Louis, MO 63110

Our objective is to understand the molecular mechanisms by which clathrin coated pits and coated vesicles regulate vesicular membrane traffic. Clathrin light chains are extended molecules tightly bound to the cytoplasmic face of the clathrin coat that surrounds coated pits and coated vesicles. Because they are composed of consecutive and independent folded domains, it is possible to target mutations that will not alter light chain binding to heavy chains but have the potential to interfere with interactions with other molecules necessary for the normal function of clathrin.

We have established CHO cell lines impaired in their clathrin-mediated vesicular traffic due to expression of a truncated rat LCB3 light chain lacking its 92-amino acid amino-terminal domain. Several of these cell lines are defective in the receptor-mediated endocytosis of transferrin and LDL and are significantly blocked in their susceptibility to infection by Sindbis and Semliki Forest virus. Growth curves show that production of virus is decreased 5-10 fold in cells expressing the truncated LCB3 compared to control cells expressing the intact LCB3. This decrease was proportional to the reduction in the activity of a CAT reporter gene included in the genome of one variant of Sindbis virus, indicating that inhibition was at an early step in virus replication. Direct measurements of the viral entry step are currently in progress.

In the future we will use these cell lines impaired in clathrin function to study in more detail how viruses enter the endocytic pathway and determine when and where the virus-uncoating step leading to viral replication happens.

N 127 AVIAN C-TYPE AND PRIMATE D-TYPE ONCORETROVIRUSES ARE MEMBERS OF THE SAME RECEPTOR INTERFERENCE GROUP, Han-Mo Koo[1,2], Jie Gu[1,2], Alfredo Varela-Echavarria[1], Yacov Ron[1] and Joseph P. Dougherty[1], Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854-5635[1], and Graduate Program in Microbiology, Rutgers University, Lipman Hall, P.O. Box 231, New Brunswick, New Jersey 08903[2]

The reticuloendotheliosis viruses (REVs) constitute a group of avian retroviruses which are more closely related to mammalian retroviruses than to other avian retroviruses. For example, the envelope glycoproteins of at least some members of the REV group display a striking amino acid sequence identity to a group of primate oncoretroviruses which belong to a single receptor interference group and include all D-type and some C-type primate oncoretroviruses. Moreover, members of the REV group have a broad host range which includes avian, rat, canine, simian and human cells. Because of the sequence similarities between their envelope glycoproteins and the broad host range of members of the REV group, the pattern of cross-interference between members of the avian and primate virus groups was investigated to determine if they utilized the same receptor. Superinfection experiments, using a vector virus containing the *E. coli lacZ* gene, showed that these avian and simian oncoretroviruses indeed constitute a single receptor interference group on both human and canine cells indicating that they bind to the same receptor to initiate infection. These results suggest that the receptor binding specificity is maintained over a wide range of cells and has led to the broad trans-species spread of these retroviruses.

N 128 VIRAL DNA IS CARRIED BY MATURE HIV-1 VIRIONS, Franco Lori*, Fulvia Veronese^, Anthony DeVico^, Paolo Lusso*, Cindy Boyer*, Andrea Cara*, Marvin S. Reitz Jr.* and Robert C. Gallo*, *Laboratory of Tumor Cell Biology, NIH, NCI, Bethesda, MD, 20892 and ^Advanced Biosciences Laboratories, Kensington, MD, 2895

Reverse transcriptase is thought to initiate DNA synthesis only after the retroviral core enters the target cell and retroviruses are thought to carry genomic information only in a RNA form. We present here evidence that viral DNA is carried by mature HIV-1 virions. This DNA appears to originate from a reverse transcription step prior to or during viral release. Analysis of this DNA revealed that: a) viral DNA is incomplete and heterogeneous; b) minus strand DNA is represented by molecules of variable length, all starting from the same origin of replication (5' of the primer binding site), although not all of them are full-length molecules; c) plus strand DNA synthesis has reached at least the primer binding site region. The presence of specific viral DNA in extracellular particles represents a novel finding in retroviruses. This DNA might play a role in the latency of HIV-1 after infecting resting cells (such as unstimulated T-lymphocytes). HIV-1 DNA might also be involved in episomal pathways of replication of HIV-1 genome in terminally differentiated cells (such as macrophages). HIV-1 replication may thus bear similarities with replication of reverse transcriptase dependent DNA viruses (hepatitis B virus and/or cauliflower mosaic virus).

N 129 STRUCTURAL STUDIES ON COXSACKIEVIRUS ANTIGEN CHIMERAS. Fiona McPhee, Birgit-Yvonne Reimann, Roland Zell and Reinhard Kandolf. Max Planck Institute for Biochemistry, D-8033 Martinsried, Germany.

Crystal structure analyses of polioviruses and human rhinoviruses have shown that there is a high degree of homology in the tertiary structure of various picornaviruses. With this in mind, molecular modelling techniques have been employed to aid the construction of intertypic antigen chimeras of coxsackie B viruses. Based on the amino acid sequence homologies of coxsackie B viruses and polioviruses, we constructed antigen chimeras using infectious coxsackievirus B3 cDNA and characterised antigenic site I (N-AgI) in the putative VP1-BC-loop of coxsackievirus B4 (CVB4). Of the original chimeras constructed, one (CVB3/4) was viable while a second chimeric cDNA construct (CVB3/4ii) was not infectious although the deduced amino acid sequence differed only in one amino acid at the beginning of the BC-loop, i.e. a serine residue replaced a glycine. Molecular modelling was employed to predict possible interactions between the BC-loop and surrounding amino acids of chimeric proteins in both CVB3/4 and CVB3/4ii. Based on these studies, a series of cDNA chimeras were constructed containing either deletions or substitutions in the sequence of the VP1-BC-loop. The antigenic and biological properties of the antigen chimeras were analysed in order to determine whether theoretically derived interactions could be experimentally verified. The results obtained indicate that molecular modelling may provide a useful tool in the rational design of viable chimeras as well as in the characterisation of antigenic sites.

N 130 CHARACTERIZATION OF A HUMAN RECEPTOR FOR MEASLES VIRUS USING A MONOCLONAL ANTIBODY, Denise Naniche[1], Fabian T. Wild[2], Chantal Rabourdin-Combe[1] & Denis Gerlier[1]. [1]Immunobiologie Moléculaire, UMR 49, CNRS-ENS Lyon, & [2]EITVM CNRS UMR05, Lyon, France.

Measles virus (MV) exhibits a very limited host-range and man is the only natural reservoir for the virus. We believe that this restricted tropism may be due to a lack of expression of cell surface receptors on non-primate cells. Indeed, human but not mouse cell lines were found to bind purified metabolically radiolabelled MV particles, and after incubation with MV, only human cells could form rosettes with hemagglutinin-reactive vervet monkey erythrocytes. The MV binding capability of human cells was destroyed after proteolytic treatment and could be regenerated after incubation at 37°C. The re-expression of the binding capability was inhibited if cells were treated with cycloheximide or tunicamicin. Human cells were used to immunize mice and monoclonal antibodies were derived. They were selected by their ability to block the measles-like cytopathic effect observed after infection of human Hela cells with a recombinant vaccinia virus coding for both measles hemagglutinin and fusion glycoproteins. Purified MVR20.6 antibody inhibited the MV binding of human cells as detected using radiolabelled binding and rosetting assays. This antibody immunoprecipitated two polypeptides with an apparent molecular mass of 57 and 67 kD from human but not from mouse cells. We are currently using this antibody to clone the MV receptor.

N 131 BINDING OF SV40 TO SURFACE CLASS I MHC PROTEINS ACTIVATES CELLULAR PROTO-ONCOGENES, Leonard C. Norkin and Walter C. Breau, Department of Microbiology, University of Massachusetts, Amherst, MA 01003

A number of our results support the conclusion that class I MHC proteins are a necessary component of the cell surface receptor for SV40. Our most compelling finding is that SV40 does not bind to cells of two different human lymphoblastoid cell lines which do not express surface class I proteins. One of these lines (Daudi) has a defect in the gene for B_2-microglobulin. The other line has genetic defects in the HLA complex. Transfection of these cell lines with cloned genes for B_2-microglobulin and HLA-B8, respectively, restored expression of their surface class I MHC proteins and resulted in SV40 binding as well.

Currently, we are looking into whether SV40 binding at the cell surface might induce a transmembrane signal. We find that SV40 binding indeed activates transcription of the proto-oncogenes c-fos and c-myc. These results imply that the interaction of SV40 with surface class I MHC proteins somehow results in the transmission of an extracellular signal.

N 132 MYRISTYLATION OF *gag-pol* POLYPROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 IS REQUIRED FOR THE OPTIMAL INCORPORATION INTO VIRION PARTICLE. Jinseu Park and Casey D. Morrow, Dep.of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294. The genome of the Human Immunodeficiency Virus type-1 (HIV-1) contains the same *gag-pol-env* organization characteristic of all retroviruses. The expression of the *pol* genes occurs via a ribosomal frameshift between the *gag* and *pol* genes to produce a *gag-pol* polyprotein at a 5-10 % level of the *gag* protein. The *gag-pol* polyprotein contains the viral protease, reverse transcriptase and integrase. Although *gag-pol* is essential for viral infectivity, the molecular mechanism by which HIV-1 *gag-pol* polyprotein is incorporated into the virion particle is unkown. To study the assembly of *gag-pol* into the virion particle, a complementation system was developed in which unprocessed *gag* and *gag-pol* were expressed from mutant proviral genomes (pGAG and pGAGPOL), respectively, in the COS-1 cells. We detected a low level of reverse transcriptase activity in the supernatant of cells transfected with pGAGPOL alone. Co-transfection of pGAG and pGAGPOL into cells resulted in a significantly higher level of reverse transcriptase activity in the supernatant. Sedimentation analysis using sucrose density gradients revealed that most of the reverse transcriptase activity was found in fractions corresponding to the density of virion particles, indicating the *gag-pol* polyprotein was released in association with the *gag* virion particle. To evaluate the effect of myrisylation, a nonmyristylated form of *gag-pol* was also expressed (pGAGPOLM(-)). The *gag-pol* (myr-) was incorporated into virion particle but at a lower efficiency than the myristylated *gag-pol*. From the cell fractionation experiments, the distributions of the *gag-pol* (myr-) and *gag-pol* in the membrane and cytosol were similar using low or high ionic strength conditions.
Taken together, these results suggest that myristylation of *gag-pol* polyprotein is necessary but may not be sufficient for the targeting into virion particle and that the gag moiety of *gag-pol* polyprotein may provide the targeting signals that direct this protein into the virion particle.

N 133 IDENTIFICATION, CHARACTERIZATION, AND SUBCELLULAR DISTRIBUTION OF THE HIV-2 VIF GENE PRODUCT, Parkin, J. S.[1], J. C. Kappes[2], and G. M. Shaw[2], [1]Department of Microbiology, The University of Pennsylvania, Philadelphia PA 19104, [2] Department of Medicine, The University of Alabama at Birmingham, Birmingham, AL 35294 Human and simian immunodeficiency viruses (HIV-1,-2, and SIV) contain a well-conserved central region open reading frame termed the virion infectivity factor (vif). We have generated a series of specific immunological reagents towards the viral gene product encoded by the HIV-2_{ROD} vif open reading frame and demonstrated their immunoreactivity towards partially purified prokaryotically expressed and eukaryotically expressed vif. We have identified and characterized the vif gene product in a chronically HIV-2-infected $CD4^+$ immortalized lymphocytic cell line (Sup-T1). The HIV-2_{ROD} vif open reading frame is capable of encoding a protein with an apparent molecular mass of 26 kDa as determined by its migration in SDS-PAGE gels. Multiple forms of the vif protein were identified in cell lysates suggesting it undergoes a posttranslational modification(s). The localization of this gene product in the virion was examined and it was demonstrated that vif is not efficiently incorporated into the virion. Western immunoblot and radioimmunoprecipitation analysis of sucrose cushion purified virus revealed similar viral protein profiles in both vif-deficient and wild-type virus, as compared to each other. Direct quantitation of viral proteins in both mutant and wild-type virus demonstrated the presence of equimolar quantities of the envelope glycoproteins (gp32 and gp120), the structural proteins (gag and pol), and the centrally encoded vpx protein. Subcellular fractionation and indirect immunofluorescence studies utilizing a transient eukaryotic expression system in Cos-1 cells demonstrated that the vif gene product is capable of displaying both nuclear and cytoplasmic, but not membrane localization. A putative nuclear localization signal, RKQRRRDYRR, was identified in the carboxyl terminus of the protein. The implications of these findings and the putative role of vif in HIV/SIV replication will be addressed.

N 134 FINE MAPPING OF DETERMINANTS OF THE CANINE HOST RANGE OF CANINE PARVOVIRUS IN THE STRUCTURE OF THE CAPSID.

Colin R. Parrish and Shwu-Fen Chang, J.A. Baker Institute for Animal Health, N.Y. State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Canine Parvovirus (CPV) and Feline Panleukopenia Virus (FPV) differ by only a small number of sequence differences. We have shown that the canine host range of these viruses is affected by residues within the capsid protein gene.

Here we define the role(s) of various residues in the CPV and FPV capsids which determine the ability of the viruses to replicate in canine cells using site directed mutagenesis and recombination mapping, using infectious plasmid clones the two viruses. This showed that two residues from CPV introduced into the genome, within the capsid protein gene, are sufficient to endow FPV with the ability to replicate in dog cells. One further CPV-specific change is required to allow the viability of viruses with those changes in the CPV background. The role of the same changes in determining the pH dependence of HA was also determined, and shown to involve coordinate changes of two residues.

A mutant of CPV which lost the canine host range was shown to involve a single sequence difference in the capsid protein gene, as well as an antigenic difference.

The common features between these changes and the specific alterations which they make in the structures of the viruses are examined by comparison with the three-dimensional structure of CPV.

N 135 PRES1-SPECIFIC BINDING PROTEINS AS PUTATIVE RECEPTORS FOR HEPATITIS B VIRUS IN HUMAN HEPATOCYTES, Marie-Anne Petit*, Francis Capel, Sylvie Dubanchet and Hélène Mabit, INSERM U 131, 32 rue des Carnets, 92140, Clamart, France

Cellular receptors play an important role in viral pathogenesis. Until now, there has been no reliable information on receptor(s) for Hepatitis B Virus (HBV). Therefore, we attempted to identify specific receptors in human hepatocytes using an immunological approach. Anti-idiotypic (Ab2) antibodies were raised in rabbits against our monoclonal antibody (MAb1) F35.25. MAb1 F35.25 (i) recognized the hepatocyte receptor binding site on HBV (located between aminoacid residues 21 and 47 of the preS1 sequence) and (ii) blocked the attachment of preS1-positive HBV particles to human hepatocytes.
The presence of Ab2 antibodies in rabbit sera was determined by the ability of antisera to inhibit Id (Ab1)/antigen (HBV) recognition. Affinity purified Ab2 IgGs to F35.25 represented an internal image for the preS1 domain 12-53. Our present studies indicate that Ab2 IgGs to F35.25 (1) recognized the membrane-associated structure of preS1-specific HBV receptor in HepG2 cell binding assay, as visualized by immunoenzymatic staining; (2) strongly bound to a major 35 kDa component and to three other related proteins of 50, 43 and 40 kDa in extracts of HepG2 cells; and (3) reacted with several soluble and membrane-associated proteins in normal human liver cells. The binding was insensitive to reduction. All preS1-binding proteins were V8-protease sensitive and endoglycosidase-H resistant. The 35 kDa species was trypsin resistant and generated a band of 32 kDa by endoglycosidase-F treatment.
Together, our results suggest that the identified preS1-specific binding proteins may be involved in putative complex structure of the hepatocyte receptor for HBV.

N 136 MECHANISM OF TROPHOBLAST INFECTION BY HUMAN IMMUNODEFICIENCY VIRUS, David M. Phillips, The Population Council, 1230 York Ave. New York, New York 10021

An in vitro model has made it possible to demonstrate HIV virus transmission from infected lymphocytes to CD4-negative placental trophoblast cells via endocytosis. Upon addition to cultured trophoblast cells (BeWo), chronically HIV-infected lymphocytic cells (MOLT-4) adhered to the epithelial cells via a complex of newly induced microvilli. Though viruses were rarely seen in the infected parental lymphocytic cell line, mature HIV virions appeared promptly and profusely in the interstices between the interdigitating microvilli of the two cell types. Virions appeared to bud from the lymphocyte donor cells at the point of cell-cell contact and to be rapidly taken up by the trophoblast acceptor cells via an endocytic mechanism involving coated pits, endosomes, and lysosomes. Electron microscopic observations suggest that HIV may later escape into the trophoblast cytoplasm by fusing with the endosome membrane or by lysing the lysosome membrane. A 1-hour period of co-incubation was sufficient to establish the HIV infection in the trophoblastic cell line. Four weeks after thoroughly washing out the donor lymphocytic cells, HIV RNA was demonstrated in clusters of BeWo cells by in situ hybridization. The HIV infection was productive as demonstrated by cocultivating the BeWo cells with indicator lymphocytes 4 weeks after the initial infection. This study, demonstrating the mechanism of HIV transmission, expands upon previous observations that trophoblast cell lines lacking the CD4 viral receptor can nevertheless be infected by HIV and can support productive infection.

N 137 THE PRODUCTION AND USE OF RECOMBINANT FELV SURFACE GLYCOPROTEIN IN THE IDENTIFICATION OF THE FELV CELLULAR RECEPTOR. I.K.Ramsey, N.Spibey, O.Jarrett. MRC Retrovirus Laboratory, Department of Veterinary Pathology, University of Glasgow, Glasgow, Great Britain.

Feline leukaemia virus (FeLV), an important pathogen of the domestic cat, is a type C retrovirus that naturally occurs in 3 subgroups as defined by an interference assay. This suggests that three FeLV cellular receptors exist, one for each subgroup. It has been well documented that the subgroup phenotypic determinants are located within the external surface glycoprotein (gp70) of the virus. The purpose of the present study is to attempt to identify the cellular receptor of the horizontally transmitted subgroup A of FeLV, using recombinant envelope proteins as ligands.

A recombinant baculovirus vector containing the entire FeLV-A gp70 and some of the transmembrane p15(E) sequence was cotransfected with baculovirus DNA into SF9 insect cells. The resulting recombinant virus produced high yields of the recombinant protein (Bgp70) which was concentrated by diafiltration.

Bgp70 has an approximate relative molecular mass of 70kD and exhibits several relevant biological properties. Monoclonal antibodies specific for FeLV gp70 bind to Bgp70, as do polyclonal immune cat sera. Bgp70 interferes with the infection by FeLV-A of a susceptible cell line (QN10). These data suggest that Bgp70 may be sufficiently similar to the wild type protein to serve as a ligand for the FeLV cellular receptor.

N 138 **Regulation of HIV1 Tropism for Macrophages** Lee Ratner and Peter Westervelt, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, 63110

HIV1 isolates differ in their tropism for primary human monocytes, in contrast to the permissiveness of primary lymphocytes for infection by most strains of HIV1. We have identified three distinct patterns for replication of HIV1 on primary monocytes, designated "productive," "silent," and "no infection" phenotypes. "Silent" infection is characterized by infection at levels that are not detectable by reverse transcriptase or p24 antigen assays; however, virus can be rescued from these infected monocytes by cocultivation with primary lymphocytes. To identify determinants for these infection phenotypes, we have constructed chimeric proviral clones between two full-length molecular clones which are incapable of infection of monocytes (NL4-3 and HXB2) and a monocyte-tropic clone (ADA). We have identified a 94 amino acid envelope domain including the V3 loop which converts the "no infection" phenotype of the HXB2 clone to "silent infec- tion." A single amino acid residue within this domain is at least partially responsible for this activity. Using sequences from three other viruses obtained from brain or spleen tissues of AIDS patients without in vitro cultivation, we have confirmed that an envelope domain including V3 sequences was critical for infection of primary human monocytes. Another region of envelope amino terminal to that containing the V3 loop modulates the level of replication of HIV1 in monocytes, and has been designated an "efficiency domain." We have also found that either functional vpr or vpu sequences can convert viruses capable of "silent infection" to those which can infect productively infect monocytes. Definition of the function of each of these genetic elements in vitro should contribute to a better understanding of in vivo phenomena such as disease latency and progression to AIDS.

N 139 ANALYSIS OF THE DOMAINS OF THE CD4 MOLECULE INVOLVED IN THE MULTI-STEP PROCESS OF HIV-1 ENTRY INTO CD4-POSITIVE CELLS, Dianne M. Rausch, Vaniambadi S. Kalyanaraman, Edward Berger, Jeffrey D. Lifson and Lee E. Eiden

Laboratory of Cell Biology, NIMH and Laboratory of Viral Diseases, NIAID, Bethesda, MD, 20892, Advanced Bioscience Laboratories, Inc., Kensington, MD 20895, and Genelabs, Incorporated, Redwood City, CA 94063

We have analyzed the role of the CDR3-like domain of CD4 in the entry of laboratory isolates of HIV-1 into a variety of CD4-positive cell lines, and the entry of primary/clinical isolates of HIV-1 into human peripheral blood mononuclear cells. This analysis has been carried out with anti-CD4 antibodies directed to the CDR3-like domain of the CD4 molecule, with synthetic peptides derived from this region (81-92) of the CD4 molecule, with soluble CD4, and with the CDR2-directed anti-CD4 antibodies anti-Leu3a and OKT4A.

The CDR3-like domain of the CD4 molecule appears to be critical in CD4/gp120 binding interactions that lead directly to release of gp120 from the gp120/gp41 complex. At the same time, the CDR3-like domain is clearly involved in the high-affinity binding of gp120 to CD4 initiating viral entry as well. A model in which processive binding of gp120 to the CDR2- and CDR3-like domains of the CD4 molecule is required for both virus binding and entry into CD4-positive cells will be presented, and used to explain the differential susceptibility of laboratory and primary/clinical isolates of HIV-1 to neutralization by soluble CD4 and CD4(81-92) peptides.

N 140 FUNCTION OF M1 PROTEIN OF INFLUENZA VIRUS IN VIRUS ASSEMBLY: ANALYSIS OF TS MUTANTS OF A/WSN/33 (H1N1) VIRUS. Osvaldo Rey and Debi P. Nayak, Department of Microbiology and Immunology, Jonsson Comprehensive Cancer Center UCLA School of Medicine Los Angeles, CA 90024-1747. The function of M1 protein in the biology of virus assembly was investigated by examining the phenotype of temperature sensitive mutants at the permissive (33^{o}C) and non permissive (39.5^{o}C) temperatures. At the nonpermissive temperature, ts mutants were found to be defective in virus replication. Mutants were more defective in MDBK cells compared to MDCK cells. A number of revertants have been isolated. We are currently using these mutants and isolated revertants to define the location of mutation(s) in the M1 protein responsible for the observed phenotype. A detailed analysis of the phenotype of the mutant viruses as well as the location of the mutation in the viral protein will be presented.

N 141 FUNCTIONAL INTERACTIONS BETWEEN CD4 AND CR2 (CD21): ROLE IN ENHANCED IN VITRO LENTIVIRUS INFECTIONS. W. Edward Robinson, Jr. and William M. Mitchell. Department of Pathology, Vanderbilt University, Nashville, TN 37232.

The complement receptor type 2 (CR2, CD21) has been demonstrated to be the receptor for Epstein-Barr Virus and the complement component C3d,g. CD4 is the primary receptor for the HIV-1 and SIV envelope glycoproteins as well as MHC-II. Recently, it has been demonstrated by several laboratories that cells bearing CD4 and CR2 can be infected by primate lentiviruses *in vitro*. Furthermore, in the presence of antibodies to HIV-1 and complement proteins, lentivirus infection can be enhanced in cells bearing both CD4 and CR2. Such enhanced infections lead to 10-100 fold increases in virus production as detected by tissue culture 50% infectious dose, RNA and protein synthesis, and reverse transcriptase release. The mechanism by which CD4 and CR2 combine to cause enhanced infections is examined herein.

Using human monoclonal antibodies, it is shown that both CD4 and CR2 are required for enhanced infections to occur. Using the lysosomal inhibitors quinine and ammonium chloride, it is demonstrated that differential routing of the virus to lysosomes with subsuquent uncoating of the lentivirus does not occur. The role of cyclic nucleotides is investigated by the use of phosphodiesterase inhibitors and cyclic nucleotide analogues. The role of protein kinase C and Ca++ will be reported using phorbol esters and calcium ionophore, respectively, and correlated with inositol triphosphate metabolism. The results of these studies in relation to pathogenesis of HIV infection and the potential importance to vaccine design and antiviral drug evaluation will be described. Supported in part by grant #AI29398 from the National Institutes of Health.

N 142 STUDIES ON HIV-1 ENTRY, H.L. Robinson, R. Fernandez-Larsson and S. Lu, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655

We have been using single cycle infections in T-cell lines and PBMCs to determine which steps in the HIV-1 life cycle determine differences in the permissiveness of infection. Each of the differences we have found has involved entry. 50% entry times have ranged from <20 minutes to 6 hours. Both the virus isolate and the cell type have affected entry. Viruses exhibiting the most rapid entry have been rapid/high patient isolates and the one exhibiting the slowest entry, a slow/low patient isolate. A lab strain, NL4-3, has intermediate entry characteristics. C8166 cells have been the most permissive for entry; PBMCs, intermediate in permissiveness and H9, A3.01 and Jurkat cells, the least permissive.

To learn which steps in entry are determining differences we have begun to screen various agents for the time course with which they block entry. The first of these studies has compared the time course with which NL4-3 escapes the ability to escape a "wash" block, block by a neutralizing antibody to the V3 loop (0.5ß), and block by an antibody to the gp120 binding domain on CD4 (leu3a). Unexpectedly, the escape from the 0.5ß block was more rapid than escape from the leu3a block. In C8166 infections 50% escape from the 0.5ß block occurred simultaneously with escape from the wash block (requiring 20 minutes) while escape from the leu3a block required 40 minutes. In H9 cells, escape from the wash block required about one hour, escape from 0.5ß, about 2 hours, and escape from leu3a about 3 hours. These studies demonstrate that anti-CD4 antibodies (such as leu3a) have a longer window of time in which they can block entry than anti-V3 loop antibodies.

N 143 ACTIVATION OF HIV FUSION BY CD4

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Infection of CD4+ cells by HIV is initiated by the high affinity binding of the outer envelope glycoprotein, gp120, to CD4. Although the regions of CD4 involved in gp120 binding have been precisely defined, the role of CD4 in events subsequent to virus binding which lead to virus entry are little understood. Using recombinant, soluble CD4 (sCD4) to model the interaction between CD4 and gp120 at the surface of virions and HIV-infected cells, we have shown that sCD4 binding induces allosteric changes in gp120, increased exposure of cryptic epitopes on gp41, and the dissociation of gp120 from gp41. Cyclised peptides synthesised from the sequence of the CDR-3-like loop of CD4 domain 1 mimic sCD4 in this activity, and block HIV infection, thus suggesting a role for the CDR-3 loop in HIV entry. Monoclonal antibodies binding to the third domain of CD4 inhibit HIV infection and syncytium formation but not virus binding, and prevent sCD4 induced conformational changes in the HIV envelope glycoproteins. Thus regions of CD4 outside domain 1 may also participate in HIV entry. We therefore conclude that CD4 is involved not only in virus binding, but is important for subsequent events leading to HIV induced membrane fusion.

N 144 ARYL-α-D-GALACTOSAMINIDES INHIBIT THE ABILITY OF ROTAVIRUS TO BIND TO AND INFECT SUSCEPTIBLE CELLS. Mary E. Schaefer and James H. Gilbert. Glycomed, Inc., Alameda, CA 94501.

We have examined the effect of aryl-α-D-N-acetyl-galactosaminides on the ability of rotavirus to infect susceptible tissue culture cells. MA104 cells were incubated with aryl-α-D-N-acetyl-galactosaminides until confluent monolayers formed. The infectivity of SA11 rotavirus on treated and untreated cells was measured by plaque assay. Treated cells showed markedly fewer plaques than untreated cells. The inhibition is concentration dependent, and requires the α-anomeric form of the N-acetyl-galactosamine. The aryl-α-anomers of glucose and N-acetyl-glucosamine are inactive, as are aryl-β-anomeric sugars. The ability of rotavirus to bind to these cells was measured using ^{35}S labelled SA11. Incubation of MA104 cells with aryl-α-D-N-acetyl-galactosaminides resulted in a significant decrease in the amount of bound virus, suggesting that the rotavirus receptor may be modified or present at lower levels. The general physiologic condition of cells treated with benzyl-α-D-N-acetyl-galactosaminide was studied by determining cell growth curves and the ability of treated cells to incorporate ^{3}H-threonine, ^{3}H-mannose, and ^{3}H-glucosamine. ^{3}H labeled benzyl-α-D-N-acetyl-galactosaminide was employed to determine if treated cells secrete oligosaccharides containing the benzyl-α-D-N-acetyl-galactosaminide into the medium, similar to mucin producing cells treated with aryl-α-D-N-acetyl-galactosaminides (Kuan, *et al*, Jour. Biol. Chem. **264**:19271-19277. 1989). Our results suggest that the rotavirus receptor on MA104 cells is glycosylated by a mechanism similar to mucin.

N 145 ASSOCIATION OF THE NSs PROTEIN OF UUKUNIEMI VIRUS WITH THE 40S RIBOSOMAL SUBUNIT, Jan Fredrik Simons, Robert Persson and Ralf F. Pettersson, Ludwig Institute for Cancer Research, Stockholm Branch, P.B. 60202, 10401 Stockholm, Sweden.

Uukuniemi virus, the prototype of the Uukuvirus genus within the Bunyaviridae family, has a tripartite negative strand RNA genome encoding four structural and one nonstructural protein. The L RNA segment encodes the RNA polymerase, the M RNA segment encodes the spike proteins G1 and G2 and the S RNA segment gives rise to the nucleocapsid protein N, and a nonstructural protein NSs, in an ambisense fashion.

The function of the NSs protein is unknown for all bunyaviruses. However, an involvement in the transcription or replication machinery has been proposed. An investigation of Punta Toro virus (Phlebovirus genus) suggested that the NSs protein is associated with the nucleocapsids in virions, and in Vero cells infected with Rift Valley Fever virus (RVFV; Phlebovirus) NSs showed a nuclear localization. Again, in plant cells infected with Tomato spotted wilt virus (TSWV; Tospovirus) NSs was either found dispersed in the cytoplasm or associated with cytoplasmic fibers. No NSs could be detected in purified TSWV particles.

To study the NSs protein of Uukuniemi virus (UUK), antiserum was raised against baculovirus expressed NSs. Using this antiserum, immunofluorescence of infected BHK cells showed a punctate pattern throughout the cytoplasm. This distribution was further confirmed by the absense of NSs in the nuclear fraction of metabolically labelled cells. Attempts to identify NSs in purified virus preparations were also performed but turned out negative. Interaction of NSs with other viral or cellular proteins was studied by subjecting infected cell lysates to sucrose gradient fractionation. No association with viral proteins was observed but instead NSs was found to cofractionate with the 40S ribosomal subunit. This cofractionation was confirmed with in vitro translated NSs and was shown to be RNase sensitive, indicating the true involvement of the ribosomal subunit. Subjecting the lysate to increasing ionic strength prior to fractionation showed that the interaction partly resists 1 M but not 1.5 M NaCl.

To further characterize the NSs protein of UUK we also studied whether it is phosphorylated, since the NSs protein of RVFV has been shown to be phosphorylated. However, for UUK no phosphorylation of NSs could be detected.

N 146 INTERACTION OF SIMIAN IMMUNODEFICIENCY VIRUS WITH CD4$^+$ AND CD4$^-$ CELLS: ENVELOPE FUSION, P27 ENTRY AND INFECTIVITY. Leonidas Stamatatos[1,3] and Nejat Düzgünes[2,3]. [1]Cancer Res. Inst. and [2]Dept. of Pharm. Chem., Univ. of California, San Francisco, CA 94143-0128; [3]Dept. of Microbiology, Univ. of the Pacific School of Dentistry, San Francisco, CA 94115.

We investigated the fusion and infectivity of SIV_{mac251} with H9, SupT1 and Raji cells. The fraction of CD4$^+$ cells was different for each cell line. The virus was grown in H9 cells, purified on sucrose gradients and labeled with Octadecyl Rhodamine (R-18). The R-18 labeled virus was incubated with the cells at 0 °C for 30 min, the temperature of the mixture was raised to 37 °C and aliquots were withdrawn at various intervals. Half of each aliquot was used to determine the extent of lipid mixing by measuring the R-18 dequenching. The other half was subjected to trypsinization to remove the bound but not fused virions from the cell surface. Following extensive washing, the trypsinized cells were lysed and the amount of SIV core protein (p27) delivered into the cytoplasm was evaluated using an antigen capture assay. Thus, we were able to correlate the extent of R-18 dequenching with the amount of SIV core proteins introduced into the cytoplasm. Although at 0 °C there was no fusion of SIV with cells (no R-18 dequenching and no p27 entry), at 37 °C, lipid mixing and p27 entry was observed both with CD4$^+$ and CD4$^-$ cells. Surprisingly, the extent of fusion did not correlate with CD4 expression on the cell surface. The virus fused almost as extensively with Raji cells (0% CD$^+$) as it did with H9 cells (55% CD4$^+$). Less fusion was observed with SupT1 cells (99% CD4$^+$) than with H9 cells. Examination of cell culture media for SIV production by the p27 capture assay revealed that H9 cells were productively infected, while Raji cells were not. For each cell line tested, the extent of fusion and the amount of internalized p27 increased with time of incubation. However, at any given time, the % of R-18 dequenching was equal to or two-fold higher than the % of p27 internalized. Only a slight increase in the extent of lipid mixing was observed with decreasing the pH of the medium. SIV_{mac}-cell fusion was inhibited by the presence of high concentrations of either plant lectins or recombinant soluble CD4. The fusion of SIV with cells was inhibited by 50% at 10 μg/ml or higher rsCD4. Trypsinization of the cell surface resulted in a 35-40% reduction in the extent of R-18 dequenching. We conclude that SIV-cell fusion can proceed by a CD4-independent mechanism, and does not necessarily lead to productive infection in established lymphoid cell lines.

N 147 INHIBITION OF HIV REPLICATION BY AJOENE: POSSIBLE INTERFERENCE WITH THE CD4--INDEPENDENT PATHWAY OF HIV ENTRY, Alexander V. Tatarintsev, Peter V. Vrzheshch, Andrey A. Schegolev, Dmitriy E. Yershov, Edward V. Karamov, Galina V. Kornilayeva, Tatyana V. Makarova, Nikolay A. Fedorov and Ali S. Turgiev, Institute of Physical and Chemical Biology, Moscow State University, Moscow 119899, USSR.

Ajoene has previously been shown to inactivate the platelet integrin, GP IIb/IIIa (Apitz-Castro R. et al. BBRC, 1986, 141:145). Structural similarity of integrins led us to propose that ajoene may inhibit integrin-mediated processes in HIV-infected cells. Ajoene inhibited fusion of intact H9 cells with H9:RF cells (IC50 ~45 nM; 16 hrs of incubation). Replication of HIV-1/H9 RF in H9 cells was inhibited with an IC50 of 25 uM (m.o.i. 0.1; 72 hrs). Assessement of HIV-1/CEM/Lav 1 Bru replication in CEM13 cells revealed more pronounced activity (IC50 ~5 uM; m.o.i. 0.1; 72 hrs). A considerable increase in the latter became evident when the compound was administered in aliquots of 50 nM per 12 hrs of incubation (CEM13-HIV-1/CEM/Lav 1 Bru system; inhibition by 30%; total concentration 0.25 uM; m.o.i. 0.1; 72 hrs). The observed anti-viral activity of ajoene might be related to its interference with integrin-mediated endocytosis of HIV. Several lines of indirect evidence make this possibility worth consideration: i) both HIV entry and integration of proviral sequences have been reported to proceed in the absence of CD4-gp160 interactions and fusion events (Zucker-Franklin D. et al., Blood, 1990, 75:1920); ii) integrins may be internalized as receptor-ligand complexes; iii) integrins are capable of interacting with HIV proteins (Brake D. et al., J. Cell Biol., 1990, 111:1275). If our hypothesis is correct, ajoene would be expected to preclude HIV incorporation into CD4-negative cells and thereby reduce the amount of potential reservoires of infection.

N 148 CYTOKINE MODULATION OF EMC-D VIRUS BINDING TO PANCREATIC β-CELLS. David J. Topham and John E. Craighead, Department of Pathology, University of Vermont, Burlington, VT 05405

The binding of a virus to its specific receptor on the surface of the target cell is the first event which determines the tropism of that virus to the tissue. Immune mechanisms involving the secretion of cytokines have been implied as a component of determining the tropism and pathogenicity of various members of the Picornaviridae family of positive strand RNA viruses. Infection of susceptible strains of mice with the diabetogenic variant of Encephalomyocarditis virus, EMC-D, has been previously demonstrated to be regulated by cells within the immune system. Using radiolabeled EMC-D we have demonstrated that the binding of the virus to the surface of a pancreatic β-cell line, BTC3, can be modulated by culturing of the cells in either recombinant murine interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α). This increase in binding is accompanied by increased expression of immunoglobulin superfamily members Class I MHC and ICAM-1 antigens, on the cell surface.

N 149 DIFFERENT REGIONS OF THE *M. DUNNI* ECOTROPIC RECEPTOR FACILITATE ENTRY OF MOLONEY MURINE LEUKEMIA VIRUS AND RAUSCHER MURINE LEUKEMIA VIRUS.

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Mus dunni fibroblasts, a cell line derived from an Asian wild mouse, are resistant to infection by the ecotropic Moloney murine leukemia virus (MoMLV), but not by other members of the ecotropic virus family (*e.g.*, Rauscher MLV). Replication-defective virions which contain a MoMLV core and genome and an amphotropic MLV envelope infect *M. dunni* cells. Therefore, the block to MoMLV infection in *M. dunni* fibroblasts occurs at the level of the envelope-receptor interaction. An ecotropic receptor mRNA species of the appropriate size and relative abundance is present in *M. dunni* fibroblasts. This mRNA presumably encodes an ecotropic receptor that functions to facilitate RaMLV, but not MoMLV, infection of *M. dunni* cells. Pretreatment of *M. dunni* fibroblasts with tunicamycin, an inhibitor of *N*-linked glycosylation, reverses resistance to MoMLV infection. Cell-specific post-translational modification of the ecotropic receptor could account for the tunicamycin-induced reversal of *M. dunni* resistance to MoMLV infection. To determine if this was the case, we expressed the functional MoMLV receptor cDNA (cloned from NIH3T3 cells) in *M. dunni* cells. MoMLV infects *M. dunni* cells expressing this MoMLV receptor, thus cell-specific modification of the MoMLV receptor is not responsible for the loss of function of the *M. dunni* ecotropic receptor. The *M. dunni* ecotropic receptor has been cloned and sequenced. We are now examining whether the *M. dunni* ecotropic receptor can be used by RaMLV to gain entry into human cells, and which amino acid changes in the *M. dunni* ecotropic receptor are critical for MoMLV infection. We propose that the altered primary amino acid sequence of the *M. dunni* ecotropic receptor results in a conformational change which masks an epitope required for infection by MoMLV but not by other ecotropic viruses.

Structure and Function of Viral Glycoprotein and Viral Induced Cell Fusion

N 200 **MEMBRANE FUSION INDUCED BY THE HIV *env* GLYCOPROTEIN.** Robert Blumenthal[1], Hana Golding[2], and Dimiter S. Dimitrov[1], NCI[1], NIH, and CBER[2], FDA, Bethesda, MD, 20892.

Membrane fusion is an essential step in the infection of permissive cells with human immunodeficiency virus (HIV). Infected cells frequently fuse with each other, and then progress to form multinucleated giant cells (syncytia). To gain insight into mechanisms of HIV*env*-mediated membrane fusion, we developed a new assay for studying the initial events. The assay is based on the redistribution of fluorescent markers between membranes and cytoplasm of adjacent cells examined by means of fluorescence video microscopy. Membrane fusion between HIV-1 envelope glycoprotein (gp120/41) expressing effector cells and $CD4^+$ target cells was observed 15-20 min after the association of cells, whereas the first syncytia only became apparent after 60-90 min. Moreover, membrane fusion events were observed under conditions where no syncytia were detected. In order to determine whether quantitative differences in receptor expression might influence the extent of membrane fusion, we used laboratory selected variants of CEM cells that differ in their expression of CD4. We found that CD4 is required on the target membrane for HIV*env*-mediated membrane fusion, but its extent is only partially dependent on CD4 surface concentration. The ability of those CEM variants to take part in HIV*env*-mediated membrane fusion did not correlate with their capacity to form syncytia. To examine the role of cell surface adhesion molecules in cell membrane fusion and syncytia formation, we performed syncytia and fluorescent dye redistribution assays using HIV-1_{env} expressed in EBV transformed lines from two leukocyte adhesion deficiency patients, whose cells do not express the surface adhesion molecule LFA-1. Experiments with $CD4^+$ lines as targets cells which express either normal or low levels of LFA-1 on their surface indicate that LFA-1 was required for syncytia formation but played no role in membrane fusion. These findings indicate that additional steps are needed to form syncytia after membrane fusion.

N 201 DIFFERENTIAL INFECTABILITY OF TWO HUMAN T-CELL LINES BY HIV-1: ROLE OF EARLY EVENTS IN THE VIRAL REPLICATION CYCLE, Robert W. Buckheit, Jr., Retrovirus Research Section, Southern Research Institute, Birmingham, AL 35255

The replication of HIV-1 in human T cells requires a complex interaction of viral and host factors. We have been examining differences in the ability of human cell lines to support productive HIV-1 infection to aid in identifying the viral and cellular factors required for efficient infection. Two CEM cell lines have been identified which are highly divergent in their ability to be infected by HIV-1. Both of the cell lines express similar levels of cell surface CD4 and are able to bind comparable levels of infectious virus. Upon infection with free virus, CEM-SS cells exhibit massive syncytium formation, produce high levels of infectious virus, and are rapidly killed within 7 days of infection. CEM-CCRF cells infected with the same quantity of infectious virus slowly become 100% infected over the course of 20-30 days, exhibit slight syncytium formation, and a transient decrease in cell viability. These cells become chronically infected with HIV-1 and produce high levels of infectious virus. Initiation of acute infection of CEM-CCRF cells with chronically infected HIV-1 producing CEM cells is also inefficient, requiring 100 to 1000 fold greater numbers of infected cells than is observed for CEM-SS cells. Utilizing single step infections of these cells with HIV-1 we have determined that the inefficient infectivity of HIV-1 in CEM-CCRF cells occurs following binding of the virions to the cell and prior to virus expression. We have also determined that CEM-CCRF cells inefficiently form syncytia when cocultivated with chronically infected CEM-SS cells, suggesting the fusion process may be involved in inefficient infection of CEM-CCRF cells. We will present the results of our ongoing efforts to identify the viral and cellular factors responsible for differential infectivity of human cells.

N 202 HEMAGGLUTININ PROTEINS FROM MEASLES VIRUSES THAT CAUSED LETHAL HUMAN DISEASES ARE DEFECTIVE IN MATURATION, TRANSPORT, OR FUSION-HELPER FUNCTION, R. Cattaneo*, P. Spielhofer#, M.A. Billeter# and J.K. Rose* **Departments of Pathology and Cell Biology, Yale University, USA, and #Institute for Molecular Biology I, University of Zurich, Switzerland*

We used the vaccinia virus-T7 RNA polymerase system to express measles virus (MV) hemagglutinin (H) cDNAs in Hela cells. MV cDNAs were obtained from brains of children who died of subacute sclerosing panencephalitis (SSPE, cases A and B), measles inclusion body encephalitis (case C), from a SSPE cell line (I), and from the MV vaccine strain Edmonston. The intracellular transport of the five H proteins was analysed by immuno-fluorescence, immunoprecipitation of total, surface, or internal protein, and pulse-chase labelling followed by endoglycosidase H or neuraminidase digestion. The H proteins of cell line I, characterized by a cluster of biased mutations resulting in many amino acid changes, of case A, in which two potential glycosylation sites are mutated, and of case B did not efficiently form disulfide-linked dimers, and were not transported efficiently at the cell surface. The H proteins of case C and of the Edmonston strain dimerized efficiently, and endoglycosidase H resistant forms were detected at the cell surface. However, only the H protein of the Edmonston strain could complement MV fusion protein and induce extensive syncitia formation after cotransfection of the corresponding plasmids in Hela cells. Thus all four H proteins from persistent MVs examined here showed defects in maturation, transport, or fusion-helper function. Three of the corresponding fusion proteins have a truncated intracellular domain, whereas one of the corresponding matrix proteins is unstable, and another matrix gene has undergone biased hypermutation. We conclude that defects in all three envelope proteins characterize persistent MV infections.

N 203 CLONING AND STRUCTURAL ANALYSIS OF THE GLYCOPROTEIN GENE OF ATTENUATED LEP-FLURY STRAIN OF RABIES VIRUS. S.Chandrasekhar, Syed, E Hasnian,P.S.Khandekar, G.P.Talwar, National Institute Of Immunology, New Delhi 110067, India.

The gene encoding the envelope glycoprotein (G) of attenuated LEP-Flury rabies virus strain was identified in a cDNA library made from infected mouse brain tissue. The gene consisted of 2042 nucleotides with an open reading frame encoding 525 amino acids, located form nucleotides 28 to 1602. The gene lacked the first stretch of poly A sequence following the coding region as was found in the genome of PV and SAD B19 strain of rabies viruses. However, the 3' non-coding region was homologous to that of HEP strain and the pseudogene as termed in other sequences. This observation and the change in the putative start site suggest that the LEP strain does not contain pseudogene instead has a long 3' noncoding region. The protein had a homology of 99.8% with HEP strain and 89-91% with the other published sequences of rabies virus. The amino acid sequences showed substitutions at positions from 180-210 representing the antigenic site II of rabies virus. These substitutions have been found to alter the secondary structure of the glycoprotein molecule and appears to be unique for Flury strain of the virus. Arginine at position 333 of antigenic site III and known as a marker for virulence, was found in the LEP sequence as compared to glutamine in the HEP strain. These alterations in the structure and amino acid substitutions offer explanation for the difference in virulence between Flury and other strains and also for the level of attenuation between LEP and HEP strains.

N 204 INHIBITION OF VIRUS-INDUCED CELL FUSION BY ANTIVIRALS, S. Chatterjee, R.J. Whitley, R.W. Compans and E.R. Kern, Departments of Microbiology and Pediatrics, University of Alabama at Birmingham, Birmingham, AL 35294

The purpose of these studies was to investigate the effect of various antiviral agents on virus-induced multinucleate cell (syncytium) formation. Syncytium formation induced by animal viruses is considered to be a primary mode by which the virus can be transmitted from cell-to-cell. Antiviral agents such as (S)-1-[(3-hydroxy-2-phosphonyl methoxy) propyl] cytosine (HPMPC) and acyclovir (ACV) significantly blocked cell fusion induced by herpes simplex virus type 1 (HSV-1) in human fibroblast and African green monkey kidney cells by inhibiting DNA and subsequent protein syntheses. Thus, the block appeared to be early in the viral replication cycle and not, therefore, directly on cell membranes or the glycoproteins present on cell membranes. Antivirals such as human alpha and beta interferons (IFNs) or an extract of culture medium of Lentinus edodes mycelia (JLS-S001), also significantly reduced the HSV-1-induced cell fusion in human fibroblast and monkey kidney cells. However, these drugs had no significant effect on DNA synthesis or expression of the major nucleocapsid proteins inside the treated cells. Thus, the block appeared to be late in the virus morphogenesis. In another system (i.e. in the absence of complete virus particles), we found that both IFNs (alpha and beta) or JLS-S001 blocked cell fusion induced by human immunodeficiency virus type 1 envelope glycoproteins expressed from a vaccinia virus recombinant. Although pretreatment of the target cells with these drugs was required for induction of a fusion-resistant state, a direct effect of these agents on the cell membrane cannot be excluded. These results suggest that the inhibition of virus-induced cell fusion (either direct or indirect) may inhibit cell-to-cell spread of virus infection in vivo.

N 205 HIV-1 glycoprotein precursor retains a CD4-p56^{lck} complex in the endoplasmic reticulum, Bruce Crise and John K. Rose *Departments of Pathology and Cell Biology, Yale School of Medicine, New Haven, CT*

The cell surface glycoprotein, CD4, is the receptor for human immunodeficiency virus (HIV) in T lymphocytes. Following HIV infection there is reduced expression of CD4 on the cell surface and this downregulation probably results at least in part from the formation of complexes containing HIV-1 glycoprotein precursor (gp160) and CD4 that are not transported from the endoplasmic reticulum (ER). At the plasma membrane of T-cells, CD4 is tightly associated with a cytoplasmic tyrosine kinase (p56^{lck}) that is involved in T-cell activation. Here we show that newly synthesized CD4 associates with p56lck before CD4 is transported from the ER. In the presence of HIV-1 gp160, the complex of gp160-CD4 and p56lck is retained in the ER. Such mislocalizaton of a tyrosine kinase to the cytoplasmic face of the ER may play a role in HIV-induced T lymphocyte killing.

N 206 THE STRUCTURAL PROTEINS OF EQUINE ARTERITIS VIRUS, Antoine A.F. de Vries, Ewan D. Chirnside, Marian C. Horzinek and Peter J.M. Rottier, Department of Virology, University of Utrecht, Yalelaan 1, 3584 CL Utrecht, The Netherlands

Equine arteritis virus (EAV) contains at least four structural proteins: a phosphorylated core protein of 12 kDa, a non-glycosylated membrane protein of 17 kDa, an envelope glycoprotein of 26 kDa and one or two heterogeneously migrating envelope glycoproteins in the size range of 28 - 40 kDa. Seven open reading frames (ORFs) have been identified by sequence analysis of cDNA clones covering the entire viral genomic RNA. On the basis of amino acid content, calculated molar mass, hydrophobicity profiles and number of predicted N-glycosylation sites, ORF 7, 6, 5 and 2 were expected to encode viral structural proteins. For this reason these open reading frames were expressed in vitro and in vivo using prokaryotic expression vectors and the vaccinia virus expression system. A polyclonal anti-virion serum which strongly recognized the expression products of ORF 7, 6 and 5 was produced in rabbits. Concurrently, monospecific antisera directed against the translation products of ORF 7, 6 and 2 were raised using bacterial fusion proteins and synthetic peptides. With these tools we proved ORF 7 to encode the nucleocapsid protein, ORF 6 to represent the unglycosylated membrane protein and ORF 5 to encode the heterogeneously glycosylated membrane protein. Subsequently, the antisera were used to study in greater detail the properties of the viral structural proteins in cells infected with EAV or with virus vectors expressing EAV genes. Experimental data regarding the post-translational modifications, the intracellular routing and the multimerization of the viral structural proteins will be presented.

N 207 CALCIUM CHELATION ALTERS THE CONFORMATION OF RECOMBINANT HSV-1-EXPRESSED ROTAVIRUS VP7. Philip R. Dormitzer and Harry B. Greenberg, Program in Cancer Biology and Division of Gastroenterology, Stanford University Medical Center, Stanford, CA 94301

Although rotavirus glycoprotein VP7 expressed by recombinant HSV-1 lacks several neutralizing epitopes, it bears epitopes recognized by non-neutralizing monoclonal antibodies (mAbs) and by a neutralizing mAb, M159. A full set of neutralizing epitopes is conferred on HSV-1-expressed VP7 by incorporation into rotavirus virions in cells coinfected with VP7-expressing recombinant HSV-1 and a heterologous rotavirus. Calcium chelation by EGTA eliminates the M159 epitope on HSV-1- or rotavirus-expressed VP7, indicating that calcium chelation alters the conformation of VP7. Because this conformational change occurs in the absence of other rotavirus proteins, it is not caused by loss of VP7 from the rotavirus outer capsid during EGTA-induced viral uncoating. Calcium chelation has no effect on the non-neutralizing epitopes examined on HSV-1-expressed VP7. The chelation-induced conformational change can be reversed by the addition of excess calcium after EGTA treatment. This restoration of conformation by calcium addition is more efficient in the presence of other rotavirus proteins, indicating that VP7 folding is facilitated by other rotavirus proteins. While VP7 in recombinant HSV-1-infected cell lysates is completely eluted from bound M159 by calcium chelation, a fraction of the VP7 in rotavirus-infected cell lysates is protected from elution. This finding indicates that M159 binding can prevent some VP7 coexpressed with other rotavirus proteins from undergoing a conformational change in response to EGTA treatment. These results have implications for the mechanisms of rotavirus assembly, uncoating, and neutralization by antibody.

N 208 THE TM SUBUNIT OF RSV ENVELOPE GLYCOPROTEIN CAN DIRECT OLIGOMER FORMATION IN THE ABSENCE OF SU, David A. Einfeld and Eric Hunter, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

The envelope glycoprotein of Rous sarcoma virus (RSV) forms an oligomer prior to cleavage of the precursor molecule to the SU and TM proteins. When the mature oligomer is treated with reducing agents, monomeric SU is released from the complex while the TM proteins remain in an oligomer. The role of the TM protein in the oligomeric structure has been examined by expression of TM as a separate protein. Sedimentation gradient analyses indicate that TM is capable of efficiently oligomerizing in the absence of SU. The oligomer is similar to the TM oligomer isolated from wild type virus in both size and resistance to reducing agents. In contrast, the SU subunit expressed independently does not form detectable oligomers although the protein is efficiently secreted from cells. Analysis of carboxy-terminal truncations of the Env protein indicate that the 129 NH_2-terminal amino acids of TM are sufficient for oligomer formation. Truncation after amino acid 105 of TM, however, has a negative effect on oligomer formation and intracellular transport.

The intracellular fate of the independently expressed TM protein has been examined. Although TM oligomerizes more rapidly than the full-length Env protein, acquisition of endoglycosidaseH resistance is not accelerated, indicating that oligomerization is not rate-limiting for export from the ER. Intracellular transport of TM has been detected by immunofluorescence and by secretion of a non-membrane anchored form of the protein. Expression of TM may be useful for further structural characterization of the envelope complex.

N 209 ANALYSIS OF COMPLEMENT COMPONENT C3B BINDING REGIONS ON HERPES SIMPLEX VIRUS GLYCOPROTEIN C. Roselyn J. Eisenberg, Shan-Ling Hung, Cynthia Seidel-Dugan, Sudha Srinivasan, Harvey M. Friedman and Gary H. Cohen. University of Pennsylvania, Philadelphia PA 19104.

Herpes simplex viruses (HSVs) encode at least ten distinct glycoproteins, which are found on the virion envelope as well as on the surface of infected cells. Several have functions that may modulate the host immune response, and hence are important for HSV pathogenesis. Glycoprotein C (gC) acts as a receptor for the C3b fragment of the third component of complement. Viruses containing gC are more resistant to complement mediated neutralization and lysis, and purified gC has been shown to interfere with at least two steps of the complement pathway. Although gC is not required for infection in cell culture, it is usually present in clinical isolates, suggesting that this activity might play a role in vivo. Furthermore, gC, though non-essential for virus replication in cultured cells, is important for attachment and there is evidence that it interacts with a specific cell receptor. Our goal is to define the regions on gC which are important for its biological activities. The strategy is to use site-directed mutagenesis to map the domains on gC which are necessary for its functions as a C3b receptor and in virus attachment. We constructed a large panel of mutants using the cloned gC gene. In transient transfection assays, most of the mutant proteins were transported to the mammalian cell surface and reacted with one or more monoclonal antibodies (MAbs) to discontinuous epitopes. Linker-insertion mutagenesis identified four regions of gC which are essential for C3b binding. Region III shares some similarities with the short consensus repeat (SCR) found in CR1, the human complement receptor. However, our results do not support the concept of a structural relationship between the SCR of CR1 and gC, as mutations of putative conserved residues, including several cysteines found in region III, had no effect on C3b binding. Several deletion mutants were still able to fold properly, were transported and were able to bind C3b. These studies show that the four C3b binding domains of gC are located within the central portion of the protein and partially overlap antigenic sites. Thus, these findings have important implications for gC structure. Several deletion mutations have been recombined back into the virus genome in order to map the parts of gC that are involved in virus attachment and in interference with the complement pathway.

N 210 Genetic Analysis of the Cytoplasmic Domain of Herpes Simplex Virus Type 1 Glycoprotein B (gB-1): Identification of a Region Affecting Both Virus Penetration and Cell Fusion. P.J. Gage, B. Sauer, M. Levine and J.C. Glorioso. University of Michigan Medical School, Ann Arbor, MI; Dupont-Merck Pharmaceutical Co., Wilmington, DE; and University of Pittsbrugh Medical Center, Pittsburgh, PA 15261.

Using two genetic approaches, we demonstrate that: i) a *syn* mutant gB cytoplasmic domain, expressed as a fusion protein with glycoprotein C (gC), is not sufficient to induce cell fusion (syncytia or *syn*) and ii) two discrete regions within the gB cytoplasmic domain influence cell fusion and one co-localizes with a determinant which influences virus penetration. Viruses which express protein chimeras consisting of the extracellular and transmembrane domains of gC fused to the cytoplasmic domain of either a wild-type or *syn* mutant gB cytoplasmic domain were constructed. Although both chimeras were expressed at high levels, neither was able to induce *syn*. Eight new and three previously described gB *syn* mutations identified two discrete primary structures within the cytoplasmic domain which play a role in cell fusion. *Syn* region I extends from residues R796 through E816, while *syn* region II extends at least from residues A855 through R858 and may begin as early as A843 and extend as far as R870. A minimum of four *syn* mutations involve loss of charge, suggesting that these regions participate in protein-protein interactions which are important modulators of cell fusion. We also describe a rate of entry mutation at residue A851, which is near or within *syn* region II. This is the first example of molecular determinants affecting virus penetration and cell fusion sharing a common region on a single viral protein.

N 211 CLASSIFICATION AND COMPARATIVE ANALYSIS OF FUSION PEPTIDE SUBFAMILIES WITHIN THE RETROVIRUS FAMILY, William R. Gallaher*, Angela M. Martin* and Eric Hunter+, *Department of Microbiology, Immunology and Parasitology, LSU Medical Center, New Orleans, LA 70112 and +Department of Microbiology, University of Alabama at Birmingham Medical Center, Birmingham AL 35294

The surface glycoproteins of retroviruses consist of heterodimers of a surface (SU) protein primarily responsible for binding to susceptible host cells, and a transmembrane (TM) protein associated with virion-cell and cell-cell fusion. Their biology is highly similar to that of influenza and paramyxoviruses, in that these proteins are derived from a precursor protein which is cleaved by a cellular enzyme to expose the hydrophobic amino-terminus of TM implicated as the "fusion peptide". That fusion by these virus families occurs by a similar mechanism is suggested bysequence similarity among disparate viral agents which cannot be explained merely on the basis of maintaining hydrophobicity.

We have compared the sequences of fusion peptides from the myxo-, paramyxo-, and retrovirus families and found that nearly all fall into two fusion peptide subfamilies. One pattern, dominated by an aromatic amino acid and typified by the Phe-X-Gly tripeptide, is found in the myxo- and paramyxovirus families of both primates and other animals, as well as in retroviruses of human/primate origin. The second pattern can be discerned by graphically arranging the fusion peptide sequence as a beta sheet. One side of the pleated sheet shows a symmetrical arrangement of aliphatic amino acids over a linear sequence of 5-9 amino acids. When so arranged, the beta symmetrical array is more similar among disparate viruses from the same species than the overall sequence. For the very distantly related feline retroviruses, the lentivirus feline immunodeficiency virus (FIV) and the oncovirus feline leukemia virus (FeLV), this array is identical. One of these two patterns can be observed over 25 disparate viruses from the three virus families. A few viruses, such as influenza and Mason-Pfizer monkjey virus (MPMV) exhibit both motifs.

We have begun to examine the sequence specificity of the apparent functional motif by mutating the *env* gene of HIV . Conservative changes were introduced, effectively creating chimeric sequences from among the natural fusion peptide variants, and effects on cell fusion were assayed in a transient assay on $CD4^+$-HeLa cells. Even limited sequence changes significantly affect fusion peptide biology, e.g. changing of Phe518 in HIV-1/HXB to Trp or Leu, while retaining some function, dramatically affect the level of fusion while retaining some function.

N 212 Similarities of viral proteins to toxins that interact with monovalent cation channels: Molecular mimicry and a possible role in cytopathogenesis. Robert F. Garry, Gebhard Koch*, David Axelos*, Jens J. Kort*,†, Friedrich Koch-Nolte**, and Johannes Koch§, Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112, *Departments of Molecular Biology and **Immunology and Allergy, University of Hamburg, Hamburg, Germany, †University of Maryland Cancer Center, Baltimore, MD 21201, §Department of Gastroenterology, University of California, San Francisco School of Medicine, San Francisco, CA 94143.

Alteration of transmembrane monovalent cation gradients appears to be involved in cytopathogenesis by a variety of viruses, but the molecular mechanisms by which lytic viruses modulate plasma membrane-mediated ion transport have not yet been elucidated. Recent studies have identified sequences in several viral proteins that have similarities to sequences in toxins that interact with monovalent cation channels. For example, both picornavirus structural protein VP1 and HIV transmembrane glycoprotein TM contain sequences that mimic sequences found in the active sites of scorpions toxins, small neuroactive proteins that interact with monovalent cation channels. In the case of HIV TM, viruses with site-directed mutations in this sequence are replication-competent, but deficient in inducing cytopathology. Synthetic peptides with the sequences of the toxin-like segments of poliovirus VP1 or HIV TM induced changes in the transmembrane fluxes of both sodium and potassium in cultured cells within seconds of addition to the medium as measured by spectrofluorometric techniques. Measurement of the activities of monovalent cation transport systems using isotopic tracers and specific inhibitors indicated that the alterations induced by the viral peptides were functionally similar to those induced by either scorpion toxin or a synthetic peptide corresponding to the active site of this neurotoxin. However, the alterations in monovalent cation fluxes induced by the poliovirus- and HIV-related peptides occurred more rapidly and were of significantly greater magnitude than the changes induced by scorpion toxin or its active site peptide. These results suggest that early interactions of poliovirus VP1 or HIV TM with plasma membrane monovalent cation channels may be involved in cytopathogenesis, particularly in the nervous system.

N 213 THE prM-E INTERACTION IN FLAVIVIRUSES: IMPLICATIONS FOR VIRUS ENTRY AND RELEASE, Franz X. Heinz, Gudrun Auer, Farshad Guirakhoo, and Christian Kunz, Institute of Virology, University of Vienna, Austria.

There is evidence that flaviviruses enter cells by receptor-mediated endocytosis, maturate at intracellular membranes and are released by exocytosis. Mature virions contain three structural proteins: Capsid (C), envelope (E), and membrane (M). The M protein is derived from a glycosylated precursor protein (prM) by the action of a cellular (Golgi or post Golgi) protease. Using Tick-borne encephalitis (TBE) virus as a model we are investigating the structural and functional role of prM as a constituent of immature virions which can be prepared by allowing the virus to replicate in the presence of NH_4Cl. These particles exhibit a significantly reduced specific infectivity and HA activity. Monoclonal antibody binding studies indicate that exposure to a pH below 6.4 causes an irreversible conformational change of protein E in mature virions which is associated with the loss of HA activtiy. In the presence of membranes however fusion occurs. No such acidic-pH-dependent structural changes nor fusion activity can be demonstrated with immature, prM containing virions. As revealed by crosslinking experiments and sedimentation analyses prM and E exist as a heterodimer which is apparently not fusion-competent. Thus the functional role of prM may be the prevention of E-protein-inactivation during its passage through acidic post-Golgi vesicles.

N 214 MUTATIONAL ANALYSIS OF THE HIV-1 PRECURSOR ENVELOPE GLYCOPROTEIN: ROLE OF THE CYTOPLASMIC DOMAIN IN INTRACELLULAR PROTEIN TRANSPORT. M. Abdul Jabbar and Martin J. Vincent, Department of Molecular Biology/Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195.

We investigated the role of the cytoplasmic domain of the HIV-1 envelope glycoprotein in intracellular transport, and sorting. Progressive deletions of the cytoplasmic domain were generated using polymerase chain reaction (PCR) techniques, and the deletion mutants were expressed in mammalian cells under control of the T7 promoter in a transient expression system that employs recombinant vaccinia virus expressing T7 RNA polymerase. Both biochemical and immunohistochemical analyses of the mutants were performed to monitor intracellular transport. The mutant that was deleted of 20 amino acids from the carboxy-terminus of the envelope glycoprotein (Env-tyrΔ20) appeared to be localized predominantly in the endoplasmic reticulum whereas the other deletion mutants, Env-tyrΔ55, Env-tyrΔ62, and Env-tyrΔ89 underwent complex sugar oligosaccharide modifications indicative of the protein movement through the Golgi apparatus. These data suggest that a structural motif in the cytoplasmic domain of the envelope glycoprotein might play an important role in the movement of the protein in the intracellular membrane compartment.

N 215 CHARACTERIZATION OF THE gB GLYCOPROTEIN OF INFECTIOUS LARYNGOTRACHEITIS VIRUS, Calvin L. Keeler, Jr. and David J. Poulsen, Department of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717

Infectious laryngotracheitis is an acute upper respiratory disease of world wide economic importance to the poultry industry. Its etiological agent is the alphaherpesvirus infectious laryngotracheitis virus (ILTV). As an initial step in characterizing the envelope glycoproteins of ILTV, the glycoprotein B (gB) gene has been cloned and sequenced (Poulsen et al., Virus Genes, 5:335-347, 1991). The four glycosylation sites and all 10 cysteine residues of the predicted translation product are conserved with HSV-1gB. This conservation of sequence and structure suggests that ILTV gB performs a function similar to other herpesvirus gB homologues. ILTV gB processing and transport are being studied with monoclonal and peptide antibodies to gB. Three glycosylated protein species of approximately 200, 100, and 80 kD are immunoprecipitated from ILTV infected chicken embryo liver cells with these antisera. Further structural and functional characterization of this, the first structural glycoprotein identified from ILTV, is in progress.

N 216 THEORETICAL AND FUNCTIONAL ANALYSIS OF THE SIV FUSION PEPTIDE.

Lambrecht B, Horth M, Lay Khim M, Bex F, Thiriart C[1,2], Ruysschaert J-M[2], Burny A and Brasseur R[2]. Laboratory of Biological Chemistry, Free University of Brussels, 1640 Rhode-St-Genèse, Belgium, [1]Department of Molecular and cellular biology, Smith-Kline-Beecham Biologicals, 1330 Rixensart, Belgium and [2]Laboratory of Macromolecules at Interfaces, Free University of Brussels, 1050 Brussels, Belgium.

The fusion domain of Simian Immunodeficiency Virus (SIV) envelope glycoproteins is a hydrophobic region located at the amino-terminal extremity of the transmembrane protein (gp32). Assuming an α helical structure for the SIV fusogenic domain of the gp32 in a lipid environment, theoretical studies have predicted that the fusion peptide would insert obliquely in the lipid bilayer. This oblique insertion could be an initial step of the fusion process by disorganizing locally the structure of the lipid bilayer. We have tested this hypothesis by selectively mutagenezing the SIV gp160 expressed via a vaccinia virus vector, to alter the theoretical angle of insertion of the fusion peptide. The fusogenic activity of the wild-type and mutant glycoproteins was tested after infection of T4 lymphocytic cell lines by the recombinant vaccinia virus. Mutations that modified the oblique orientation reduced the fusogenic activity. On contrast, mutations that conserve the oblique orientation did not alter the fusogenic properties. our results support the hypothesis that oblique orientation is important for fusogenic activity.

N 217 FUSION DOMAIN MUTANTS OF SEMLIKI FOREST VIRUS. Pnina Levy-Mintz and Margaret Kielian, Department of Cell Biology, Albert Einstein College of Medicine, Bronx NY 10461.

Membrane fusion is an obligatory step in the entry of enveloped viruses into their host cells. A highly conserved hydrophobic peptide is often found at or near the amino terminus of one of the viral envelope proteins, and is thought to mediate fusion between the viral and host cell membranes. The entry of Semliki Forest virus (SFV), an enveloped alphavirus, occurs in the early endosome, where the mildly acidic environment of pH < 6.5 activates membrane fusion. Fusion is mediated by the viral spike protein, a heterotrimer containing two transmembrane subunits, E1 and E2. The SFV spike protein contains a hydrophobic domain that is highly conserved among all alphaviruses and extends from residues 75 to 97 of the E1 subunit. Surprisingly, a homologous region is also found in the VP5 outer capsid protein of the non-enveloped rotaviruses. We have previously shown that single amino acid changes in the putative fusion peptide of SFV alter membrane fusion. Mutation of Asp75, Gly83, or Gly91 to Ala shifted the fusion pH threshold to a more acidic range (pH 5.9-5.3). A change of Gly91 to Asp completely blocked cell-cell fusion at all acidic pH values (pH 7.0-4.7), without affecting spike protein assembly or transport.

We set out to isolate other fusion block mutants by targetting residues that gave a pH shift phenotype. Wild type and mutant spike proteins were expressed in COS cells using an SV40 vector, and low pH-dependent cell-cell fusion was visualized and quantitated by immunoflourescence and nuclear staining. We now report two additional mutants with a more acidic threshold for fusion. An Asp75 to Glu, or an Asp75Glu, Lys79Arg double mutant showed a pH threshold of 5.5 and 5.3 respectively. Importantly, mutation of Gly83 to Val completely blocked fusion from pH 7.0-5.0. Thus, mutations at two different positions in the E1 hydrophobic domain can abolish spike protein fusion activity. Both Gly83 and Gly 91 are conserved between alpha and rotaviruses, suggesting that these residues may also play a role in rotavirus membrane penetration. We are currently defining the mechanism of these fusion defects by analysis of these mutations in an infectious clone of SFV.

N 218 INFLUENZA VIRUS-CELL FUSION: OBSERVATIONS OF PROTEIN AND LIPID MOVEMENTS USING LOW-LIGHT-LEVEL FLUORESCENT VIDEO MICROSCOPY R. Joel Lowy, Debi P. Sarkar, and Robert Blumenthal, Physiology Dept., Armed Forces Radiobiology Research Inst., Bethesda, MD 20889 and LMMB, National Cancer Inst., Bethesda, MD 20892

We have been developing methods to use intensified video fluorescent microscopy and digital image processing to observe and quantitate influenza virus (A/Japan or X:31) fusion processes, activated by low pH, using human red blood cell membranes (HRBC) as targets. Initial studies demonstrated the feasibility of using video methods to study viral fusion, including single particles (PNAS 87:1850, 1990), labeled with the lipid analog octadecylrhodamine (R18). The measured half-time of 46 s at pH 5.0 for R18 virus-to-cell transfer for single particles was much longer than would be predicted from 2-D or 3-D diffusion calculations. Video methods have also allowed observation of protein redistribution during fusion by monitoring changes in the pattern of fluorescence from live virus protein-FITC conjugates. Experiments comparing low pH (4.8) induction of fusion at 11 or 28 ^{o}C found that the lipid (R18) readily redistributed, but at a slower rate at 11 ^{o}C. These observations confirm reports that viral fusion can occur below 20 ^{o}C and provide a means of slowing fusion kinetics for more detailed examinations of single particle kinetics. The FITC-protein showed a different temperature dependent pattern, redistribution occurring at 28 ^{o}C, but with no detectable change over the same 30-40 min time course at low temperature. This unexpected observation is consistent with a temperature dependent decoupling of viral lipid and protein dispersion into the target HRBC membrane. Acridine orange (AO) staining (RNA) subsequent to low pH induction of fusion of multiple virions at low temperature results in a viral fusion dependent fluorescence which shows two patterns, a punctate one and dispersed volume filling one. The volume pattern is likely due to release of viral RNA throughout the HRBC. The RNA punctate pattern is suggestive that some RNA remains within viral envelopes or is attached to the HRBC membrane at the point of fusion. At elevated temperature only the volume AO pattern is observed. Combined, all of these results are consistent with the idea that additional persistent structural states of the virus-cell fusion complex exist, subsequent to fusion pore formation, and the mobility of molecules is partially restricted within these structures.

N 219 BINDING OF MATRIX PROTEIN OF VESICULAR STOMATITIS VIRUS TO MONOMERIC AND OLIGOMERIC FORMS OF THE ENVELOPE GLYCOPROTEIN. Douglas S. Lyles, Margie McKenzie, and J. Wallace Parce, Departments of Microbiology and Immunology and Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1064.

The envelope glycoprotein (G protein) of vesicular stomatitis virus, Indiana serotype (VSV-IND) can exist as trimers, dimers, or monomers of identical subunits, in the presence of the detergent octyl glucoside. In contrast, the G protein of VSV, New Jersey serotype (VSV-NJ) is monomeric in octyl glucoside. Fluorescent probes covalently attached to the G protein near the membrane anchor sequence are capable of detecting changes in the G protein oligomeric structure. Dilution of the VSV-IND G protein below approximately 5 μg/ml in octyl glucoside results in partial dissociation of G protein trimers that is reversible. Addition of purified M protein or nucleocapsid-M protein complexes to partially dissociated G protein resulted in rapid reassociation of G protein oligomers, while nucleocapsids lacking M protein had little effect. This indicates that binding of M protein enhances the stability of G protein oligomers. The binding of M protein to the G protein *in vitro* had the specificity, reversibility, and affinity necessary to account for virus assembly *in vivo*. M protein had no effect on the subunit interaction of the VSV-NJ G protein. Mixing the VSV-IND G protein with nucleocapsid-M protein complexes isolated from VSV-IND or VSV-NJ gave similar results in both homologous and heterologous combinations, indicating that the interaction between the G and M proteins was not serotype-specific. Kinetic analysis of subunit exchange indicated that the effect of M protein was mediated by binding to monomer subunits and enhancement of their association rate. Addition of excess monomeric G protein from VSV-NJ competitively inhibited the binding of M protein to the VSV-IND G protein. These data indicate that M protein can bind to both monomeric and oligomeric forms of the G protein and that M protein binding enhances the subunit interactions of the G protein. (Supported by NIH grants AI15892 and AI20778)

N 220 PRECISE DEFINITION OF THE ANTIGENIC DOMAIN 1 (AD-1) ON GLYCOPROTEIN GP58 OF HUMAN CYTOMEGALOVIRUS. M. Mach[1], B. Wagner[1], V.-A. Sundquist[2] and W. Britt[3]. [1]Institut für Klinische und Molekulare Virologie, Erlangen, FRG, [2]The National Bacteriological Laboratory S-105 21, Stockholm, Sweden, [3]Dept. of Pediatrics and Microbiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294

The envelope glycoprotein gp58/116 of human cytomegalovirus (HCMV) is capable of inducing neutralizing antibodies during natural infection. An immunodominant sequential antibody binding site (AD-1) has been located on gp58 between amino acids 589-645 (1). The site is capable of binding neutralizing as well as non-neutralizing antibodies in a competitive fashion. Using bacterially derived fusion proteins as well as synthetic peptides, we have further characterized the antibody binding site. A continuous sequence of more than seventy amino acids between residues 550 and 630 have been identified to be essential for binding of monoclonal antibodies as well as antibodies developed during natural infection in humans. The binding regions for neutralizing and non-neutralizing antibodies were found to be indistinguishable.

(1) Utz et al. (1989) J. Virol. 63, 1995-2001.

N 221 EXPRESSION OF THE MEMBRANE GLYCOPROTEINS G1 AND G2 OF UUKUNIEMI VIRUS FROM CLONED cDNAs, Lars Melin, Robert Persson, Masha Fridman, Ragna Rönnholm and Ralf F. Pettersson, Ludwig Institute for Cancer Research, Stockholm Branch, Stockholm, Sweden.

Uukuniemi virus, a member of the Bunyaviridae family, matures into the Golgi complex (GC) by a budding process. This site of maturation is probably determined by the accumulation of the two membrane glycoproteins G1 and G2 in this organelle. Previous work has indicated that G1 and G2 form heterodimers in the ER in virus-infected cells. G1 was found to fold rapidly (<10 min), while G2 forms its correct disulfide bonds very slowly (T1/2 about 45 min) (Persson and Pettersson, J.Cell.Biol. 112:257-266, 1991). During folding, G1 and G2 were associated with BiP. The results further showed that G2 cannot exit the ER unless dimerized to G1. Whether G1 can leave the ER alone was not analyzed and therefore remained unclear.

We have now expressed G1 and G2 in BHK21 cells together from the same mRNA, or separately from different mRNAs using the T7 RNA polymerase-driven vaccinia virus system. Proteins of the correct size were synthesized in expressing cells. When expressed together both G1 and G2 localized to the GC. Likewise, when G1 was expressed alone it also localized to the GC. In contrast, G2 remained in the ER when expressed alone. This is in conformity with the notion that G2 requires interaction with G1 in order to become transport competent. G1 apparently is able to attain a transport competent conformation in the absence of G2. With none of the constracts were the glycoproteins expressed on the cell surface.

Our aim is to identify a retention signal responsible for the Golgi-localization. To this end we are in the process of mutagenizing G1 to see if the Golgi-retention can be abolished, allowing the protein to be transported to the plasma membrane. The results of these experiments will be presented.

N 222 ROLE OF HERPESVIRUS (PSEUDORABIES VIRUS) GLYCOPROTEINS IN ADSORPTION, ENTRY AND CELL-CELL FUSION, Thomas C. Mettenleiter, Isabella Rauh, Axel Karger, Annegret Kopp and Barbara Klupp, Federal Research Center for Virus Diseases of Animals, D-7400 Tübingen, Germany

Herpesviruses, after initial adsorption, enter target cells by fusion of the viral envelope with the cellular cytoplasmic membrane. Virus spread then occurs either through repeated cycles of release of enveloped particles and re-entry or by direct cell-cell spread after virus induced fusion of infected cells with adjacent noninfected cells. These processes are mediated by viral glycoproteins. The alphaherpesvirus Pseudorabies Virus (PrV) encodes at least seven glycoproteins that all exhibit homologies to herpes simplex virus (HSV) glycoproteins. Three of them, gH, gp50 (gD) and gII (gB) are essential for viral replication. We isolated specific PrV mutants deficient in expression of several essential and non-essential glycoproteins. Analysis in vitro showed that virions devoid of the non-essential gIII (gC) are impaired in adsorption and exhibit a delayed penetration. Virions lacking gp50 also showed less efficient adsorption whereas virions lacking gI, gII or gp63 adsorbed normally. Virions devoid of gII or gp50 proved to be non-infectious due to a defect in penetration indicating that gII and gp50 are essential for viral entry. gII was essential also for cell-cell fusion whereas gp50 was dispensible for this process. Our results establish that (i) gIII and gp50 are involved in mediating adsorption, (ii) gII(PrV),as gB(HSV), is essential for both viral entry and cell-cell fusion, (iii) whereas gD(HSV) is also essential for both processes gp50(PrV) is dispensible for cell-cell fusion. We conclude that virus entry and direct cell-cell spread, although both involving membrane fusion events, are distinct processes that can be separated in the PrV system.

N 223 VIRUS NEUTRALIZATION EPITOPES ON THE EPSTEIN-BARR VIRUS ENVELOPE GLYCOPROTEIN VACCINE MOLECULE GP340 Andrew J. Morgan and Richard J. Pither, Department of Pathology & Microbiology, University of Bristol, U.K.

Epstein-Barr virus (EBV) is strongly associated with certain important human cancers including endemic Burkitt's lymphoma, lymphomas in immunodepressed individuals and in undifferentiated nasopharyngeal carcinoma which is a significant world health problem, there being more than 80,000 new cases reported each year. A vaccine has been developed based on the EBV envelope glycoprotein and receptor ligand gp340. This molecule induces virus-neutralizing antibodies, T cell responses and protective immunity in the cottontop tamarin which is susceptible to EBV-induced malignant lymphomas. Bacterial β-galactosidase fusion protein fragments of the gp340 gene along with synthetic peptides have been used to map important epitopes on the gp340 molecule. Naturally-occurring human sera containing anti gp340 antibodies were found to recognize particular regions of the molecule in Western blots expressed as fusion proteins. The pattern of recognition was the same with antibodies from healthy subjects or from subjects with EBV-associated disease. Antibodies specific for the major epitope-containing region were found not to be virus-neutralizing but bound to native gp340 on the surface of live cells. No reactivity was detected in ELISA against a series of overlapping synthetic peptides (15 mer) spanning the whole gene, suggesting that natural infection generates anti-gp340 antibodies which recognize only discontinuous or conformational epitopes or carbohydrate related epitopes.

EBV-neutralizing sera from rabbits immunized with gp340 in various immunogenic forms were examined for reactivity to these gp340-fusion protein fragments in Western blot assays. It was found that fragments from nearly all regions of the gp340-polypeptide chain were bound by such sera. The existence of linear epitopes within these areas was examined using a series of overlapping peptides in an ELISA. The only linear epitopes detected using this method were located within the amino terminal region of the protein between amino acids 236 and 327. A gp340-fusion protein containing amino acids 9 to 323 of the gp340 polypeptide chain was used to produce an affinity column to isolate antibody from two EBV-neutralizing sera. Affinity purified antibody bound both gp340 and gp340 synthetic peptides in ELISAs and was able to substantially reduce the infectivity of EBV *in vitro*. These results suggest that neutralizing epitopes are present within this region of gp340, which is also the virus receptor binding region, and that some of these epitopes may be linear in nature.

N 224MEMBRANE FUSION MEDIATED BY HUMAN PARAINFLUENZA TYPE 3: DIFFERENT REQUIREMENTS FOR VIRAL INFECTION AND CELL-CELL FUSION. Anne Moscona[1] and Richard Peluso[2], Departments of [1]Pediatrics and [2]Microbiology, Mt Sinai School of Medicine, NY, NY 10029.

Human parainfluenza virus type 3 (HPF3) establishes persistent infection in cell culture following high (>5 pfu/cell) multiplicity infection. The persistently infected (pi) cells do not fuse with each other, yet rapidly fuse when seeded with uninfected cells. We have shown that the failure of the pi cells to fuse with each other is due to a lack of a receptor on these cells for the viral HN glycoprotein, and established that both F and HN proteins are needed for cell fusion mediated by HPF3. We now use this information to investigate parameters of persistent infection by HPF3. Low multiplicity infection with HPF3 normally results in extensive cell fusion. However, syncytium formation can be prevented by the addition of neuraminidase to remove sialic acid, the receptor for the viral HN glycoprotein. While the infected cells do not fuse, the virus still spreads throughout the culture, and the cells become persistently infected. Defective-interfering (DI) particle genomes are not detected until at least seven passages of these cells, suggesting that DI particles are not required for establishment of persistent infection by HPF3. The finding that neuraminidase treatment of cells infected at a low moi allowed spread of the virus without cell fusion suggests that there are different sialic acid requirements for a virus to infect a cell (by fusing with the plasma membrane) than for fusion between cells. To investigate this, cells infected with low moi were treated with several different amounts of bacterial neuraminidase. With low level neuraminidase treatment, there is sufficient sialic acid for virus to infect a cell but not enough to allow cell fusion, while with higher level neuraminidase treatment there is insufficient sialic acid even for virus infection. Our interpretation of these data is that there are different neuraminic acid requirements for a virus particle to infect a cell by fusion of the viral envelope with the plasma membrane than for fusion of an infected cell with an uninfected cell.

N 225 MAPPING OF A FUNCTIONAL SITE ON HERPES SIMPLEX VIRUS GLYCOPROTEIN D. Martin I. Muggeridge, Hsien-Yuan Chiang, Gary H. Cohen and Roselyn J. Eisenberg, University of Pennsylvania, Philadelphia, PA 19104.

Herpes simplex virus glycoprotein D (gD) is essential for penetration of virions into the cell, but its precise role has not yet been defined. The observations that some anti-gD MAbs can neutralize virus even after it has adsorbed to the cell, and that viruses lacking gD have no defect in adsorption, indicate that gD does not function at this stage. Nevertheless, there is considerable evidence that gD interacts with a cellular receptor; presumably, this occurs after adsorption but before fusion of the virion envelope with the plasma membrane. We have used panels of deletion and linker-insertion mutants to map a functional site on gD. The assay for functional activity takes advantage of a gD-minus virus, F-gDβ, which replicates normally only in the gD-expressing cell line VD60; F-gDβ virions produced in Vero cells lack gD and are noninfectious. The defect can be complemented by prior transfection of Vero cells with plasmids expressing either wild-type or functional mutants of gD. We first constructed a series of deletion mutants, each lacking three residues, and found that aa222-254 are required for gD function. In agreement with this result, insertion of four residues at positions 235, 243 or 246, but not at 257, also abolished function. Furthermore, preliminary analysis of other mutants suggests that the functional site may be composed of two or three discontinuous elements, since activity is abolished by an insertion at position 43 or by deletion of aa277-310. Parallel studies on MAb binding indicate overlap between functional and antigenic sites. Antigenic site Ib of gD is a major target for virus-neutralizing antibodies and contains at least two discontinuous elements, one of which comprises aa222-230. This suggests that neutralization is due to blocking of the functional site.

N 226 STRUCTURAL FEATURES IN THE TRANSMEMBRANE SPANNING DOMAIN OF THE HIV ENVELOPE GLYCOPROTEIN REQUIRED FOR FUSION ACTIVITY, Randall J. Owens, Christine L. Burke, and John K. Rose, Departments of Cell Biology and Pathology, Yale University School of Medicine, New Haven, CT 06510.

We are studying the structural features important for the membrane fusion function of the human immunodeficiency virus (HIV) envelope glycoprotein. As with many viral fusion proteins, the HIV env protein is synthesized as a precursor polypeptide (gp160) that is proteolitically processed into two subunits (gp120 and gp41). The newly created amino terminus of gp41 contains a stretch of hydrophobic amino acids that is referred to as the "fusion peptide", and is required for membrane fusion activity. There is a second stretch of hydrophobic amino acids in gp41 that spans the viral envelope and serves to anchor the envelope glycoprotein complex on the surface of the virion. We made hybrid proteins between HIV env and vesicular stomatitis virus (VSV) G and found that certain constructs dramatically affected HIV env fusion activity. In particular, when we replaced the transmembrane anchor and cytoplasmic tail of the HIV env protein with that of VSV G, no fusion was observed in CD4+ HeLa cells, although transport and processing of this hybrid protein were normal. In contrast, fusion activity was unaffected in other hybrid constructs that retained the HIV env transmembrane domain. These results suggest that structural features of the HIV env transmembrane domain are required for fusion activity. We have also constructed several other HIV env transmembrane domain mutants (truncations, point mutations, deletions, and hybrids), and analyzed their fusion activity; these results will be presented in detail.

N 227 DOMAINS OF HERPES SIMPLEX VIRUS I GLYCOPROTEIN B THAT FUNCTION IN VIRUS PENETRATION, CELL-TO-CELL SPREAD, AND CELL FUSION, Lenore Pereira, David Navarro, and Pedro Paz, Division of Oral Biology, School of Dentistry, University of California San Francisco, San Francisco, CA 94143-0512

Herpes simplex virus 1 (HSV-1) glycoprotein B (gB) is one of ten glycoproteins in the virion envelope and in the membranes of infected cells. It is required for infection of cells in culture and functions in penetration of the cell by fusing the virion envelope with the plasma membrane. In studies to map the functional domains on HSV-1 gB, we reported that epitopes of potent neutralizing antibodies cluster in three major antigenic domains. We have now analyzed the function of these domains in virion infectivity by a detailed examination of the effects of 16 neutralizing antibodies. Ten antibodies with complement-independent neutralizing activity blocked penetration of virions into cells but not their adsorption to the cell surface. Treating cell-bound, neutralized virus with the fusogenic agent polyethylene glycol promoted their entry into cells. Ten antibodies with complement-dependent and -independent neutralizing activity interfered with plaque development by preventing spread of virus from infected to neighboring uninfected cells. Nine neutralizing antibodies, all complement-independent, prevented cell fusion induced by strain HFEM *syn*. We conclude that domains mapping in three regions of gB function in penetration of virions into cells, and that most neutralizing antibodies to these domains also block cell-to-cell spread of virus and cell fusion. The findings that three complement-independent neutralizing antibodies that blocked penetration did not inhibit plaque development, and that only one of these blocked cell fusion, indicate that the cell-to-cell spread of virus and cell fusion are related processes, but not identical to the penetration function.

N 228 ISOLATION OF SEMLIKI FOREST VIRUS MUTANTS WITH AN ALTERED STEROL REQUIREMENT FOR FUSION. Thomas E. Phalen and Margaret Kielian, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461.

The entry of viruses into their host cells is a key step in the virus infection pathway. The enveloped alphavirus, Semliki Forest virus (SFV), infects cells via endocytic entry followed by a membrane fusion reaction triggered by the acid pH present in intracellular vacuoles. We have previously shown that SFV fusion and infection are strictly dependent on the presence of cholesterol in the target membrane. Sterol is not required for virus-receptor binding, endocytosis, acidification, or virus replication. Since the sterol requirement is specific for the virus fusion step, it has enabled a selection scheme for generating virus mutants with altered sterol specificity. Our selection takes advantage of the fact that insect cells are cholesterol auxotrophs, and can be virtually completely depleted of cholesterol by growth in low density lipoprotein-deficient serum. SFV was mutagenized to 18% viability with N-methyl-N-nitro-N'-nitrosoguanidine, and grown for one cycle on normal BHK cells to permit expression of any mutant phenotypes. The mutant stock was then selected on a mosquito cell line that was depleted of cholesterol and enriched with 3β-chlorocholesterol. Repletion with steroid was accomplished by incubation of depleted cells with chlorocholesterol-BSA complexes for five hours. Selection on chlorocholesterol-repleted cells yielded a population of viruses that can replicate using this normally non-permissive sterol. Mutants were then isolated by limiting dilution on chlorocholesterol-repleted cells, and positives were identified by immunostaining. Five isolates have been shown to infect 3β-chlorocholesterol-enriched cells at ratios from 40-500 fold higher than wild type SFV. We propose naming these isolates srf mutants, for sterol requirement in fusion. We are now determining the sterol specificity for membrane fusion of the srf mutants. Ultimately, characterization of the amino acid changes that permit use of 3β-chlorocholesterol should provide important information on the spike protein site that interacts with cholesterol during fusion.

N 229 MAPPING THE EXPRESSION OF MURINE AND HUMAN RETROVIRUS ANTIGENS ON THE CELL SURFACE USING IMMUNOTOXINS AND FLOW CYTOMETRY, Seth H. Pincus, NIAID, Rocky Mountain Laboratories, Hamilton, MT 59840

The topology of retrovirus antigen expression on the surfaces of infected cell lines has been comparatively mapped using immunotoxins and flow cytometry. This is a biologic approach to studying the structure of retroviral proteins as they appear on the surface of viable cells.

The following antibodies were conjugated to ricin A-chain (RAC): six MAbs recognizing different epitopes on the HIV envelope, four MAbs binding to MuLV envelopes, two MAbs reacting with MuLV p30 Gag protein (a known superantigen), and a control antibody. Cell surface antigen expression was tested by flow cytometry. The efficacy of the immunotoxin was measured in tissue culture cell lines that were acutely or persistently infected with different HIV's and MuLV's and with mutant viruses.

The efficacy of the immunotoxins varied dramatically depending upon the epitope recognized. Activity of the immunotoxin was not always a direct function of the binding of the antibody to the surface of the infected cells. Interactions between the host cell and specific viruses resulted in differential susceptibility to the panel of immunotoxins that surprisingly was often inversely related to antigen expression on the cell surface.

Because immunotoxin efficacy requires internalization of the bound antibody and is related to the proximity of the epitope to the cell surface, comparative flow cytometry and immunotoxin efficacy data provide information regarding the dynamics of retrovirus envelope and core protein expression on the surface of infected cells. Our data demonstrate that there are both epitope-specific and virus-specific effects.

N 230 BIOSYNTHESIS OF HIV-1 ENVELOPE PROTEINS IN NORMAL AND NEOPLASTIC CELLS OF DIFFERENT LINEAGE: IMPLICATIONS FOR NEUTRALIZATION AND PATHOGENESIS,
Victoria R. Polonis, Robert R. Redfield, *Yiling Liu and *Suzanne Gartner, Walter Reed, Washington, D.C. and *Henry M. Jackson Foundation, Rockville, MD 20850
The HIV-1 envelope gp160 precursor protein is post-translationally processed by a host cell protease to produce the gp120 surface and gp41 transmembrane glycoproteins. This cleavage event is proposed to occur during the transport of gp160 to the cell surface; gp120 is present on the surface of virions as well as infected cells. We have investigated the processing of the HIV-1 envelope in specific cell lineages. H9 T cells acutely infected with HIV-1(MN, IIIB, or RF) cleave a significant amount of newly synthesized gp160 to produce cell associated gp120. In contrast, acutely infected CEMss T cells have a predominance of cell associated gp160, suggesting a difference in intracellular transport and processing of envelope. Moreover, differences were observed between ACH.2 and 8E5, two T cell clones derived from the same HIV-1 infected parental (A3.01) line. While ACH.2 cells cleave gp160 to produce cell associated gp120, a predominance of gp160 is found associated with 8E5 cells. This increased ratio of gp160:gp120 is also seen in U-1, a promonocytic cell line chronically infected with HIV-1. We have also investigated the biosynthesis of HIV-1 envelope in normal human T lymphocytes and monocyte-macrophages. While significant levels of cell associated gp160 and gp120 can be detected in T lymphocytes, only gp160 can be detected in macrophages. T lymphocytes and macrophages are primary targets for HIV in vivo; elucidation of the mechanisms responsible for differences in envelope biogenesis will be critical in understanding the immunopathogenesis of HIV. These studies will help in defining differences in the virus life cycle in these distinct cellular targets and in resolving the apparent disparities among HIV neutralization assays using these cell types.

N 231 CHARACTERIZATION OF MACROPHAGE-COMPETENT BIOLOGICALLY ACTIVE MOLECULAR CLONES OF HIV-1,
M.S.Reitz, F.Lori, L.Hall, F.Michaels, P.Lusso, P.Markham*, M.Popovic, and R.C.Gallo, LTCB, NCI, NIH, Bethesda MD 20892 and *Advanced Biosciences Lab., Kensington MD 20895.
HIV-1 strains differ greatly in their ability to infect macrophages. We have characterized two molecular clones which grow efficiently in macrophages. One, called pMN-ST1, is derived from an early passage of HIV-1(MN). Although long established MN cultures do not grow in macrophages, MN-ST1 grows well in these cells. MN-ST1 varies very little from other clones of HIV-1(MN), suggesting that very slight differences can strongly influence cell tropism, and that macrophage tropic viruses are easily lost during passage in T cell lines. The second clone, pLW12.3, was derived from a laboratory worker (LW) accidentally infected with HIV-1 (HTLV-IIIB). The virus derived from the LW grew well in macrophages, in marked contrast to IIIB. The pLW12.3 clone was shown to have a small deletion of the carboxy terminus of vif and the amino terminus of vpr, and derived virus only grew in the SupT1 T cell line. After adding the missing region using an analogous seqment from IIIB, the pLW12.3 grew well in macrophages, in spite of only minimal differences from the non-macrophage competent IIIB.

N 232 MUTATIONAL ANALYSIS OF THE MOLONEY MURINE LEUKEMIA VIRUS ENVELOPE PROTEIN,
David Avram Sanders and Richard C. Mulligan, Whitehead Institute for Biomedical Research, Cambridge, MA 01242
In order to enhance our understanding of the process by which retroviruses bind receptors and infect cells we are attempting to elucidate the roles that particular polypeptide domains, specific amino acids, and post-translational modifications play in the activity of the Moloney leukemia virus envelope protein. In particular, we wish to identify those regions and residues that are critical for the structure of the envelope protein, for assembly of the protein into virions, for binding to cellular receptors, and for fusion of viral and cellular membranes. We are undertaking this investigation through the examination of the processing and activity of artificial mutant envelope proteins.

The mutations introduced into the envelope protein were selected on the basis of a model of the protein that resulted from analysis of the pattern of conserved amino-acid residues in a family of closely related envelope proteins. The mutations fall into a number of classes:
1. Mutations that examine the roles of post-translational modifications, such as signal peptide cleavage, cleavage of gp70 from the transmembrane p15E, and the removal of a 2Kd domain from the C-terminus of p15E .
2. Mutations that test the structural model through disruption of amino acid residues that are predicted to play critical roles in particular functions, eg., proper folding, subunit association, assembly into virions, receptor binding, and membrane fusion.
3. Mutations that may enhance the resolution of the structural model (replacement of cysteine residues).
4. Mutations that probe the malleability of the envelope structure.
The fourth class includes mutations that test the tolerance of nonconserved regions to insertions and deletions and the possibility of assembly of functional gp70/p15E heterodimers from subunits synthesized separately.

We are assaying the proteins for biological function through the determination of whether viral particles released from cells containing the mutant envelope genes are infectious. The subcellular localization and degree of posttranslational processing of the envelope proteins are being determined through immunological techniques, while their ability to promote viral interference and syncytia formation is also being measured.

N 233 GLYCOSYLATED FORMS OF GAG (GCSA) ALLOW BETTER SPREADING AND INCREASED VIRULENCE OF THE FRIEND MURINE LEUKEMIA VIRUS.
Marc Sitbon[1], Antoine Corbin[1], Anne-Catherine Prats[2], and Jean-Luc Darlix[3]. [1]ICGM, INSERM U152, 75674 Paris Cedex; [2]CRBGC du CNRS, 31062 Toulouse; [3]LaboRetro, ENS, 69364 Lyon, France.
The *gag* region of murine leukemia retroviruses (MuLV) encodes for a precursor yielding the non-glycosylated matrix, capsid, and nucleocapsid proteins, and a phosphorylated protein of unknown function. Upstream of the canonic AUG Gag initiation codon, a CUG initiation codon has been identified (Prats *et al.*, 1989, J.Mol.Biol. 205:363-372). This CUG adds an 88 amino acid-long peptide to the *gag* coding frame, and the corresponding precursor protein matures through the endoplasmic reticulum. This translocated Gag precursor leads to glycosylated forms of Gag expressed at the cell surface and absent from mature virions. To evaluate the importance of this *gag*-encoded cell surface antigen (GCSA), we derived a GCSA$^-$ mutant of the Friend-MuLV. The GCSA$^-$ mutant produced infectious virions with no detected alteration of the other viral structural proteins. However, we observed that the GCSA$^-$ virus had delayed *ex vivo* and *in vivo* spreading abilities. Consequently, severe early hemolytic anemia observed in animals inoculated with wt Friend-MuLV, was no more observed with the GCSA$^-$ mutant. Also, *in vivo*-production of *env*-recombinant viruses (MCF viruses) was delayed after inoculation of the GCSA$^-$ mutant as compared to wt F-MuLV. Nevertheless, similarly to wt, erythroleukemia was observed with both the wt and the GCSA$^-$ mutant. The mutated region was evaluated *in vivo* by PCR of DNA from animals at different ages after inoculation. *In vivo* presence of GCSA proteins was also monitored. Preliminary results indicate that significant amount of replicating viral DNA from animals inoculated with the GCSA$^-$ mutant had a reverted phenotype, suggesting *in vivo* selection pressure for a re-established GCSA coding ability. Role of GCSA proteins in efficient viral spreading will be discussed.

N 234 NEUTRALIZING ANTIBODIES DIRECTED AGAINST THE HIV-1 gp120 GLYCOPROTEIN: EPITOPE CHARACTERIZATION, COOPERATIVITY AND MECHANISMS OF NEUTRALIZATION ESCAPE.

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We have mapped the discontinuous epitopes of human immunodeficiency virus type 1 gp120 recognized by various broadly neutralizing human monoclonal antibodies. These antibodies recognize epitopes that overlap the CD4-binding region of gp120. We investigated the cooperative interaction of monoclonal antibodies directed against linear determinants within the third variable (V3) loop of gp120 and antibodies directed against the discontinuous CD4 binding region. Furthermore, we will present results of experiments aimed at the understanding how certain virus mutants can escape neutralization by these different groups of antibodies.

N 235 Implications from Inhibition Studies for the Mechanism of Fusion of Sendai Virus. Philip L. Yeagle and Thomas Flanagan, Departments of Biochemistry and Microbiology, University at Buffalo School of Medicine (SUNY), Buffalo, NY, 14214.

Recent work with specific peptide inhibitors of viral infection, such as Z-D-Phe-L-PheGly, revealed that these hydrophobic peptides inhibited the fusion step in enveloped virus infection rather than inhibiting interaction between the viral fusion protein and a putative receptor on the cell surface (*J. Biol. Chem. 265*, 12178-12183 1990). These inhibitory peptides also inhibited the fusion of a simple phospholipid vesicle fusion system whose pathway of fusion was better understood (*Virology*, 182, 690-702, 1991). Studies on the mechanism of inhibition by one of the more potent peptide inhibitors, Z-D-Phe-L-PheGly, indicated that this peptide inhibited the formation of phospholipid structures with short radii of curvature that were suggested previously to be involved in the fusion of the above mentioned phospholipid vesicles, thereby inhibiting the fusion of these vesicles. Other studies suggested that the portion of the fusion protein of measles virus, referred to as the fusion peptide, promoted the formation of structures by phospholipids with a small radius of curvature that were implicated in the fusion of the above mentioned vesicles and stimulated fusion (*Biochim. Biophys. Acta* 1065, 49-53 1991). Taken together these data suggested that the fusion of some enveloped viruses, such as Sendai and measles, may follow a pathway involving intermediates in which phospholipids formed structures with a short radius of curvature. This work was supported by a grant from the NIH (AI 26800).

Virus Infection of the Nervous System and Major Histocompatibility Glycoprotein Interaction

N 300 INTERACTION OF THE HUMAN POLYOMAVIRUS JCV WITH HUMAN B-LYMPHOCYTES, Atwood W.J., Amemiya K., Tornatore C., Traub R., and Eugene O. Major, National Institute of Neurological Disorder and Stroke, National Institutes of Health, Bethesda, MD 20892

JC Virus (JCV) is known to have a very restricted host range, growing well principally in human fetal glial cells, HFGC. Our laboratory has identified the human B-lymphocyte as another site of JCV multiplication. JCV positive B-lymphocytes were identified by in situ hybridization in bone marrow, spleen and brain from patients with the central nervous system demyelinating disease, progressive multifocal leukoencephalopathy (PML). In addition, JCV DNA was found associated with lymphocytes in the peripheral blood of these patients by PCR. In an effort to define cell-type specific regulation of the JCV genome in these very different cell types, we have analyzed the binding of nuclear proteins from glial cells, B-lymphocytes and two non-permissive cell types, HeLa and the A3.01 line of human T-lymphocyte to the JCV regulatory region. DNase footprinting analysis reveals that similar regions in the JCV regulatory region are protected by nuclear extracts prepared from each of the cell types. We have previously identified this protected region as a nuclear factor type 1 (NF-1) binding site. Analysis of nuclear protein binding to the JCV regulatory region by gel retention assays, however, reveals an additional shifted band in glial and B lymphocytes which is not present in the HeLa or T cells. A oligonucleotide containing an NF-1 binding site effectively competes this band away. This indicates that the NF-1 present in glial cells and B cells is either different than the NF-1 found in T cells and HeLa cells or that NF-1 is associated with a complex of proteins which is similar in glial cells and B cells but different in T cells and HeLa cells.

N 301 EFFECT OF HCMV INFECTION ON MHC CLASS I PROTEINS.

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Human cytomegalovirus (HCMV) has been reported to downregulate MHC class I expression without affecting the level of heavy chain mRNA in infected cells. This virus expresses a glycoprotein with sequence similarity (20%) to class I heavy chain, which is coprecipitable with β_2m when both are expressed in vaccinia virus. Therefore, it has been suggested that HCMV interferes with class I expression by competing with the heavy chain for β_2m binding. If true, this should result in a surplus of nonassembled heavy chains in HCMV infected cells.

Contrary to this expectation, we observed a drastically reduced level of free heavy chains in HCMV infected cells. The levels of free β_2m were comparable to those in uninfected cells. We could not detect viral proteins coprecipitated with either β_2m or heavy chains.

RNA blot analysis and in vitro translation of poly-A RNA showed equivalent levels of translatable class I mRNA in HCMV infected cells and control cells, suggesting a posttranslational effect of the virus. Pulse chase analysis of HCMV infected cells showed that free heavy chains as well as class I complexes could be detected after a short pulse. However, these proteins have a very short half life when compared to the situation in uninfected control cells.

Our results suggest a new mechanism by which a virus downregulates class I proteins to escape immune surveillance, namely by disturbing an early step in the assembly of class I complexes. Adenovirus, the only other virus for which a posttranslational regulation of class I expression has been described, acts in a completely different way: the viral protein E19 contains an ER-retention signal and strongly binds to class I complexes, thereby preventing their transport to the cell surface. By coinfection with HCMV and adenovirus we could show that the HCMV effect is dominant over that of Adenovirus.

N 302 IDENTIFICATION OF AMINO ACIDS WITHIN THE MHC MOLECULE IMPORTANT FOR BINDING TO THE ADENOVIRUS PROTEIN E3/19K, Hans-Gerhard Burgert and Dominik Feuerbach, Max-Planck-Institut für Immunbiologie, W-7800 Freiburg, Germany

The early non structural glycoprotein E3/19K of adenoviruses (Ad) is not essential for virus growth in tissue culture cells. Its relative conservation in most Ad serotypes together with its biologic activity argues for an important role of this protein in adenovirus pathogenesis and persistence. We previously showed that the E3/19K protein of Ad2 binds to human class I histocompatibility antigens (human MHC or HLA) in the rough ER inhibiting their transport to the cell surface. Moreover, T cell recognition of E3/19K$^+$ cells is drastically reduced. The structural requirements of MHC antigens for binding E3/19K are not yet understood. In order to determine these we took advantage of the differential binding properties of the murine MHC antigens K^d and K^k: the K^d molecule binds E3/19K whereas K^k does not. To identify the domains that are important for this interaction K^d/K^k hybrid molecules were transfected into E3/19K$^+$ cells and binding was monitored in immunoprecipitation experiments. Hybrid MHC antigens with the two N- terminal domains of K^d bind to E3/19K while those with only one of the two of K^d do not. Therefore, the α1 and α2 domains comprising the peptide binding pocket are essential. At present, we are using site directed mutagenesis to identify amino acids within the α2 domain that contribute to the complex formation.

N 303 HERPES SIMPLEX VIRUS 1 (HSV-1) GENE $\gamma_1$34.5 BLOCKS THE NEUROBLASTOMA CELLS FROM CELL DEATH (APOPTOSIS) INDUCED BY VIRAL INFECTION, Joany Chou and Bernard Roizman, Marjorie B Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, IL 60637

CNS cells are highly permissive for HSV-1 and direct inoculation of virus into the brain causes CNS destruction and death. Viral deletion mutants lacking a gene designated as $\gamma_1$34.5 do not replicate in or destroy CNS cells. This gene maps in the inverted repeats and is present in two copies per genome. The gene product is made in relatively small amounts. In the course of studies to determine the function of the $\gamma_1$34.5 gene, we discovered that a neuroblastoma cell lines of human neuronal origin replicated wild type virus but that cells infected with the $\gamma_1$34.5 minus mutant ceased all protein synthesis between 7 and 12 h post infection even though viral DNA was made and mRNA accumulated in these cells. The stimulus that activatd the cessation of protein synthesis in the absence of the $\gamma_1$34.5 gene was related to the onset of viral DNA synthesis. Thus, cells infected with the $\gamma_1$34.5 deletion mutant and treated with phosphonoacetate at concentrations sufficient to block viral DNA synthesis continued to synthesize proteins as late as 24 hours post infection.

Our data suggest that in this and one other cell line of neuronal origin, HSV-1 infection induces a stress which causes the cells to cease all protein synthesis, and that the function of the $\gamma_1$34.5 gene is to preclude the cessation of protein synthesis in order to allow viral replication to proceed. This gene does not appear to be essential for maintenance of protein synthesis in cells derived from other organs.

N 304 MOLECULAR CHARACTERIZATION OF A POLIOVIRUS TYPE 1 MUTANT NATURALLY NEUROVIRULENT FOR MOUSE

Thérèse Couderc, Bruno Blondel & Florian Horaud.
Medical Virology Unit, Pasteur Institute, 75015 Paris.

Wild strains of poliovirus type 2 (PV-2), like Lansing strain, are known to induce poliomyelitis in mice after intracerebral inoculation. In contrast, inoculation of mice with poliovirus type 1 (PV-1) fails to result in disease. It was shown that the ability of PV-2 Lansing strain to infect mice can be transferred to the PV-1 Mahoney strain by exchange of the 9 amino acid sequence in VP1 corresponding to the neutralization antigenic site 1 (Martin *et al.*, 1988, EMBO J., 7: 2839; Murray *et al.*, 1988, Science, 241: 213). In order to obtain further information on the molecular determinants implicated in the mouse-adapted phenotype of PV-1, we isolated a PV-1 natural mutant, called NK13, from passage of Mahoney strain directly into mice. The phenotypic markers of the mouse-adapted NK13 mutant were determined. To identify the sequence changes that lead to adaptation of NK13 to mice, the entire genome of both the NK13 mutant and the mouse avirulent parental Mahoney strain were sequenced. Only 6 point mutations, among three which are silent, were detected in the NK13 genome. None of them was localized in the antigenic site 1 considered so far as a critical sequence for mouse neurovirulence. Studies are under way to determine the role of each mutation in adaptation of PV-1 to mice.

N 305 **PSEUDORABIES VIRUS GLYCOPROTEIN gI (THE HSV-1 gE HOMOLOG) INFLUENCES TARGET CELL RECOGNITION AND VIRULENCE AFTER INFECTION OF THE RAT VISUAL SYSTEM.** L.W. Enquist, M.E. Whealy, A.K. Robbins and J.P. Card. DuPont Merck Pharmaceutical Co., Wilmington, Delaware 19880.

We have demonstrated that intraocular injection of wild type (PRV-Be; Becker) and attenuated (PRV-Ba; Bartha) strains of pseudorabies virus produce transneuronal infection of functionally distinct circuits in the rodent visual system (Card et. al. Neuron 6, 957-969, '91). PRV-Be induces two temporally separated waves of infection that ultimately target all known visual centers in the brain. In contrast, PRV-Ba only infects a functionally distinct subset of these neurons that control circadian rhythms. This present analysis localized the genetic defect responsible for this phenotype. Glycoproteins gI, gIII and gp63 are all significantly reduced (gIII) or absent (gI & gp63) in the envelope of PRV-Ba. We systematically examined the role of these defects by constructing 7 mutant viruses. Reduction in concentration or elimination of gIII from the envelope did not alter the pattern of viral infectivity produced by intraocular injection of virus. In contrast, specific deletion of only gI from the wild type strain or a rescued strain of PRV-Ba converted the phenotypic pattern of infectivity from wild type to attenuated virus; ie. neurons targeted in the first wave of wild type infection were spared. In addition, mutants lacking gI exhibited reduced virulence. Thus a single glycoprotein, not essential in tissue culture, dramatically affects neurotropism and neurovirulence.

N 306 NEUROVIRULENCE OF SEMLIKI FOREST VIRUS IS RELATED TO DEVELOPMENTAL CHANGES IN NEURONES

John K. Fazakerley, Department of Pathology, Cambridge University, England, UK.

Following intraperitoneal infection of mice, Semliki Forest virus (SFV) establishes an infection in muscles giving rise to a high titer plasma viraemia. Infection of the CNS occurs following virus passage accross cerebral endothelial cells. The A7 strain of SFV is highly virulent in neonatal mice but avirulent in adult mice (>28 days of age). Between these times, animals are progressively less susceptible with increasing age. The L10 strain of SFV is virulent in mice of all age groups. Immunosuppression (8.5 Gy, total body irradiation, cyclosporin-A, cyclophospahmide, cycloleucine) of adult mice does not render the A7 infection lethal. Studies on the CNS spread and tropism of these two viruses in neonatal and adult mice by *in situ* hybridization demonstrate that both viruses cross cerebral endothelial cells and infect adjacent neurones. The L10 virus, in mice of all ages and the A7 virus in neonatal mice spread rapidly from the point of entry to involve increasing numbers of neurones and the animals die of massive neuronal destruction within a few days of infection. In contrast, in adult mice infected with A7 virus, infection is limited to a few neurones around vessels and is not disseminated further, neuronal involvement is limited and the mouse survives. Electron microscopical study indicates that the replication of the A7 strain is restricted in adult mouse neurones. The molecular mechansim for this age-related, neuronal restriction of virus replication is currently under investigation.

N 307 DESIGN OF HIGH-AFFINITY D^b-SPECIFIC ANTAGONIST PEPTIDES THAT INHIBIT CTL ACTIVITY. IMPLICATION FOR CONTROL OF VIRAL AND AUTOIMMUNE DISEASE, Jean Edouard Gairin and Michael B. A. Oldstone, Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA.

T cells recognize peptides from foreign (viral) or autoantigens presented by the cells in context of their major histocompatibility glycoproteins (MHC). Killing of virus infected cells by cytotoxic T lymphocytes (CTLs) is a prominent host mechanism for controlling viral infection. However, in that process, lysis and death of non-dividing and/or vital cells can cause irreversible damage. Also, T lymphocyte activity against cells presenting autoantigens is implicated in the pathogenesis of autoimmune disease. In both situations, search for antagonists of CTL activity is an important goal. As described in this report, we have designed a family of high-affinity, MHC-D^b allele-specific antagonist peptides. Two specific binding assays on living cells (the murine lymphoma mutant cell line RMA-S) and an *in vitro* biological assay (lymphocytic choriomeningitis virus (LCMV) model in ^{51}Cr release assay) were used. The results show that these synthetic peptides: i) bind to D^b molecules and compete with D^b restricted viral epitopes with high affinity; ii) do not sensitise H-2b target cells for H-2 restricted CTL lysis; and iii) can protect selectively LCMV peptide-coated uninfected target cells or LCMV-infected target cells from H-2 restricted CTL activity.

N 308 HIGH EFFICIENCY OF MHC CLASS II PRESENTATION OF ENDOGENOUS MEASLES VIRUS HEMAGGLUTININ, Denis Gerlier, Suzanne Lombard-Platet, Patrick Bertolino, Isabelle Chrétien, and Chantal Rabourdin-Combe. Immunobiologie moléculaire, UMR 49, CNRS-ENS Lyon, France.

APC transfected with a recombinant expression vector coding for transmembrane measles virus hemagglutinin (HA) expressed HA at their cell-surface, and efficiently processed and presented endogenous HA to class II-restricted T hybridoma cells. Coculture experiments revealed that the presentation of transmembrane HA was not due to recapture of shed HA. However, the presentation of endogenous HA was very efficient compared to that of exogenous HA, since less than 10^3 HA-expressing APC were sufficient to significantly stimulate 10^5 T hybridoma cells, whereas 3 x 10^4 naive APC pulsed with 3 µg/ml of exogenous HA were necessary. This efficiency correlated with the amount of HA expressed at the cell-surface of the APC. Sensitivity to BFA and chloroquine could be revealed when APC were fixed by paraformaldehyde, indicating that the processing of endogenous HA is likely to occur within the endosomal compartment. The formation of HA peptides-MHC class II complexes in fibroblastic cells expressing a high amount of HA was found to be independent of the presence of the invariant chain, whereas the presentation of a limited amount of exogenous HA required Ii expression in the APC. Taken together, these data show that peptides derived from an endogenous transmembrane molecule are very efficiently presented by MHC class II through an endosomal pathway and that endogenous HA must be able to reach the endosomal compartment very efficiently.

N 309 ALPHAVIRUS INFECTION OF NEURONS Diane E. Griffin, Beth Levine, Pamela Tucker & Sukathida Ubol, Departments of Medicine & Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Alphaviruses cause acute mosquito-borne encephalitis in the New World. Sindbis virus (SV) is the prototype alphavirus and causes acute age-dependent encephalitis in mice. In the central nervous system (CNS) neurons are the primary cell infected. For young mice infection is fatal while mature mice infected with the prototype AR339 strain recover uneventfully despite widespread CNS infection. SV strains have been identified that are both more virulent and less virulent than AR339. Our studies have shown that the virulence of virus strains is determined in part by an amino acid change in the E2 glycoprotein (residue 172) that affects the efficiency of binding to a neuronal receptor. This receptor has been identified using an antiidiotypic antibody to a neutralizing anti E2 MAb as a 74Kd protein expressed on all CNS cells at birth. Expression is terminated on 50% of cells within 48h of birth and this down regulation may be an important determinant of decreasing susceptibility with increasing age. Other amino acid changes (residue 55) in E2 affect the efficiency of replication in mature neurons and the ability of virus to cause fatal infection in 1-3 week old mice. Amino acid changes in the E1 glycoprotein that affect the fusion of virus with neuroblastoma cells are also correlated with virulence for mice. Four week old mice survive infection with most strains and clear virus from the CNS. *Scid* mice become persistently infected. Using *scid* mice and neuronal cultures in vitro we have shown that clearance from neurons is effected by antibody by inhibiting synthesis of viral RNA. Although infectious virus is rapidly eliminated viral RNA is cleared more slowly and can be detected by PCR for months after recovery. Prevention of relapse may provide a rationale for prolonged intrathecal antibody synthesis after recovery from CNS viral infections.

N 310 POLYGENIC CONTROL OF NEUROINVASIVENESS OF ATTENUATED CALIFORNIA BUNYAVIRUS CLONES

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We have described two different attenuated clones of California bunyavirus, both of which exhibit reduced neuroinvasiveness upon sc injection of suckling mice. Clone B1-1a bears an attenuated middle RNA segment (virulence genotype VAV) and clone B.5 bears an attenuated large RNA segment (virulence genotype AVV). We have now constructed a complete panel of reassortants between these two clones and tested them in suckling mice by a comparison of the ratio PFU/LD50 (neuroinvasiveness index). Reassortants with genotype VVV are highly neuroinvasive, ie, are much more virulent than either parent clone. Reassortants with genotype AAV are more attenuated than either parent clone. These results indicate that (i) attenuation in either the M or L RNA segments may lead to reduced neuroinvasiveness; (ii) certain reassortants between two non-neuroinvasive clones may be more neuroinvasive than either parent; and (iii) reassortants with two attenuated RNA segments may show even greater reduction in neuroinvasiveness than either parental clone. These results contrast with our prior studies of neurovirulence (ic injection of adult mice) which show that clone B.5 is non-neurovirulent while clone B1-1a is highly neurovirulent, suggesting that attenuating mutations may or may not simultaneously affect neuroinvasiveness and neurovirulence.

N 311 ANALYSIS OF T CELL RECEPTOR (TCR) VB REPERTOIRE RESPONDING TO HERPES SIMPLEX VIRUS (HSV) IN MURINE MODEL OF HERPES STROMAL KERATITIS (HSK), Soon Jin Lee, Arnd Heiligenhaus, C. Stephen Foster, Departmnet of Ophthalmology, Mass. Eye & Ear Infirmary, Harvard Medical School, Boston, MA 02114

Genetic factors influencing immune response of the host contribute to the generation of HSK and T cells were involved in this process. T cell response was studied in Balb/c congenic mice that show differential susceptibility to HSK and are different at the Igh-1 locus that is known to influence T cell repertoire. T cell subsets in spleen, lymph nodes and eye were analysed by PCR technique using synthetic oligonucleotide primers that are specific for 18 T cell subsets defined by their Vβ gene usage of TCR at different time points after corneal infection with HSV-1 KOS strain. Kinetics of each subsets of T cells responding to herpes antigen were different among susceptible and resistant strains of mice. Vβ 6, 4, 14 and 16 were the major subsets responding to infection in resistant strain C.B 17 while Vβ 2, 6, 13 and 14 were the major ones in susceptible strain C.AL 20. Kinetics of the expression of the total α and β chains of TCR and certain Vβ subsets implied immune hyperresponse at the earlier times of infection and immune suppression in the later times. FACS analysis of T cells in spleen confirmed previous studies that CD8 cells are preferentially expressed in resistant mice while CD4 cells were prevalent in susceptible mice. TCR regulation patterns shown in this study implies HSV as superantigen.

N 312 PROCESSING PATHWAYS FOR THE PRESENTATION OF ENDOGENOUS ANTIGENS TO MHC CLASS II-RESTRICTED T CELLS, Eric O. Long, Mauro S. Malnati, Merce Marti and Robert DeMars, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, and Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

Strong cellular immune responses are usually elicited upon virus infection. Recognition of processed viral antigens by $CD4^+$ T cells is essential for the establishment of cellular immune responses, as well as antibody production. Cytotoxic effector T cells include both $CD8^+$ and $CD4^+$ cells, restricted by class I and class II MHC molecules, respectively. Although class II MHC molecules are unique in their ability to efficiently present exogenous antigens that have been endocytosed into acidic compartments, they can also present endogenous antigens. Using mutant human B-cell lines and T cells specific for the H3 hemagglutinin of influenza A virus, we have demonstrated the existence of multiple endogenous pathways for processing and presentation of cytosolic antigen by HLA-DR1. A cytosolic form of H3, expressed by a recombinant vaccinia virus, was efficiently presented to DR1-restricted T cells, even in a B-cell line deficient in the presumed peptide transporter necessary for class I-restricted presentation. A short cytosolic peptide, expressed from a mini-gene encoding the DR1-restricted H3 epitope, was also presented, albeit less efficiently than the cytosolic protein. In contrast to the cytosolic H3 protein, the short peptide was not presented in cells deficient in the presumed peptide transporter. Helper T cell epitopes can thus be generated from cytosolic proteins by several mechanisms, one of which is totally distinct from the class I pathway of antigen presentation.

N 313 MUTATIONAL ANALYSIS OF A VIRAL CYTOTOXIC T LYMPHOCYTE EPITOPE, EFFECTS ON RECOGNITION AND PROTECTION. Thomas A McKee, Michael B A Oldstone and J Lindsay Whitton, Dept of Neuropharmacology, The Scripps Research Institute, La Jolla CA 92037.

Cytotoxic T lymphocytes (CTLs) are known to be critical effectors in the immunological response to viral infections and certain tumours. In mice infected with lymphocytic choriomeningitis virus (LCMV) these cells are known to be essential for viral clearance, responsible for pathology and mortality and functionally absent in viral persistence. In Balb/c ($H\text{-}2^d$) mice the major CTL epitope is located between amino acids 116 and 129 of the LCMV nucleoprotein gene, this epitope is also recognised on the $H\text{-}2^q$ and $H\text{-}2^u$ backgrounds. We have undertaken a mutational analysis of the epitope by expressing wild type and mutant sequences from recombinant vaccinia viruses in the form of short polypeptides (minigenes). Initial experiments revealed that a 16 amino acid polypeptide of wild type sequence encoded by a recombinant vaccinia virus allows efficient, MHC restricted, recognition of infected cells by immune CTLs. Vaccinia viruses encoding minigenes with mutations through the epitope were produced and the recognition of cells infected by them analysed in CTL assays. Mutations tested thus far fall into two groups resulting either in complete failure of killing by CTLs induced by LCMV or an 80% reduction of killing. These data differ from a mutational analysis of the same epitope using synthetic peptides. Current studies are aimed to extend these data to the $H\text{-}2^q$ and $H\text{-}2^u$ backgrounds and to determine whether the failure of recognition results from defective interaction with MHC or, by exclusion, with the T-cell receptor.

Immunisation with vaccinia virus containing the wild type epitope has been shown to protect animals from a lethal dose of LCMV. Selected mutants are currently being tested to determine the correlation between recognition in CTL assays and protection *in vivo*.

N 314 ORGANIZATION OF BORNA DISEASE VIRUS RNAs, Joanna M. Pyper and Janice E. Clements, Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205,

Borna disease virus (BDV) is a viral agent that causes profound disturbances in motor function and behavior in a wide range of animal species possibly including humans. The virus is highly neurotropic depending on neurons for infection and dissemination in the infected animal. The infectious nature of BDV has long been established but the virus has not been isolated or classified. The isolation of BDV-specific cDNA clones using subtractive libraries have enabled us to identify BDV as a RNA virus. The BDV RNAs are organized as an overlapping 3' nested set of RNAs (0.85,2.1,3.6 and 10.5 kb) with the largest RNA presumably the genomic RNA. Both positive and negative polarity RNA exist for the three largest RNA species; only positive polarity RNA has been detected for the 0.85 kb RNA. The organization of the BDV viral RNAs as well as the presence of both positive and negative polarity RNAs is similar to the coronavirus superfamily. Experiments have been done to determine if the BDV RNAs have leader sequences analogous to the coronavirus RNAs.

Oligonucleotides were synthesized that were complementary to sequences at the 5' end of the BDV cDNA clone B8 and used for primer extension experiments. The results from the primer extension experiment suggest that there are two sets of sequences present 5' of the sequences found in the B8 cDNA clone. Potentially, two sets of sequences could be leader and genomic sequences. Further experiments are underway to characterize the nature and location of these 5' specific sequences in the BDV RNAs.

N 315 CYTOTOXIC T LYMPHOCYTE RECOGNITION OF THE VESICULAR STOMATITIS VIRUS NUCLEOPROTEIN ANTIGEN IN A ß-GALACTOSIDASE FUSION PROTEIN. Donna M. Roscoe and Douglas S. Lyles, Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1064.

Major histocompatibility complex (MHC) class I molecules present peptides derived from the proteolysis of endogenous proteins to cytotoxic T lymphocytes (CTL). Efficiency of proteolytic processing may be restricted by flanking amino acid sequences as well as the antigenic sequence itself. Anti-VSV CTL from H-2^b mice primarily recognize an epitope derived from the viral nucleoprotein (N protein) in association with H-$2K^b$. This epitope was determined to consist of amino acids 52-59 using a series of vaccinia virus recombinants expressing deletion fragments of the N gene and chemically synthesized peptides, and by analysis of an endogenous peptide bound to H-$2K^b$ in VSV-infected cells (van Bleek and Nathenson, Nature 384, 213, 1990). The coding sequence for the N protein epitope was inserted into the ß-galactosidase gene to test the effects of flanking amino acid sequences on processing of the antigenic peptide. Expression of enzymatic activity indicated that a native protein structure was retained by the resulting fusion protein. EL4 cells infected with a recombinant vaccinia virus expressing the chimeric ß-galactosidase gene were efficiently recognized and lysed by anti-VSV CTL. These results indicate that either the processing protease(s) does not specifically recognize the sequences flanking the epitope or else that alternative cleavage sites can substitute for those found in the N protein. These possibilities will be addressed by analysis of endogenous peptides generated from the fusion protein. (Supported by NIH grant AI20778)

N 316 HUMAN CD8$^+$ HERPES SIMPLEX VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTE CLONES RECOGNIZE DIVERSE VIRION PROTEIN ANTIGENS

Michael A. Tigges[1], David Koelle[2], Karin Hartog[1], Rose E. Sekulovich[1], Lawrence Corey[2] and Rae Lyn Burke[1]
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The role of the HLA class I restricted, CD8+, HSV-specific CTL in the control of human HSV infections is controversial as isolation of such cells from humans with HSV infections has been difficult. Seven HSV-specific CD8$^+$ CTL clones have been isolated from a patient with recurrent genital herpes who is HSV-2 solely seropositive. Six of the seven clones lysed only HSV-2 infected targets; one lysed both HSV-1 and HSV-2 infected targets. A series of drug-block/release protocols to limit the repertoire of viral gene expression to select transcriptional classes was employed to determine the antigens recognized by these HSV-specific CTL. Five of the clones exhibited different patterns of cytotoxicity suggesting that each recognized a distinct HSV antigen. One of the clones appears to be directed against an immediate early antigen; six of the clones recognize virion proteins. Five of these clones recognized internal virion proteins that could be introduced into target cells by HSV infection in the absence of virus gene expression. Antigen specificity was further tested by using vaccinia virus vectors that express glycoproteins gD2 and gB2 or the tegument protein VP16. One clone lysed vac/gD2-infected target cells; the remaining clones did not recognize any of these gene products. Although the CD8+ response from this individual is diverse, these results suggest that the CTL response is primarily type specific and focused on internal virion proteins rather than envelope glycoproteins.

Protein Trafficking in Infected Cells, Viral Persistence and Pathogenesis Transgenic Models

N 400 USE OF AN INTERFERON-INDUCIBLE PROMOTER FOR CONTROLLED EXPRESSION OF GENES ENCODING DISEASE RESISTANCE FACTORS IN TRANSGENIC ANIMALS. Margaret H. Abel, Matthew H. Lewis, Aldwyn Haven, John W. McCauley and Christopher J. Bostock, Division of Molecular Biology, AFRC Institute for Animal Health, Compton, Newbury, Berkshire, RG16 0NN, U.K.

Transgenic mice carrying the LacZ reporter gene under the interferon-inducible promoter from the human 6-16 gene express β-galactosidase predominantly in heart, brain and smooth muscle 24 hrs after stimulation with ds.RNA (poly I/poly C). Inoculation of these mice with Influenza A virus (X.31) induced β-galactosidase in tracheal and bronchial epithelium 6 hrs after infection. By 24 hrs expression was no longer seen in these tissues but β-galactosidase was detected in sub-epithelial tissues and cardiac muscle. The pattern of expression in vivo of the human 6-16 gene is not known. A cDNA copy of the mouse Mx gene encoding the Mx protein known to confer resistance to influenza virus in wild type mice has been linked to the interferon-inducible 6-16 promoter. A stable mouse fibroblast line expressing this construct and pre-treated with mouse interferon α/β (500 U/ml) shows decreased synthesis of virus polypeptides 5 hrs after infection with influenza virus when compared with a control line not exposed to interferon. Transgenic mice carrying the 6-16.Mx construct have been generated and are being tested for resistance to influenza virus in vivo. The interferon-inducible 6-16 promoter has also been linked to specific cDNA copies of influenza virus genes coding for nucleoprotein (segment 5) or neuraminidase (segment 6). Stable mouse fibroblast lines carrying these constructs and transgenic mice with either 6-16 segment 5 or 6-16 segment 6 stably integrated are being tested for resistance to infection with influenza virus.

N 401 A TRANSGENIC APPROACH TO STUDY THE PATHOGENESIS OF THE IMMUNOSUPPRESSIVE STRAIN OF THE PARVOVIRUS MINUTE VIRUS OF MICE (MVMi), Jose M. Almendral, Juan C. Ramirez, Jose C. Segovia, A. Ramirez and Juan A. Bueren, CIEMAT Department of Molecular and Cell Biology and Centro de Biologia Molecular (UAM-CSIC). Madrid, Spain.

Parvovirus are nuclear single-stranded DNA virus, that require for their replication functions expressed in proliferating cells. Cell permissiveness to MVM life cycle in vitro, relies also on the interaction between differentiation factors and strain specific virus determinants, what restricts infection at unidentified post-entry step(s). Productive interactions are monitored early in the infection by viral P4 promoter activity and the cytotoxic NS-1 protein expression.

As a first step to understand the basis of the MVMi pathogenesis, we have previously explored the susceptibility of the mouse bone marrow to virus infection in vitro and in vivo. The MVMi was severely myelosuppressive in vitro for the committed progenitors(CFU-GM,BFU-E) and for primitive haemopoietic cells of the stem compartment (CFU-S12d) (Blood 77,980.1991), and it was able to complete a productive cycle in myeloid cultures as well as in purified haemopoietic progenitors. Nevertheless, a limited viral multiplication occurs in the haemopoietic organs of intranasally inoculated newborn mice. Virus titers reach a maximum 6 days post-infection but are kept at low level and eventually cleared by an early and effective immune response.

To gain a whole picture of the main MVMi tissue tropism, we produced transgenic mice bearing the late P38 promoter of the virus governing the lac Z reporter gene encoding bacterial β-galactosidase. This promoter is constitutively silent in normal cells, but can be transactivated by the nonstructural proteins in permissive proliferating cells. So β-galactosidase positive tissues should indicate preferent sites of virus multiplication. Transgenic offspring were infected at birth and sacrified 6 days afterward, the whole body sliced and processed for β-galactosidase expression. Most animals of one P38-Z transgenic line out of three analyzed, showed high β-galactosidase activity in the kidney parenchyma and a compartmentalized staining pattern in the Central Nervous System (CNS). Lac Z expression was restricted to the cerebellum and other regions of the encephalon which in the mouse undergo much of their development after birth. The possibility that Parvovirus might be used as developmental markers to identify regions of the CNS with proliferation capacity is currently being evaluated.

N 402 IN VIVO TRAFFIC OF ENTEROVIRUSES: UTILITY OF MONOCLONAL REAGENTS. Daniel R. Anderson, Greg A. Perry, James M. Gulizia, Bruce M. McManus. Departments of Pathology & Microbiology, & Anatomy & Cell Biology, University Nebraska Medical Center, Omaha, NE.

Recent observations in our laboratory have suggested a significant viral trafficking and reservoir role for the immune system in the enteroviral murine model of myocarditis/pancreatitis. Fluorescence-activated cell sorting (FACS) analysis detected both surface and cytoplasmic coxsackievirus B3 (CVB3) viral capsid antigens on infected HeLa cells using monoclonal antibodies (Mabs). Both MAbs were originally characterized in another laboratory as $IgG_{1\kappa}$. Further characterization of these antibodies by the UNMC's Monoclonal Antibody Facility several months later demonstrated that one Mab was IgM_{κ}, while the other Mab displayed three isotypes, ($IgG_{1\kappa}$, $IgG_{2a\kappa}$ and IgM_{λ}). Although these Mabs reacted with 88.2% and 93.1% of the infected HeLa cells, respectively, the monoclonality and specificity of the Mabs were questioned. On this basis we fully characterized the growth and antibody production of five original hybridomas. Hybrid viability ranged from 8 to 56% and all lines were subcultured. Supernatants were collected in serum-containing (1 - 10%) and serum free media. Analysis of the supernatants by ELISA and SDS-Page demonstrated that only two of the original clones produced antibody, and only in the presence of 10% fetal bovine serum. Both of these produced only IgM MAbs. One of these two was described originally as an IgM secretor, while the other was an IgG. These results demonstrate the absolute requirement for ongoing evaluation of clones and antibodies used in these types of assays and suggest that significant characterization must be an initial step in investigation prior to applications. New Abs against the viral capsid antigens of CVB3 (VP 1, 2/4/3) are being developed and will be utilized along with immune cell differentiation and activation markers in FACS and anchored cell analysis as we pursue our original pathological questions about in vivo viral localization and persistence.

N 403 THE U_L20 GENE OF HERPES SIMPLEX VIRUS ENCODES A FUNCTION NECESSARY FOR VIRAL EGRESS, Joel D. Baines, Patricial L. Ward, Gabriella Campadelli-Fiume, and Bernard Roizman, The Marjorie Kovler Oncology Laboratories, University of Chicago, Chicago, IL 60637. In order to determine the function of the U_L20 gene of herpes simplex virus, we constructed a viral deletion mutant lacking the start codon and 53% of the U_L20 open reading frame. We report the following: (i) The U_L20- mutant formed small plaques in 143 cells but failed to form plaques in Vero cells. Viral yields were reduced in all cell lines tested. (ii) electron microscopic examination of infected Vero cells revealed that U_L20 - virions accumulated in the cytoplasm in a perinuclear compartment, possibly between the inner and outer lamellae of the nuclear membrane. (iii) Glycoproteins B, C, D, E, H and I recovered from lysates of cells infected with the U_L20- cells were indistinguishable from those obtained from cells infected with wild type virus by electrophoretic mobility of mature or precursor forms. (iv) Repair of the deleted sequences restored the wild type phenotype. (v) The U_L20 gene product was shown to be associated with cellular membranes and to possess characteristics of integral membrane proteins. We conclude that the U_L20 gene encodes a hitherto unrecognized function in that it enables transport of virions to the extracellular space and that the function of the U_L20 gene is complemented by some cell lines but not by Vero cells. These data indicate that herpesvirions are transported in a manner distinct from normal cellular secretory proteins.

N 404 Role of the major outer envelope protein in cell-to-cell transmission and release of vaccinia virus.

Rafael Blasco and Bernard Moss. Laboratory of Viral Diseases. National Institute of Allergy and Infectious Diseases. National Institutes of Health.

Vaccinia virus infection gives rise to two different types of infectious particles. The best characterized is named intracellular naked virus (INV). INV virions assemble in perinuclear areas and, after assembly, are found free in the cytosol. Extracellular enveloped virus (EEV) contain an additional membrane (outer envelope) with respect to the INV. The process leading to virus release involves wrapping of mature INV particles by a double membrane, and subsequent fusion of the outer layer with the plasma membrane. We have isolated a deletion mutant in the gene coding for the major outer envelope protein . The deletion mutant was affected in EEV formation and cell-to-cell transmission, suggesting that the pressence of enveloped virus is an absolute requirement for virus transmission. Electron microscopic examination of cells infected by the mutant virus revealed a block in the EEV pathway at the level of INV wrapping. Also, cells infected with the mutant virus did not show acid-dependent fusion activity, despite of the presence of viral glycoprotein in the cell membrane. Our results suggest that virus transmission, as well as cell fusion, is mediated by enveloped virions attached to the cell membrane.

N 405 DIFFERENTIAL REGULATION OF CYTOKINE GENE PRODUCT EXPRESSION IN MACROPHAGES INFECTED WITH WILD TYPE VERSUS AN IMMUNOSUPPRESSIVE VARIANT LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV),

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Intravenous inoculation of adult immunocompetent mice with wild-type LCMV (Arm 53b) provokes a vigorous $CD8^+$-mediated cytotoxic T lymphocyte (CTL) response that clears the infection. On the other hand similar inoculation with a variant LCMV (Cl. 13) does not provoke a CTL response, induces a more generalized immune suppression and is associated with persistent infection. In the present study we have investigated the possibility that the dichotomous $CD8^+$ CTL responses seen between these highly homologous viruses is the result of differential effects on cytokine gene expression in infected macrophages.

At a multiplicity of infection (moi) of 1, Arm 53b and Cl. 13 productively infected >90% of cultured peritoneal macrophages isolated from Balb/cBy mice. Marked induction of both IL-1β and TNF-α mRNA and protein secretion were found in Arm 53b but not Cl. 13 infected macrophages at 24h and 48h post-infection respectively. The ability of Arm 53b to induce IL-1β and TNF-α mRNA was (i) not due to endotoxin contamination of the virus stock, and (ii) abolished by prior UV-irradiation inactivation of the virus, indicating a dependence on viral replication. The inability of Cl. 13 to induce IL-1β or TNF-α gene product expression was maintained over a range of moi (0.002-2), and time points (8-72h).

These findings indicate differential regulation of cytokine gene product expression in macrophages when infected with Arm 53b versus Cl. 13. The inability of Cl. 13, in contrast to Arm 53b, to induce IL-1β and TNF-α in these primary antigen presenting cells may provide a molecular basis for the $CTL^{-}P^{+}$ phenotype of this LCMV variant.

N 406 THE ROLE OF DIFFERENT HIV LTRs IN GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM,

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AIDS and the AIDS Dementia complex (ADC) is caused by infection with the human immunodeficiency virus (HIV-1). However, it has become increasingly clear that many different strains of HIV exist in the population as well as in individuals with AIDS. The contribution of different strains of HIV to the development of ADC is not clear. Transgenic mice provide an animal model to examine the role of individual HIV genes on cell tropism and CNS pathogenesis. The LTR of HIV contains the viral transcriptional elements of the virus; the promoter element of other retroviruses have been shown to play a role in cell tropism and disease potential of the virus.

To investigate the role of different HIV strains in infection in the central nervous system, transgenic mice were constructed using the LTRs from three different strains of HIV (HIV_{IIIB}, $HIV_{JR\text{-}CSF}$, $HIV_{JR\text{-}FL}$) linked to the bacterial reporter gene lac Z. Two of these strains of HIV were isolated from one individual who had the ADC. Expression in adult transgenic mice was monitored by X-gal staining of thymus and the level of expression was quantitated by an RNase protection assay. The three HIV LTRs expressed to high levels in the thymus of the mice and showed differential expression in the brain. The $HIV_{JR\text{-}FL}$ mice expressed B-gal strongly in regions of the brain (septal nuclei, hippocampus, reticular thalamic nuclei, superior colliculus and optic tract) and to a lesser extent in the cerebellum and brain stem. The $HIV_{JR\text{-}CSF}$ mice expressed B-gal strongly in the cerebrum, thalamus, hypothalamus and LGN and to a lesser extent in the hippocampus and cerebellum. HIV_{IIIB} LTR expressed to a very low extent in the hippocampus and endopiriform nucleus. Currently, the specific cells in these regions which express B-gal are being identified.

N 407 MOLECULAR BASIS OF THE GROWTH HORMONE DEFICIENCY SYNDROME CAUSED BY LCMV,

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We have previously described that C3H mice persistently infected with the noncytopathic lymphocytic choriomeniingitis virus (LCMV) develop a growth hormone (GH) deficiency syndrome manifested as retarded growth and hypogycemia. Despite high levels of virus replication in the GH producing cells of the anterior pituitary, there is no evidence of structural damage or inflamation, but production of GH is significantly diminished at both the RNA and protein levels. To examine the molecular mechanisms whereby GH is turned down without cellular functions being affected, we have established a tissue culture model allowing the study of the consequences of LCMV infection on a somatotroph cell line, PC-cells, which expresses GH and prolactin (PL). PC-cells proved to support LCMV replication without signs of structural damage or impairment in cell physiology. More interestingly, a dramatic decrease (5-10 fold) in GH mRNA steady state level was seen only in LCMV-infected cells, whereas no differences in mRNA levels of actin and some other housekeeping genes were observed between uninfected and infected PC-cells. Here we present evidence that LCMV replication in somatotroph cells causes a decrease in the transcription factor GHF1, which correlates with lower levels of GH promoter activity in the infected cells. This effect of LCMV infection on GHF1 is likely to be the cause of the GH syndrome which is exhibited by C3H mice persistently infected with LCMV.

N 408 INTERACTIONS OF A MUTANT INFLUENZA VIRUS HEMAGGLUTININ PROTEIN WITH COATED PITS MEASURED *IN INTACT CELLS BY LATERAL MOBILITY STUDIES*, Ella Fire[1], Michael G. Roth[2], and Yoav I. Henis[1], [1]Tel Aviv University, Tel Aviv 69978, Israel; [2]University of Texas, Southern Medical Center, Dallas, Texas 75235, USA

Replacement of cysteine at position 543 by tyrosine in the influenza virus hemagglutinin (HA) protein enables the endocytosis of the mutant protein (Tyr 543) through coated pits (Lazarovits, J. and M.G. Roth, 1988, Cell 53:743-752). To investigate the interactions between Tyr 543 and coated pits in the plasma membrane of live cells, we performed fluorescence photobleaching recovery measurements comparing the lateral mobilities of Tyr 543 (which enters coated pits) and wild-type HA (HA wt, which is excluded from coated pits), following their expression in CV-1 cells by SV-40 vectors. While both proteins exhibited the same high mobile fractions, the lateral diffusion rate of Tyr 543 was significantly slower than that of HA wt. Incubation of the cells in a sucrose-containing hypertonic medium, a treatment which disperses the membrane-associated coated pits, resulted in similar lateral mobilities for Tyr 543 and HA wt. These findings indicate that the lateral motion of Tyr 543 (but not of HA wt) is inhibited by transient interactions with coated pits (which are essentially immobile on the time scale of the lateral mobility measurements). Acidification of the cytoplasm by prepulsing the cells with NH_4Cl (a treatment which arrests the pinching-off of coated vesicles from the plasma membrane and alters the clathrin lattice morphology) led to immobilization of a significant part of the Tyr 543 molecules, presumably due to their entrapment in coated pits for the entire duration of the lateral mobility measurement. Furthermore, in both untreated and cytosol-acidified cells, the restrictions on Tyr 543 mobility were less pronounced in the cold, suggesting that the interactions with coated pit-associated components are temperature dependent and become weaker at low temperatures.

N 409 THE ROLE OF POLYOMAVIRUS MIDDLE T-ANTIGEN AND A ENHANCER DOMAIN IN INFECTIONS OF MICE. Michele M. Fluck, Julie Wirth, Andrea Amafitano, Larry Martin and Ming Chu Chen. Department of Microbiology, Michigan State University, East Lansing, MI 48823-1101.

We have studied replication of polyomavirus wild type A2 in mice infected as neonates or as adults and have demonstrated an organ-specific and age-dependent pattern of replication. We have also established a correlation between the presence of specific enhancer domains and replication in specific organs. Furthermore, we have shown that, in tissue culture, middle T-antigen, the Py viral oncogene, has a major role in controlling viral DNA replication through the A enhancer domain. Thus, it appears that, in Group I organs (the mammary gland, the skin and the bone), replication is dependent upon the A enhancer domain, requires middle T and occurs in adult as as well as neonate organs. In contrast, in group II organs (the kidney, the liver, and the lung), replication is dependent upon the B enhancer domain, independent of middle T and does not occur in adult in contrast to neonate organs. Taken together, the results suggest that middle T antigen has a major role in viral persistence.

N 410 REPLICATION OF ENTEROVIRUS IN MUSCLE FROM POSTVIRAL FATIGUE SYNDROME PATIENTS

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We previously have demonstrated the presence of enteroviral sequences in the muscle of postviral fatigue syndrome (PFS) patients. The continuing presence of enterovirus in these patients may have been due to either abnormal/mutant virus or host genetic factors. Kandolf et al demonstrated restricted viral replication in the development of persistent enteroviral infections of mice. In contrast to the one hundred fold excess of viral plus-strand RNA found in acute infections, persistently infected myocardial cells were found to contain equal amounts of plus and minus strands of RNA. Similarly, Cunningham et al, suggested that both strands of enteroviral RNA were present in equal amounts in four out of eight PFS muscle samples.

We examined the relative amounts of plus and minus RNA strands in twenty PFS patients using slot blot hybridisation and complimentary oligonucleotide probes. We detected a higher abundance of positive strand compared to negative strand RNA in all cases. To verify this result, a novel quantitative PCR method was developed to determine relative amounts of template. cDNA was synthesised from equal aliquots of template RNA with either a plus strand or a minus strand primer. These cDNA populations were then amplified with a nested primer set and the products visualised on agarose gels. As previously, we found that the enterovirus RNA was present in much higher levels as positive strand template rather than negative strand template. These results strongly suggest that at least at the level of transcription, the enterovirus present in our PFS patient population was normal. The implications of these results will be discussed.

N 411 DETECTION OF HUMAN CYTOMEGALOVIRUS ANTIGENS BY IMMUNOHISTOCHEMISTRY. Gerhard Jahn[1], Christian Sinzger[1], Hartmut Stöss[2] and Bodo Plachter[1]. [1]Institute for Clinical and Molecular Virology, University of Erlangen-Nürnberg, Germany; [2]Institute for Pathology, University of Erlangen-Nürnberg, Germany.

The mechanism of pathogenesis of human cytomegalovirus (HCMV) is up to now poorly understood. During infection, HCMV nucleic acids have been found in a variety of organs by in-situ hybridization. These investigations, however, could not discriminate between chronic silent, abortive or permissive infection of HCMV. Viral antigen detection in tissue sections can provide information about the state of infection in particular organs of an infected individual. We have characterized a number of monoclonal antibodies that are directed against immediate early, early and late viral antigens. Some of these antibodies can detect their antigen in formalin-fixed and paraffin-embedded tissue sections. The monoclonal antibodies taken for immunohistochemistry are directed against the proteins made from immediate early regions 1 and 2, against the early-late DNA-binding protein p52 and against the late tegument protein pp150. Different cytomegalic and non-cytomegalic cells in a variety of organs were found positive for HCMV antigens.

N 412 PATHOGENESIS OF MOLECULARLY CLONED AVIRULENT MUTANTS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS, F. B. Grieder, N. L. Davis, G. F. Greenwald and R. E. Johnston, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599-7290.

Venezuelan equine encephalitis virus (VEE), an alphavirus, induces a fatal encephalitis in mice. To examine the molecular genetics of VEE pathogenesis, virus stocks differing by a single substitution at either E2 glycoprotein amino acid 76 (glu to lys) or 209 (glu to lys) were generated by site-directed mutagenesis of a cDNA clone of wild-type VEE. Molecularly cloned wild-type VEE caused 100% mortality after inoculation into the left rear footpad (fp) or after intracerebral (ic) inoculation. In contrast, the E2 209 mutant was avirulent by fp inoculation yet gave 100% mortality when administered ic. The E2 76 mutant was attenuated by both fp and ic routes (0% and 20% mortality, respectively). After fp inoculation of either wild-type or E2 209, mice were sacrificed at intervals and examined for distribution of virus, histopathology and immunocytochemistry. Within 6 hr post-inoculation (pi), wild-type replication in the draining lymph node was detected, followed by viremia at 12 hr and replication in lymphoid and other organs by 18 hr. Viremia and peripheral organ involvement decreased to low levels by 3-4 d pi. Invasion of the brain occurred 2-3 d pi, and high titer replication in the brain continued through the end of the experiment (6 d). Appearance of the E2 209 mutant was delayed, and its organ distribution was restricted. E2 209 was first detected at 24 hr pi in the draining lymph node. In some animals, at 24 and 48 hr pi, virus was detected in draining and opposing lymph nodes and in spleen but in no other organ systems or serum. Assessment of virulence and RNA sequence of isolates from E2 209 infected mice demonstrated that spread beyond the draining lymph node was accompanied by either same-site or second-site reversion to a more virulent phenotype. Similar experiments with the E2 76 mutant are in progress. Production of infectious E2 209 and E2 76 virions was restricted in cultured peritoneal macrophages relative to wild-type. The correlation between attenuation in mice and growth restriction in cultured macrophages is being examined using the E2 209 revertants.

N 413 CHARACTERIZATION OF THE $\alpha\beta$ T CELL RESPONSE TO PARAINFLUENZA (SENDAI) VIRUS IN THE RESPIRATORY TRACT OF MICE, Jacqueline M. Katz[1], Samuel Hou[2], and Peter C. Doherty[2], Departments of [1]Virology/Molecular Biology and [2]Immunology, St. Jude Children's Research Hospital, Memphis, TN 38101

In order to better understand the requirements for effective immunity to parainfluenza type 1 (PIV-1) viruses, the role and interdependence of $CD8^+$ and $CD4^+$ $\alpha\beta$ T cells in the response of mice acutely infected with Sendai virus has been examined. In immunologically intact C57BL/6 mice an enrichment of $CD8^+$ virus-specific cytotoxic T lymphocyte (CTL) effectors in the lung coincided with the elimination of the virus from the lung by day 10 post-infection. This CTL effector populations recognized predominantly an epitope derived from the C-terminus of Sendai virus nucleoprotein (NP) in assocation with the class I MHC K^b glycoprotein. *In vivo* depletion of $CD4^+$ T cells by treatment with specific mAbs prior to infection of mice with Sendai virus, did not affect appreciably either the recruitment of $CD8^+$ T cells to the infected lung or their development into specific cytotoxic effectors at this site. In contrast, *in vivo* depletion of the $CD8^+$ T cell subset delayed substantially the clearance of virus from the lung. Nevertheless, the majority of mice recovered completely from infection. When transgenic mice homozygous (-/-) for β2 microglobulin (β 2-m) gene disruption, which lack both class I MHC glycoproteins and mature $CD8^+$ $\alpha\beta$ T cells, were infected with Sendai virus, they too cleared virus from their lungs, over a similar time period to the mice depleted of $CD8^+$ T cells *in vivo*. Class II but not class I MHC-restricted virus-specific cytotoxic effectors were detected in the MLN of the homozygous (-/-) β 2-m transgenics which had been restimulated *in vitro*. Investigations into the nature of the viral epitopes recognized by class II MHC-restricted CTL as well as Sendai virus-specific class I MHC-restricted CTL from mice of different H-2 haplotypes are currently underway. These studies will provide insight into the critical elements of cell-mediated immunity to PIV-1 necessary for the rational design of vaccines.

N 414 **INTRACELLULAR TRANSPORT OF INFLUENZA VIRUS RIBONUCLEOPROTEINS,** Iris Kemler, Kelsey Martin and Ari Helenius, Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, USA

Influenza virus, after binding to the cell surface, is internalized and delivered to endosomes. The low pH of this compartment results in acidification of the virions, probably mediated by ion flow through channels in the viral envelope composed of the M2 protein. The low pH also triggers a membrane fusion reaction, and penetration into the cytoplasm. Since transcription and replication of the viral genome, consisting of eight separate RNA segments coated with the viral nucleoprotein (NP) and forming ribonucleoproteins (vRNPs), occurs in the nucleus, the vRNPs have to migrate to this compartment. We have recently shown that once the vRNPs are released into the cytoplasm they loose the matrix protein (M1) and rapidly enter the nucleus through the nuclear pores by an active process (Martin and Helenius, J. Virol. 65:232 (1991)). Subsequently, in order to form new vRNPs, newly synthesized NP molecules enter the nucleus where they associate with viral RNAs. The newly formed vRNPs have now to leave the nucleus, and we previously presented evidence that newly synthesized M1 protein enters the nucleus and associates with vRNPs to directly promote their nuclear exit (Martin and Helenius, Cell 67:117 (1991)). Therefore, M1 seems to modulate the directionality of vRNP transport into and out of the nucleus. Experiments are in progress which further investigate the mechanism of the two-way-traffic of influenza vRNPs across the nuclear membrane *in vivo* and *in vitro*, as well as the effect of the drug amantadine on virion acidification in the endosome.

N 415 CLONING OF HSV-SPECIFIC CD4+ AND CD8+ T CELLS AND NK-LIKE CELLS DIRECTLY FROM A HERPETIC LESION, David M. Koelle, Hiyam Abbo, Michael Remington, Lawrence Corey, Departments of Medicine and Laboratory Medicine, University of Washington, Seattle, WA 98105.

Cells with CD4, CD8, and NK markers infiltrate herpetic lesions, but their relative functional importance in recovery from human disease is not known. CD4+ and CD8+ T cell clones with CTL activity against HSV-infected target cells and clones with NK-like activity against HSV-infected target cells have previously been recovered from peripheral blood, however, their direct role in clearing mucocutaneous HSV is unknown. With gentle unroofing and scraping of vesicles from a patient with frequent buttock recurrences, 420,000 mononuclear cells were recovered. Cells were cloned without secondary *in vitro* stimulation with HSV. Of 103 clones, 60 were screened in ^{51}Cr release assays, five had reproducible cytotoxicity against autologous HSV-infected B-LCL but not uninfected autologous B-LCL, and two have been characterized in detail. One is CD3+, CD8+, and negative for CD4, TCR gamma-delta, and CD16. This clone selectively lysis HSV-2 but not HSV-1 infected autologous B-LCL. HLA class I-mismatched, HSV-2 infected B-LCL are not lysed unless they share possess the B45 allele in common with the patient. Lysis is blocked 97% by mAb against HLA class I but only 40% by mAb against HLA class II and is unaffected by inclusion of acyclovir during target cell infection and assay. The second clone is CD2+ and negative for CD16, CD3, CD4, CD8, and TCR gamma-delta. It lyses autologous and 3 separate HLA class I and II mismatched LCL equally well after HSV-2 infection, as well as K562 cells. Two of 22 clones screened in proliferative assays incorporate ^3H-thymidine in response to UV-treated HSV-2 with stimulation indices of 38 and 90. Both are CD3+, CD4+, and CD8 negative. This is the first direct demonstration of HSV-specific CD8+ and CD4+ lymphocytes in a herpetic lesion and suggests that several types of lymphoid effector cells are recruited to the site of HSV recurrences.

N 416 CORONAVIRUS INDUCED ENCEPHALOMYELITIS IN THE LEWIS RAT: $CD4^+$ T CELLS SPECIFIC FOR VIRAL PROTEINS PROTECT FROM DISEASE IN THE ABSENCE OF $CD8^+$ T CELLS.

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Coronavirus MHV-JHM infection of mice and rats causes a demyelinating disease of the central nervous system. The outcome of infection depends on factors such as age, genetic background, and the virus isolate used. In Lewis rats two forms of disease have been described. The acute form (AE) is a rapidly progressing encephalomyelitis and leads to the death of the animal within 6-15 days after infection. The subacute form (SDE) is a paralytic disease characterized by selective loss of myelin in the white matter of the CNS and inflammatory lesions consisting of T cells, monocytes, and macrophages. T cell responses against viral antigen and myelin have been observed in SDE.

To study the cellular immune response to the virus we established T cell lines against two major viral proteins (spike-protein and nucleocapsid). The T cells were of the CD4 subtype and produced gamma-interferon and interleukin 2. Adoptive transfer of $5x10^6$ T cells into young, immunologically immature rats protected the animals from lethal disease and led to rapid clearence of virus from the brain. Furthermore we could show in additional experiments, that this process occurred in the abscence of neutralizing antiviral antibodies and after depletion of $CD8^+$ cytotoxic T cells.

N 417 EFFECTS OF PROTEIN KINASE INHIBITORS ON VESICULAR STOMATITIS VIRUS TRANSCRIPTION IN VIVO AND IN VITRO.

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Staurosporine, an inhibitor of protein kinase C, and genistein, an inhibitor of tyrosine protein kinases, both inhibited plaque production in BHK cells by vesicular stomatitis virus (VSV) by >90%. Inhibition occurred at an early step in infection, since primary transcription in these cells ("in vivo") was virtually abolished by staurosporine (<10 uM) or genistein (<50 uM). Transcription by nucleocapsids prepared from purified VS virions ("in vitro") was unaffected by either inhibitor, although staurosporine abolished the phosphorylation of L protein completely, and partially inhibited phosphorylation of NS protein. Since the levels of inhibition of primary transcription in vivo were the same whether the inhibitors were added at t-0 or at t-1h after infection, inhibition did not occur at the uncoating step. However, staurosporine did inhibit internalization of VSV by about 35%. Addition of high speed uninfected BHK cytosol to the in vitro transcription reaction failed to confer inhibition by either drug. It appears that either: (i) in vivo transcription requires a phosphorylated protein that is present in an active form in vitro, but is lost or modified after uncoating; or (ii) there is an intracellular inhibitor of transcription, absent from the in vitro preparation, that is inactivated by phosphorylation in vivo.

N 418 GENERATION OF TRANSGENIC MICE CARRYING THE HIV-1 NEF GENE UNDER CONTROL OF A T-CELL SPECIFIC PROMOTOR.

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The main objective of this project is to investigate in vivo the physiological consequences of the HIV-1 NEF protein expression on T-cell activation and maturation. As a membrane-associated putative GTP-binding protein NEF may influence the signal transduction pathways utilized in T-cells. To study the effects of NEF production on T-cell function we chose NEF transgenic mice as an in vivo model system. In order to direct NEF expression to lymphoid cells, the normal taget cells of HIV, the HIV-1 NEF gene was put under control of a mouse TCR β-chain promotor-enhancer.

16 transgenic lines with different copy numbers were generated by standard microinjection techniques. The founders showed no evident change in T-cell phenotype as judged by blood analysis. Breeding resulted in 10 transmissive lines which produced transgenic offspring. Two lines showed reduced CD4 expression in thymus and lymphnodes. A detailed analysis of thymocyte and peripheral T-cell subsets and analysis for tissue specific NEF expression will be presented.

N 419 Recombinant vaccinia viruses expressing the flavivirus prM and E genes induce the synthesis of flavivirus-like particles.

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Recombinant vaccinia viruses expressing the prM and E genes of Japanese encephalitis virus (JEV) induce the synthesis of extracellular particles. These particles contain the membrane (M) and envelope (E) structural proteins of JEV embedded in a lipid envelope, and behave like empty viral envelopes in sucrose density gradients. The particles are devoid of flavivirus nucleic acid and are immunogenic in mice. The sucrose gradient-purified particles have a diameter of 20 nm, and can be labeled with a monoclonal antibody to the E protein using immuno-electron microscopy. The particles are retained by infected cells in an immature form, and then slowly released into the extracellular fluid in a mature form. Their release is further retarded by the co-synthesis of the JEV nonstructural glycoprotein NS1, and is inhibited completely by co-synthesis of the flavivirus capsid protein C. The properties of these recombinants are discussed in terms of the mechanism of flavivirus virion assembly and maturation, and flavivirus vaccine production.

N 420 PERSISTENT ENTEROVIRAL INFECTIONS AND THE RELATION TO VIRAL TROPISM

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Evidence is accumulating that enteroviruses may persist in chronic diseases as polymyositis and ideopathic dilated cardiomyopathy. However, it is still unknown how the virus can persist and what are the underlying causes of viral tropism.
To study the role of enterovirus persistence in the pathogenesis of these diseases, and to investigate enteroviral tropism, we use an experimentally induced polymyositis model in newborn outbred Swiss mice infected with a myotropic Coxsackievirus B1 variant. Although the virus can be isolated from different organs and tissues in the acute phase of the infection, it can no longer be isolated in the chronic stage of the disease. However, using an enteroviral specific PCR assay, the viral RNA could still be detected in the skeletal muscles and the brains in this chronic phase. The presence of enteroviral RNA in muscle indicates enteroviral persistence with a preference for skeletal muscle.
We are currently cloning the complete viral RNA genome of this myotropic Coxsackievirus B1 variant to generate an infectious cDNA clone for the study of viral persistence and tropism in this model system.

N 421 MURINE HERPESVIRUS-68 ESTABLISHES LATENT INFECTION IN B CELLS *IN VITRO* AND *IN VIVO*,

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Murine herpes virus-68 is a natural pathogen of small, free living rodents. The virus is genetically related to the gamma herpesviruses EB virus and HVS. Administration of virus intranasally results in infection of the lung, with virus detected in alveolar epithelial cells and in mononuclear cells. As with other members of this virus group, MHV-68 was recovered from lymphoid tissue during the acute and latent periods of infection. In the spleen of latently infected mice, virus was detected in 1 in 10^5 cells using a co-cultivation technique. The cell harbouring the latent virus was identified as a B lymphocyte. The tropism of this virus for B lymphocytes was further highlighted in vitro with a persistent/latent infection established in a myeloma (B cell) cell line, but not in a thymoma (T cell) cell line. The viral genome exists in both linear and circular forms during a productive infection, but only the circular form is found in latently infected myeloma cells. These data highlight the molecular and pathogenetic similarities between MHV-68 and EBV.

N 422 THE HUMAN CYTOMEGALOVIRUS IMMEDIATE EARLY PROMOTER TARGETS EXPRESSION TO THE BRAIN, SALIVARY GLAND, PANCREAS, INTESTINE, AND TESTES OF TRANSGENIC MICE, Jay A. Nelson[1], Edgardo Baracchini[1], Susan Gould[1], Peter Ghazal[1], Richard Stenberg[2] and Clayton Wiley[3], [1]The Scripps Research Institute, La Jolla, CA 92037, [2]Eastern Virginia Medical School, Norfolk, VA 23501, and [3]University of California San Diego, La Jolla, CA 92037.
We have established 6 transgenic lines containing the β galactosidase (β-gal) gene regulated by the human cytomegalovirus (HCMV) major immediate early promoter (MIEP). By immunohistochemical analyses of mouse tissues for the presence of β-gal, we find that the pattern of expression in 3 mouse lines is similar to human tissues naturally infected by HCMV *in vivo*. We have morphologically identified the cells in these tissues which include 1) oligodendrocytes in the brain, 2) salivary gland duct, 3) alpha or delta cells in the Islets of Langerhans in the pancreas, 4) the differentiated epithelium of the jejunum in the gut, and 5) Leydig cells in the testes. The prediction that optimal activity of the MIEP is a critical determinant in the outcome of a productive HCMV infection in the human appears to correlate with our transgenic mouse data. Thus, these transgenic mice offer an ideal model to study tissue specific expression and pathogenic mechanisms of HCMV *in vivo*.

N 423 REPLICATION OF HEPATITIS DELTA VIRUS RNA IN CULTURED Cos AND HeLa CELLS

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The Rockefeller University, New York, NY 10021

In order to study replication of hepatitis delta virus (HDV) RNA we have developed an in vivo transfection assay. Plasmid DNAs containing HDV sequences cloned downstream of the SV40 promoter producing both genomic and antigenomic polarity transcripts were introduced in parallel into Cos and HeLa cells. Replication of RNA genome at various time points was detected by RNase protection assay. In addition, HeLa cell lines stably expressing delta antigen were established and tested for replication of wild type as well as several mutant HDV sequences. Cos cells supported replication of wild type delta virus irrespective of orientation of the HDV sequences. In contrast, transfection of HeLa cells with plasmid DNA expressing antigenomic polarity resulted in replication of wild type delta RNA only in cell lines that stably express delta antigen. Thus, HeLa cell line provides a better system to study specific requirements for HDV RNA replication.

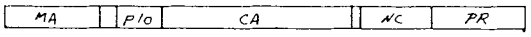
Cotransfection into Cos cells of plasmid DNAs producing delta antigen and a mutant virus containing an out-of-frame mutation in the antigen coding region resulted in replication of the virus (Kuo et. al., 1989. J.Virol. 63:1945-50). In contrast, similar experiments using HeLa cell lines stably expressing delta antigen did not result in HDV replication. Further experiments will be needed to clarify the role of delta antigen in the replication process.

N 424 SUBCELLULAR LOCALIZATION OF TRUNCATED AVIAN LEUKOSIS VIRUS (ALV) GAG PROTEINS, Laurel E. Southard and Volker M. Vogt, Department of Biochemistry, Molecular and Cell Biology, Cornell Univ, Ithaca, N.Y 14853.

Assembly and budding of avian leukosis viruses (ALV) occurs at the plasma membrane of infected cells. The gag polyprotein precursor (Pr76) is translated on free polysomes and is targeted by an unknown mechanism to the plasma membrane. There it associates with the envelope glycoproteins and virion RNA to initiate formation of an infectious virus particle. Previous studies have shown that gag polyprotein alone is able to assemble into a particle and bud from the cell. In most other retroviral systems, gag precursor polypeptides are myristoylated at an N-terminal glycine, which is part of the MA (matrix) protein domain, and this modification is necessary for targeting and final assembly. By contrast, the ALV gag precursor is not myristoylated; the initiating methionine is retained and acetylated. The mechanism of targeting thus may differ from that in other systems. Nevertheless, as shown by our previous lipid-protein cross linking studies, in ALV as in MULV the N-terminal stretch of ca. 40 amino acids of the MA protein is closely apposed to the lipid bilayer in mature virions(JV 52:145).

We are attempting to identify the sequences in gag that are required for association of Pr76 with the plasma membrane. Our approach is to analyze the subcellular localization of partially deleted gag proteins expressed in quail cell lines. The cells are lysed in hypotonic buffer, and the resulting extracts fractionated by centrifugation into crude nuclei, crude membranes and cytosol. Analysis of gag protein is performed by Western blotting with specific antisera. In some cases, the crude membranes are fractionated further by floating in sucrose gradients. We have established that a protein encoding the entire MA domain remains entirely in the cytosol, as does a naturally occurring deleted gag protein that includes MA, and part of p10. A fraction of all proteins truncated past the nucleocapsid domain sediment with crude membranes, float up in sucrose gradients and form insoluble intracellular aggregates.

Immunofluorescence (IF) analysis of the subcellular localizations of these truncated proteins shows that many of the proteins are associated with cytoplasmic vesicles. This suggests that gag proteins may travel to the plasma membrane on exocytic vesicles. Hansen, et al.(JV64:5306) using myristoylated MuLV constructs have obtained similar IF results and have further shown that monensin inhibits transport of gag proteins out of the cell We are now examining if non-myristoylated avian gag molecules are transported to the plasma membrane by a similar mechanism.



N 425 DEFINING AND MAPPING THE CANINE AND FELINE HOST RANGES OF FELINE PANLEUKOPENIA VIRUS AND CANINE PARVOVIRUS.

Uwe Truyen* and Colin R. Parrish, J.A. Baker Institute for Animal Health, N.Y. State College of Veterinary Medicine, Cornell University, Ithaca, New York, 14853.

Canine Parvovirus (CPV) and Feline Panleukopenia Virus (FPV) are natural variants, differing in <1% of their DNA sequences, but having diverse host ranges. Here we define the *in vivo* and *in vitro* host ranges of both viruses.

Inoculation of permanent or primary canine or feline cell lines showed that FPV replicated in all the feline cell lines, but none of the canine cells. CPV replicated in most canine and feline cell lines. Mitogen stimulated canine and feline peripheral blood lymphocytes (PBL) show the same *in vitro* susceptibility.

In vivo host ranges were examined by inoculation of dogs and cats. FPV replicated to high titers in the canine thymus after intranasal or intravenous inoculation, but little replication was detected in other canine tissues in which CPV replicates (e.g., mesenteric lymphnodes and small intestine). FPV inoculated dogs also show a PBL-associated viremia, although canine PBL stimulated *in vitro* are not permissive.

The feline host range is being mapped by viral recombination analysis to genetically define the specific determinants of the host ranges. Together with the previously defined canine host range determinants of these viruses, the feline-specific determinants will be defined. The host-specific mechanisms of virus restriction are being examined by identifying the target cells in the homologous and heterologous hosts - e.g. to define the thymocyte subsets which replicate the virus. This will allow the evolution and pathogenesis of FPV and CPV to be defined.

Late Abstracts

Characterization and Comparison of the Binding Site on CD4 for gp120 Proteins of Divergent HIV-1 and HIV-2 Isolates. M. Chaikin, K. Deen, P. Tsui, P. Ryan, D. Krawczyk, A. Truneh, M. Rosenberg and R. Sweet. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19004.

The design of small molecule antagonists of the interaction of gp120 with CD4 is predicated on the molecular definition of this binding site. The success of these molecules may ultimately depend on the elucidation of a universal binding site on CD4 by different isolates of HIV-1 and HIV-2. Previous studies localized the primary determinants of the gp120 binding site on CD4 to residues 40-60 within the first extracellular domain (D1) using HIV-1 prototypic viruses. Overlay of the recent mutational data onto the structure of the D1D2 domains of CD4, solved in collaboration with the laboratory of Wayne Hendrickson, presented a topological puzzle in which putative components of the gp120 binding site were widely separated in space. Against this background, we introduced 60 substitution mutations into the D1 domain of soluble CD4 and quantitatively assessed their effect on recognition of gp120 from the 3B/BH10 isolate and of several α-CD4 antibodies. Locally disruptive mutants with decreased, increased, and unaltered binding were observed which defined the closely apposed side chains of phe43 and arg59 as probable contact sites.

The envelope glycoproteins of different HIV-1 and HIV-2 isolates vary considerably in their affinity for CD4. We have used a panel of our soluble CD4 mutants to further define these affinity differences at a molecular level. While although the envelope proteins of different HIV-1 and HIV-2 isolates share a common CD4 binding site, there are a number of differences in the fine specificity of this interaction.

CLEAVAGE OF VP1 OF SOME ENTEROVIRUSES RESULTS IN ALTERED EARLY VIRUS-CELL INTERACTIONS, Tapani Hovi, Merja Roivainen, Liisa Piirainen, Antero Airaksinen, Enterovirus Laboratory and Molecular Biology Unit, National Public Health Institute, Helsinki, Finland

Most strains of type 2 and 3 polioviruses are known to be sensitive to host proteolytic enzymes, trypsin in intestinal fluid and plasmin. Protease-cleaved virus retains its infectivity while the antigenic properties are drastically altered. The cleavage takes place in vivo during poliovirus infection in man and the cleaved virus markedly contributes to the immunogenicity of the infection. We have now studied the rate of uncoating by analyzing the rate of disintegration of radiolabeled cell-associated virions by velocity sedimentation on sucrose gradients. Poliovirus type 3/Saukett showed a very slow rate of disintegration with most of the label sedimenting at the rate of an infectious virus still after 90 min. In contrast, most of cell-associated type 1/Mahoney label showed an altered rate of sedimentation already at 30 min. Trypsin-treatment of type 3/Saukett virus remarkably enhanced the rate of cell-induced virion disintegration with more than 50 % of virions being altered by 60 min. A similar trypsin-treatment-induced enhancement of the apparent rate of uncoating was seen with poliovirus type 3/Leon. Poliovirus type 1/Sabin and type 2/MEF, which are cleaved by trypsin not unlike type 3/Saukett, had a Mahoney-like fast rate of disintegration that was not further enhanced by trypsin treatment. These results may explain our previous results revealing a serotype 3 specific effect of trypsin treatment on one step growth curve of poliovirus: In spite of identical theoretical multiplicity of infection the trypsin-cleaved type 3 polioviruses regularly had a lower nadir of the curve than the corresponding intact virus. Recently we observed that VP1 of another enterovirus, coxsackievirus A9 (CAV-9) is also cleaved by intestinal trypsin. However, the site of cleavage is close to the C-terminus of the protein rather than the BC-loop. This results in an enhanced rate of host cell-induced uncoating like in the case of type 3 polioviruses. In addition, the receptor specificity of the virus is altered after cleavage (M. Roivainen, T. Hyypiä, L. Piirainen, N. Kalkkinen, G. Stanway, T. Hovi, J. Virol.65:4735-4740, 1991).

THE IMMUNOSUPPRESSIVE PEPTIDE OF HIV-1: FUNCTIONAL DOMAINS AND INTERFERENCE WITH HIV INFECTION OR REPLICATION, Reinhard Kurth, Joachim Denner, Stephen Norley, Paul-Ehrlich-Institut, D-6070 Langen, Fed. Rep. of Germany

The immunosuppressive peptide of HIV-1 (ISU-peptide), a 17-mer peptide corresponding to the amino acid domain 583-599 of the transmembrane glycoprotein gp41, exhibits sequence homology to the highly conserved immunosuppressive peptide of type B, C and D retroviruses. Both peptides have similar properties: They inhibit in vitro T-cell mitogen and lymphokine-induced lymphocyte proliferation, they are interspecies-reactive, they have to be conjugated to a carrier protein in order to be immunosuppressive and their N-terminal octamers represent the minimal immunosuppressive domain.

Most HIV-infected individuals produce antibodies against an epitope located at the C-terminal end of this peptide and the number of responders and the antibody titres decrease during progression of AIDS. Both the decrease of antibody response and the increase of the titre of HIV-1 during progression of AIDS would lead to an increasing concentration of the immunosuppressive domain which might contribute to the development of AIDS.

In addition to its immunosuppressive activity, the ISU-peptide conjugated to a carrier protein inhibited the cytopathic effect of HIV-1 on human MT4 cells, suggesting that it may interfere with virus entry by binding to the postulated secondary receptor for HIV-1.

EBV INFECTION IN HIV-ASSOCIATED HAIRY LEUKOPLAKIA Nancy Raab-Traub[1], Dennis M. Walling[1], Rachel McCoy[1], John W. Sixbey[2], and Lionel Resnick[3]. [1]Departments of Microbiology and Immunology and Medicine, University of North Carolina, Chapel Hill, N.C. 27599-7295, [2]Departments of Infectious Diseases and Virology/Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, 38101, [3]Department of Research, Mount Sinai Medical Center, Miami Beach, FL, 33140

Hairy leukoplakia (HLP), a condition which develops in individuals who are infected with HIV, is a permissive EBV infection. In HLP, all classes of EBV replicative mRNAs are expressed with multiple transcripts detected from the sequences which encode the EBV replication activator gene, ZEBRA. Two forms of mRNA which can encode the latent membrane protein (LMP) mRNA are detected, however, the small nuclear RNAs, the EBERs, are not transcribed. These data indicate that the EBERs do not function during viral replication and suggest that EBER expression may be considered a marker for latent infection. In HLP, multiple strains of EBV can be detected in a single lesion without detectable episomal DNA, suggesting that exogenous infection or superinfection is a common occurrence. The simultaneous presence of both EBV-1 and EBV-2 was demonstrated by Southern blot analysis and polymerase chain reaction assay. The viral strains in HLP differ in physically separate lesions and change over time in a dynamic, evolving process. The replicating viral genomes recombine in and around the EBNA-2 coding sequence, generating new strains that are defective or deleted for the EBNA-2 gene. The EBNA-2 defective strains apparently replicate with high efficiency and in some cases predominate. These data suggest that recombination during viral replication generates novel strains which may replicate with increased efficiency.

ENTEROVIRUS-RECEPTOR INTERACTIONS, Merja Roivainen[1], Timo Hyypiä[2], Liisa Piirainen[1], Sirpa Jalkanen[2], Tuuli Rysä[1], Jyrki Heino[2], Leif Laaksonen[3], Tapani Hovi[1], [1]National Public Health Institute, Helsinki, [2]University of Turku, Turku, [3]VTT Biotechnology Laboratory, Espoo, Finland

The arginine-glycine-aspartic acid (RGD) motif close to the C-terminal end of the VP1 protein of coxsackievirus A9 (CAV-9) is needed for attachment of the virus to its cellular receptor as shown by a blocking test using synthetic oligopeptides Exposure of CAV-9 to intestinal trypsin resulted in selective cleavage of a small fragment, including the RGD sequence, from the C-terminal end of VP1. The cleaved virus was still infectious and in this case the RGD containing oligopeptides did not interfere with infectivity. This indicates that cleaved virus, unlike intact CAV-9, was able to infect the cells through a mechanism independent from RGD (M. Roivainen, T. Hyypiä, L. Piirainen, N. Kalkkinen, G. Stanway, T. Hovi, J. Virol. 65:4735,1991). The bivalent receptor specificity was also demonstrated by using putative receptor antibodies. A monoclonal antibody reacting with human synovial membrane cells effectively protected GMK cells from infection by intact CAV-9 but not at all from that by trypsin-cleaved virus. A rabbit antiserum to integrin component B_1 protected the cells from both virus preparations but more efficiently from infection by the intact virus. The binding of CAV-9 to GMK cells was blocked by echovirus 22 and coxsackievirus B4 (CBV-4). Studies are in progress to identify CAV-9 binding proteins in host cell lysates. The location of the RGD-containing C-terminus of VP1 in the structure of CAV-9 virion is not known. The canyon surrounding the 5'-fold vertices in the 3-dimensional crystallographic model is a likely receptor binding site of rhinoviruses but in the case of enteroviruses there is no direct evidence for this. Furthermore, the parts of the capsid proteins presenting for receptor interaction are difficult to define, as we have evidence that amino acids exposed at the virion surface in solution may partially differ from those seen in the crystal structure. Synthetic peptide-induced antibodies to a "hidden" immunodominant region in the N-terminal part of VP1 of poliovirus readily precipitate radiolabelled virions. The corresponding sequence in CAV-9 shows great homology with that of polioviruses and, accordingly, these peptide antibodies were able to precipitate radiolabelled CAV-9 as well.

A VIRAL PROTON CHANNEL

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It has been shown that isolated nucleocapsids of Semliki Forest virus (SFV) contract upon low pH exposure (Soederlund et.al., Virology 47, 753-760, 1972). This contraction of the nucleocapsids has been used as an indicator to demonstrate that the spike proteins of SFV can translocate protons into the interior of the virus particle upon low pH (5.8) exposure. Spikeless virus particles obtained after bromelain digestion at neutral pH, which were used as a control, did not translocate protons. Bromelain digestion of SFV renders particles which contain only the anchor sequences of the envelope proteins, as confirmed by protein sequence analysis. This implies that the ectodomain of the spike plays a crucial role for the proton translocation. Furthermore, digestion of SFV which has been exposed to pH 5.8 with bromelain yields an additional peptide of apparent molecular weight of 17kD associated with the virus envelope. Thus, it is tempting to speculate that part of the ectodomain of the viral spike, folds back into the viral envelope to function as proton channel. A model based on these data and on theoretical considerations as well as more recent data will be presented and a possible role of the described process for SFV-induced fusion from within and virus-endosome fusion and uncoating of the viral RNA will be discussed.