MOLECULAR BIOLOGY OF HUMAN PATHOGENIC VIRUSES

Organizers: Arnold Levine and Peter Howley

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Molecular Biology of Human Pathogenic Viruses

Herpes Viruses

M 001 EPSTEIN-BARR VIRUS REPLICATION AND EXPRESSION IN VIVO, Nancy Raab-Traub, Department of Microbiology & Immunology, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC, 27599-7295

To characterize EBV pathogenesis molecularly, EBV DNA structure and expression has been analyzed in infected tissues. Nasopharyngeal carcinoma (NPC) and lymphoproliferative conditions are predominantly latently infected, although low levels of EBV replication have been detected in some specimens. In contrast, oral hairy leukoplakia (HLP), a condition which develops in individuals who are infected with HIV, is a permissive EBV 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. In HLP, the latent membrane protein (LMP) mRNA and all classes of EBV replicative mRNAs are expressed; however, the small nuclear RNAs, the EBERs, are not detected. 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.

EBV expression has been analyzed in NPC using cDNA cloning, *in situ* hybridization, and polymerase chain reaction (PCR) amplification of cDNA. The data indicate that the EBER RNAs and LMP mRNA are consistently expressed in all NPC specimens. In addition, cDNAs homologous to the BamHI A fragment, which is not expressed in latently infected lymphoid cell lines, were identified in a cDNA library synthesized from the C15 NPC, which has been established in nude mice. Northern blot hybridizations, PCR analysis, and hybridization *in situ* identified transcription from BamHI A in all specimens of NPC examined and in carcinoma of the parotid gland. Transcription of the EBERs, LMP, and EBNA2 was also detected in non-Hodgkins lymphoma; however, transcription from BamHI A was not detected in the lymphoma specimens, suggesting that BamHI A may be preferentially transcribed in latently infected malignant epithelial tissues.

Sequence analysis of the cDNAs revealed a previously undescribed open reading frame (BARF0). The BamHI A cDNA was transcribed and translated *in vitro*. The *in vitro* translation products were precipitable with serum from patients with NPC, indicating that the putative protein product of BARF0 is antigenic and expressed *in vivo*.

These comparisons of EBV expression reveal that expression of the EBER RNAs distinguishes latent and permissive infections. Moreover, viral expression is distinct in infected epithelial and lymphoid tissues, in that the BARF0 open reading frame is consistently expressed in epithelial malignancies but not in lymphoid tissue. Lymphoid proliferations are characterized by expression of the EBERs, LMP and EBNA2.

M 002 THE MOLECULAR BASIS OF HERPES SIMPLEX VIRUSES (HSV) INFECTIONS
Bernard Roizman, The University of Chicago, Chicago Il 60637 USA

The HSV genome consists of >150 Kbp arranged in two stretches of quasi unique sequences each flanked inverted repeats. After infection, viral DNA circularizes. The HSV genome contains at least 74 open reading frames encoding 71 polypeptides including 8 surface glycoproteins, and numerous enzymes and other proteins involved in nucleic acid metabolism and post translational processing of proteins. Viral gene expression is tightly regulated in a cascade fashion by several viral trans-activating factors at least two of which act by interacting with cis-acting sites in viral DNA. Viral DNA is replicated as a rolling circle by proteins specified by the virus. The capsids containing DNA are enveloped at the nuclear membrane and are transported to extracellular space. The functional complexity of HSV genomes is staggering. By means of genetic engineering of deletion mutants, it has been established that the HSV genes form (a) a minimal essential set required for DNA replication, packaging and virion maturation in restricted repertory of cells, and (b) a supplementary essential set which provides precursors and substrates that may be missing in some tissue cells or factors which make the process of replication more efficient. A fringe benefit of the analyses of HSV gene functions by genetic engineering of novel genomes is the development of attenuated, immunogenic viruses for potential use in prevention of diseases caused by HSV.

In humans and in experimental animal models, HSV multiplies at the portal of entry and then infects and establishes a latent infection in sensory neurons. The latent virus becomes periodically activated and is transported to a site at or near the portal of entry where it can cause lesions. The manifestation of human infections are first infections of the mouth and genitals, recurrent lesions at the portal of entry, in the cornea and CNS, adult herpes encephalitis and devastating infections of the newborn acquired during birth. Studies on deletion mutants have led to the identification of several genes whose products are required for viral replication in the central nervous system. Several other genes which are required for the initiation of replication of latent virus have also been identified. As yet no viral gene or sequence has been demonstrated to be essential for the establishment of the latent state. The specific question as to why viral gene functions related to replication on not expressed routinely in sensory neurons remains unanswered.

M 003 IMMORTALIZING FUNCTIONS OF EPSTEIN-BARR VIRUS, Bill Sugden, Jennifer Martin, and Tim Middleton, McArdle Laboratory, University of Wisconsin, Madison, WI 53706

The efficient immortalization of human B-lymphocytes by Epstein-Barr virus (EBV) is presumably accomplished by several viral genes. One of them, the E.B. nuclear antigen-2 (EBNA-2) has been shown genetically to be required for immortalization. Two additional viral genes likely to be required include that encoding the latent membrane protein (LMP) and EBNA-1. We have found that LMP shares many biochemical properties with receptors for growth-factors. It homes to the plasma membrane, turns over rapidly, its turnover is inhibited by treatment of cells with cycloheximide and is probably preceded by its internalization from the cell surface. Functional mutants of LMP have these properties; non-functional ones do not. We suggest that these properties support a model in which LMP is a receptor-like protein or affects the function of a cellular receptor to alter the growth characteristics of cells in which it is expressed.

It is known that EBNA-1 both activates transcription and mediates plasmid replication by binding specifically to elements within *oriP*, the origin of plasmid replication of EBV. In an attempt to dissect EBNA-1's contributions to these two functions we constructed a chimeric protein that consists of the estrogen receptor with the DNA binding domain of EBNA-1 in place of its own. This protein activates the enhancer within *oriP* in the presence of its ligand but does not support the replication of *oriP*. We suggest that EBNA-1 contributes an activity to DNA replication distinct from those required for transcriptional activation.

Hepatitis B and Delta Agent

M 004 MULTIPLE PATHWAYS OF HEPATITIS B VIRUS PATHOGENESIS. Francis V. Chisari, Takashi Moriyama and Patrick N. Gilles, Division of Experimental Pathology, Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, CA 92037

The mechanisms responsible for viral clearance and hepatocellular injury in hepatitis B virus (HBV) infection are not clearly understood, although it is generally believed that a cellular immune response to one or more HBV encoded antigens plays an important role in these processes. Using transgenic mice which express some or all of the gene products of the HBV genome we have identified multiple, direct and indirect, pathways for clearance of the HBV positive hepatocyte. First, we have observed liver cell injury after transfer of HBsAg primed spleen cells and cloned, MHC class I restricted, $CD8^+$, cytotoxic T lymphocytes into syngeneic mice that express the HBV envelope antigens. These results suggest that HBsAg is a potential target antigen on the surface of HBV positive hepatocytes and provide the first definitive evidence that a CTL response to a defined subregion of this antigen can cause liver cell injury in vivo. Second, we have shown that HBsAg positive hepatocytes are exquisitely sensitive to the hepatocytotoxic effects of bacterial lipopolysaccharide (LPS), that LPS-induced liver cell injury can be prevented by the prior administration of antibodies to tumor necrosis factor alpha (TNF-alpha) and to gamma interferon (IFN-gamma), and that the effect can be reproduced by the administration of recombinant TNF-alpha and IFN-gamma, but not by recombinant interleukin 1 or interleukin 6. We have also shown that the same cytokines modulate the steady state levels of all of the HBV-encoded RNA transcripts in this model. These results suggest that the release of these cytokines by HBV antigen specific and nonspecific infiltrating inflammatory cells may influence not only the expression of the HBV genome by the hepatocyte, but also may lead to its elimination in an antigen nonspecific manner. Finally, dysregulated overexpression of the HBV large envelope polypeptide by the hepatocyte leads to the production of long, filamentous, nonsecretable HBsAg particles which accumulate in the endoplasmic reticulum, causing it to become hyperplastic, and eventually kill the cell. The prolonged liver cell injury characteristic of this model eventually leads to the development of hepatocellular carcinoma. Preliminary evidence suggests that reactive oxygen intermediates may play a central role in the pathogenesis of hepatocellular injury in this lesion. In aggregate, these results suggest that several mechanisms may be involved in clearance of the HBV-infected hepatocyte and in the pathogenesis of HBV-induced liver disease, they indicate directions for further study of the molecular and cellular basis of the chronic carrier state, and they lay the groundwork for the development of therapeutic strategies to terminate chronic HBV infection and prevent its progression to hepatocellular carcinoma.

M 005 MOLECULAR BIOLOGY OF HEPATITIS B VIRUS AND LIVER CANCER,
Kenichi Matsubara, Institute for Molecular and Cellular Bioilogy, Osaka University, Yamada-oka, Suita, 565 Japan

Hepatitis B virus (HBV), a small DNA virus carrying only 3.2 kb size genome, is a causative agent for human hepatitis. Some 200 million people in the world suffer from this virus, and these people constitute a high risk group for future development of liver cancer. (The risk rate is 100 times higher than normal). We have engaged in elucidating the link between infection of this virus and formation of liver cancer.

For many years since its discovery, HBV does not infected any experimental animals or cultured human cells, rendering its molecular biological study almost impossible. We have developed a hepatoblastoma-derived cell line that has integrated tandemly arranged HBV genome, and showed that this cell line produces and releases virions. Through use of this cell line and related hepatocellular carcinoma-derived cell lines that can be transfected by HBV DNA (but not by virions), much has been learned as to gene expressions, regulations and replication of the HBV genome. In the first part of this talk, I shall briefly summarize the outline of these topics.

We then developed a system using fetal hepatocytes that can be infected by HBV virions. In only few days of infection, some of the HBV DNA is integrated into cellular DNA. The structure of these integrants, as well as those from liver cancers allows us to infer integration process of the virus genome. The replication and transcription origins of HBV genome often appears at the viral-cellular DNA junctions, suggesting that replication intermediates may be the frequently used substrates for the integration reaction. The target cellular site for integration seems not to be unique, but the integration results in insertional and deletion mutageneses. In addition, there seems to be many secondary chromosomal rearrangements. Some of the integrants show transactivation of many cellular genes. These features will be discussed in connection with the role of HBV infection and liver cancer formation.

The later development of cancer may be brought about as a result of continuous selection and enrichment of better growing cells in the hepatocyte population. Mutagenesis by integration may enhance appearance of such cells. We are now attempting to elucidate how many genetic changes can be found in liver cancers, and what fraction of them might be shared in common. Studies along this line, using precancerous cells in addition, may shed light on the major process of hepatocarcinogenesis.

M 006 STRUCTURE AND REPLICATION OF HUMAN HEPATITIS DELTA VIRUS, J. Taylor, M. Chao, S.-Y. Hsieh and W.-S. Ryu, Fox Chase Cancer Center, Philadelphia, PA 19111

Strictly speaking, HDV is not a virus but a subviral satellite. It replicates in patients along with hepatitis B virus, HBV, which provides the envelope for the packaging of the HDV genome. HDV is usually pathogenic in that it increases the liver damage relative to HBV alone, and in a significant fraction of cases, leads to fulminant hepatitis and death. The single-stranded RNA genome of HDV is quite different from those of other animal viruses. The special features include: (i) size (only 1700 nucleotide in length); (ii) conformation (covalently-closed circular); (iii) structure (ability to fold on itself by Watson and Crick pairing to form an unbranched rod-like structure); and (iv) ribozyme activity (ability to undergo in vitro, specific and efficient self-cleavage and self-ligation). Unlike HBV, HDV replicates via RNA-directed RNA synthesis, probably using the host RNA polymerase II. Thus the genomic HDV RNA yields a complementary RNA, referred to as the antigenomic RNA. We now have data indicative of a site on the genomic RNA that is promotor-like, in that it is recognized for the initiation of RNA-directed synthesis of antigenomic RNA. Antigenomic RNA encodes the only known protein of HDV, a 195 amino acid species, designated the delta antigen. This antigen accumulates in the nucleus and is essential for genome replication. It is also present in virions and has RNA binding ability. We have used recombinant protein to investigate the sequence specificity of this RNA binding. Also, we have information on the nature and stoichiometry of the binding that occurs within virus particles. Finally, from cotransfection studies, we have been able to assemble HDV RNA into virions, with the envelope proteins of either HBV or the related woodchuck hepatitis virus.

Mixoviruses-Paramyxoviruses

M 007 STRUCTURE AND INTRACELLULAR TRANSPORT OF VIRAL INTEGRAL MEMBRANE PROTEINS. Robert A. Lamb, Leslie J. Holsinger, Curt M. Horvath, Davis T.W. Ng, Griffith D. Parks, Reay G. Paterson and David A. Simpson. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL. 60208-3500.

The paramyxovirus SV5 encodes three integral membrane proteins, the hemagglutinin-neuraminidase (HN), the fusion protein (F) and a small hydrophobic protein (SH). The influenza A and B viruses each encode a total of three integral membrane proteins, the hemagglutinin (HA), the neuraminidase (NA) and either the small integral membrane protein M_2 (influenza A virus) or NB (influenza B virus). Our laboratory is interested in the basic mechanisms by which these proteins are inserted into the membrane of the endoplasmic reticulum, the rules that control the adoption of their final orientation, the mechanism of folding of their polypeptide chains and the nature of their oligomeric form. We have also been studying the requirements of these processes on the ability of the molecules to be exported from the endoplasmic reticulum. Specific examples of some of these aspects will described in detail.

M 008 THE POLY-A ADDITION SIGNAL OF INFLUENZA VIRUS RNA CONSISTS OF A STRETCH OF U RESIDUES IN JUXTAPOSITION TO A dsRNA STRUCTURE. *Peter Palese, Guangxiang Luo, Willem Luytjes and Masayoshi Enami, Department of Microbiology, Mt. Sinai School of Medicine, NY, NY*

We succeeded in introducing cDNA-derived RNA into the genome of influenza viruses. This was achieved by reconstituting a biologically active ribonucleoprotein (RNP) complex *in vitro* using RNA and a purified influenza virus polymerase preparation (1, 2). After transfection of the RNP into cells and infection with helper virus, appropriate RNAs are transcribed and amplified and proteins are expressed. This system then permits us to study the signals involved in transcription, replication and packaging of influenza virus RNAs. For the analysis of the poly adenylation signal, we used an RNA containing the reporter gene chloramphenicol acetyltransferase (CAT) flanked by 22 nucleotides at the 5' end and 26 nucleotides at the 3' end, which were derived from the NS RNA segment of influenza A/WSN/33 virus. Mutations were then introduced into both the 5' and 3' ends and the resulting RNAs were studied: (a) by transcription *in vitro*, (b) for CAT expression activity, in the RNA transfection assay system, and (c) for the addition of poly A, in RNP-transfected cells using PCR. The results reveal that a stretch of uninterrupted uridines at the 5' end of the negative strand RNA is essential for poly A addition. Also, a ds RNA "panhandle" structure made up of the 5' and 3' terminal nucleotides is required for polyadenylation, since opening up of these basepairs destroys the poly A addition--but not amplification--of the RNA. Finally, it was shown that this ds RNA structural requirement is not sequence-specific, since a synthetic GC clamp can replace the virus-coded RNA duplex. The data suggest that the viral RNA polymerase adds poly A by a slippage (stuttering) mechanism which occurs when it hits the ds RNA barrier next to the stretch of uridines.

1. Luytjes et al., Cell 59, 1107, 1989. 2. Enami et al., Proc. Natl. Acad. Sci. USA 87, 3802, 1990.

M 009 TRANSGENIC MICE EXPRESSING INFLUENZA VIRUS HEMAGGLUTININ ON PANCREATIC CELLS DEVELOP AUTOIMMUNE DIABETES

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Insulin-dependent diabetes (IDDM) in humans is characterized by a cellular and humoral assault on pancreatic cells by components of the immune system. The target antigens(s) involved in this immunopathological process have not been identified. Our strategy was to determine whether the expression of a novel surface antigen on the surface of murine pancreatic cells would result in IDDM. We have generated lines of transgenic mice (RIP-HA) that express the hemagglutinin (HA) of the Japan A/Japan/305/57 strain of influenza virus on the insulin-producing cells. Hyperglycemia and ketosis developed in mice derived from three independent founder lines and was associated with lymphocytic infiltration of the islets and a humoral response to against -cell antigens, including HA. When challenged by infection with influenza virus, the mice develop high titres of antibodies of HA. The transgenic animals are therefore not immunologically tolerant against HA. These results suggest that the pancreas may be an immunologically privileged site and that IDDM in these animals involves breakdown of this privilege. The RIP-HA mice should therefore prove to be a useful system in which to study the cellular interactions involved in the induction of self-tolerance and immunity.

Chemotherapy, Vaccines and Interferon

M 010 SIGNAL TRANSDUCTION BY HUMAN INTERFERON-ALPHA. David. E. Levy, Department of Pathology and Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.

We have been studying the mechanism by which human interferon-α (IFNα) induces expression of a discrete set of genes. The signal transduction pathway used by IFNα has been traced by first identifying primary response genes, studying the physiology of their induction, identification of their regulatory elements, and analysis of the proteins mediating this regulation. We are now studying the biochemical mechanisms responsible for delivering to the nucleus the signal produced by IFNα binding to its specific cell surface receptor.

IFNα stimulated genes are directly induced at the transcriptional level in response to IFNα treatment but not by any other cytokine tested. This process is mediated by pre-existing proteins with no requirement for ongoing protein synthesis. Transcriptional induction is mediated by a *cis*-acting DNA sequence found in the promoters of all IFNα-stimulated genes. This DNA sequence serves as the binding site for a complex of proteins, termed ISGF3, which is activated by IFNα treatment and required for transcriptional induction. These proteins are sequestered in the cytoplasm of untreated cells where they are acted upon following IFNα binding to its cell surface receptor. This activation event stimulates their translocation to the nucleus and assembly of a transcriptionally active complex on DNA. The ultimate transcription factor complex is composed of two distinct components: a 48 kDa DNA binding protein and a regulatory complex composed of three polypeptides of 84, 91, and 113 kDa. These regulatory proteins are the target for activation in response to the liganded receptor, being stimulated to translocate to the nucleus, where they modulate the DNA binding affinity of the 48 kDa DNA-binding protein. One or more of the regulatory polypeptides of ISGF3 is phosphorylated by a kinase activity which can be inhibited by staurosporine or K-252a. However, it is unlikely that a global activation of protein kinase C or calcium- or cAMP-dependent kinases is involved in IFNα signalling. Direct transfer of receptor-activated transcription factor subunits from cytoplasm to nucleus maintains the specificity and rapidity of the IFNα response in the absence of a traditional second messenger signalling system.

Viral Pathogenesis

M 011 ANATOMY OF VIRAL PERSISTENCE AND DISEASE, Michael B.A. Oldstone, Department of Neuropharmacology, Division of Virology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Viral persistence consists of two essential components: a failure of the immune system to recognize and clear virus and a successful replicative strategy of the virus. One manner for viruses to initiate and maintain persistence is by direct infection of lymphocytes, cells that ordinarily detect and eliminate the foreign agent and the cells' it infects. Thus, viruses can infect lymphocytes resulting in immunosuppression against the infecting agent thereby providing the virus a selective advantage for survival in a hostile immunologic environment. Virus specific MHC restricted cytotoxic T lymphocytes (CTL) play a cardinal role in clearance of several viruses. Using genetic and biochemical approaches, epitopes on viral protein(s) recognized by CTL can be mapped. These epitopes can be utilized in vaccines that offer protection from both acute and persistent infections.

Once virus evades immune surveillance, based on its tropism, it can set up a strategy of replication in specialized differentiated cells. This can lead to altered homeostasis in the absence of an inflammatory response and signals a novel way viruses cause disease.

M 012 THE ORGAN SPECIFICITY AND PERSISTENCE OF A DNA VIRUS: POLYOMA VIRUS

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The ability of cis-regulatory DNA sequences to alter or control the organ specificity and persistence of polyoma in mice is examined. We have previously shown that either deletions or substitutions of cis regulatory can either restrict or expand the acute organ specificity of virus replication and that this effect is generally cis-restricted for DNA replication. We further examine the genetics of acute infections and establish the importance of the Py A enhancer in this specificity. We have also examined the relationship between cell division and Acute Py infections with the aim to understand why Py infections are normally restricted to newborn animals. Host cell division in target organs is not detectably induced by Py virus replication but may be necessary. In addition, Py variants with in vivo pancreas organ specificity are not also specific for replication in transformed cells from the pancreas. Thus cellular DNA replication control appears to be important for in vivo organ specificity of Py replication. We also examine the cis-regulatory genetics of Py persistent infections. There is a significant variability in the persistent levels of wild type Py DNA, which appears to be due to variable reactivation rates. We propose that there may be an underlying but low and stable level of persistent viral DNA. We also show the elements within the B enhancer have significant affects on levels and cell types of persistent infections.

M 013 NEOPLASIA IN TRANSGENIC MICE EXPRESSING THE EPSTEIN-BARR VIRUS LATENT PROTEINS. Joanna B. Wilson, Department of Genetics, University of Glasgow, Glasgow, G11 5JS, U.K.

The Epstein-Barr Virus (EBV) nuclear antigen-1 (EBNA-1) is required for the latent mode of replication by the virus. It is consistently expressed in all EBV-associated Burkitt lymphomas, nasopharyngeal carcinomas and other EBV associated disorders. A viral latent membrane protein (LMP) expressed in EBV *in vitro* immortalised B-cells, is not expressed in newly explanted Burkitt lymphoma cells, but is detected in many nasopharyngeal carcinomas (NPC) and in the effected tongue epithelium of patients suffering from the EBV and HIV associated syndrome oral hairy leukoplakia (OHL). In order to elucidate the individual *in vivo* action of each of these two candidate viral oncogenes, they have been expressed in transgenic mice. The expression of EBNA-1 was targeted to B-cells while that of the LMP to epithelial cells (in particular the mouse epidermis). In two lines of mice (with different transgene integration sites), expression of LMP in the epidermis caused epithelial hyperplasia. Terminal differentiation was inhibited in these cell layers and growth induced, as evidenced by the aberrant expression of a hyperproliferative cytokeratin (1). This phenotypic condition is very similar to that observed in the tongue epithelium of OHL sufferers, as well as representing a preneoplastic state implicating the LMP as factorial in both OHL and NPC. Further to these results, the LMP induces a chronic inflammatory infiltrate where expressed, which, in transgenic mice expressing LMP in a global fashion, is ultimately lethal. This observation may reflect the ability of LMP to induce the expression of adhesion molecules (the latter being crucial in the recruitment of leukocytes). Expression of EBNA-1 in B-cells has so far only been achieved in one line of transgenic mice. Nevertheless, the mice of this one line succumb to a poorly differentiated, highly invasive, monoclonal B-cell lymphoma. These results suggest that as well as functioning to maintain the viral genome, EBNA-1 may indeed play a direct role in the onset of EBV associated lymphomas.

1. Wilson *et. al.* (1990). Cell 61 1315-1327

Polivirus

M 014 RECOMBINATION, EDITING AND PACKAGING OF THE POLIOVIRUS RNA GENOME, Karla Kirkegaard, Thale C. Jarvis, Louisa M. Morrissey, Constance I. Nugent and Janice D. Pata, Department of Molecular, Cellular and Developmental Biology and Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309.

Several different kinds of RNA-protein interactions can be studied in detail during poliovirus infection; some of these may be shared with its mammalian host and some of them are undoubtedly unique to the specialized lifestyle of a positive-strand RNA virus. Here we will discuss progress in understanding genetic recombination on the RNA level among polivirus genomes, dsRNA editing in human cells using poliovirus as an assay, and the packaging of poliovirus RNA during viral assembly.

Genetic evidence has suggested that RNA recombination of poliovirus genomes occurs during negative strand RNA synthesis, by a template-switching mechanism. We have developed a quantitative PCR method to detect RNA recombinants, thus obviating the need for genetic markers and viable progeny. The timing and strand-specificity of RNA recombination during the poliovirus infectious cycle and the development of totally *in vitro* RNA recombination assays will be presented.

A dsRNA-specific RNA modification activity has been identified in a variety of mammalian cells. The purpose of this RNA modification activity, which specifically converts adenosine to inosine residues in dsRNA, is unknown, but its proposed functions have ranged from natural antisense regulation to antiviral functions. We have found that this activity is *inhibited* in cells during the antiviral state that ensues following dsRNA treatment, suggesting that it is unlikely that the dsRNA modification activity is itself antiviral. We have investigated the frequency of A-to-I modification in poliovirus infections initiated with both single- and double-stranded RNA, using the phenotypic reversion of a temperature-sensitive poliovirus mutant as a sensitive biological assay for this conversion.

The final biological activity of poliovirus RNA in infected cells is to be specifically packaged into viral particles. However, which of the various candidate morphogenic intermediates in poliovirus assembly is reponsible for packaging poliovirus RNA has remained mysterious. We have quantified the binding of various subviral particles to biotinylated poliovirus RNA; the binding characteristics of particles from both wild-type poliovirus and a poliovirus mutant defective in RNA packaging will be discussed.

M 015 TRANSGENIC MICE EXPRESSING HUMAN POLIOVIRUS RECEPTORS: A NEW MODEL FOR POLIOMYELITIS,
Vincent R. Racaniello*, Ruibao Ren*, Frank Costantini†, Edward J. Gorgacz+, James. J. Lee†, *Dept. of Microbiology and †Dept. of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032, and +Lederle-Praxis Biologicals, Pearl River, N.Y. 10965
Poliovirus infection is limited to primates, and within the infected animal, the virus replicates at very few sites, including lymphoid tissues of the gut and pharynx, and motor neurons within the central nervous system. Examination of the expression of the cell receptor for poliovirus (PVR) revealed the presence of PVR RNA and protein in a wide variety of human tissues, including those that are not susceptible to poliovirus infection. To further study the relationship between PVR expression and poliovirus tissue tropism, a human PVR gene was isolated and used to generate transgenic mice. Several PVR transgenic mouse lines were established that express PVR transcripts and poliovirus binding sites in a wide variety of tissues, including brain, spinal cord, kidney, liver, lung and intestine. Intracerebral inoculation of PVR transgenic mice with poliovirus type 1 Mahoney resulted in viral replication in the brain and spinal cord and development of paralytic poliomyelitis. In contrast, nontransgenic mice did not develop disease after inoculation with this strain. PVR transgenic mice also developed poliomyelitis after intravenous, intraperitoneal, and intramuscular inoculation with type 1 poliovirus. After intravenous inoculation with poliovirus, viral replication was confined to the brain and spinal cord of transgenic mice. When PVR expression was examined by *in situ* hybridization, RNA was detected in many transgenic mouse organs, but largely in specific cell types. Poliovirus infection is therefore restricted in the PVR transgenic mice, despite widespread expression of virus binding sites. These results suggest that poliovirus tissue tropism may be controlled by other factors required for cell entry or replication. However, the PVR is clearly the major determinant of poliovirus host range. PVR transgenic mice should be useful for studying poliovirus tissue tropism, neurovirulence, and attenuation, and for the development and perhaps testing of poliovirus vaccine strains.

M 016 GENETIC DETERMINANTS OF PICORNAVIRUS REPLICATION
Bert L. Semler, Cristina Giachetti, William A. Charini, Sandra L. Dildine, & Aurelia A. Haller, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717.
We have used molecular genetic approaches to define the protein and RNA determinants of picornavirus replication. The first approach involves a study of the polypeptide requirements for poliovirus RNA synthesis. Our work has focused on the role of polypeptide 3AB (a membrane-associated precursor of the genome-linked protein, VPg) in the initiation of poliovirus RNA synthesis. We employed site-directed mutagenesis to generate a number of amino acid replacement and insertion mutants in the hydrophobic domain contained within polypeptide 3AB. One 3AB mutation produced a mutant virus (Se1-3AB-310/4) that was temperature sensitive for viral RNA synthesis. Biochemical analysis of *in vivo* and *in vitro* RNA synthesis of this mutant indicated that it is defective in initiation of (+) strand RNA synthesis and in synthesis of VPg-pU(pU), a nucleotidyl protein that has been proposed as a primer for the viral polymerase. Our results provide evidence in support of a model in which polypeptide 3AB serves as carrier for VPg to initiate (+) strand RNA synthesis. They also suggest that a separate mechanism could be used in the initiation of (-) strand RNA synthesis. We have extended our study of picornavirus RNA replication in an attempt to define the *cis* versus *trans* nature of replication functions supplied by specific viral polypeptides. We have engineered several deletion and insertion mutations into a bacteriophage T7 *in vitro* transcription vector containing poliovirus cDNA sequences. We have employed complementation analysis using either cotransfected pairs of mutant RNAs or rescue of a single transfected mutant RNA by a superinfecting virus that contains coxsackievirus B3 sequences in the 5' noncoding region of its genome. Slot blot analysis using template-specific oligonucleotide probes allows one replicating template to be distinguished from the other. One of the mutant viruses we have recovered, Se1-3D-18, contains an amino acid insertion in the viral RNA polymerase and is ts for RNA synthesis. Our data demonstrate that the RNA synthesis defect of 3D-18 at 39°C can be rescued in *trans*. A second approach that we have initiated to determine the viral sequences required for successful completion of a picornavirus life cycle involves the genetic alteration of sequences in the 5' *noncoding* region (5' NCR) of poliovirus RNA. We have produced deletion and substitution mutations as well as a limited set of linker scanning mutations within the poliovirus 5' NCR. Genetic and biochemical analysis suggests that lesions which disrupt predicted secondary structures within this 742 nucleotide region of viral RNA produce deleterious effects on virus growth. Some of these effects result from defective translation initiation and may be attributable to altered RNA-protein interactions within the 5' NCR of the poliovirus genome. Understanding the nature of such RNA-protein complexes will provide insights into the mechanisms employed by picornaviruses to insure efficient replication of their genetic information.

M 017 STUDIES OF EARLY EVENTS IN PICORNAVIRUS REPLICATION: RECEPTOR/POLIOVIRUS INTERACTION AND INITIATION OF PICORNAVIRAL POLYPROTEIN SYNTHESIS. H.C. Selinka, A. Zibert, J.J. Harber, S.K. Jang, T. Pestova, G.W. Witherell, C.U.T. Hellen, A. Molla, and E. Wimmer; Dept. of Microbiology, School of Medicine, SUNY at Stony Brook, Stony Brook, N.Y. 11794

The first event in viral infection is receptor mediated uptake of the viral particle. We have studied properties of the poliovirus receptor (PVR), an Ig-like glycoprotein of 67 kDa containing three Ig domains with the general structure V-C2-C2/transmembrane region/ cytoplasmic tail. Deletion of the two C2-domains reduces but does not abolish complete receptor activity (defined as binding leading to infection). Expression in mouse cells of the V-domain spliced onto a truncated ICAM-1 rhinovirus receptor molecule leads to infection. Thus poliovirus can enter the cell by an ICAM-1 pathway. Computer modeling (in collaboration with M.de Crombrugghe, D.Oren, and E.Arnold) and site-directed mutagenesis have further defined the interaction between PVR and poliovirion. We have found a sequence mapping to the V-domain of PVR which down-regulates PVR translation in vivo and in vitro. This negative control element may play a role in the expression of PVR.

Following uptake and uncoating, plus-stranded picornavirus genomes, which contain unusually long 5' nontranslated regions (5'NTR), function as mRNAs. Studying the 5'NTR of encephalomyocarditis virus (EMCV) RNA, we have discovered a 400 nt long segment, called internal ribosomal entry site (IRES), that serves as positive translational control element and renders translation cap-independent. A similar element in poliovirus RNA has been defined by others. Two cellular proteins have been found to bind specifically to EMCV IRES: p57, which attaches to a stem-loop at the 5' border, and p52, which attaches to the 3' border of IRES. Binding of p57 appears to be required for IRES function in vitro. An oligopyrimidine-rich region within the IRES of EMCV and poliovirus has an important cis function as shown by mutational analyses in vitro and in vivo. We have developed a model explaining IRES function even though these elements appear to be structurally quite different amongst picornavirus mRNAs.

Transcription

M 018 REGULATION OF c-JUN TRANSCRIPTIONAL ACTIVITY BY INTERACTION OF A CELL-SPECIFIC INHIBITORY ACTIVITY WITH A REGULATORY DOMAIN: DIFFERENCES BETWEEN v- AND c-JUN, Vijay R. Baichwal and Robert Tjian, Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

Transcription factor AP-1 plays a pivotal role in control of cell growth and proliferation, and its activity is tightly regulated in cells. Stimulation of quiescent cells by exposure to mitogens, growth factors, or other extracellular stimuli causes a rapid increase in AP-1 activity. Transfection of a number of oncogenes including *ras*, *raf*, *src*, and *mos* also enhances AP-1 activity, suggesting that at least part of the effect of these oncogenes on gene expression is mediated by changes in AP-1 activity. The two major components of AP-1 purified from HeLa cells are the products of the two proto-oncogenes c-jun and c-fos. Both c-Jun and c-Fos proteins have a bipartite DNA binding domain, which consisits of a region rich in basic amino acids that is involved in sequence-specific DNA binding and an adjacently located leucine repeat that mediates dimer formation. c-Jun forms homodimers that binds DNA in a sequence specific manner and activate transcription from templates containing AP-1 sites. c-Jun can also form a heterodimer with c-Fos that binds DNA more tightly than the c-jun homodimer and is more potent at activating transcription.

To analyze the regulation of c-Jun transcriptional activity we have constructed chimeric proteins in which the DNA binding and dimerization domains of c-Jun are replaced by DNA binding domains of the yeast GAL4 transcriptional activator, or the bovine papilloma virus transactivator E2. The transcriptional properties of these chimeric proteins have been determined by co-transfection assays in several cell-lines. Our analysis reveals that c-Jun contains two transcriptional activation domains (A1 and A2) and a negative regulatory region (δ). The A2 domain located near the DNA binding domain is constitutive. A1 located within the amino terminal half is a regulated activation domain which is active in some cell-types but not others. In vivo competition experiments suggest that A1 is regulated by interaction with an inhibitory activity that is cell-type specific and therefore A1 is a cell-type specific activation domain. The δ region located adjacent to the A1 domain regulates it by facilitating or stabilizing the inhibitory activity. The cell-specific repression of the δ and A1 domain is recapitulated with in vitro transcription experiments. Our findings may help explain how the retroviral homolog, v-jun, is transforming. Transforming activity of the v-jun coding sequences has been traced to the lack of a δ region in v-jun. The δ region is a negative regulatory region and therefore v-Jun transcriptional activity is not as effectively inhibited as that of c-Jun. Consequently, v-jun is a transforming protein probably because it is a more potent transcriptional activator than c-jun. The results of these and other experiments examining the role of the A1 domain in modulating c-jun transcriptional activity in response to physiolgical cues will be discussed.

M 019 ACTIVATION AND REPRESSION OF TRANSCRIPTION BY THE FULL LENGTH PAPILLOMAVIRUS E2 PROTEIN

Jean-Michel Gauthier*, Nathalie Dostatni*, Jonathan Ham*, Françoise Thierry*, Paul Lambert°, Peter Howley+ and Moshe Yaniv*, * Department of Biotechnology, Pasteur Institute, 25, rue du Docteur Roux, 75724 Paris Cedex 15., France, ° Mc Ardle Laboratory for cancer research, University of Wisconsin, Madison, Wisconsin, + Laboratory of Tumor Virus Biology, NCI, Bethesda, Maryland 20892.

Papillomaviruses encode a DNA binding protein -E2- that binds to the palindromic sequence ACCGN$_4$CGGT present in multiple copies in the viral genome, predominantly in the long control region (LCR). A strong synergy in transcriptional activation is observed between two such palindromic sites when cloned upstream of an exogenous promoter suggesting that the active form may be a tetramer. The same potential tetrameric structure is required in human epithelial cells for more than additive cooperation with other transcription factors that interact with the viral LCR.

In contrast to the behaviour of other promoter or enhancer binding factors, activation by E2 is optimal when one to several binding sites for transcription binding factors are present between tne TATA box and the E2 binding sites.

The full length BPV1 E2 protein, overproduced in yeast, activates in vitro transcription of the HSV TK promoter containing one to several E2 binding sites at position -109. In contrast to the observations made in vivo, no synergy is observed between several E2 binding sites. Finally, when E2 site(s) are present just upstream of the TATA box, as is the case for human genital papillomaviruses, the viral E2 protein strongly represses transcription by preventing the formation of the initiation complex. This inhibition may occur by direct interference with TFIID binding to the TATA box.

Transformation

M 020 THE P53 TUMOR SUPPRESSOR GENE AND PRODUCT, Robin S. Quartin, Cathy A. Finlay, Gerard P. Zambetti, Jesse D. Martinez, Diane Harvey and Arnold J. Levine, Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014.

The great majority of the p53 cDNAs obtained from colon carcinomas contain missense mutations that map at one of four hot spots, amino acid residues 175, 248, 273 and 281 out of a total of 393 amino acids. DNA clones of these p53 mutations cooperate with the *ras* oncogene to transform primary rat embryo fibroblasts. When the mutant human p53 is regulated by an inducible expression vector and is turned off in these cells, the cells change morphology and are no longer transformed, demonstrating the need for a continued presence of mutant p53 protein to maintain the transformed phenotype. A temperature sensitive p53 protein that is wild-type at 32°C and mutant at 39.5°C regulates these transformed cells so as to stop growth, at the G_1-S border, at 32°C. At 37°C, half of the p53 is wild-type and half is a mutant form and the cells are transformed. In G_1, the wild-type p53 is kept in a complex with hsc70 and mutant p53 protein in the cell cytoplasm. This prevents the wild-type p53 from functioning in the nucleus in G_1 to block the entry of the cell into S-phase. In this way, mutant p53 protein might act as a dominant loss of function mutation.

M 021 The RB Gene Product Can Interact Specifcally With Multiple Cellular Proteins. W. Kaelin, J. DeCaprio, F. Kaye[1], D. Pallas, and D. M. Livingston. Dana Farber Cancer Institute and Harvard Medical School, Boston, Mass. and [1]The National Cancer Institute-NIH, Bethesda, MD.

The RB-1 product is a 928 aa nuclear protein, which has growth and tumor suppression activity. The mechanisms by which it serves these functions are not known. What is clear is that RB exists in a variety of forms, some overtly phosphorylated and most likely not identical in the state of their phosphorylation. At least one additional RB species is un- or underphosphorylated by comparison with the aforementioned forms. RB phosphorylation occurs in a cell cycle-dependent manner, with no apparent action in G1 and the initiation of phosphorylation occurring in a clock-work manner at the G1/S boundary. The protein is also enzymatically dephosphorylated in M. RB is a known specific binding target of at least three, different transforming gene products of DNA tumor viruses - adenovirus E1A, papovaviral large T Ag (T), and the E7 product of transforming strains of HPV.

RB contains a discrete domain, extending over ~400 residues of its sequence, which can, as an independent unit, bind to T and E1A with the same specificity as the intact protein. Naturally occurring loss of function RB-1 mutations in human tumors which give rise to inactive, but stable, products frequently map to this sequence. These two findings suggest that this domain normally contributes to RB function and that it does so, at least in part, by binding to one or more cellular proteins.

With this as background, we have searched for RB-binding cellular proteins by fusing this domain to a 'biological hook' and then using the chimeric protein bound to a specific insoluble support as an affinity chromatographic reagent. The results indicate that a set of ~7 different proteins can be identified in extracts of a variety of human cells, and their binding, as measured in vitro, fully mimics the specificity noted for the interaction of the domain with E1A and T. These data suggest that one aspect of RB action is to interact with a set of cellular proteins as part of the performance of its growth suppression function. Since T competed with these proteins for binding to the isolated domain, at least part of the mechanism used by T to disrupt RB function may be to block RB access to one or more of its normal cellular targets.

M 022 THE ONCOGENICITY OF JUN, Peter K. Vogt[1], Timothy Bos[2], Heyun Su[1], Martin Breitman[3], Iain Morgan[1], Yoshiaki Ito[4], Luc Vanhamme[1], [1]University of Southern California, School of Medicine, Department of Microbiology, Los Angeles, CA 90033-1054, [2]Department of Microbiology and Immunology, Eastern Virginia Medical School, Box 1980, Norfolk, VA 23501, [3]Division of Cancer and Cell Biology, Mount Sinai Hospital Research Institute, Toronto, Ontario, Canada M5G 1X5, [4]Kyoto University, 53 Kawahara-Machi, Shogoin, Sakyo-ku, Kyoto, Japan 606. The chicken cellular Jun protein has a low capacity for inducing transformation in chicken embryo fibroblast cultures, while the viral Jun is an effective oncogenic transformer. Two changes are needed to turn the poorly transforming celluar jun gene into an active oncogene: a deletion in the amino proximal region, presumably interfering with the binding of a cellular inhibitor, and removal of 3' untranslated sequences that cause mRNA instability. Besides activating transcription Jun can also stimulate DNA synthesis. Mutants and recombinants of viral and various cellular Jun proteins show a rough correlation between their ability to transform chicken embryo fibroblasts and stimulation of DNA synthesis as well as transactivation. The viral Jun also effectively interferes with differentiation of avian myoblasts in vitro. Myogenic chicken and quail cultures expressing viral Jun show little fusion into multinucleated postmitotic myotubes and fail to express muscle specific proteins. The few myotubes that do form in these cultures fail to overexpress Jun. Initiation of the myogenic program and overexpression of Jun are mutually exclusive. V-*jun* transgenic mice do not show an increased incidence of spontaneous tumors. However, upon full depth wounding these animals develop persistent hypertrophic lesions at the site of the wound, some of these lesions become sarcomatous . Cell cultures derived from sub sarcomas are uniquely responsive to select growth factors which may play a role in secondary genetic or epigenetic events that are required for tumor development

Nucleic Acid Replication

M 023 BIOCHEMICAL ANALYSIS OF THE VIRUS-ENCODED PROTEINS INVOLVED IN HERPES SIMPLEX VIRUS DNA SYNTHESIS, Mark D. Challberg, Daniel Fierer, John Gottlieb, and Glenn Sherman, Laboratory of Viral Diseases, NIH, Bethesda, MD 20892

Genetic experiments have shown that seven HSV genes are both necessary and sufficient to support the replication of viral DNA in infected cells. To facilitate the biochemical analysis of these proteins we have expressed each polypeptide using the baculovirus expression system. The activities of the overexpressed proteins togehter comprise the core reactions thought to be necessary for the semi-conservative synthesis of duplex DNA.

The UL9 protein binds specifically to the viral origins of DNA replication. Hydrodynamic measurements have shown that UL9 exists predominantly as a homodimeric structure. The interaction of purified UL9 with wild-type and mutated versions of the origin has been studied in some detail. Our results suggest that: 1) the binding of UL9 to each of the two binding sites correlates well with the ability of the origin to function in vivo, and 2) there is a cooperative interaction between UL9 bound at both arms of the origin palindrome. In addition to origin binding, the purified UL9 protein possesses an intrinsic 3' to 5' helicase activity that is capable of unwinding duplex DNA segments in an ATP-dependent reaction.

The HSV DNA polymerase consists of a stable complex of UL30 (the catalytic subunit) and UL42. This complex can be reconstituted either by mixing of the two purified subunits, or by co-infection of insect cells with recombinant baculoviruses that express each subunit. Experiments using defined primer-templates have shown that the UL42 subunit increases the processivity of the DNA polymerase. Gel mobility shift and DNase footprint experiments suggest that the UL42 increases the affinity of the enzyme for primer termini by anchoring the polymerase to the double-stranded DNA behind a growing strand, thereby decreasing the probability that the polymerase will dissociate from the template after each cycle of catalysis.

The UL5, UL8, and UL52 genes encode the components of a helicase-primase complex. When expressed individually, the three polypeptides are either not soluble or have no apparent enzymatic acitivty.. Co-expression of all three polypepetides, or of the UL5 and UL52 polypeptides, results in the formation of a soluble complex having both helicase and primase activities. The role of the UL8 polypeptide is therefore currently unknown. We have purified both the UL5/UL8/UL52 complex and he UL5/UL52 complex to homogeneity and are beginning investigate the activity of these proteins on templates that more closely approximate the structure of authentic HSV DNA replication intermediates.

M 024 FUNCTIONAL ANALYSIS OF SV40 AND POLYOMA LARGE T ANTIGENS AND THE P53 TUMOR SUPPRESSOR PROTEIN C. Prives, P. Friedman, H. Lorimer, K. Moses, I. Reynisdottir, and E. Wang, Department of Biological Sciences, Columbia University, N.Y., N.Y. 10027

We have characterized the interactions of various forms of p53 with SV40 large T antigen (T Ag). It was determined that both murine and human wild type p53 proteins block the DNA replication initiation functions of SV40 T Ag *in vitro*. Two mutant p53 phenotypes were observed: mutant p53 that fails to inhibit DNA synthesis at all concentrations tested, and mutant p53 that inhibits this process only at low but not at higher concentrations. This latter phenotype suggests that such p53 may preferentially self-associate at higher concentrations. Interestingly wild type human p53 synthesized in bacteria, and thus presumably unmodified, behaves like the second type of mutant suggesting that phosphorylation of p53 may regulate p53:T vs p53:p53 interactions. This hypothesis is currently being tested. Understanding the processes by which wild type and mutant p53 proteins interact with T antigen and with eachother may provide insight into the roles of p53 within cells.

Our studies with polyoma T antigen (Py T Ag) have revealed similarities and differences when compared to its SV40 counterpart. We have established that ATP induces the assembly of Py T Ag into hexamers and alters its interactions with the viral replication origin. Py T Ag, like SV40 T Ag, is a DNA helicase that can unwind duplex DNA. However, unlike SV40 T Ag, Py T Ag displays little sequence specificity for DNA unwinding under normal replication conditions suggesting that additional activities of Py T Ag are involved in its replication initiation functions. Related to this Py T Ag displays a specific interaction with murine but not human DNA polymerase. Another marked difference between the two T antigens is related to the effects of phosphorylation upon their replication function. Treatment of SV40 T Ag with alkaline phosphatase (CIAP) activates its replication function by increasing its binding to the SV40 origin. By contrast, removal of phosphates from Py T Ag by CIAP blocks Py ori-DNA replication in a manner that is apparently unrelated to its binding to the Py origin.

M 025 MECHANISM AND REGULATION OF DNA REPLICATION: LESSONS FROM SV40, **Bruce Stillman, Anindya Dutta, Thomas Melendy and Toshiki Tsurimoto.** Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, New York 11724.

The replication of plasmid DNAs containing the Simian Virus 40 origin of DNA replication has been reconstituted *in vitro* with highly purified proteins. In addition to the virus encoded large tumor antigen (TAg), which functions as an origin recognition protein and DNA helicase, seven cellular proteins have been identified as essential components. These include replication factor A (RF-A), a multi-subunit single strand DNA binding protein (SSB) that is required for initiation and elongation; RF-C, a multi-subunit DNA-dependent ATPase and primer binding protein; PCNA, a co-factor for RF-C and DNA polymerase δ; polymerase α/ primase complex; DNA polymerase δ and the topoisomerases I and II. The mechanism of DNA replication using these proteins will be discussed.

To begin to understand the regulation of DNA replication in eukaryotic cells, we have focused our attention on the initiation and *ori* unwinding events, both of which require RF-A. RF-A consists of three protein subunits with molecular weights of 70K, 34K, and 11K in both human and yeast cells. The 70K subunit is the SSB, but phosphorylation of the 34K subunit enhances RF-A DNA binding and function. The RF-A kinase has been purified to homogeneity and was identified as the cell-cycle regulated cdc2-cyclin kinase complex. The 34K subunit is phosphorylated in a cell-cycle dependent manner, being hypo-phosphorylated in the G1 phase and hyper-phosphorylated in the S and G2 phases. These observations provide a direct link between regulation of DNA replication and cell-cycle control.

Papilloma Viruses

M 026 GENETIC AND MOLECULAR ANALYSIS OF CELL TRANSFORMATION BY PAPILLOMAVIRUS E5 PROTEINS, Daniel DiMaio, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510

The primary bovine papillomavirus (BPV) gene responsible for morphologic transformation of established lines of rodent fibroblasts is the E5 gene which encodes a 44-amino acid, very hydrophobic protein that is among the smallest transforming proteins known. Efficient transformation by the E5 protein requires a very short "active site" that may consist of as few as seven amino acids. The overall hydrophobicity of the molecule is also important although many random hydrophobic sequences can functionally substitute for the wild type one. We have found that the receptor for platelet-derived growth factor is specifically and constitutively activated in fibroblasts transformed by the BPV and deer papillomavirus (DPV) E5 genes. When the E5 protein is delivered acutely into cells, receptor activation precedes the proliferative response to this protein. Moreover, PDGF receptor activation by PDGF treatment or expression of the PDGF gene is sufficient to induce DNA synthesis and stable growth transformation. We propose that in fibroblasts the PDGF receptor is a crucial intermediate in delivering the proliferative signal generated by the E5 protein. This model is supported by genetic experiments that suggest that the integrity of the PDGF signaling pathway is required for E5-mediated transformation of fibroblasts. There is striking amino acid sequence similarity between the active site of the fibropapillomavirus E5 proteins and PDGF, suggesting that both the E5 protein and PDGF may interact directly with the receptor. In contrast, the human papillomaviruses (HPV) do not efficiently transform fibroblasts, and their E5 proteins do not resemble PDGF. However, introduction of the HPV16 E5 gene into established lines of cultured murine keratinocytes results in tumorigenic transformation, indicating that the HPV16 E5 gene is a transforming gene in cells similar to its natural host cell. This suggests that the HPV E5 genes may play a role in the development of the proliferative lesions associated with HPV infection.

M 027 HPV TRANSFORMING PROTEINS, E6 AND E7

P. Howley, K. Münger, C. Yee, J. Huibregtse, and M. Scheffner, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland

A variety of studies have indicated that the papillomaviruses associated with cervical carcinoma, HPV-16 and HPV-18, contain two genes (E6 and E7) which have transforming properties. The HPV-16 E7 protein is a multifunctional protein with transcriptional modulatory and cellular transformation properties similar to the adenovirus E1A proteins (CELL 53:539, 1988). The N-terminal 37 amino acids of the E7 proteins encoded by the HPVs associated with genital tract lesions are highly conserved and are similar to the portions of Ad E1A conserved domains 1 and 2 involved in binding pRB. These E7 proteins can all bind pRB (Science 243:934, 1989; EMBO J. 8:4099, 1989) although the E7 proteins of HPV-16 and HPV-18 have a higher binding affinity than the E7 proteins encoded by HPV-6 and HPV-11. Thus, the ability of E7 to bind pRB per se does not allow for the qualitative discrimination between the biologic properties of the "high risk" viruses, such as HPV-16 and HPV-18, and "low risk" viruses, such as HPV-6 and HPV-11. Immortalization of primary human keratinocytes requires both E6 and E7 (J. Virol. 63:4417, 1989; EMBO J. 8:3905, 1989). In recent collaborative studies with A. Levine, we have shown that the E6 proteins of HPV-16 and HPV-18 are able to complex p53 in vitro. (Science 248:76-79, 1990). The wild type p53 gene has tumor suppressor properties and is a target for several oncoproteins encoded by DNA tumor viruses. We have recently shown that the E6 proteins of the "high risk" HPVs which bind p53 stimulate the degradation of p53 (Cell December, 1990). The E6 promoted degradation of p53 is ATP dependent and involves the ubiquitin-dependent protease system. The selective degradation of cellular proteins, such as p53 with negative regulatory functions, provides a novel mechanism of action for dominant acting oncoproteins. Thus the papillomaviruses appear to exert some of their proliferative and oncogenic effects through the interactions of their encoded proteins with key cellular proteins.

Human Retroviruses

M 028 THE ROLE OF LTR REGULATORY ELEMENTS IN HIV REPLICATION

Arnold B. Rabson[1], Elizabeth K. Ross[2], Carmen Parrott[2,3], Todd Seidner[2,3], John Leonard[2], Elia Duh[2], Alicia Buckler-White[4], and Malcolm A. Martin[2]. [1]Center for Advanced Biotechnology and Medicine, Piscataway, NJ. 08854, [2]NIAID, NIH, Bethesda, MD 20892, [3]Howard Hughes Medical Institute, Bethesda, MD 20814, and [4]Georgetown University, Rockville, MD 20852

The long terminal repeat (LTR) of HIV-1 contains multiple cis-acting regulatory elements that modulate the degree of HIV RNA synthesis and viral replication. These include the target for tat transactivation, the TAR region, as well as two binding sites for the cellular transcriptional factor NF-κB and three binding sites for Sp1. Previous studies have demonstrated the role of the NF-κB sites in mediating LTR activation by phorbol ester and cytokines in both transient transfection assays and in chronically-infected T cell and promonocytic cell lines. The NF-κB sites are not required for HIV infection of T lymphocytes however, as deletion of these sites from an infectious molecular clone of HIV does not block viral replication. Deletion of the three LTR Sp1 binding sites results in a virus that is capable of infecting some, but not all, T cells. The Sp1 deleted virus replicated efficiently in human T cells containing NF-κB binding activity such as periperal blood lymphocytes and MT4 cells, however replicated poorly, or not at all in cells lacking NF-κB activity such as A3.01 cells. Activators of NF-κB could induce replication of the Sp- deleted virus in A3.01 cells, suggesting that NF-κB can functionally substitute for Sp1 in activating HIV replication. Deletion of both NF-κB sites and all three Sp1 sites resulted in loss of viral infectivity as did a mutation in the TAR region that eliminated tat transactivation. In recent studies, the roles of the individual NF-κB and Sp1 binding sites have been evaluated by reconstructing LTRs containing these sites individually and in combination. Viruses containing juxtaposed NF-κB and Sp1 binding sites are more potent activators of HIV replication than those containing two copies of either site alone; two copies of either site are more efficient in supporting viral replication than are single sites. These studies suggest that the HIV LTR is similar to the promoter/enhancer elements of other viruses in that it is composed of multiple functional elements that contribute differentially to viral replication in different cell types.

M 029 REGULATORY MECHANISMS OF GENE-EXPRESSION IN HUMAN T CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1), Mitsuaki Yoshida, Hiroshi Hirai, Masami Toita and Jun-ichi Fujisawa, Department of Cellular and Molecular Biology, Institute of Medical Science, The University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan

Human T cell leukemia virus type 1 (HTLV-1) is an etiologic agent of adult T cell leukemia (ATL) and also associated with tropical spastic paraparesia (TSP). Viral replication and gene expression are regulated by at least two trans-acting regulators, tax and rex, that are encoded by its own genome.
Tax protein is a transcriptional activator that activates the viral enhancer consisting of 21 bp. Cellular proteins (TREB) that bind to the 21 bp viral enhancer and their cDNA were characterized. TREB proteins contain leucine zipper and basic amino acid domain structure and are member of a CREB family. TREB proteins were found to activate or suppress the gene expression in cell line-specific fashion. Interaction of tax protein with these TREB proteins and also with the DNA are currently investigated. Tax also activates cellular genes such as IL-2, IL-2 receptor, GM-CSF, FOS, PTHrP and MHC class I. A cellular DNA binding NF-κB was proposed to be involved in the activation of IL-2 receptor α gene. These activations of cellular gene may explain some phenotypes of ATL cells and ATL patients, however, may not be sufficient to explain malignancy of ATL cells..The other trans-regulator rex modulates RNA processing and stimulates the expression of unspliced mRNA that codes gag, pol and env. On the other hand, rex repress the expression of fully spliced tax/rex mRNA. For these regulation, rex require the specific sequence in the 3'LTR that forms a very stable secondary structure, and rex recognizes the secondary structure.
Combination of transcriptional activation with tax and its repression by rex modulating RNA processing makes the viral gene expression transient and keeps it lower level, thus allow HTLV-1 infected cells to escape from the host immune response.

Late Abstract

THE INTERACTION OF INFLUENZA VIRUS WITH THE IMMUNE SYSTEM, Young S. Hahn, Chang S. Hahn*, David Kittlesen, Vivian L. Braciale, Charles Rice*, and Thomas J. Braciale, Departments of Pathology and Molecular Microbiology*, Washington University School of Medicine, St. Louis, MO 63110.
The influenza viruses are major human pathogens which cause morbidity on a world-wide scale. Because of the detailed information on the structure, replication and host response to this important virus group, influenza has also proved to be a powerful tool to analyze the interaction of protein antigens with the immune system, particularly T lymphocytes. A large body of evidence now strongly suggests that the antigen receptor on T lymphocytes recognizes fragments of protein antigens like influenza polypeptides in association with class I or class II MHC molecules. This presentation will review recent evidence on the intracellular compartments where influenza protein antigens are targeted for presentation to MHC class I and class II restricted T lymphocytes. In particular, we will focus on recent data on T lymphocyte recognition of minigenes encoding antigenic epitopes expressed in cells using a novel Sindbis virus expression system.

Molecular Biology of Human Pathogenic Viruses

Retroviruses

M 100 ANALYSIS OF THE HIV-1 V3 DOMAIN DURING EMERGENCE OF NEUTRALIZATION-RESISTANT VIRUS VARIANTS IN VIVO,
Jan Albert[1,2], Johan Wahlberg[3], Mathias Uhlén[3], Eva Maria Fenyö[2], Departments of Virology, [1]National Bacteriological Laboratory and [2]Karolinska Institute, Stockholm, Sweden, [3]Department of Biochemistry and Biotechnology, Royal Institute of Technology, Sweden
Objective: To study the molecular basis for neutralization-resistance during natural HIV-1 infection in man.
Methods: We have previously documented the emergence *in vivo* of HIV-1 variants with reduced sensitivity to neutralization by autologous sera. The V3 domain, which has been proposed as the principal target for neutralization, was amplified from sequential virus isolates from four patients by a polymerase chain reaction with nested primers and directly sequenced by a novel semi-automated DNA sequencing method.
Results: The deduced amino acid sequence of the V3 domain remained unchanged over time in three patients, despite the emergence of neutralization-resistant virus variants in two patients. In one patient only the emergence of neutralization-resistant virus variants coincided with mutations in the V3 domain.
Conclusions: We show that resistance of HIV-1 to neutralization by autologous sera may be caused by mutations both inside and outside the V3 domain. The complete envelope gene will be sequenced to establish the mechanism for virus escape from neutralization in all four patients. These findings have important implications for the understanding of HIV-1 pathogenesis and the attempts to develop a vaccine against HIV-1 infection.

M 101 ENDOGENOUS ORIGIN OF DEFECTIVE, RETROVIRUS-LIKE PARTICLES FROM A RECOMBINANT CHINESE HAMSTER OVARY CELL LINE.
Kevin P. Anderson, Yolanda S. Lie, Mari-Anne L. Low, and Elicia M. Penuel. Department of Medicinal and Analytical Chemistry, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080, U.S.A.
The presence of budding C-type and intracytoplasmic A-type particles in Chinese hamster ovary (CHO) cells is well documented. However, extensive screening has failed to detect any evidence of infectivity. Continuous-flow ultracentrifugation has been used to concentrate extracellular particles from culture fluid of a recombinant CHO cell subclone for molecular characterization. Particles exhibiting reverse transcriptase activity and associated with mammalian C-type retrovirus structural antigens banded in sucrose gradients at a density characteristic of retroviruses. Double-gradient purified particles contained RNA which hybridized to probes for murine leukemia virus, and endogenous Chinese hamster intracisternal A-particle (IAP) elements. DNA sequence analysis of a cDNA clone isolated from purified particles revealed multiple interruptions of the endonuclease reading frame, providing one possible explanation for the non-infectious nature of the observed particles. Sequences present as RNA in purified particles were also present as conserved, repetitive, provirus sequences in genomic DNA of all CHO cell lines examined and in Chinese hamster liver DNA. The observed particles are therefore likely to be the products of endogenous retroviruslike elements present in the germline of Chinese hamsters.

M 102 TRANSACTIVATION OF THE HIV-LTR IN HUMAN CD4+ T LYMPHOCYTES DEPENDS ON THE QUALITY OF THE SIGNAL INDUCING CELL ACTIVATION,
Arenzana, F. Hazan, U., Alcami, J. Paya, C., Michelson, S. and Virelizier, J-L., Institut Pasteur, Paris, France
We analyzed immunological signals and molecular events leading to transactivation of the HIV-1-LTR in human IL-2 dependent, cloned T lymphocytes transiently transfected with a luciferase gene under the control of various regions of the HIV-1-LTR. Specific antigen presentation (tetanus toxoid) by autologous macrophages to a specific T cell clone led to clear transactivation of the HIV-LTR. PMA similarly transactivated the whole HIV-LTR or the HIV-enhancer and translocated NFkB-like proteins to the nucleus. Anti-CD3 antibody induced TNF and lymphotoxin production in the cells and promoted massive nuclear translocation of NFkB-like proteins, however, this did not lead to HIV-LTR transactivaiton. Neither did IL-2 transactivate LTR activity despite stimulation of T cell proliferation.

These findings suggest that events linked to T cell activation, triggered by potent inducers (antigen recognition, PMA), but not by TNF or anti-CD3 antibodies, are necessary to induce functional NFkB-HIV-enhancer complexes. Efficient transactivation in human T cells may rely on post-translational modifications of one or more proteins of this complex.

M 103 UNINTEGRATED HIV-1 DNA IN CHRONICALLY INFECTED CELL LINES IS NOT CORRELATED WITH SURFACE CD4 EXPRESSION, N.J. Besansky, S.T. Butera, S. Sinha and T.M. Folks, Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333

Infection with HIV-1 may lead either to destruction of the host cell or to a persistant, noncytopathic infection. The outcome has been correlated with the amount of unintegrated viral DNA present in the host cell. While accumulation of large quantities of unintegrated viral DNA is a hallmark of an acute, cytopathic infection, significant amounts of unintegrated viral DNA are not generally observed in persistent HIV-1 infections. This has been ascribed to immunity from reinfection due to the loss or down-modulation of the CD4 receptor, a feature of most continuously infected cell lines. Using PCR of crude cell lysates, we have detected unintegrated HIV-1 DNA in chronically infected T lymphocytic (ACH-2, J1) and promyelocytic (OM-10.1) cell lines. Treatment with AZT or soluble CD4 inhibited accumulation of unintegrated viral DNA at least tenfold within 72 hours; removal of AZT permitted recovery to pre-treatment levels within 72 hours. Our results indicate that unintegrated HIV-1 DNA is unstable in these cell lines and originates from a continuous process of reinfection. Interestingly, the accumulation of unintegrated viral DNA was not correlated with the level of CD4 surface expression. By flow cytometry, OM-10.1 cells had relatively high levels of both surface CD4 expression and unintegrated viral DNA. ACH-2 and J1 cells were CD4-negative by flow cytometry, yet ACH-2 cells had a very low level of unintegrated viral DNA and J1 cells had a level of unintegrated viral DNA similar to OM-10.1 cells. This implies that the number of CD4 receptors is not rate-limiting for reinfection. We have been able to detect unintegrated HIV-1 DNA in PBLs from asymptomatic individuals as well as AIDS patients. Clinical studies are underway in our laboratory to establish a correlation between unintegrated DNA and disease progression.

M 104 IDENTIFICATION AND CHARACTERIZATION OF A TYPE D RETROVIRUS ISOLATED FROM AN AIDS PATIENT, Robert C. Bohannon[1,2], Lawrence A. Donehower[1], and Richard J. Ford[2]; [1]Baylor College of Medicine, Division of Molecular Virology, 1 Baylor Plaza, Houston, TX 77030; [2] M.D. Anderson Cancer Center, Department of Molecular Pathology, 1515 Holcombe, Houston, TX 77030

AIDS patients have a marked propensity to develop malignant non-Hodgkin's lymphomas. An AIDS patient presented with atypical syncytial variants of high grade B-cell lymphomas of the Burkitt's type. Electron microscopic examination of two established lymphoma cell lines from the patient indicated that large quantities of a type D retrovirus were being produced. Reverse transcriptase template-primer preferences, Southern hybridizations, immunoblot analysis and PCR amplification confirmed that the isolate was not HIV-1, HIV-2, HTLV-I, or HLTV-II. Using degenerate primers to a conserved region of *pol* in all known retroviruses, we cloned a 135 bp fragment of the isolate and DNA sequence analysis of this fragment indicated that the isolate was nearly identical to the Mason-Pfizer monkey virus (MPMV), a type D simian retrovirus that causes an AIDS-like syndrome in rhesus monkeys. Amino acid analysis of the p27 and p14 proteins of the isolate, along with Southern hybridization and immunoblot analysis, confirmed the relatedness of the the isolate to MPMV. The patient's serum recognized the viral isolate by immunoblot analysis, ELISA techniques and by immunoprecipitation of the p27 major capsid protein of MPMV. Additionally, PCR analysis of the patient's original diagnostic bone marrow biopsy with MPMV-specific primers demonstrated the patient was infected with the isolate. This is the first confirmed report of a human infected with a simian type D retrovirus. The serologic prevelance of this isolate and its possible pathologic effects will be discussed.

M 105 EQUINE INFECTIOUS ANEMIA VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS DNA SYNTHESIS IN VITRO: CHARACTERIZATION OF THE ENDOGENOUS REVERSE TRANSCRIPTASE REACTION, Katyna Borroto-Esoda and Lawrence R. Boone, Wellcome Research Laboratories, Research Triangle Park, NC 27709

The endogenous reverse transcriptase reaction of equine infectious anemia virus (EIAV) has been characterized. In contrast to results reported for other retroviruses, synthesis of EIAV full length (-) strand DNA was not impaired by high concentrations of NP-40, the nonionic detergent used to make the virion envelope permeable. Other components of the reaction were titrated for maximum synthesis of complete (-) strands and a time course was determined. Minor subgenomic bands were observed and both size and proportion varied with reaction conditions. Conditions established for EIAV DNA synthesis produced only modest yields of full genome length human immunodeficiency virus-1 (HIV-1) DNA synthesis. Prominent subgenomic reverse transcripts were observed in the HIV endogenous reaction, suggesting premature termination was a frequent event. Also with HIV-1, in contrast to EIAV, the synthesis of high molecular weight DNA was drastically reduced at NP-40 concentrations above 0.02%. These results indicated that a detergent stable core is not a property shared by all lentiviruses. The EIAV virion synthetic machinery is unusually stable and provides a convenient system for further study of in vitro replication of retroviruses.

M 106 STRUCTURAL CHANGES IN THE HIV-1 ENVELOPE GLYCOPROTEIN INDUCED BY CD4 BINDING
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HIV-1 infects susceptible T-cells by binding to CD4 molecules at the cell surface and then directly fusing with the cell membrane. The binding function is mediated by specific association of the external subunit of the viral envelope glycoprotein (gp120) with CD4, whereas the fusion event involves interaction of the transmembrane subunit (gp41) with the target cell membrane. A similar type of interaction is believed to mediate fusion between HIV-infected cells and CD4+ cells. We are investigating the possibility that CD4 binding might induce fusion-related structural changes in the envelope glycoprotein (gp120/gp41) complex. Soluble CD4 (sCD4) was found to promote dissociation of gp120 from the HIV-1 envelope glycoprotein complex expressed on the surface of cells infected with a vaccinia virus vector. sCD4-induced release of gp120 was dependent on time, temperature, and sCD4 concentration, and was associated with specific binding between these molecules. Release of gp120 was also induced by synthetic peptide derivatives corresponding to residues 81-92 of human CD4 (the CDR3-like region), which have previously been shown to inhibit HIV-mediated cell-cell fusion. A peptide containing the substitution of gly for glu at the postion correponding to CD4 residue 87 had greatly reduced gp120 release activity, consistent with the effect of this substitution on the cell fusion inhibitory activity of the peptide, as well as on the ability of membrane-associated CD4 to mediate cell fusion. We are currently investigating the region(s) of the CD4 and gp120 molecules involved in CD4-induced dissociation of the gp120/gp41 complex, as well as developing methods to test whether the fusogenic N-terminus of gp41 becomes exposed upon CD4 binding.

M 107 THE ROLE OF PROTEIN N-MYRISTOYLATION IN GAG-POL PRECURSOR PROCESSING AND VIRUS REPLICATION, ML Bryant[1,4], L Ratner[2], and J Gordon[2,3], [1]Pediatrics, [2]Medicine and Molecular Microbiology, [3]Biochemistry and Molecular Biophysics, Washington University School of Medicine and [4]G.D. Searle/Monsanto Company, St. Louis, MO 63110
Replication of HIV requires the production of myristoylated polyprotein precursors which when specifically cleaved by the viral protease form the structural components and replicative enzymes of the infectious virus particle. Substitution of the Gly^2 residue of Pr180gag-pol with Ala in a functional clone of HIV-1 blocks N-myristoylation and prevents virus replication in T-lymphoid cell lines and stably transfected HeLa cell clones. The mutant nonmyristoylated polyproteins are only weakly associated with the cellular membrane fraction, are not proteolytically processed, and fail to assemble into mature virions. Analogs of myristic acid, with oxygen and sulfur for methylene substitutions (similar chain length as myristate but marked reduction in hydrophobicity) when converted to CoA thioesters, serve as alternative substrates for NMT _in vitro_ and _in vivo_. 12-Methoxydodecanoic (13-oxamyristic acid) produces a concentration-dependent decrease in RT, p24 antigen, and syncytia formation in both acute and chronic virus replication assays. Metabolic labeling studies using $[^3H]$13-oxamyristic acid demonstrated cellular uptake and incorporation into HIV gag precursor(s) and into nef. In addition, recreation of eukaryotic protein N-myristoylation of HIV GAG-POL gene products in _E. coli_ has provided a unique way to analyze the physical-chemical features of myristate which are essential for virus assembly and in the specific activation of the replicative enzymes involved in human retrovirus infection.

M 108 VARIABLE PATHOGENESIS OF HIV-1 ON HUMAN T-CELLS: THE ROLE OF VIRAL AND CELLULAR FACTORS IN PATHOGENESIS, Robert W. Buckheit, Jr., D. Adam Plier,and Cynthia Hayslette-Doggett
Retrovirus Research Laboratory, Southern Research Institute, Birmingham, AL 35255
The pathogenesis of infection of human T cells by HIV-1 is quite heterogeneous. This heterogeneity is in part derived from differences which exist among the various HIV isolates and in part from differences which exist among the various human cell lines. We have been examining differences in the capacity of a panel of cultured human T cell lines to be infected with HIV-1 in order to identify the viral and cellular factors which are important in HIV replication and pathogenesis. We have identified two CEM cell lines which are divergent in their ability to be infected with HIV. Both of these lines express equivalent levels of cell surface CD4 and grow at the same rate in cell culture. Upon infection with 100 TCID50 units of virus, the HIV-sensitive cell line (CEM-SS) exhibit massive syncytia formation, produce high levels of infectious virus and are completely killed within 7 days of infection. A resistant CEM cell line (CEM-CCRF) infected with the same quantity of infectious virus slowly becomes 100% infected over the course of 20-30 days. These cells exhibit low level syncytia formation and only a slight, transient decrease in cell viability (5-10%). Virus production in CEM-CCRF cells, as measured by supernatant reverse transcriptase and intracellular p24 antigen expression, is much lower at any given time post-infection. Following the acute infection period, the CEM-CCRF cell line becomes chronically infected and produces high levels of infectious virus. Inefficient infectability is also observed upon cocultivation of the CEM-CCRF cell line with chronically infected, HIV-producing cells. In order to infect CEM-CCRF cells it requires 100 to 1000 fold greater numbers of infected cells than is observed for CEM-SS cells. We will present the results of our ongoing efforts to identify the viral and cellular factors responsible for differential infectivity of human cells.

M 109 MECHANISMS CONTROLLING LATENCY IN AN HIV-1 INFECTED PROMYELOCYTIC CLONE.
Salvatore T. Butera and Thomas M. Folks. Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA 30333.

Using the CD4+, latently-HIV-1-infected promyelocytic clone, OM-10.1, we have investigated the mechanisms whereby these cells become CD4- and HIV-1+ (by FACS and IFA respectively) within 24 hours following TNF-α treatment. When TNF-α is removed from these cultures, surface CD4 antigen returns and HIV-1 expression subsides by 3 days.

In TNF-α pulse experiments, where OM-10.1 cells were treated for brief periods with TNF-α and then placed back into medium for 24 hours, only a subpopulation of cells were found to undergo HIV-1 activation. The percentage of activated OM-10.1 cells, based on CD4 down-modulation, continued to increase during the 24 hour incubation and was variable depending upon the length of the initial TNF-α treatment (25% CD4- following a 15 minute treatment vs 64% CD4- following a 4 hour treatment). To better understand this control of latency, OM-10.1 cells were again treated for brief periods with TNF-α and then cultured in the presence of either anti-TNF-α antibodies or the PK inhibitor H-7 for 24 hours. Following TNF-α treatment, culture of OM-10.1 cells in the presence of anti-TNF-α antibodies was found to prevent the increasing HIV-1 activation. Yet, once the OM-10.1 cells had committed to activation during the treatment period, anti-TNF-α antibodies could not return these cells to a state of latency. However, H-7 treatment of OM-10.1 cells after TNF-α induction prevented further activation and quickly returned the committed cells to a latent HIV-1 state (within 24 hours) as compared to medium or HA1004 control cultures (72 hours).

In conclusion, anti-TNF-α antibody treatment was found to prevent the progressive increase towards HIV-1 activation while H-7 treatment caused a reversion of the committed cells and a return to HIV-1 latency in TNF-α-pulsed OM-10.1 cultures. The cellular model presented here permits further investigations into the role of cell cycle and second messenger pathway involvement on HIV-1 regulation.

M 110 FUNCTIONAL EXPRESSION OF HIV-1 INTEGRASE IN _E. COLI_: PROPERTIES AND SUBSTRATE REQUIREMENTS _IN VITRO_, Michael G. Cordingley, Pia L. Callahan and Robert LaFemina. Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486
The integrase gene of HIV-1 was expressed in _E. coli_ as the mature 32kD polypeptide (IN) but with a single additional N-terminal methionine residue. Purified recombinant IN protein was functional _in vitro_, exhibiting both endonuclease and integration functions. The Mn^{2+}-dependent endonuclease activity cleaved 2 nucleotides from the 3' end of substrate oligonucleotides representing HIV-1 proviral termini but did not cleave heterologous retroviral termini. These oligonucleotides served as both substrates and targets for integration by IN since molecules characteristic of integration products were generated in these reactions. Recombinant IN protein specifically bound LTR termini and formed a stable complex with substrate oligonucleotides in gel mobility shift assays. Mutational analysis of the U5 LTR substrate has been utilized to locate critical residues for substrate binding and/or cleavage by HIV-1 IN _in vitro_. These results suggest that the IN protein is capable of mediating all steps of retroviral integration in the absence of other viral or cellular factors. The integrase gene of HIV-1 represents an attractive antiviral target.

M 111 EXPRESSION, CHARACTERIZATION AND PURIFICATION OF HIV I PROTEASE CODED FROM A SYNTHETIC A/T- RICH GENE
ALBERT G. DEE, DENNIS P. SMITH, TOM C. HASSELL, HANSEN M. HSIUNG, RICHARD M. VAN FRANK AND MEI T. LAI
Lilly Research Labs, Eli Lilly & Co., Indianapolis, IN. 46285

An A/T- rich synthetic gene encoding HIV I protease was synthesized and cloned into an expression vector controlled by the lambda pL promoter and regulated by the c1857 thermolabile repressor. This plasmid was transformed into E. coli RV308 cells. SDS-PAGE and Western blot analysis of the heat induced plasmid revealed a greatly elevated level of expression as compared to the natural HIV I protease sequence in the same expression vector. The expressed protease was primarily in the form of inclusion bodies (granules). The protease has been purified and refolded to an active form from these granules. This active form of the protease cleaved recombinant HIV I GAG polyprotein properly. Amino acid sequence analysis of the purified protein showed that the expressed protein was indeed HIV I protease and that greater than 95% of the initiator methionine was cleaved off. Possible factors that may lead to the increased synthesis of protease will be discussed.

M 112 CONSTRUCTION OF RECOMBINANT ANTIBODY MOLECULES WHICH INTERFERE WITH HIV REPLICATION, Patrick J. Dillon, Chein-Hwa Chen, Craig A. Rosen, Department of Molecular Oncology and Virology, Roche Institute of Molecular Biology, Nutley, NJ 07110

The human immunodeficiency virus (HIV) is the primary etiological agent of acquired immunodeficiency syndrome (AIDS). Current anti-viral therapies against HIV have shown only limited success. In this study, we have examined the possibility of using recombinant antibody molecules to inhibit the functions of the essential viral regulatory proteins, Tat and Rev. We have isolated monoclonal antibodies which interfere with the ability of Tat and Rev to bind to their respective RNA targets, TAR and RRE. Using a PCR approach, we have cloned cDNAs encoding the heavy and light chain variable domains from these "neutralizing" antibodies and have constructed murine-human chimeric antibody molecules and single chain antibodies which retain the ability to bind Tat and Rev. A single chain antibody against the Rev protein has been synthesized in *E. coli* and is capable of interfering with Rev-RRE interaction in vitro. Modified recombinant antibody molecules capable of localizing to the nucleus will be tested for their ability to interfere with tat and rev function and will be discussed. In addition, recombinant antibodies against gp120 and CD4 have been constructed and their ability to prevent viral replication will be discussed.

M 113 STRONG DENATURING CONDITIONS PERMIT SPECIFIC BINDING OF HIV-1 TAT TO TAR RNA IN VITRO. Steven E. Fong, Patricia A. Smanik, Theresa W. Thais, Michele C. Smith and S. Richard Jaskunas. Department of Virology, MC619, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

The HIV-1 tat protein functions as a potent transactivator of viral gene expression, requiring for this activity a 59 base RNA stem-loop structure, TAR, located at the 5' terminus of the HIV-1 transcript. To determine whether tat can bind TAR RNA in vitro, we have conducted gel retardation assays using ^{32}P-labelled RNA and highly purified tat in which cysteine residues were blocked by sulfitolysis. Under nondenaturing binding and gel electrophoresis conditions tat was found to bind nonspecifically to non-TAR as well as TAR RNA species. In contrast, specific TAR-tat complexes were observed on 7 M urea acrylamide gels following binding in the presence of 0.1% to 1% SDS, 4 M urea or 1 M sodium iodide. Mutations in the lower stem, upper stem and bulge of the TAR- stem-loop resulted in reduced binding under these conditions, but binding was unaffected by mutations in the loop. These data suggest that strong denaturing conditions can reveal protein and/or RNA regions required for formation of specific complexes, and that under these conditions tat cysteine residues are not directly required for RNA binding. The denaturation conditions used may induce protein or RNA conformational changes that mimic changes induced in vivo by cellular factors.

M 114 REV ACTIVATES EXPRESSION OF THE HIV-1 VIF AND VPR GENE PRODUCTS, Elizabeth D. Garrett, Laurence S. Tiley and Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710.

The gene products encoded by HIV-1 can be divided into two, temporally regulated classes. Early, regulatory gene products are encoded by multiply spliced mRNA species and are expressed constitutively. In contrast, the late, structural proteins of HIV-1 are encoded by an unspliced or singly spliced class of viral transcripts whose cytoplasmic expression is dependent upon the viral Rev *trans*-activator. We will present data demonstrating that the viral Vif and Vpr gene products are encoded by singly-spliced viral mRNAs whose expression is activated by Rev. This activation is shown to result from the reduced utilization of splice sites adjacent to or within the vif and vpr coding sequences. The observation that vif and vpr belong to the late class of HIV-1 gene products is consistent with the hypothesis that Rev activates the expression of incompletely spliced HIV-1 transcripts by circumventing a cellular barrier to nuclear RNA export that is imposed by the presence of intact splice sites.

M 115 HUMAN CHROMOSOME 12 IS REQUIRED FOR HOMOLOGOUS BUT NOT HETEROLOGOUS *TAT*-TRANS-ACTIVATION OF THE HIV-1 & 2 LTR. Clyde Hart, Judy Galphin, Mark Westhafer, Chin-Yih Ou, and Gerald Schochetman. Centers for Disease Control, Atlanta, GA 30333.

Host cell factors are believed to be involved in the interaction of *tat* with TAR. We investigated whether human chromosome 12 in human-hamster hybrid cells, which supports high level HIV-1 trans-activation [Hart et al., Science 246, 488-491 (1989)], may encode a trans-activation pathway used by other human retroviruses. Human-hamster hybrid cell clones with defined sets of human chromosomes were assayed by transient DNA transfection for support of HIV-1 and -2 and HTLV I LTR-CAT gene expression. Of 17 hybrid clones tested, the 4 hybrid clones with chromosome 12 showed the highest HIV-2 *tat* (tat_2)-induced trans-activation of the HIV-2 LTR (LTR2). Trans-activation of the HTLV I LTR or HIV-1 LTR by the HTLV I trans-activator gene (tax_1) was not increased by the presence of chromosome 12. Cells without chromosome 12 supported heterologous tat_1 trans-activation of the HIV-2 LTR 4.7 to 5.7 fold higher than the homologous HIV-1 trans-activation; chromosome 12-containing cells had a 0.9 to 3.4 fold increase in tat_1 trans-activation of the HIV-2 LTR compared to the homologous HIV-1 trans-activation. Our results suggest that a complete heterologous trans-activation of the HIV-2 LTR may be supported by a combination of two or more cellular mechanisms; the chromosome 12 pathway and an additional less efficient secondary mechanism. Furthermore, chromosome 12 supports a homologous HIV-1 and -2 trans-activation mechanism but not a universal trans-activation mechanism for human retroviruses.

M 116 A NEW AND RAPID METHOD FOR THE QUANTITATIVE DETECTION OF HIV-1 SPECIFIC DNA IN PLASMA/SERUM Denis R. Henrard, William E. Mehaffey and Jean-Pierre Allain
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OBJECTIVE: To develop a rapid and sensitive method, not involving organic solvents for the extraction of nucleic acids from serum/plasma.
METHODS: We analyzed normal serum/plasma for the presence of the single-copy β-globin gene, and normal plasma spiked with titrations of HIV-1 containing plasmid (puc23) or genomic DNA prepared from H9-IIIB infected cells. After bringing the sample to pH 10.0 in 1% SDS, the proteins are selectively precipitated with a 5.0 M acetate solution. The DNA was then precipitated with isopropanol in the presence of carrier and directly analyzed by PCR. Human β-globin specific and HIV-1 specific gag and LTR primers were used. PCR products were analyzed by liquid hybridization with labeled oligonucleotides followed by PAGE and autoradiography.
RESULTS: β-globin sequences were detected in 100% of the 20μl samples. Fewer than 10 copies of HIV-1 DNA could be routinely detected in 150 μl of spiked serum/plasma. No HIV-1 reactivity was observed in unspiked normal samples.
CONCLUSION: This rapid and sensitive method could provide an alternative to the preparation of PBM cells for the detection of HIV-1 and could be applied to the direct detection of many pathogenic DNA viruses.

M 117 **HIV1 CYTOPATHICITY - A MULTIGENIC PHENOMENON : GENETIC DIFFERENCE BETWEEN DIRECT KILLING AND SYNCYTIA FORMATION EFFECT.** Ivan Hirsch, Bruno Spire, Danielle Salaun, Yasuki Yokota and Jean-Claude Chermann.
Unité de Recherches sur les Rétrovirus et Maladies Associées (INSERM U 322), BP 33, 13273 Marseille Cedex 9, France.

Highly cytopathic Zaïrian virus HIV1 NDK was compared with HIV1 BRU prototype to perform genetic analysis of phenotypic differences in growth properties, cell killing ability and fusogenic potential. Recombinant provirus molecules derived from DNA cloned HIV1 BRU and HIV1 NDK were constructed using conservative *Bss*HII, *Spe*I, *Eco*RI and *Xho*I sites. Recombinant viruses obtained after transfection were standardized according to RT activity and end point dilution. Their replication and killing characteristics were measured by RT and MTT tests, respectively, in MT4 and CEM cells ; fusogenicity was determined in MT4 cells. Formation of large syncytia, rapid cell killing and early onset of replication were characteristics of HIV1 NDK. HIV1 NDK derived *Spe*I/*Eco*RI fragment covering part of the *gag* and *pol* genes conferred the early onset of replication to recombinant viruses. All those recombinants were also rapidly killing the target cells. However not all of them were highly fusogenic. Combination of HIV1 NDK derived fragments *Bss*/HII/SpeI and *Eco*RI/*Xho*I was necessary for the full expression of HIV1 NDK fusonegic phenotype ; the HIV1 NDK *env* and *gag* genes alone were not sufficient to obtain high titer of large syncytia. Different regions of HIV1 genome were responsible for the direct-killing and fusogenic effect. LTR and regulatory genes *vpr*, *tat* and *rev* were not responsible for the variation of HIV1 replication kinetic.

M 118 BIOCHEMICAL CHARACTERIZATION AND CRYSTALLIZATION OF THE RNase H DOMAIN OF HIV-1 REVERSE TRANSCRIPTASE,

Zuzana Hostomska, Zdenek Hostomsky, Jay Davies and David Matthews, *Agouron Pharmaceuticals, Inc., 505 Coast Blvd., La Jolla, CA 92037*

HIV-1 reverse transcriptase forms a heterodimer composed of two subunits (p51 and p66), which have identical N-termini. Compared to p51, the p66 subunit has an additional C-terminal domain, p15. The amino acid sequence of the p15 domain shows homology with RNase H of other retroviruses as well as with *E. coli* RNase H. To examine the functional interaction between the domains of HIV-1 RT, we expressed them separately and purified both to homogeneity. The N-terminal domain (p51) behaves as a monomeric protein exhibiting salt sensitive DNA polymerase activity. The isolated C-terminal domain (p15) has no detectable RNase H activity. However, purified p51 and p15 combined *in vitro* reconstitutes RNase H activity on a defined substrate. These results demonstrate that domains of HIV-1 RT, although structurally distinct, are functionally interdependent. The p15 domain has been crystallized as large trigonal prisms which diffract X-rays to a resolution of at least 2.5 Å. A full three dimensional structural elucidation of the p15 domain is now underway.

M 119 CIRCULATING CD8+ CYTOTOXIC T LYMPHOCYTES SPECIIFC FOR HTLV-I pX IN PATIENTS WITH HTLV-I NEUROLOGICAL DISEASE, Steven Jacobson*, Hisatoshi Shida@, Dale E. McFarlin*, Anthony S. Fauci# and Scott Koenig#. *Neuroimmunology Branch NINDS/NIH, Bethesda, MD. USA; @Institute of Virology, Kyoto University, Kyoto, Japan; #Laboratory of Immunoregulation, NIAID/NIH, Bethesda, MD. USA.

HTLV-I is associated with adult T cell leukemia and a chronic-progressive disease of the central nervous system (HAM/TSP). Why one group of HTLV-I seropositive individuals develops a neoplastic condition, another develops neurologic symptoms, and a third group remains as asymptomatic carriers of the virus is not known. The various mainfestations of an HTLV-I infection may be related to differences in genetic backgrounds of individuals, infection with variant strains of HTLV-I or differences in target tissue tropism of the virus. Another possibility is that differences in host immune responses to HTLV-I contribute to these varied HTLV-I associated conditions. While the humoral response to HTLV-I is well characterized, little is known about the human cellular immune response, such as cytotoxic T cells (CTL) particularly in patients with neurological diseases associated with HTLV-I. Here we report the presence of high levels of circulating HTLV-I specific CTL in patients with HTLV-I associated neurologic disease but not in HTLV-I seropositive individuals without neurologic involvement. These CTL are CD8+, HLA class I restricted and predominantly recognize the HTLV-I gene products encoded in the HTLV-I regulatory region, pX. These findings suggest that HTLV-I specific CTL may contribute to the pathogenesis of HTLV-I associated neurological disorders.

M 120 HIV *GAG* PARTICLE MORPHOGENESIS STUDIED USING RECOMBINANT BACULOVIRUSES

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When the p55 *gag* protein of HIV is expressed in insect cells using recombinant baculoviruses it assembles into HIV core like particles (1,2,3). Preliminary evidence, however, indicates that p160 *gag-pol* proteins (formed by frameshifting of the *gag* and *pol* reading frames) do not form particulate structures (4). However, it is unclear if the inability of p160 *gag-pol* to form particles is the result of the truncation of the *gag* open reading frame by the frameshifting event or the addition of the *pol* domain to the *gag* protein. To address this question we have constructed a recombinant baculovirus that expresses a p46 *gag* representing the HIV *gag* open reading frame truncated at the frameshift site and lacking the carboxy terminal p6 domain. We show that this recombinant produces high levels of p46 *gag* antigen which is secreted into the culture media as an HIV core like particle. We conclude that the carboxy terminal p6 domain of *gag* plays no role in particle formation suggesting that failure of p160 *gag-pol* to assemble is a consequence of the presence of the *pol* domain. In addition, these results suggest that replacement of the p6 domain with foreign antigens may be possible in order to use truncated *gag* antigens as particulate carriers.

1)Overton *et al.*, Virology 170; p107 (1989)
2)Gheysen *et al.*, Cell 59; p103 (1989)
3)Delchambre *et al.*, EMBO J 8; 2653 (1989)
4)Kojima *et al.*, Abstracts of the VIIIth International congress of Virology, Berlin (1990)

M 121 EFFECTS OF CELL DIFFERENTIATION ON HIV GROWTH: USE OF PROMYELOCYTIC CELL LINE HL-60 AS A MODEL SYSTEM.
Sunyoung Kim[1], Mark Feinberg[2], and Paula Cannon[1], [1]New England Deaconess Hospital, and Harvard Medical School, Boston, MA 02215;[2]Whitehead Institute, Cambridge, MA 02142.

One possibility to account for HIV infection of macrophages is that HIV initially infects cells of early myeloid lineage, which later differentiate into infected macrophages. We have investigated this possibility using the multipotent cell line HL-60, which can be induced to differentiate into various cell types under different conditions. First, we have tested the effects of cell differentiation on gene expression driven by the long terminal repeat (LTR) of HIV. When cells were treated with PMA, which causes differentiation into macrophage-like cells, CAT activity increased by 10 to 20 fold. This induction appears to be mediated through as yet unidentified inducible factor(s), in addition to NF-kB. Also, we have subcloned HL-60 cells infected with HIV. Our preliminary data suggest that viral production increases upon treatment of these cells with PMA, but only transiently. We are currently analyzing the kinetics of RNA expression upon PMA induction. Data will also be presented concerning the effect of HIV infection on cell differentiation, assayed by the expression of several cell surface proteins.

M 122 HIV-1 *vpu* PROTEIN REGULATES ENVELOPE GLYCOPROTEIN ACCUMULATION AND PROCESSING IN THE GOLGI,
Tominori Kimura and Jonathan Karn, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England
Production of high levels of infectious HIV-1 particles requires the expression of three poorly understood proteins encoded by the *vpu*, *vif* and *vpr* genes. The products of these genes are not required for regulation of HIV gene expression nor are they found in mature virus particles. It seems likely that these proteins play a role in regulating virus assembly either by regulating virion protein biosynthesis or, alternatively, by acting as "molecular chaperones" that help direct virion proteins towards the budding particle on the cellular membrane. Viruses carrying mutations in the *vpu* gene show delayed release of progeny virus, and an accumulation of characteristic virion particles with an "immature" morphology and an unusual pattern of budding. *Vpu* also shows a homology to a number of membrane-bound proteins including two other viral proteins known to influence virion assembly, the foot-and-mouth disease virus p3A protein, and the influenza virus M2 protein. We have found that *vpu* plays a role in envelope glycoprotein levels and processing. Efficient production of gp160, processing of gp160 to gp120 and gp41, and secretion of gp120, is only observed when the *vpu* and *env* genes are co-expressed in COS-1 cells. The major effect of *vpu* is to increase the intracellular levels of both gp160 and gp120. Fourteen times more gp160 is produced from HIV plasmids carrying intact *env* and *vpu* genes than from *vpu* minus plasmids. *Vpu* is also active *in trans*, and increases gp160 expression 3- to 4-fold. Cleavage of gp160 to gp120/gp41 takes place prior to the acquisition of endo-H resistance by the addition of complex sugars in the *trans* Golgi, and is independent of *vpu*. Treatment of cells expressing *vpu*, with NH_4Cl increases the levels of gp160 and gp120, presumably by inhibiting lysozomal degradation of the envelope glycoproteins. NH_4Cl also partially inhibits cleavage of gp160 to gp120/gp41. However, in the absence of *vpu* NH_4Cl has little effect on either the level of gp160 expression or the rate of gp160 cleavage. Immunofluorescence microscopy has demonstrated that both *vpu* and gp160 accumulate in wheat germ agglutinin-stained vesicles of the Golgi, but neither protein is transported to the cell surface. In contrast, gp120 and *gag* proteins are found associated with cytoplasmic membranes which are not stained by wheat germ agglutinin, and a small amount of gp120 can also be detected on the cell surface. It is proposed that *vpu* acts as an ion channel which regulates the pH of the Golgi and thereby indirectly influences envelope protein processsing and accumulation.

M 123 TNF-ALPHA INDUCES CIRCULAR FORMS OF HIV-1 DNA IN A LATENT CELL LINE.
K. Kitamura, N.J. Besansky, S. Sinha, P. Mautner*, J.P. Spadoro* and T.M. Folks, Retrovirus Disease Branch, DVRD, CDC, Atlanta, GA 30333, *ENZO Biochem, New York, NY 10013.
The latent T cell line, ACH-2, was used to investigate the accumulation of the 2-LTR circular form of (unintegrated) HIV-1 DNA after TNF-alpha (TNF-a) induction. Using PCR to amplify across the 2-LTR junction, we have determined the cellular location and relative abundance of circular HIV-1 DNA following TNF-a stimulation. A ten-fold increase in HIV-1 p24 antigen was detected in the 48-hour supernatant of TNF-a induced ACH-2 cultures (500 Ng/ml) over unstimulated controls (<50Ng/ml). Concommitantly a 10-fold increase in circular HIV DNA was detected in the treated cells by PCR analysis following limiting dilution. To identify the cellular compartmental location of the circular DNA accumulation, the cells were fractionated into cytoplasmic and nuclear components and analyzed by PCR. Southern analysis of 2-LTR amplified product from the treated cells indicated that the nucleus was the source of the 2-LTR HIV-I DNA. To support the PCR analysis, fluorescent in situ hybridization was performed on ACH-2 cells following TNF-a induction. Using a biotinylated HIV-1 DNA probe, increased nuclear fluorescence was observed in the TNF-a induced cells over controls. These findings indicate that 2-LTR HIV DNA accumulates in the nucleus of TNF-a induced latent cells and offers insight into the role of unintegrated viral DNA in pathogenesis.

M 124 STUDIES INTO THE BASIS FOR THE APATHOGENICITY OF SIMIAN IMMUNODEFICIENCY VIRUS FROM AFRICAN GREEN MONKEYS, Reinhard Kurth, Stephen G. Norley, Joachim Ennen, Herbert König and Klaus Cichutek,
Paul-Ehrlich-Institute, D-6070 Langen/Frankfurt, FRG
Potential reasons for the lack of pathogenicity of SIVagm in its natural host, the African green monkey (AGM), were investigated with respect to immunological and other mechanisms. It was shown that the functional immune response of monkeys to infection was similar (though not identical) to that of humans to HIV-1 infection. In the sera of infected animals, levels of neutralizing antibody were found to be low or absent and in particular there was no neutralization of different isolates by homologous sera. There was no detectable antibody/complement cytotoxicity (ACC), though AGM sera were able to initiate antibody dependent cellular cytolysis (ADCC) and cytotoxic T-cell responses to infected cells. Thus, the immune response to SIVagm in AGM is neither qualitatively nor quantitatively very different from the response in HIV-infected individuals.
As the tropism for HIV and SIVagm is also indistinguishable, other host or viral factors must account for the lack of SIVagm pathogenicity. We have found that the reverse transcriptase (RT) of SIVagm is less error-prone than the RT of HIV, reducing the rate of SIVagm variant formation. In addition, the SIVagm load in PBLs is 10- to 500-fold lower than in ARC/AIDS patients, suggesting an intracellular suppression of virus expression in AGM.

M 125 CHARACTERIZATION OF A HTLV-II ISOLATE FROM A CD4+/CDw29+ CELL LINE DERIVED FROM A GUAYMI INDIAN FROM PANAMA. Michael D. Lairmore[1], R. Lal[2], B. Roberts[2], K. Hadlock[3], G. Keyes[3], Thomas Folks[2], et al. The Ohio State University[1], Columbus, OH; Centers For Disease Control[2], Atlanta, GA; GeneLabs, Inc.[3], Redwood City, CA.

To characterize a unique Guaymi Indian HTLV-II isolate (PNAS, Nov. 15, 1990) a peripheral blood lymphocyte (PBL) cell line (G 12.1) was established by co-culture methods. A continuous IL-2 dependent cell line has been maintained independent of normal feeder PBLs for 10 months of culture. The cell line has sustained production of soluble and cell surface HTLV-II p24 capsid proteins (assessed by antigen capture assay and avidin-biotin-complex assays), contains approximately 10 copies of proviral HTLV-II DNA per cell as determined Southern blot analysis, budding HTLV particles has been observed by electron microscopy, and lethally irradiated G12.1 cells transmitted the HTLV-II infection to rabbits. Western blot analysis using type specific monoclonal antibodies and PCR analysis using strain specific oligonucleotide primers and probes have confirmed the virus type to be HTLV-II. Serum derived from the Guaymi Indian donor was positive for antibody reactivity in HTLV-II specific env peptide-based assays and failed to react with HTLV-I env and gag peptides. Phenotype analysis by FACS indicated the cell line to be CD4+ (Leu 3a) and CDw29+ (4B4) with high expression of CD25 (IL-2R, L243) and CD71 (transfer receptor); the cell line was negative for CD8, CD14, CD16, and CD19 antigens. In addition, the cell line produced the cytokines gamma interferon and GM-CSF, but is negative for IL-4 and TNF-alpha. A G12.1 genomic DNA library has been constructed and 4 molecular clones which hybridize to both HTLV-II env and gag specific DNA probes have been isolated. These clones are currently being exploited for nucleotide sequencing and other studies. The G12.1 cell line will allow continued characterization of this unique HTLV-II isolate.

M 126 MOLECULAR CLONING AND ANALYSIS OF REPLICATION COMPETENT HIV-1 PROVIRUSES FROM UNCULTURED HUMAN BRAIN IN AIDS DEMENTIA COMPLEX. Yuexia Li[1], Huxiong Hui[1], John C. Kappes[1], Joan Conway[1], Richard W. Price[2], Beatrice H. Hahn[1], and George M. Shaw[1]. [1]University of Alabama at Birmingham, AL 35294, [2]University of Minnesoda, Minneapolis, MN 55455.
All replication-competent proviral clones of HIV-1 available to date have been obtained from cell cultures of amplified virus. In this project, we sought to molecularly clone and genetically and biologically characterize full-length HIV-1 proviruses directly from uncultured human brain tissue of a patient with AIDS Dementia Complex (ADC). Specific objectives were (i) to generate replication competent proviral clones from uncultured human tissue thereby allowing an analysis of genome organization and gene structure-function relationships of virus not subjected to *in vitro* selection pressures, (ii) to generate transfection-derived HIV-1 strains without interim cell culture for analysis of replicative DNA intermediate forms, integration status, and the possible existence of defective and/or helper forms, (iii) to compare and characterize genotypic variation of proviral clones obtained by direct lambda phage cloning to clones obained by PCR amplification. High molecular weight DNA from a brain specimen obtained at necropsy was subjected to lambda phage cloning and 10 HIV-1 proviral clones were obtained out of 8×10^6 recombinants. These proviral clones contained integrated and unintegrated forms, forms with one or two LTRs, and genomes with large deletions and self-integrated reversed LTR sequences. Eight HIV-1 proviral clones (in lambda) and 11 PCR derived clones from the same brain were sequenced in a 525 bp hypervariable envelope region. All 19 clones were highly related yet distinct with nucleotide variation between 0.1 and 0.3%. Four proviral clones in lambda were full-length by restriction mapping, and one of these was subsequently shown to be transfection-competent in Cos-1 cells and replication competent in human T cells and monocytes after cell free passage. The genomic organization, structure-function characteristics of specific gene products, and the biological properties of these HIV-1 proviral clones derived from uncultured human brain are under study.

M 127 **The Role of the Human *rel* Oncogene Product in Control of Transcription by the Human Immunodeficiency Virus Long-Terminal Repeat (HIV1-LTR) κB Elements,** Yuchi Li, JooHun Lee, Gilda Mak, Janet Ross, and B. Robert Franza, Jr., Freeman Laboratory of Cancer Cell Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724.

Several years ago we identified a set of cellular proteins that interact specifically with the κB sites within the HIV1-LTR. One of these proteins demonstrates different levels of constitutive expression in human B and T lymphoblast cell lines. It is significantly inducible by mitogenic lectins, tumor promoters and the protein synthesis inhibitor cycloheximide. We designated the protein HIVEN86A. Other proteins shared some of the properties of HIVEN86A. In addition, we identified proteins that were apparently cell-type specific. Recently, we have demonstrated (with W. Greene and colleagues, Duke University) that HIVEN86A is the product of the human *rel* proto-oncogene. We will discuss immunologic analysis of Rel and other proteins that associate with the κB site that are related to Rel. We will also discuss the fact that at least one protein is immunologically related to Rel but demonstrates different binding site requirements than Rel. Functional characterization of the κB sites within the HIV1-LTR by *in vitro* transcription analysis of extracts derived from cells at different moments within the first two hours of stimulation by mitogenic lectin or tumor promoter reveals that eventhough they contribute to activation of transcription they are not sufficient. The role of Rel and other proteins that interact with the κB site in activation are being characterized biochemically and results of these studies will be presented.

M 128 Induction of Nuclear NF-kB DNA Binding Activity After Exposure of Lymphoid Cells to Soluble Tax_1 Protein. Paul F. Lindholm, Susan J. Marriott, Scott D. Gitlin, Cindy A. Bohan, John N. Brady, Laboratory of Molecular Virology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

We have demonstrated that purified HTLV-I Tax_1 protein can be taken up by 70Z/3 lymphoid cells and localized to both the nuclear and cytoplasmic compartments. Introduction of the Tax_1 protein into the growth medium of 70Z/3 cells resulted in the rapid and transient induction of NF-kB binding activity in the nuclear fraction. Tax_1 activation of NF-kB was not sensitive to either staurosporin or prolonged stimulation with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate, suggesting that Tax_1-dependent NF-kB activation did not require the protein kinase C pathway. Purified Tax_1 did not directly increase NF-kB binding activity in 70Z/3 cytoplasmic extracts, suggesting that NF-kB induction may require cellular factors. Western blot and competitive radioimmunoassays demonstrated that Tax_1 protein was present in the tissue culture media of HTLV-I-transformed cell lines. These results show that extracellular Tax_1 may regulate cellular gene expression in noninfected cells. We are currently investigating the possibilities that Tax_1 may induce NF-kB through a receptor mediated signal transduction event or via direct effects of internalized Tax_1 on the stability of the NF-kB-IkB cytoplasmic complex.

M 129 Soluble HTLV-I Tax_1 Stimulates Proliferation of Competent Human Peripheral Blood Lymphocytes. Susan J. Marriott, John Brady, Laboratory of Molecular Virology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

The human T-cell leukemia/lymphoma virus type I encodes a regulatory protein Tax_1 which activates expression of both viral and cellular genes and has been implicated in the transforming potential of the virus. Since antibodies to Tax_1 are often found in the serum of patients infected with the virus, it is interesting to consider that Tax_1 may be present as an extracellular protein and may play a role in progression of HTLV-I-associated disease. We have recently demonstrated that extracellular Tax_1 is taken up by a variety of cell types including human peripheral blood lymphocytes (PBLs) and transported to the nucleus as an intact 40 kD protein. To investigate the potential role of extracellular Tax_1 in cell growth regulation, human peripheral blood lymphocytes (PBLs) were cultured from healthy HIV and HTLV-I negative donors. Cultured PBLs were treated with phytohemagglutinin (PHA) followed by interleukin-2 (IL-2), purified Tax_1 protein, or a mock bacterial extract containing no Tax_1. An increase in cell proliferation was observed in both the Tax_1 or IL-2 treated, but not in the control treated cells. PBLs exposed to Tax_1 exhibited prolonged sensitivity to interleukin-2-induced proliferation. It has previously been suggested that stimulation of cell growth could result from the transcriptional activation of IL-2 and IL-2 receptor alpha chain by Tax_1. Our studies suggest a new biological role of Tax_1 which may contribute to the leukemogenesis and neurologic complications observed in HTLV-I infected individuals.

M 130 SITE-DIRECTED MUTAGENESIS OF LYS^{263} OF HUMAN IMMUNODEFICIENCY VIRUS-1 REVERSE TRANSCRIPTASE,

J. Louise Martin, Jeanne E. Wilson, Samuel E. Hopkins, and Phillip A. Furman, Division of Virology, Burroughs Wellcome Co, Research Triangle Park, NC 27709

Site-directed mutagenesis has been used to assess the importance of lysine^{263} in substrate binding of human immunodeficiency virus-1 reverse transcriptase. Previous studies have indicated that lysine^{263} functions in the binding of 2'-deoxynucleoside-5'-triphosphate (dNTP) substrates (Basu *et al*, JBC, 1988). We have chosen to study this interaction directly by using site-specific mutagenesis to change lysine^{263} to a serine and an isoleucine. Characterization of highly purified mutant enzymes has shown that K263S and K263I bind natural dNTP substrates and primed polynucleic acid substrates with equal affinity when compared to the wild-type reverse transcriptase. No difference was observed in the binding of AZTTP to either mutant on the basis of K_m and K_i determinations. Neither substitution had any effect on RNase H activity. These results indicate that lysine^{263} is not essential in the binding of substrates to HIV-1 reverse transcriptase.

M 131 **CIS- AND TRANS-ACTING ELEMENTS REGULATING EXPRESSION OF SIMIAN FOAMY VIRUS TYPE-1,**

Ayalew Mergia, Karen E. S. Shaw, Elissa Pratt-Lowe, Peter A. Barry, and Paul A. Luciw, Department of Medical Pathology, University of California, Davis, CA 95616

The three subfamilies of retroviruses are oncoviruses, lentiviruses, and spumaviruses. The latter, also designated foamy or syncytium-forming viruses, are found in a variety of species, including humans, and are highly cytopathic in tissue culture systems. In the host, spumaviruses appear to establish latency; a clear connection with disease has not yet been established. We have molecularly cloned and sequenced the genome of simian foamy virus type-1 (SFV-1), an isolate obtained from rhesus monkeys. Sequence analysis reveals that SFV-1 is 60% to 80% related to the human foamy virus (HFV) in the gag, pol, and env genes. Both SFV-1 and HFV contain open reading frames (ORFs) which extend from the end of the env gene into the 3'LTR. The ORFs of SFV-1 and HFV show only 39% similarity. Analysis of the long terminal repeats (LTR) of both viruses reveals that the R-U5 and the U3 regions are about 85% and 30% related, respectively. Using transient expression assays in tissue culture systems, we have demonstrated that SFV-1 encodes a transactivator that acts through sequences in the LTR. This transactivator functions at the transcriptional level to augment initiation of viral transcripts. A cis-acting target element for the SFV-1 transactivator has been localized to the U3 region of the LTR upstream from the cap site that marks the initiation site for viral transcripts. RNA transcripts from the ORF region have been detected in infected cells and cDNA libraries have been analyzed; multiple splicing events give rise to these viral transcripts. Transient expression assays identified the first ORF (ORF1) as the transcriptional transacivator of foamy virus, designated *taf*. Current efforts are directed at (i) precisely defining the the cis-acting element, and (ii) elucidating the mechanism of transactivation. Thus, members of each of the three retrovirus subfamilies (e.g., the oncovirus human T-lymphotropic virus type-1, the lentivirus human immunodeficiency virus type 1, and the spumavirus SFV-1) encode transactivator genes that act through elements in the LTR to regulate viral gene expression. These investigations provide a basis for comparing pathogenic and non-pathogenic primate retroviruses and for determining the molecular mechanisms that may account for retroviral latency and disease.

M 132 DEMONSTRATION OF HIV-1 INFECTION IN AT-RISK SERONEGATIVE CHILDREN BY REGULATORY PEPTIDE-ELISA (reg-PE), STRUCTURAL PEPTIDE-ELISA (struct-PE) AND POLYMERASE CHAIN REACTION (PCR).

Viviana Moschese, Valter Lombardi, Carlo Fundarò, Anna Plebani, Pierangelo Tovo, Britta Wahren and Paolo Rossi. Dept of Immunology, Karolinska Institute and Dept. of Virology, NBL, Stockholm, Sweden; Depts. of Pediatrics, Catholic University of Rome, University of Milan and University of Turin, Italy.

OBJECTIVE: To investigate the occurrence of HIV-1 infection in seronegative children born to HIV-1 infected mothers by reg-PE, struct-PE and PCR and compare the differing techniques. PATIENTS AND METHODS: 36 at-risk children negative to standard HIV-1 serology were studied. A panel of env and gag synthetic peptides (SP) and of nef, rev and tat SP were used, respectively, for reg-PE and struct-PE. PCR analysis was performed using three sets of primers specific for select HIV-1 viral DNA sequences. 20 age-matched HIV-1 positive children and 15 non at-risk HIV-1 negative children were tested as positive and negative controls. RESULTS: 1/36 at-risk seronegative child (3%) was positive to all used techniques, 1/36 was seroreacting to both reg-PE and struct-PE but negative to PCR and 1/36 was only positive to reg-PE and PCR . As to the positive controls, 20/20 (100%) seroreacted to struct-PE, 17/20 (85%) to reg-PE and 19/20 (95%) were positive to PCR. CONCLUSIONS: This study suggests the need of further HIV-1 diagnostic evaluation in at-risk seronegative children but also indicates that the accuracy of each single technique has still to be ascertained.

M 133 CONTRIBUTION OF PRIMARY NUCLEOTIDE SEQUENCE FOR INTERACTION OF HIV-1 REV PROTEIN WITH RRE RNA, Henrik S. Olsen, Alan Cochrane, Patrick J. Dillon, and Craig A. Rosen, Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Interaction of the human immunodeficiency virus Rev protein with a structured region within *env* mRNA (termed RRE) mediates the export of virus structural mRNAs from the nucleus to the cytoplasm. We have shown in previous studies using a combined gel retardation RNase protection assay, that purified Rev protein forms a specific interaction with an RNA secondary structure generated within the RRE. Furthermore, these studies indicate that secondary structure, rather than primary nucleotide sequences, is the major determinant for Rev interaction with RNA.

To extend our analysis of Rev-RRE interaction, we have used *in vitro* mutagenesis of the RRE to mutate specific nucleotides adjacent to a stem loop structure previously shown to be critical for Rev-RRE RNA binding. We find that *in vitro* Rev-RRE interaction as well as *in vivo* function of Rev requires the presence of certain nucleotides. These results suggest that highly structured regions of RRE RNA may serve as a guide to bring Rev in contact with a primary nucleotide sequence required for stable protein-RNA interaction.

M 134 MUTATIONAL AND FUNCTIONAL ANALYSIS OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 2 VIRION INFECTIVITY FACTOR UTILIZING A QUANTITATIVE POLYMERASE CHAIN REACTION METHOD, Jeffrey S. Parkin, John C. Kappes, Beatrice H. Hahn, and George M. Shaw, Departments of Biochemistry, Microbiology, and Medicine, The University of Alabama at Birmingham, Birmingham, AL 35259.
Human and simian immunodeficiency viruses (HIV/SIVs) contain a well-conserved open reading frame (ORF) capable of encoding a 23-25 kDa protein designated the virion infectivity factor (vif; previously termed sor, A, Q, P, or ORF-1). In order to more thoroughly analyze the role of the vif gene product in viral replication, a series of biochemical and molecular studies were undertaken. Mutational analysis of the HIV-2_{ROD} vif ORF resulted in the production of progeny virions that were significantly impaired (100-1,000 X) in cell-free viral transmission in a variety of $CD4^+$ immortalized lymphocytic cell lines (Sup-T1, H9, CEM, CEMx174), as well as in peripheral blood lymphocytes (PBL). Western analysis of HIV-2_{ROD} infected cell lysates utilizing anti-vif polyclonal rabbit antisera demonstrated the presence of two co-migrating forms of the protein at 26 and 27 kDA, respectively. Western and radioimmunoprecipitation analysis of sucrose gradient purified HIV-2_{ROD} virions demonstrated that the vif protein is not an integral virion protein. Electron microscopic analysis of vif^- infected cultures revealed the presence of morphologically normal virions 100-120 nm in diameter with cylindrical core structures, the presence of mature gp120-gp41 complexes on the virion surface, and the ability of mature virions to assemble and budd from the cytoplasmic membrane. Quantitative polymerase chain reaction (PCR) analysis of genomic DNA extracted from different immortalized lymphocytic cultures and PBL following cell-free viral transmission demonstrated that vif^- viruses were capable of initiating reverse transcription, complementary DNA synthesis, and completion of full-length viral DNA synthesis. Temporal quantitative PCR experiments between wildtype and vif mutants demonstrated equivalent levels of viral DNA at 1hr, minimal differences at 24 hr (3-8 X), and significant differences at 48 hr (10-30 X). Further characterization of the role of the vif gene product in viral replication is currently underway.

M 135 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.

M 136 THE HTLV-1 TAX PROTEIN CAN ACT AS BOTH A SUPPRESSOR AND ACTIVATOR OF NFKB IN T-CELLS, Steven M. Ruben and Craig A. Rosen, Department of Oncology & Virology, Roche Research Center, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The Tax protein of HTLV-I is capable of activating both viral and cellular gene expression including IL-2 and IL-2 Ra. While Tax does not interact directly with target sequences, transcriptional activation by Tax may be mediated by interaction with host proteins. The regulatory protein NFkB has been implicated in activation of IL-2Ra Tax. We have demonstrated the presence of a specific lymphoid factor, LkB-A, in a Tax expressing B-cell line necessary for activation of NFkB in this cell line. However, in a Jurkat T cell line expressing Tax, NFkB is not activated, although, activation of viral sequences is normal. Also, NFkB is not activated by mitogens or cytokines known to activate NFkB binding activity suggesting that the pathways leading to NFkB activation are specifically suppressed following the long term expression of Tax. Consistent with these findings is the fact that induction of *fos* and *jun* proteins is normal in these cells, suggesting that activation of protein kinase C is not blocked. Thus, depending on the cellular environment, Tax can have both short and long-term effects on specific transduction systems.

M 137 **ALTERNATIVE INDUCIBLE PATHWAY OF HIV GENE EXPRESSION.** Mamoru Sakaguchi, Jerome Groopman, and Sunyoung Kim, New England Deaconess Hospital, and Harvard Medical School, Boston, MA 02215.

The long terminal repeat (LTR) of HIV interacts with many cellular factors. Among these, the transcription factor NF-kB has received much attention because it is a potent inducible activator of the HIV LTR. Based on this observation, it has often been proposed that NF-kB might be involved in activation of the dormant HIV provirus, resulting in the switch from the latent state to the productive stage of viral infection. However, it has recently been reported that NF-kB sequences are not essential for viral growth. Therefore, we have looked for the presence of inducible transcription factors other than NF-kB that could act on the LTR. We have consistently found that in certain cell types, there is a significant level of CAT activity even when NF-kB binding sequences are mutated or deleted. Moreover, this expression increases by 10 to 20 fold upon PMA induction. Our preliminary data suggest that the sequence responsible for this induction is located downstream from the NF-kB binding sites, but upstream from the TAT-responsive element. We are determining the exact location of this cis-acting sequence by gel retardation assay as well as mutational analysis.

M 138 PURIFICATION OF LBP1: A CELLULAR FACTOR THAT BINDS TO DNA SEQUENCES CRITICAL FOR TRANSACTIVATION OF THE HIV-1, CMV AND HSV PROMOTERS. P. L. Sheridan and K. A. Jones, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA. 92037.

The HIV-1 promoter can be transactivated by regulatory proteins encoded by several pathogenic viruses which may act as critical co-factors in the pathogenesis of AIDS. Recent evidence suggests that the immediate early (IE) proteins of the Herpes Simplex (HSV) and Cytomegaloviruses (CMV) may function indirectly by modifying host cell transcription factors, one of which may be LBP1. LBP1 binds cooperatively to a large region downstream from the RNA start site, a region that contributes to the basal activity of the promoter and, more recently, has been shown to be critical for responsiveness of the HIV-1 promoter to transactivation by CMV-IE proteins. We purified LBP1 to near homogeneity from HeLa cells by sequential chromatography of nuclear extracts on heparin-agarose, DEAE-sepharose, and DNA-affinity resins containing multiple copies of the HIV-1 LBP1 binding site. Purified LBP1 migrates as a 63kDa peptide in SDS-gels and was used in DNA-footprinting and gel-shift assays to identify recognition sites in well characterized cellular and viral genes and possibly elucidate a functional role for this factor. LBP1 was found to bind to transcriptionally important sites in the murine Thy-1 intronic enhancer and the human c-myc P2 and CMV-IE promoters; all regions downstream of the RNA start site. In addition, LBP1 specifically recognizes a distal site in the HSV-IE3 promoter required for regulation of IE genes by the HSV-1 VP16 transactivator protein. Thus, while LBP1 is involved in the activation of HIV-1, it may also be an inducible (regulated) factor responsible for transactivation of HIV-1 by heterologous viruses.

M 139 A NOVEL SYSTEM FOR THE EVALUATION OF RETROVIRAL PROTEINASE INHIBITORS, Monica Torruella, Giuliana Molaro and Robert Cozens, Department of Oncology and Virology, CIBA-GEIGY Ltd., 4002 Basel, Switzerland.

Feline Leukemia Virus (FeLV) was shown to be associated with immunodeficiency syndrome in cats. As most of the retroviruses, **Gag** gene is translated into the polyprotein precursor Pr65, which is further processed and the **Pol** gene encodes a protein with several enzymatic domains.The proteinase cleaves itself out of the **Pol** precursor and is responsable of the processing of Pr65. In an attempt to develop a system where we could evaluate the activity of protease inhibitors against retroviral proteinases, a protease substrate plasmid was constructed to contain, under beta actin promoter control and in frame, part of FeLV **Gag** including a proteinase cleavage site and the Hepatitis B Virus surface antigen (HBVsAg) coding sequences. Upon transfeccion of cat fibroblasts, the levels of HBVsAg in the tissue culture was not detectable, due to the N-terminal fusion of the polyprotein produced which blocks the secretion. However if this plasmid was cotransfected with a plasmid carring the protease coding region, HBVsAg was observed. When the substrate plasmid was introduced in FeLV infected cat cells, HBVsAg was also detectable. Immunoblotting, using antibodies against HBVsAg, confirmed the proteolytic cleavage.To test if the murine retrovirus can recognize feline **Gag** as substrate, murine fibroblasts infected with the virus LP-BM5 were transfected with pGag-HBV and the results clearly showed that the viral protease is able to process FeLV **Gag**. During analysis of the effects of inhibitors of HIV-1 protease,an intracellular accumulation of **Gag-HBV** was observed. These studies show this system to be of potential use for testing compounds that may inhibit retroviral proteinases. Attempts to apply this work to the evaluation of proteinase inhibitors *in vivo* are under way.

M 140 **INDUCTION OF HIV-1 IN CHRONICALLY INFECTED T-CELLS AND MONOCYTES BY CYTOKINES,** **Jaromir Vlach and Paula M. Pitha, The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD 21205**

We have been studying the molecular mechanisms by which cytokines affect HIV-1 replication in chronically infected T-cells and monocytes. The activation of HIV replication (measured by the virus production and by the level of HIV RNAs) by these stimuli was compared with the relative levels and numbers of nuclear proteins binding to the enhancer region of HIV-LTR. The results indicate that, while in T-cells, the activation of HIV replication by these compounds correlates with the binding of nuclear proteins to the NF-κB site; in monocytes, we have not observed a direct correlation between the relative levels of NF-κB in the nucleus and the activation of HIV-1 replication. By the detailed analysis of the distribution of the NF-κB-binding activity in nucleus and cytoplasma following the treatment of cells with various compounds, we have shown that some stimuli led only to translocation of NF-κB from cytoplasma to nucleus and other in addition to the nuclear translocation, induced synthesis of NF-κB de novo.

M 141 **Detection and Analysis of the HIV-1 Envelope Gene in Clinical Samples by Automated Solid Phase Sequencing.**

Johan Wahlberg[1], Jan Albert[2,3], Eva Maria Fenyö[3] and Mathias Uhlén[1],
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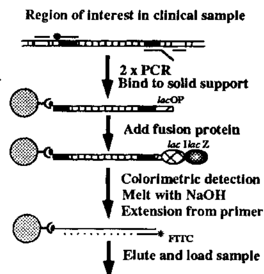
Department of Virology, [2]National Bacteriological Laboratory, [3]Karolinska Institute, Stockholm, Sweden

For detection of pathogens, *in vitro* amplification techniques have many advantages compared to conventional methods due to the generality and sensitivity. We have developed an automated solid phase PCR technique which allowes for colorimetric detection and subsequently sequencing of positive samples. This approach makes it possible to perform epidemiological and drug response studies without the need of time consuming cloning procedures. The biotin streptavidin system was used to capture the amplified fragment on magnetic solid support. The amplified material was subsequently detected using a fusion protein *lacI*lacZ (Fig). The entire procedure can be performed within a working day. By using a direct genomic sequencing method (no cloning or subcloning) the problems with *Taq* misincorporation is avoided. Results will be presented on solid phase sequence of the envelope (the V3 domain) and RT gene of HIV-1 of virus isolates from several patients.



M 142 TRANSACTIVATION OF HIV-2 LTR BY VARIOUS HIV-2 TAT GENES. Mark Westhafer, Clyde Hart, Chin-Yih Ou, Mark Rayfield, and Gerald Schochetman. Division of HIV/AIDS, Centers for Disease Control, Atlanta, GA 30333.

The human immunodeficiency virus type 2 (HIV-2) genome encodes a regulatory gene (tat) which is required for viral gene expression and replication. Strain-specific changes in the tat gene product may contribute to differing levels of LTR-driven gene expression. To study the tat activity of different HIV-2 strains, RNA was isolated from HIV-2 infected tissue culture cells and the tat cDNA amplified using the polymerase chain reaction procedure. Two species of tat cDNA were isolated from HIV-2_{Rod} RNA (Genebank accession M15390); one message with the expected coding sequence and one species with an altered splice junction between exons 2 and 3. This alteration resulted in an insertion of four amino acids. This clone also has a single base deletion which alters amino acids 91-99. These tat cDNA's were cloned into the eukaryotic expression vector pSV2-neo, and co-transfected into RD cells with a plasmid containing the chloroamphenicol acetyl transferase gene downstream of an LTR derived from HIV-2_{NJ} (Lombardo, et al. Arch. Path. Lab. Med. 113:1245, 1989). The tat gene products from these two species transactivated the HIV-2_{NJ} LTR to an equal degree; approximately 180 fold. A truncate tat cDNA was isolated from HIV-2_{618}, a cytopathic HIV-2 strain, (Rayfield, et al. J. Infect. Dis. 158:1170, 1988). This gene product contains only a portion of exon 2 and transactivates the HIV-2_{NJ} at a greatly reduced level. We are currently investigating the biological activity of this truncate tat gene and tat genes from other HIV-2 sources.

M 143 **GENOMIC REPRESENTATION AND TRANSCRIPTIONAL ACTIVITY OF A NOVEL HTLV-1 RELATED ENDOGENOUS RETROVIRUS,** Katharine A. Whartenby[2], George N. Abraham[1,2,4], Richard A. Insel[2,3], and Richard J. Looney[1], Departments of [1]Medicine, [2]Microbiology & Immunology, [3]Pediatrics, and [4]Oncology, University of Rochester, Rochester, NY 14642
Endogenous retroviruses (ERS) have been implicated in the pathogenesis of human and murine immune disease. Mechanisms of action for this role have not been elucidated. We have been investigating a recently described human ERS (HRES) with significant homology to both HTLV-1 and Hrab4, a ras-related gene, and have assessed the representation of this sequence in the genome, its similarities to other sequences, and its transcriptional state. Southern blots of numerous cell lines were probed with restriction fragments and PCR products to define regions of HRES which were represented more than once in the genome. While there is a great degree of structural overlap between HRES and Hrab4, they are separate genes, implying a potential gene duplication. Possible significance of this duplication will be discussed. To determine whether HRES was transcribed, Northern blots were probed with an RNA sequence homologous to the coding strand of HRES. Transcripts of different molecular weights were detected. To insure that these transcripts were derived from the same gene, the RNA sequence was used to probe the Southern blots. The transcripts from HRES and Hrab4 were distinct. Investigation is ongoing to clarify a role for HRES in immunologic disease.

M 144 REACTIVITY TO BOVINE LENTIVIRUS AS A POSSIBLE SOURCE OF FALSE POSITIVE HIV SEROLOGY, C. A. Whetstone[1], M. J. Van Der Maaten[1], K. R. Sayre[2], N. L. Docks[3], S. S. Alexander[4], USDA, ARS, National Animal Disease Center, Ames, IA 50010[1], Ortho Diagnostic Systems, Raritan, NJ[2], American Red Cross, Syracuse, NY[3], Biotech Research Laboratories, Inc., Rockville, MD[4]
Bovine lentivirus, known as bovine immunodeficiency-like virus (BIV), is genetically, structurally, and antigenically related to human immunodeficiency virus (HIV). This causes concern because humans consume beef and dairy products and fetal bovine serum is a component of most vaccines prepared in cell cultures. It is not known whether exposure to BIV proteins cause human sera to either become HIV seropositive or display indeterminate antibody (Aby) reaction on HIV tests. We developed and used a BIV Western blot (WB) to examine sera from people "at risk" for BIV exposure and found no evidence of seroconversion to BIV-specific proteins. We examined 24 human sera that were characterized by HIV WB as Aby positive (4), Aby negative (4), or indeterminate (16). None of these sera were positive by WB to BIV-specific proteins. Many of these sera, however, displayed a strong reactivity to both mock-infected and BIV-infected bovine cell culture antigens. Finally, we tested 371 samples of fetal bovine serum, each sample representing serum pooled from 1-3 fetuses. All samples were negative on BIV WB. This agrees with our observation that calves born to BIV-infected, Aby-positive cows are negative for detectable BIV Aby at birth. To date, we have not detected any human Aby response to BIV-specific proteins. Our data suggest that human Aby response to BIV proteins is not a source of positive or indeterminate results on HIV WB.

M 145 RAPID DETECTION OF HIV INFECTIVITY AND MEASUREMENT OF ANTIVIRAL ACTIVITY USING THE POLYMERASE CHAIN REACTION (PCR). Mark A. Winters, Mark Holodniy, David A. Katzenstein, and Thomas C. Merigan, Center for AIDS Research, Stanford University, Stanford, CA 94305

The use of PCR techniques to detect HIV infectivity in cell culture and assess the effect of nucleoside analogs on HIV replication was compared with in vitro p24 antigen production. PHA-stimulated normal PBMC were infected in vitro with 0.1 to 1000 tissue-culture infectious doses (TCID) of HIV IIIb in the presence of 0 to 10 μM AZT. Cellular DNA and RNA were prepared from cells collected at various time points and amplified using HIV gag primers. Results of these experiments showed that HIV gag RNA and DNA were detected after 24 hours in cells infected with >1000 TCID while p24 antigen could not be measured until at least 48 hours. A significant antiviral effect could be seen after 48 hours by PCR, and in 3-4 days by p24 antigen production. In cells infected with <1000 TCID, HIV gag DNA or RNA could be detected in 48-72 hours while p24 antigen could not be detected for at least 4 days. Significant antiviral activity could be measured in these cultures in 72 hours by PCR, but required 5-7 days when measuring p24 antigen production. These results suggest that PCR techniques may be useful to rapidly monitor patient viremia, strain-specific responses to antiviral therapy, and the development of drug resistance.

M 146 LINKER SCANNING MUTATIONAL ANALYSIS OF THE TRANSCRIPTIONAL ACTIVITY OF THE HUMAN IMMUNODEFICIENCY VIRUS LONG TERMINAL REPEAT. Steven L. Zeichner[1], John Y.H. Kim and James C. Alwine[2]. [1]Division of Infectious Diseases, Children's Hospital of Philadelphia and [2]Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

We have compared the relative importance of transcription regulatory regions in the HIV LTR using linker-scanning mutational analysis. 26 mutant LTR-CAT plasmids were prepared where consecutive 18 bp regions of wild type LTR were replaces with an Nde I-Xho I-Sal I (NXS) polylinker. The mutant LTR-CAT plasmids were transfected into unstimulated, TPA/PHA-stimulated and tat-expressing Jurkat cells. Transcriptional activity was measured by analysis of CAT activity. The activity of many of the mutants was predictable, based on current knowledge of LTR regulatory elements. However, some of the mutants indicated additional regulatory sites. In addition, the fine structure analysis highlights differences in utilization of known regulatory regions, depending on the cellular conditions. We find that the NF-kB sites are necessary for transcription under all cellular conditions; however, there is a marked difference in the requirement for one or two sites comparing stimulated Jurkat cells with unstimulated or tat-expressing Jurkat cells. In all cases there is a dramatic decrease in activity with mutants that covered the TATA and Spl elements. Mutants on the 3'-side of the TATA element caused a more significant decrease in activity in tat-expressing Jurkat cells compared to the others. A significant regulatory region was indicated by linker scanning mutants between nucleotides -183 and -130. These mutations caused marked decreases in activity of the LTR in unstimulated and stimulated Jurkat cells but had no effect in tat-expressing Jurkat cells. Mutants within this region (-147 to -130) appeared to have a greater effect in the stimulated cells. DNA mobility shift studies using the mutants within this region indicated that the altered CAT activity corresponds to alterations in DNA factor binding. Quite modest evidence of negative regulatory regions (1.5 fold or less) appeared in the unstimulated Jurkat cells only. Overall, a comparison of the patterns of regulatory element utilization between the three cellular conditions shows not only expected similarities but also significant differences.

M 147 INCREASED EXPRESSION OF RECOMBINANT HIV-1 INTEGRASE IN E.COLI BY OLIGONUCLEOTIDE DIRECTED MUTAGENISIS OF A "SHINE-DELGARNO ' LIKE SEQUENCE

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Lilly Research Labs, Eli lilly and Co., Indianapolis IN. 46250

Expression of the HIV-1 Integrase(IN) gene in E. coli produces an expected 32Kda protein and a 15Kda protein. Using amino terminal sequence analysis we demonstated that the 15Kda protein is the product of de novo protein synthesis initiated at an internal methionine codon at position 154 of the IN gene just downstream of a "Shine-Delgarno " like sequence in the IN gene. Oligonucleotide directed mutagensis was used to disrupt the "Shine-Delgarno" like sequence in order to increase expression of the 32Kda IN protein. Expression, purification and DNA binding studies of the IN protein produced by this vector will be discussed.

Parvoviruses, Papovaviruses, Adenoviruses

M 200 TRANSCRIPTIONAL ACTIVATION BY SV40 LARGE T ANTIGEN: REQUIREMENTS FOR A CANONICAL PROMOTER STRUCTURE AND MECHANISTIC IMPLICATIONS FOR THE SV40 LATE PROMOTER. James C. Alwine, Maryann C. Gruda, Joseph Manuppello and Gwen Gilinger. Department of Microbiology, 560 Clinical Research Building, 422 Curie Blvd., University of Pennsylvania, Philadelphia, PA 19104-6148.

Simian virus 40 (SV40) large T antigen is a promiscuous transcriptional activator of many viral and cellular promoters. Previous studies from this laboratory have shown that mutants of T antigen which are unable to bind to DNA maintain the ability to transcriptionally activate promoters. These and other data suggest that T antigen mediates transcriptional activation indirectly through the utilization of cellular transcription factors. We have found that the promoter structure necessary for T antigen mediated transcriptional activation is very simple. A TATA or initiator element is required in addition to an upstream element which can be quite variable. We have found that SP1, ATF, AP1 and SPH (TEF-1) binding sites can all function as upstream elements for transcriptional activation mediated by large T antigen. This simple and variable promoter structure can account for T antigen's promiscuous activation of promoters. The SV40 late promoter, a primary target for T antigen transcriptional activation, fits this promoter structure. It contains a cluster of initiator elements (SV40 nucs. 250 to 335) downstream of a previously described element, the T antigen activated binding site (TABS, SV40 nucs. 186 to 225), known to be necessary for the activation of the late promoter in the presence of T antigen. The TABS element functions as an upstream component of the promoter. It contains overlapping octamer (OCT) and SPH (or TEF-1) binding sites. We have found that the SPH binding sites are necessary for transcriptional activation by T antigen, while octamer factor binding mediates negative regulation of the late promoter. When Oct 1 is over-produced, it can eliminate T antigen mediated transcriptional activation of the late promoter. This suggests that one function of T antigen in transcriptional activation of the late promoter is to alter factor binding at the OCT/SPH region to favor binding of factors to the SPH sites.

M 201 EXPRESSION OF HUMAN P53 IN FISSION YEAST, James R. Bischoff, David Casso, and David Beach, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

The human tumor suppressor gene, p53, has been shown to be involved in several types of carcinomas. We have expressed the wild-type human gene in *S. pombe*. In *S. pombe* human p53 seems to behave in a similar manner as it does in mammalian cells. The protein is phosphorylated, it is localized to the nucleus, and its expression inhibits growth. So-called oncogenic mutants of p53 were also expressed in *S. pombe*. The mutants also inhibit growth, but less severely than the wild-type gene. One oncogenic mutant, a change of an arginine to a histidine at residue 175, was found to be dominant to the wild-type allele, thus providing direct evidence for dominant negative alleles of p53.

M 202 Simian Virus-40 small-t antigen inhibits DNA replication in vitro.

M. Carbone#, J. Hauser#, M. Carty#, K. Rundell*, K. Dixon#, and A. S. Levine#.
#Section on Viruses and Cellular Biology, NICHD, Bethesda, MD 20892 and Department of Microbiology, Northwestern University, Chicago IL 60611-3008.

The SV40 large T (Tumor) antigen is a multifunctional protein essential for the expression and replication of the viral genome. To initiate DNA replication, T must be phosphorylated at threonine-124, but the protein has to be dephosphorylated at serine sites. Phosphatase 2A (PP2A) dephosphorylates T at these serine sites and enhances the ability of T to support DNA replication. SV40 small t-antigen interacts with PP2A by binding to the regulatory subunit. This observation suggested to us that the interaction of small t with PP2A might modulate T-mediated DNA replication. To test this hypothesis, we used an in vitro DNA replication system which included an Hela cell extract, and as substrate the plasmid pZ189 (which contains the SV40 replication origin). Plasmid replication in this in vitro system is completely dependent on SV40 T-antigen. When purified small t (400 ng) was added to this system, replication was inhibited ~ 70%. The inhibitory effect was observed under various experimental conditions and using different T and t antigen preparations. We demonstrated by immunoblotting that t-antigen co-precipitated with PP2A in this in vitro system. Our results indicate that small t inhibits DNA replication in vitro, and that this inhibitory effect may be mediated by the interaction of small t with PP2A.

M 203 **DETECTION OF EPSTEIN-BARR VIRUS AND HUMAN PAPILLOMA VIRUS IN THE HEAD AND NECK TUMORS BY ENZYMATIC DNA AMPLIFICATION,** Yu-sun Chang and Mong-liang Chen, Department of Microbiology & Immunology, Chang-Gung Medical College, Kwei-shan, Taoyuan, Taiwan, R.O.C.

Fifty DNA samples of Head and Neck tumors were screened for the presence of Epstein-Barr virus (EBV) and human papilloma virus (HPV) by the polymerase chain reaction (PCR) method. The PCR primer sets for EBV and HPV were designed according to the reported sequences of B95-8 strain of EBV and HPV type 16, respectively. The reaction condition was optimized for the primer sequences of both viruses. EBV sequences were detected in 24 of 50 specimens (48%) and HPV DNA sequences were detected in 15 of 50 samples (30%). The coexistence of EBV and HPV DNA sequences were detected in 10 of 50 specimens (20%). The significance of dual infection of EBV and HPV in those tumors will be discussed.

M 204 HOW DO THE TRANSFORMATION AND IMMORTALIZATION ACTIVITIES OF SV40 LARGE T ANTIGEN CORRELATE WITH ITS OTHER PROPERTIES? Charles N. Cole, Philip W. Rice, Jiyue Zhu, Michele Chamberlain, and Lisa Gorsch, Department of Biochemistry and The Molecular Genetics Center, Dartmouth Medical School, Hanover, NH 03756

We have been studying the ability of a series of SV40 T antigen mutants to immortalize primary mouse embryo fibroblasts, to transform established rodent cell lines, to transactivate gene expression from various promoters in monkey kidney cells, and to bind the tumor supressor gene products, p53 and Rb. We found that transactivation activity was reduced by mutations within the first 85 amino acids and that 40% of wildtype activity was retained by an N-terminal fragment of 138 amino acids. Some mutants with defects in origin-specific binding were completely defective for trans-activation. The data suggest that trans-activation is a function of the first 85-100 amino acids of large T, that the structure and orientation of this domain are essential for it to activate gene expression. Mutations at distal sites that reduce trans-activation could do so by altering the conformation of T antigen. We found no correlation between transactivation and immortalization, transformation, Rb binding or p53 binding. Most of T antigen was required for transformation of REF-52 cells and immortalization of primary MEFs. Small insertions or deletions at many sites had no effect on either of these activities, but two mutants, with insertions at amino acid 409 and 424, were defective for both activities, even though both mutants retained the ability to bind Rb and p53. A mutant that was unable to bind p53 was defective for both immortalization of primary MEFs and transformation of REF-52 cells. Overall, these studies suggest that Rb and p53 binding are important for immortalization and transformation but that other T antigen: host protein interactions likely also play a key role in these processes.

M 205 DOWN REGULATION OF MHC-CLASS I EXPRESSION IN ONCOGENIC Ad12 TRANSFORMED CELLS. Jean-Claude D'Halluin, Chantal Cousin, Valérie Leclère and Isabelle Huvent. Molecular Virology Laboratory, INSERM U.233, 2 place de Verdun, 59045 Lille Cédex, FRANCE.

All adenoviruses transform rodent cells *in vitro*, but only transformed cells by oncogenic Ads are tumorigenic for immuno-competent animals. The MHC class I antigens are repressed in Ad12 and Ad3 transformed cells and might permit them to escape from the recognition by cytotoxic T cells of the host. In Ad40 and 41 transformed cells the MHC class I were also down regulated but these cells were not tumorigenic for animals. The MHC class I was not inhibited in transformed cells by a plasmid carrying the Ad12 12S cDNA. The class I genes were only inhibited in cells expressing the Ad12 13S mRNA product. Using chimeric E1A region between Ad2 and Ad12, we have analyzed the specific domain responsible for inhibition of MHC class I expression.

The target(s) on the H-2K^b promoter was(were) also determined in different transformed cell lines. In transient expression, in primary BRK cells, the transcription from the H-2K^b promoter was not down regulated by the Ad12 13S mRNA product. These results suggest that the MHC class I expression was not directly repressed by E1A gene but was an indirect effect of cellular transformation.

M 206 E1A REPRESSION OF POLYOMA VIRUS DNA REPLICATION: MAPPING OF FUNCTIONAL DOMAINS AND CONSERVED REGIONS NECESSARY FOR THIS ACTIVITY AND ANALYSIS OF E1A MEDIATED PERSISTENT INFECTION STATES,
N. J. DePolo and L. P. Villarreal, Department of Molecular Biology and Biochemistry, University of California, Irvine; Irvine, Ca. 92717.

We have shown previously that the adenovirus 289 amino acid E1A protein strongly represses both wild-type and F9 EC cell-selected polyomavirus (Py) DNA replication. This strong repression is not mediated by simply lowering T-Ag mRNA levels, and therefore is not due to the previously described transcriptional repression activity of E1A. We have demonstrated the repression with both transient co-transfection assays and with viral infection or transfection of E1A expressing cell lines (DePolo et al., 1989, p. 239-53, in Common Mechanisms of Transformation by Small DNA Tumor Viruses, L. P. Villarreal, ed., A.S.M. Press, Washington, DC). In the latter case, E1A protected against all apparent cytopathic effect on 3T3 cells (2G cell line; generously provided by B. Roberts, et al., 1985 J. Virol. 56:404-413), and seems to promote long term episomal maintenance in an inapparent infection state. We continue to actively investigate this apparently controlled replication state of a "runaway replicon" and will present analysis of individual cell replication states using *in situ* hybridization and other assays. The low level inapparent infection state strongly resembles that seen in undifferentiated EC cells but enhancer variants resistant to E1A have still not been found despite many attempts. We will also present results of our protein mapping analysis of this new E1A activity relative to specific conserved regions and to the presence or absence of binding sites for individual E1A associated cellular proteins such as p105 RB and p300.

M 207 THE HUMAN PAPILLOMAVIRUS 16 E6 PROTEIN ACTIVATES TRANSCRIPTION FROM SEVERAL VIRAL PROMOTERS. **Christian Desaintes, Sophie Hallez, Patrick Van Alphen and Arsène Burny. Laboratoire de Chimie Biologique, Université Libre de Bruxelles, 1640 Rhode St-Genèse, Belgium.**
Human Papillomavirus 16 (HPV16) is a DNA tumor virus that contributes to the development and maintenance of anogenital carcinomas. It encodes two transforming proteins, E6 and E7. We previously observed that the HPV 16 E6 protein trans-activated the HSV thymidine kinase promoter. In this study, we sought to delimit the E6 responsive elements by using mutated and deleted forms of the TK promoter. Since deletions leaving only 25 bp upstream of the TATA-box did not abolish the E6 responsiveness of the promoter, we investigated whether the TATA element was responsible for the E6 trans-activating activity, and if functional distinctions could be found between different TATA sequences. Six minimal viral promoters with a slightly different TATA-box were inserted upstream of the chloramphenicol acetyltransferase (CAT) gene. These included the HSV TK promoter spanning from -50 to +51 relative to the transcription initiation site, the HPV 16 P97 from -72 to +15, the Ad ML promoter from -44 to +11, the HIV LTR from -57 to +85, the SV 40 early promoter from -140 to +85, and the Ad E2P from -285 to +40. Reporter plasmids were co-transfected in triplicate into NIH3T3 cells with a vector expressing E6 from the SV 40 early promoter or, as a negative control, the same plasmid containing the E6 ORF in an anti-sense orientation. In the presence of E6, CAT activity was increased about 6 fold with all the viral promoters tested except with the Ad E2P whose activity was induced 13 fold. The same level of induction was observed at the RNA level. These results show that E6 activates transcription from several minimal viral promoters which share only a TATA element in common, and that apparently different TATA types are equally effective for the E6 mediated trans-activation.

M 208 INTEGRATED AND EPISOMAL FORMS OF HUMAN PAPILLOMAVIRUS TYPE 16 DNA IN THREE DISTINCT LESIONS FROM A FEMALE LOWER GENITAL TRACT.
D. Galehouse, E. Jenison and A. DeLucia, Department of Microbiology/Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

Human papillomavirus type 16 DNA was found in three separate lesions within a female patient. The physical form of the viral DNA in each lesion was determined by two dimensional agarose gel electrophoresis. The primary cervical tumor (papillary squamous carcinoma) contained large amounts of episomal viral DNA as well as integrated HPV DNA. The episomal DNA was resolved into at least five forms equivalent to monomers and larger multimers of viral genomes. Metastatic tumor tissue found in the vagina had reduced levels of episomal DNA and a pattern of integrated DNA distinct from that of the primary tumor. The episomal DNA in the metastatic tissue was larger than dimers of the viral genome. The vulvar carcinoma in situ (intraepithelial neoplasia VIN III) contained both free and integrated forms of viral DNA. The results suggest that both free and integrated forms of viral DNA can be found in tumor tissue and that metastatic cells contain further rearrangements of integrated DNA. Our work is consistent with the idea that mechanisms responsible for HPV 16 DNA integration may also be involved in maintaining episomal viral DNA within the tumor cell.

M 209 THE ROLE OF THE E6 AND E7 GENES OF HPV 16 AND HPV 6 IMMORTALIZATION OF HUMAN EPITHELIAL CELLS. Denise A. Galloway, Christine L. Halbert, G.William Demers, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

To investigate the contribution of the E6 and E7 genes of human papillomavirus (HPV) types 6 and 16 in the immortalization of primary human epithelial foreskin cells (HFE), amphotropic retroviruses were constructed containing the genes singly or the contiguous E6/E7 region, and expression was driven by the retroviral LTR. Immortalization was achieved readily in cells containing the HPV 16 E6/E7 sequences and more rarely in cells containing only HPV 16 E7. Those cells transformed by 16 E7 alone contained multiple copies of proviral DNA and expressed high levels of E7 protein. Early after infection with the 16 E7 recombinant retrovirus, the cells demonstrated an altered phenotype in organotypic culture. Experiments are in progress to examine the state of the p53 gene product in immortalized cells which lack HPV 16 E6.

To determine whether the E6 gene of HPV 6 could augment the efficiency of immortalization of the HPV 16 E7 gene, co-infections were performed using viruses containing HPV 6 E6 + HPV 16 E7, HPV 16 E6 + HPV 6 E7, HPV 6 E6 + HPV 6 E7, and HPV 16 E6 + HPV 16 E7. These results will be presented.

M 210 GENOMIC HOMOLOGIES BETWEEN ADENOVIRUS SEROTYPE 3 (G B STRAIN) GENOMIC SEGMENTS AND THE DNA FROM OTHER HUMAN ADENOVIRUSES, García Felipe, Gallego Gerardo, Domínguez Martha and Borrero Isabella. Department of Biology and Microbiology, Universidad del Valle, Cali, Colombia, A.A. 25360.

Human Adenovirus 3 (GB strain) DNA, was cloned into the tetracicline resistance gene of pBR322 cloning vector. The recombinant plasmids obtained were screened according with their phenotype $Amp^R Tet^S$. Eight groups of recombinant clones were submitted to restriction analysis with HindIII, showing that recombinants studied represent 92.5% of the human adenovirus serotypes 3 (GB strain) genome used as cloning source.

Recombinant clones pAd3-2 (7363bp HindIII fragment inserted) pAd3-14 (4743bp HindIII fragment inserted) and pAd3-69.5 (5841bp HindIII fragment cloned) were used in dot blot hybridization experiment as probes. The clone pAd3-2 containing genetic information for fragment A, hybridized with DNA from serotype 12 (subgenus A) 1, 2, 5, 6 (subgenus C); 3, 7, 11, 14 (subgenus B); 8, 13, 25, 26, 27 (subgenus D) and 4 (subgenus E). By the other hand recombinant pAd3-14 containing the fragment C, showed homology with serotypes above cited except with serotype 12 DNA. Finally the clon pAd3-69.5 wich contain the fragment B, hybridized preferentially with DNA from serotypes belonging to subgenus B (3, 7, 11). The results obtained enable us to conclude that genomic segment B wich has a genetic information for penton fiber protein, exhibit a high homology level between human adenovirus serotypes assayed.

This work was supported with funds from Research Vicepresidency of the Universidad del Valle/ICFES (Grant number 7052) and BOSTID Branch NIH.

M 211 **Cell-Cycle Dependent Modulation of an E1A-immune complex Histone H1 Specific Kinase Activity,** Antonio Giordano, Judith Scheppler, JooHun Lee, Janet Ross, Gilda Mak and B. Robert Franza, Jr., Freeman Laboratory of Cancer Cell Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 11724.

We have identified apparently specific isoforms of the product of the human *cdc2* gene in adenovirus early region 1A (E1A) immune complexes. The activity is present in E1A immune complexes isolated from adenovirus infected cells and from cells that constitutively express the E1A gene. These isoforms of $p34^{cdc2}$ are also present in p60/Cyclin A immune complexes derived from cells that express E1A protein or do not. The E1A immune complex demonstrates an *in vitro* kinase activity that is specific for the Histone H1 substrate. Other kinase activities may be present in the E1A immune complex, but for now we have demonstrated that the phosphorylation of Histone H1 by the E1A immune complex is modulated during the cell-division cycle. Details of these experiments will be presented.

M 212 HUMAN PAPILLOMAVIRUS TYPE 16 TRANSCRIPTION IS MODULATED BY THE ADENOVIRUS 5 E1A ANTIGEN BUT NOT BY ITS OWN E6 PROTEIN.**Sophie Hallez, Patrick Van Alphen, Arsène Burny and Christian Desaintes. laboratoire de chimie biologique, Université Libre de Bruxelles, 1640 Rhode-St-Genèse, Belgium.**

All papillomavirus (PV) genomes contain a non coding region responsible for viral DNA replication and gene expression. This region termed Upstream Regulatory Region (URR) harbors constitutive as well as inducible enhancer elements, some of which are dependent on PV encoded E2 or, as it has been shown for HPV 18, E6 products. In this study, we sought to determine if the HPV 16 URR could respond to the HPV 16 E6 or the Adenovirus E1a proteins. The whole 800 bp URR was fractionated into 2 sub-fragments. CAT 1 and CAT 97 were generated by inserting respectively a 300 bp distal and a 500 bp proximal URR fragments upstream of the TK promoter into the enhancer tester plasmid pBLCAT2. NIH3T3 cells were co-transfected with these reporter vectors and a plasmid expressing E6 from the SV 40 early promoter, or as a negative control, the same plasmid containing the E6 ORF in an anti-sense orientation. E1a 13S and 12S were expressed from their own promoters. E6 activated pBLCAT2 10 fold. No further induction was observed with CAT 1 or CAT 97. E1a 12S failed to increase the basal activity of the 3 reporter plasmids. E1a 13S could induce 2 fold pBLCAT 2 and CAT 1, whereas it activated 15 fold the CAT 97 Vector. To test if similar results could be observed on the HPV 16 homologous promoter, the whole URR comprising the p97 promoter (CAT 9), and a 5' 300 bp deleted URR (CAT 8) were inserted each in a promoter configuration into pBLCAT 3. Co-transfections were performed into a human keratinocyte cell line (HACaT). No induction was observed on both reporter plasmids in the presence of E6, whereas CAT 8 could be activated several fold by E1a 13S. These results show that the HPV 16 URR contains an E1a inducible element located within a 500 bp proximal fragment, but no E6 specific responsive element.

M 213 SYNERGISTIC COOPERATION BETWEEN THE BOVINE PAPILLOMAVIRUS-1 E2 TRANSACTIVATOR AND CELLULAR FACTORS INVOLVES A COMMON TITRATABLE TARGET, **T.H. Haugen[1], S. Parkkinen[1], P. Angel[2], and L.P. Turek[1], [1]VAMC and University of Iowa College of Medicine, Iowa City, Iowa 52242, USA and [2]KFZ, Karlsruhe, FRG**

The BPV-1 E2 transactivator protein binds as a dimer to ACC(N)$_6$GGT (E2P) palindromes, and strongly activates transcription at promoters with multiple E2P sites. Although a single E2P site was activated in response to E2, the presence of two or more E2Ps resulted in synergistic (rather than additive) cooperative activation. We have therefore designed experiments to determine the contribution of E2 domains to cooperative activation, and to test whether the E2 protein cooperates with cellular factors. The 410 AA E2 protein has a tripartite structure: a C-terminal DNA binding/dimerization domain (DBD), an N-terminal transcription activation domain (TAD), and a central "hinge" domain of ~100 AA. Cotransfection experiments using E2 mutants indicated that the central "hinge" facilitates synergistic cooperation between E2P sites located at a distance from each other. To determine whether synergistic cooperation was limited to, and therefore a special function of the E2 transcription activation domain, we have tested the E2 protein for cooperation with cellular factors. The E2 activation domain was found to cooperate synergistically with the AP-1 complex of Jun/Fos and with a chimeric c-Jun TAD/GHF-1 DBD construction. Synergistic cooperation is thus not limited to multiple E2 dimers: the TAD of E2 can cooperate with those of cellular factors. Coexpression of the N-terminal acidic helix domains of E2 or c-Jun interfered with transactivation of either AP-1 or E2P sites by their cognate factors. Since this "squelching" is thought to occur upon the depletion of a critical factor in the cell, we conclude that the transcriptional activation by the E2 and Jun proteins involves at least one common protein target.

M 214 BIOCHEMICAL CHARACTERIZATION OF THE HUMAN PAPILLOMAVIRUS E6-P53 COMPLEX, Jon M. Huibregtse, Martin Scheffner, and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Human papillomaviruses (HPVs) that infect the anogenital region can be classified as either high or low risk based on their association with carcinomas. It has been shown previously that the E6 proteins of high risk HPV types (HPV-16, 18) form a stable complex with p53 *in vitro*, and that complex formation can lead to targeted degradation of p53 in an ATP and ubiquitin-dependent reaction. Recent experiments have demonstrated that an additional cellular factor is required for E6-p53 complex formation. This factor forms a stable complex with HPV-16 or 18 E6 in the absence of p53, but not with p53 in the absence of E6. The factor is being purified from reticulocyte lysate, which contains large amounts of this factor. A bacterially produced HPV-16 E6 protein has been utilized for affinity purification of the factor. Additionally, ubiquitin affinity chromatography has been used to fractionate the enzymes involved in the ubiquitin-dependent proteolysis sytem in order to determine if the factor is directly involved in this system.

M 215 PURIFICATION AND CHARACTERIZATION OF HPV16 E7 PROTEIN EXPRESSED IN *E. COLI*, Mary S. Kasher, Mark Wakulchik, James A. Cook and Michele C. Smith, Virology Research Division, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285-0438

We have molecularly cloned the HPV16 E7 gene into a bacterial expression vector driven by a thermoinducible bacteriophage lambda p_L promoter. Expression of the plasmid, designated p16E7, at permissive temperatures in *E. coli* resulted in an induced protein of approximately 15 kd. This 15 kd protein reacted with a monoclonal antibody to HPV16 E7 in Western blot analysis. We also constructed several derivatives of p16E7, each of which encoded a six residue peptide at the amino terminus of the E7 protein. Each of the peptides contained amino acid residues capable of chelating metal ions, for use in subsequent purification of the protein by chelating peptide-immobilized metal ion affinity chromatography (CP-IMAC). The chelating peptides (CPs) varied in the number of histidine and tryptophan residues. The CP-E7 proteins were expressed in *E. coli*; expression levels varied among the constructs. This series of HPV16 E7 proteins containing various N-terminal CP extensions was purified using IMAC. The efficiency of purifying E7 with this series of chelating peptides was compared. One construct was used to prepare E7 for further biochemical and functional analyses. The results of these analyses will be presented.

M 216 AMPLIFICATION OF HUMAN PAPILLOMAVIRUS GENOMES DEPENDENT UPON EPITHELIAL DIFFERENTIATION, Laimonis A. Laimins[1,2], Mary A. Bedell[1,4], John B. Hudson[2], Todd R. Golub[1,5], Mary E. Turyk[3], Margaret Hoskins[3], George D. Wilbanks[3] [1]Department of Molecular Genetics and Cell Biology, [2]Howard Hughes Medical Institute, University of Chicago, Chicago, Illinois 60637, [3]Department of Obstetrics and Gynecology, Rush-Presbyterian St. Luke's Medical Center, Chicago, Illinois 60616

Human papillomaviruses (HPV) infect squamous epithelium and establish their genomes as stable episomes in proliferating basal cells. As infected cells differentiate, the viral DNA is amplified to high copy number and capsid proteins are expressed Viral production has not yet been observed in vitro due to the inability of standard culture methods to duplicate most stages of epithelial differentiation. In this report, we have examined a cell line derived from a CIN I lesion and found it to contain episomal copies of an HPV31 subtype, HPV31b at approximately 50 copies per cell. This cell line differentiates in a manner histologically similar to that of low grade cervical lesions (CIN I) in vivo when allowed to stratify at the air-liquid interface of in vitro raft cultures. Amplification of HPV31b genomes was observed in the upper portion of the in vitro stratified epithelium similar to what is observed in productive HPV infections in vivo. Distinct foci of genome amplification are observed in raft cultures similar to the in vivo situation. Transcripts from the late region of HPV31b were also detected specifically in stratified raft cultures, but no capsid protein was found either by immunoprecipitation or by immunohistochemical staining of raft cultures. These in vitro studies demonstrate that differentiation-specific factors regulate HPV-genome amplification.

M 217 MUTATIONAL ANALYSIS OF FUNCTIONAL REGIONS OF THE BOVINE PAPILLOMAVIRUS E1 PROTEIN. Michael Lentz, Lauren Thorner, Paul MacPherson, and Michael Botchan. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

We have been investigating biochemical properties of the bovine papillomavirus E1 protein by site-directed mutagenesis. Mutations were made in sites conserved between E1 and the SV40 large T antigen. Mutations between amino acids 100 and 113 have defined the boundaries of the nuclear localization signal for the E1 protein as assayed by expressing mutant proteins from a heterologous promoter. We are presently testing the ability of the mutations to interfere with viral DNA replication when built back into the BPV genome. This region also contains a threonine analogous to Thr 124 in SV40 T Ag whose phosphorylation regulates the replication functions of T Ag. We are analyzing mutants at the coresponding Thr in E1 to determine if it is phosphorylated and plays a similar regulatory role for BPV replication. Mutations in other regions of the protein are being used to analyze enzymatic activities of E1 such as ATP hydrolysis.

M 218 EFFECTS OF ADENOVIRUS E1A ON THE EXPRESSION OF INTERFERON-STIMULATED GENES. Debra G.B. Leonard and David E. Levy, Department of Pathology and Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.

Interferons (IFNs) exert potent effects on cellular physiology, including an inhibition of cellular proliferation, by transcriptional activation of a set of genes in target cells. The molecular mechanisms for gene activation by IFNα have been well studied. IFNα binds to its cell surface receptor and activates one subunit (ISGF3α) of a mutimeric transcriptional activator (ISGF3). Following activation, ISGF3α translocates to the nucleus and forms a complex with the DNA-binding subunit of ISGF3 (designated ISGF3γ). ISGF3 binds a specific promoter element (ISRE) in the regulatory region of the IFNα–stimulated genes and increases the transcription of these genes from undetectable to very abundant. This transcriptional activation is rapid and occurs independent of protein synthesis. Previous studies have demonstrated that adenovirus E1a protein can block the transcriptional stimulation of genes by IFNα. It is specifically the 12s form of E1a which causes this repression. We are investigating the effects of E1a on the localization, abundance, and activation of the subunits of ISGF3. In addition, the domains of the 12s E1a protein necessary for disruption of the IFN signal pathway are being defined by viral mutants of E1a.

M 219 OVERLAPPING NF-1 AND AP-1 BINDING SITES IN THE REGULATORY REGION OF THE HUMAN NEUROTROPIC POLYOMAVIRUS, JCV. Eugene O. Major and Kei Amemiya, Laboratory of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, Md. 20892

The human polyomavirus, JCV, is the cause of a demyelinating disease, progressive multi-focal leucoencephalopathy, through lytic infection of oligodendrocytes, the myelin producing cell of the central nervous system. The promoter-enhancer region of the viral genome is considered responsible for the neurotropic nature of JCV. We have been examining nuclear extracts prepared from human brain for proteins which interact with the regulatory region of JCV. We have now identified at least five sites which are recognized by nuclear factor 1 (NF-1). One of the sites is located within each of the two 98 bp repeat units in the regulatory region and the other three sites are located toward the late side of the regulatory region. All the NF-1 binding sites contain the concensus sequence 5'-TGGNNNNNN G/ACCAA/G-3'. Nuclear protein preparations from HeLa cells are able to profect all five NF-1 sites from DNase I digestion, but similar preparations from primary human fetal brain cells protect only four of these sites. A purified CTF/NF-1 protein preparation from HeLa cells, however, recognizes the same four sites as the fetal brain extract. In addition to the NF-1 sites in the regulatory region, we have identified two AP-1 binding sites. These AP-1 binding sites either overlap part of a NF-1 binding site or are within a NF-1 site. These results were determined by 1) DNase I protection analysis with a purified c-jun preparation, and 2) competitive binding assays with an oligonucleotide containing a NF-1 recognition sequence. These results suggest that NF-1 and c-jun may have a cooperative effect in the central nervous system.

M 220 ANALYSIS OF BPV-1 MUTANTS WITH AMINO ACID SUBSTITUTIONS AT THE MAJOR PHOSPHORYLATION SITES OF THE E2 TRANSCRIPTIONAL REGULATORY PROTEINS. **Alison A. McBride and Peter M. Howley**, Laboratory of Tumor Virus Biology, National Cancer Institute, NIH, Bethesda, MD 20892, USA.

The E2 open reading frame (ORF) of BPV-1 is capable of encoding at least three polypeptides with transcriptional regulatory properties. The entire E2 ORF encodes a 410 amino acid protein that functions as a transcriptional transactivator and the 3' half of the ORF has been shown to encode two smaller polypeptides that repress E2-mediated transactivation. All three E2 proteins share a common carboxy-terminal domain that encodes a specific DNA binding function and which contains sequences which promote E2 dimer formation. An amino terminal domain, which is unique to the full-length transactivator polypeptide, encodes the transcriptional activation function.

We have recently demonstrated that the BPV-1 E2 polypeptides are phosphorylated primarily on two serine residues at a site adjacent to the carboxy-terminal DNA binding domain, which is common to all three E2 proteins (McBride *et al.,* 1989. J.Virol. 63: 5076). These serine residues, at amino acid positions 298 and 301, were substituted with alanine residues and the mutated E2 ORFs substituted in the entire BPV-1 genome. The mutated BPV-1 genomes were transfected into rodent cell lines and assayed for focus formation, viral gene expression and extrachromosomal viral DNA replication. The mutated viruses were able to transform C127 cells with wild-type efficiency. However, the viral genome containing the serine to alanine substitution at position 301 of the E2 polypeptide is present at very high copy number within these cells.

M 221 HPV16 E7 MUTATIONS WHICH INHIBIT RB BINDING CAN IMMORTALIZE HUMAN KERATINOCYTES.

DENNIS J. McCANCE, RICHARD JEWERS, PETRA HILDEBRANDT, DAKSHA PATEL. DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, UNIVERSITY OF ROCHESTER, ROCHESTER, N.Y. 14642.

E7 is the major transforming gene of HPV 16 and 18. It can bind the retinoblastoma gene product (RB) through a region (2-37 amino acids), which has homology with the RB binding regions of adenovirus E1a and SV40 large T (LT). When certain amino acids in the region of RB binding are altered, the RB/E7 interaction is inhibited and E7, unlike the wild type protein, is unable to transform primary rat embryo fibroblasts in cooperation with an activated ras. However, these mutations in the background of the full length HPV 16 genome are able to immortalize human epithelial cells, the natural host cell. Mutations outside the RB binding domain such as in the cyst-x-x-cyst motifs have been shown to abrogate the ability of the viral genome to immortalize keratinocytes.

M 222 **BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF THE HPV E7 ONCOPROTEINS,**

Karl Münger[1], Carole L. Yee[1], Donald V. Heck[1], William C. Phelps[2] & Peter M. Howley[1]. [1]Laboratory of Tumor Virus Biology, National Cancer Institute Bethesda MD 20892, [2]Burroughs Wellcome Co., Research Triangle Park, NC 27709.

The E7 proteins encoded by the human papillomaviruses (HPVs) associated with anogenital lesions share significant amino acid sequence homology with the Ad E1A proteins and the large T antigens of polyomaviruses. Each of these viral oncoproteins interacts with the product of the retinoblastoma susceptibility gene, pRB. Mutagenesis studies have shown that these conserved amino acid sequences are involved in binding pRB. The E7 proteins of the high risk HPV types 16 and 18 bind pRB with an affinity 5 to 10 fold higher than that of the E7 proteins of the low risk HPV types (HPV 6 and HPV 11). The biochemical and biological properties of a series of HPV-6/HPV16 E7 chimeric proteins were studied. The amino-terminal portion of the E7 protein determines the affinity to pRB, the ability to cooperate with an activated *ras* oncogene to transform baby rat kidney cells and the aberrant mobility of HPV-16 E7 on SDS polyacrylamide gels. Studies done in collaboration with J. A. Pietenpol and H. L. Moses (Vanderbilt University, Nashville TN) have revealed that this portion of the E7 sequence also governs the ability to abrogate the TGFβ mediated repression of the c-*myc* promoter. The E7 proteins of the high risk and the low risk HPVs were able to transactivate the Ad E2 promoter with similar efficiencies indicating that this property must be mediated by a different mechanism.

M 223 THE SMALL NONSTRUCTURAL PROTEIN (NS2) OF THE PARVOVIRUS MINUTE VIRUS OF MICE IS REQUIRED FOR EFFICIENT VIRAL RNA PROCESSING, DNA REPLICATION AND INFECTIOUS VIRUS PRODUCTION IN A CELL-TYPE-SPECIFIC MANNER, Lisa Kay Naeger, Jean Cater, and David J. Pintel, Department of Molecular Microbiology and Immunology, University of Missouri, School of Medicine, Columbia, MO 65212.

Minute virus of mice (MVM) is an autonomous parvovirus, one of a group of small single-stranded DNA viruses that are pathogenic for many vertebrate species, including man, and exhibit teratogenic, immunosuppressive, and tumor suppressive activity *in vivo*.

A series of mutations that only affect the small nonstructural protein (NS2) were constructed and introduced into the infectious clone of MVM(p). Analysis of these mutants indicates that NS2 participates in MVM DNA replication and is required for efficient viral growth in a cell-type specific manner. The majority of the NS2 mutants were severely defective for replication following transfection of normal host murine A9 fibroblasts; however, all were found to replicate more efficiently and produce infectious virus in certain other cell types such as human NB324K cells. The isolation of viral stocks from NB324K cells permitted a more detailed analysis of the mutant defect on A9 cells. NS2 mutant NS2-2018 was shown to be approximately 10-fold deficient for viral monomer replicative-form DNA production within a single burst cycle in infected A9 cells and produced a reduced amount of progeny single strand. Recent results indicate that NS2 plays a role in the regulation of the accumulation of spliced viral transcripts which encode the two viral nonstructural proteins.

Analyses of NS2 mutant DNA replication, transcript and protein accumulation will be presented.

M 224 SPECIFIC BINDING OF SV40 TO CELL SURFACE CLASS I MAJOR HISTOCOMPATIBILITY PROTEINS, Leonard C. Norkin, Walter C. Breau, and Walter J. Atwood, Department of Microbiology, University of Massachusetts, Amherst, MA 01003.
Earlier results implied that class I MHC proteins are the major cell surface receptors for SV40 (Atwood and Norkin, J. Virol. 63, 4474-4477). First, preadsorption of SV40 to cells specifically inhibited binding of anti-HLA monoclonal antibodies. Second, pretreatment, but not post-treatment, of cells with anti-HLA blocked infection by SV40. The ability of anti-HLA to block virus infection, rather than virus binding, was measured because of concern that some viral binding might be nonspecific and not lead to infection. It is shown here that the binding of labelled SV40 can be blocked by preadsorption of an excess of unlabelled SV40, indicating that SV40 binding is specific. Furthermore, SV40 binding can be blocked by pretreatment of cells with anti-HLA. In addition, SV40 does not bind to Daudi cells, which do not express surface HLA due to a defect in B_2 microglobulin expression. Transfection of Daudi cells with the cloned gene for B_2 microglobulin resulted in surface HLA expression and binding by SV40. Other results suggest tht SV40 can specifically bind to both classical and non-classical MHC class I gene products.

M 225 IDENTIFICATION OF HUMAN PAPILLOMAVIRUS DNA SEQUENCES IN PERIPHERAL BLOOD MONONUCLEAR CELLS. Chia C Pao, Ph.D., Shyh-Shyan Lin, M.S., Chieh-Yu Lin, B.S., Juehn-Shin Maa, Ph.D., Chyong-Huey Lai, M.D. Department of Biochemistry and Obstetrics and Gynecology, Chang Gung Medical College and Chang Gung Memorial Hospital, Taipei, Taiwan, Republic of China

Polymerase chain reaction (PCR) was used to amplify and identify the presence of the DNA of human papillomavirus types 6, 11, 16 and 18 in peripheral blood mononuclear cells (PBMC) of women with and without urogenital HPV infections. Among 25 patients who have confirmed urogenital HPV infections, thirteen or 52.0% also had HPV DNA found in their PBMC. All these 13 patients who were positive for both cervicovaginal and PBMC HPV DNA had at least one type of HPV DNA in common between HPV DNA found in cervicovaginal and in PBMC. Furthermore, seven of the 25 cervicovaginal HPV DNA positive patients had evidence of histopathological proven vulvar Condylomata acuminata of which two were PBMC HPV DNA positive and five were PBMC HPV DNA negative. HPV DNA was not found in PBMC of anyone of the nineteen normal control women who were free of urogenital HPV infection.

The presence of HPV DNA in PBMC may perhaps impair the immunological functions of the lymphocytes, and also may play a role in the epidemiology of HPV infections as a source of reservoir of virus in the infection to others or recurrent infections to the same patient. PBMC-associated HPV may also be important in the pathogenesis of HPV-induced diseases.

M 226 INTERACTION OF THE ADENOVIRUS VA RNA WITH THE INTERFERON-INDUCED KINASE DAI, T. Pe'ery, K. H. Mellits and M.B. Mathews, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724
Virus infection induces the production of interferon in host cells probably by generating dsRNA as a by-product of viral metabolism. Interferon induces the synthesis of DAI and other enzymes which comprise the cell's antiviral response. DAI, the dsRNA-activated inhibitor of protein synthesis, is a kinase which is activated by autophosphorylation and phosphorylates the initiation factor eIF-2 causing the cessation of protein synthesis. To preserve translation in the infected cell, adenovirus has elaborated a defense against this aspect of the antiviral response. The key viral product is a small (160 nt) RNA molecule, VA RNA_I, which is produced abundantly in cells at late times of infection. VA RNA is a highly structured molecule and competes with dsRNA for binding to DAI to counteract its activation by dsRNA.

We are studying the features of VA RNA that are important for its function. Structure analysis of VA RNA_I suggested a complex stem-loop model structure which contains an apical stem loop, a central domain (CD) and a terminal stem. Introduction of deletion and substitution mutations into VA RNA_I defined two distinct regions: a duplex structure which is necessary for binding to DAI and the CD essential for blocking DAI activation. To further study the structure and function of the CD and to verify the suggested model we made a variety of mutations in this region. Changes in the CD altered the RNase protection pattern of this region but did not affect the structure of the apical stem-loop or the terminal stem. However, the analysis suggested the existence of tertiary interactions between the CD and a neighboring interior loop. Some mutants containing deletions and substitutions in the CD are functional in kinase inhibition assays despite having altered CD structure. The data begin to define the structural requirement of the CD and suggest that an alternative conformation may be important for function.

M 227 TRANSACTIVATION BY THE HPV16 E7 PROTEIN IS MEDIATED THROUGH THE E2F TRANSCRIPTION FACTOR, W.C. Phelps[1], S. Bagchi[2], J.A. Barnes[1], P. Raychaudhuri[2], K. Munger[3], P.M. Howley[3], and J.R. Nevins[2]. Div of Virology, Burroughs Wellcome Co., RTP, N.C. 27709[1], HHMI, Duke Univ Med Ctr, Durham, N.C. 27710[2] and LTVB, NCI, Bethesda, Md. 20892[3].
The E7 protein of the human papillomaviruses is a multifunctional oncoprotein encoding transforming and transcriptional functions and has substantial amino acid sequence similarity to Ad E1A (Phelps et al.Cell 53:539). The transcriptional activating function of E7 has been demonstrated using the Ad E2 promoter and recent analysis of a series of E2 promoter mutants reveals that the same sequences are required for activation by E7 and E1A. Despite these similarities, other promoters known to be E1A responsive are not activated by E7 including the Ad E3, Ad E4 and the HSP70 promoters; moreover, the amino acid sequences in E7 critical for trans-activation of the E2 promoter do not include sequences homologous to the previously defined trans-activating domain 3 of E1A. Recent experiments suggest that the 12S E1A product independent of domain 3, can activate the E2 promoter; however, neither the E3 nor E4 promoters is activated, similar to results with E7. Finally, biochemical studies have suggested a mechanism for this activation that involves the ability of E1A to dissociate complexes involving the E2F factor (Bagchi et al., Cell 62:659). We now find that the E7 protein can biochemically mimic the activity of the 12S E1A product.

M 228 COMPLEMENTATION OF TRANSFORMING DOMAINS IN E1A/MYC CHIMERAS SUGGESTS CONSERVED REGION 1 FUNCTIONS HAVE A CELLULAR COUNTERPART. Robert Ralston & Kent Thudium, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608,
The transforming functions of adenovirus-5 E1a have been mapped to two regions which are highly conserved among E1a proteins (CR1 and CR2). CR2 is involved in binding to the product of the "recessive oncogene" Rb, and this feature is shared with transforming proteins of several other DNA viruses. The function of CR1 is less well characterized, although it appears to have a major role in *trans*-repression of transcription and also shows structural homology to papovavirus and papilloma virus transforming proteins. Although release of a block to cell replication *via* Rb binding can account for part of its oncogenic potential, other biological effects of E1a are similar to those produced by "dominant" oncogenes such as *myc*. In order to investigate the relationship between E1a and *myc* transforming activities, we constructed E1a/*myc* chimeras to ask whether essential transforming domains of E1a could complement *myc* transforming domains. We found that the N-terminal domain of E1a could complement the C-terminal domain of v-*myc* for immortalization of primary REFs and collaboration with EJ *ras*. Chimeras constructed using domains from transformation-defective mutants of either E1a (eg. ΔCR1) or *myc* were inactive, suggesting that both domains contribute to the function of the chimera. The reciprocal recombinant (*myc*/E1a) also immortalized primary REFs and collaborated with EJ *ras*. We were unable to transform or immortalize REFs by complementation of E1a and *myc* domains in *trans*, although this could reflect either a strict requirement for complementation in *cis* or inefficiency in transfection or *trans*-complementation. Although work by other groups has shown that any functional similarity between E1a and *myc* probably is not due to any structural "homology", our experiments with E1a/*myc* chimeras suggest that part of the transformation potential of E1a may arise through mimicry of *myc* functions.

M 229 THE MOLECULAR BASIS OF HUMAN PAPILLOMAVIRUS TYPE 6 PATHOGENICITY, Ann Roman, Abigail Farr, and He Wang, Department of Microbiology and Immunology and The Walther Oncology Center, Indiana University School of Medicine, Indianapolis, IN 46202-5120

Human papillomavirus type 6 (HPV 6) is the cause of the most prevalent viral sexually transmitted disease, condyloma acuminata (genital warts). Such lesions are characterized by a) a change in viral gene expression as the target cell, the keratinocyte, differentiates, and b) increased cellular proliferation. We have been investigating both of these aspects using HPV6-W50, an HPV 6 genome cloned from a condyloma and verified to be an authentic representative of the genome present in the tissue. The entire long control region, LCR, of the genome and subfragments thereof have been cloned into pSVEcat, an enhancerless plasmid that contains the *cat* gene driven by the SV40 early promoter. Experiments in HeLa cells indicate that both silencer and enhancer elements are located in the LCR. Silencer activity is detected as the ability of a subfragment of the LCR, when inserted into pSVEcat, to decrease expression of CAT activity, and as an increase in CAT expression when this subfragment is removed from the entire LCR. The silencer activity is also detected in undifferentiated keratinocytes. Insertion of the silencer into pSV2cat, a plasmid containing the SV40 enhancer and promoter upstream of the *cat* gene, decreases CAT activity in both HeLa cells and keratinocytes. We propose that interactions with this silencer may change during cellular differentiation altering viral transcription and/or replication. In a second biological assay, the HPV6-W50 genome alone is sufficient to stimulate rat embryo fibroblast colony formation. Insertion of stop codons into individual viral open reading frames suggests that more than one gene is capable of stimulating cellular proliferation.

M 230 DISRUPTION OF THE E1 OR E2 GENES OF HPV-16 INCREASES THE EFFICIENCY OF IMMORTALIZATION OF EPITHELIAL CELLS. Helen Romanczuk and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
The HPV-16 P_{97} promoter directs the transcription of the viral E6 and E7 transforming genes. In primary human epithelial cells, the BPV-1 E2 gene product can act as a transcriptional repressor of this promoter through interactions with E2 binding sites in the viral regulatory region. The mutation of the E2 binding site proximal to the P_{97} promoter relieves that repression, and the mutation of an additional binding site results in the transcriptional transactivation of the promoter (J.Virol. 64: 2849, 1990). In order to assess the effect of HPV-16 E2 in the context of the full viral genome, mutations were created either in the E2 binding sites upstream of the P_{97} promoter or in the E2 gene itself. These mutants were then examined in a quantitative primary human epithelial cell immortalization assay. Mutations of E2 or of its cognate sites adjacent to the P_{97} promoter resulted in an increase in the immortalization capacity of the HPV-16 genome, confirming that E2 repression of the P_{97} promoter occurs in vivo. We have also determined that the disruption of the E1 ORF of HPV-16 results in an increased immortalization efficiency. The effect of E1 and E2 mutations on DNA copy number and viral transforming gene expression are being examined.

M 231 MOLECULAR AND BIOLOGIC STUDIES OF HPV-51: A NOVEL HUMAN PAPILLOMAVIRUS ISOLATE, Saul Silverstein and Octavian Lungu. Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.
Human papillomaviruses are readily typed on the basis of their segregation pattern with specific disease states. Recently, we identified and cloned HPV-51 from a benign condyloma. Molecular epidemiologic analysis of cervical lesions revealed that it was present in approximately 5% of all cases examined and that it was found in both cervical intraepithelial neoplasms and cancers. The complete nucleotide sequence of the genome of this isolate was determined and compared with other HPVs associated with the development of both benign and progressive lesions. Sequence analysis reveals a genome of 7808bp that is readily aligned with other HPVs and composed of eight major open reading frames which are all encoded by the same strand. The ORF encoding E6 has a potential zinc finger binding domain and the E7 ORF contains the amino acid sequence required for interaction with the Retinoblastoma gene product. Furthermore, HPV-51 as with other HPVs associated with anogenital cancer, and in contrast with those associated with benign condylomata possesses a splice donor and acceptor in E6 which would permit the synthesis of an E6* protein. These structural characteristics place HPV-51 with the oncogenic members of this family. Matrix homology comparisons show that HPV-51 is most closely related to HPV-31, a member of the highly oncogenic class of these viruses. Morphologic transformation experiments comparing this virus with HPVs 16 and 18 are currently underway to determine its oncogenic potential

M 232 FIBROEPITHELIOMAS IN BPV-1 E2 TRANSGENIC MICE
J. Skowronski[1], I. Seidman[2], G. Yelnikolopov. [1]. [1]Cold Spring Harbor Laboratory,[2]NYU Medical Center, NY.

Bovine papilloma virus type 1 (BPV-1) induces fibropapillomas in cattle and fibrosarcomas in transgenic mice. Three of the early ORFs: E2, E5 and E6, are necessary for viral transformation of permissive murine cell lines. Overexpression of the E5 and E6 ORF alone, but not the E2 ORF, results in transformation in vitro. E2 ORF encodes a set of transcription factors which interact with the BPV-1 enhancer to regulate expression of the BPV-1 early genes. Although in vitro experiments failed to prove a direct involvment of E2 in BPV-1 transformation of cultured cells, the E2 proteins, as transcription factors, have a potential to perturb expression of cellular genes and may act synergistically with the E5 and E6 oncogenes to effect cellular transformation and tumorigenesis.

Seven independent lines of transgenic mice harbouring the E2 ORF fused to the β actin promoter (pCBAE2) were generated. About 10% of mice of four of transgenic lines develop focal hyperplastic growth in the upper portions of urethers. Polypoidal projections into the lumen of urethers were caused by abnormal proliferation of both the stromal (fibroblastic) and epithelial (transitional epithelium of the urether) components of the urether wall. These hyperplastic changes were histologically benign in most of examined cases. pCBAE2 transcripts, correctly initiated at the β-actin cap site are detectable in several tissues with the highest levels of expression in the urinary tract, specifically bladders and urethers prior to development of overt macro- or microscopical lesions. Therefore it appears that E2 gene products can induce abnormal proliferation of fibroblasts and/or epithelial cells.

E2 proteins and their DNA binding specificities are conserved among different members of papilloma virus family, which includes several viruses implicated in human skin and genital premalignant and malignant lesions. This conservation of E2 may be important not only in the context of viral functions, but also relevant to the oncogenic potential of human papilloma viruses.

M 233 CHARACTERIZATION OF BOVINE PAPILLOMAVIRUS P_{2443} PROMOTER MUTATIONS AFFECTING THE EXPRESSION OF THE E2 TRANSCRIPTIONAL TRANSACTIVATOR,

Barbara, A. Spalholz, Jesse Quintero and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892.

The papillomavirus E2 proteins are important regulatory factors which affect viral transcription, episomal replication and transformation. In the bovine papillomavirus type 1 (BPV-1), several viral promoters can be transactivated by E2 through E2 dependent enhancer elements located in the viral long control region (LCR), including promoters involved in E2 expression itself. Characterization of the cis elements involved in the regulation of the major E2 promoter, P_{2443}, indicated that a binding site for the transcription factor Spl exists directly upstream of the P_{2443} TATA box which is critical for the basal level of transcription from this promoter. Point mutations in this Spl site eliminated P_{2443} promoter activity in transient expression assays for E2 and resulted in a loss of transforming activity when introduced into the full viral genome. These mutations also resulted in a reduction in the E2 transactivation potential consistent with a loss in the production of E2. RNA analysis from cell lines stably expressing these mutated viral genomes confirmed that the P_{2443} promoter was nonfunctional. Surprisingly, however the viral genome was present as a stable plasmid, although at reduced levels compared to the levels in cells harboring the wild-type viral genome. Studies investigating the levels of the E2 proteins in these cell lines as well as the activities of the other viral promoters are in progress.

M 234 CIS AND TRANS REQUIREMENTS FOR BPV DNA REPLICATION,

Arne Stenlund and Mart Ustav, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 117 24.

To study BPV DNA replication we have developed a transient replication assay based on a highly efficient electroporation procedure. Using this assay we have determined the gene requirement for viral DNA replication. Two viral polypeptides are necessary and sufficient for replication of BPV DNA in mouse C 127 cells. One of these polypeptides is a 72 kD phosphoprotein encoded from the entire E1 open reading frame, and the other is the 48 kD E2 transcriptional trans activator. The sequences required in cis for replication are limited to a 105 nucleotide long fragment which is different than the previously characterized PMS sequences. The fragment contains two binding sites for the E2 transactivator, at least one of which is required for replication.

The common requirements for the amplification and maintenance stages of viral DNA replication argue for a common mechanism of regulation for the two stages. We are currently investigating a copy number control system as responsible for the control of both stages of replication.

M 235 A 56 KILO-DALTON NUCLEAR PROTEIN INTERACTS WITH A PURINE-RICH DNA SEQUENCE TO NEGATIVELY REGULATE JC VIRUS LATE GENE EXPRESSION IN GLIAL CELLS,

Hiroomi Tada and Kamel Khalili, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA. 19130

We are studying the role of brain-specific nuclear proteins which regulate the lytic cycle of JC virus (JCV), a small neurotropic papovavirus identified as the cause of the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy. We have shown by promoter deletion analysis that sequences within the 98 base pair repeat downregulate the expression of the late promoter in glial cells. We have localized this "silencer" to a region which contains a poly-dA tract adjacent to a pentanucleotide repeat sequence (PRS) AGGGAAGGGA. This region, when present within a heterologous promoter, can also function as a negative regulator of gene expression. Using gel shift and UV-crosslinking assays, we have found that the PRS interacts specifically with a nuclear protein of approximately 56 kDa (p56). We are presently purifying p56 to homogeneity. Recent evidence suggests that p56 is capable of interacting with several factors present in brain nuclear extract. We will present experiments to determine whether p56, either alone or in concert with accessory factors, regulates the JCV late promoter in vitro. We suggest that unique interactions between p56 and other nearby regulatory factors may determine the cell type-specific expression of JCV in glial cells and function to temporally regulate early and late gene expression during the viral lytic cycle.

M 236 DIFFERENCES IN TRANSFORMATION ACTIVITY BETWEEN HPV-18 AND HPV-16 MAP TO THE VIRAL LCR-E6-E7 REGION, Luisa Lina Villa and Richard Schlegel, Ludwig Institute for Cancer Research, São Paulo, Brasil and Lab. Tumor Virus Biology, NCI, NIH, Bethesda, and, Georgetown University, Medical School, Dept. Pathology, Washington, D.C. 20007, USA.

Homologous, subgenomic fragments of the viral LCR and E6/E7 transforming genes of Human Papillomavirus (HPV) types 18 and 16 were amplified from several primary cervical, penile, and vulvar tumors and cloned into a pUC-18 derived vector. When assayed by a quantitative transformation assay using primary human keratinocytes (Schlegel R. et al. EMBO J. 7(10):3181, 1988), the subgenomic regions of HPV-16 and HPV-18 exhibited transforming activities similar to that of the full-length, prototype HPV genomes. More importantly, the HPV-18 LCR-E6-E7 region was approximately 10-50 fold more active than that of HPV-16. These studies demonstrate that the transforming activity differences previously observed between prototype HPV-16 and HPV-18 map to the LCR-E6-E7 region, and that individual and independent isolates of HPV-16 and HPV-18 exhibit consistent differences in transforming potential, even when isolated from different anatomic sites.

M 237 CHARACTERIZATION OF A BOVINE PAPILLOMAVIRUS E1-RELATED PROTEIN EXPRESSED IN E. COLI, Van G. Wilson, John Ludes-Meyers, and Genevieve Schuller, Department of Medical Microbiology and Immunology, Texas A&M University College of Medicine, College Station, TX 77843

The bovine papillomavirus (BPV) E1 open reading frame has been expressed in _E. coli_ as a fusion with the bacterial recA protein. The amino terminus of the resulting hybrid protein consists of the first 35 amino acids of recA followed by 8 amino acids derived from vector sequences. The E1 encoded portion of the fusion begins with the amino acid after the first methionine in the E1 reading frame (nucleotide 852) and contains all of the open reading frame except the carboxyl terminal 19 amino acids. Using an in vitro immunoprecipitation assay, the recA/E1 fusion protein has been shown to bind specifically to a single region on BPV DNA (nucleotides 7819-93 on the BPV map). Furthermore, recA/E1 can be covalently labeled with oxidized-^{32}P-ATP, suggesting the presence of a specific ATP binding site. Demonstration of these two activities by the bacterially expressed product indicates that recA/E1 and similar fusions should be useful for characterizing the biochemical activities associated with authentic E1 protein(s).

M 238 The three dimensional structure of canine parvovirus and its functional implications.
Hao Wu[1], Jun Tsao[1], Michael Chapman[1], Mavis Agbandje[1], Walter Keller[1], Kathy Smith[1], Ming Luo[1], Thomas Smith[1], Michael Rossmann[1], Richard Compans[2] and Collin Parrish[3]. [1]Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907. [2]Department of Microbiology, University of Alabama, University Station, Birmingham, AL, 35294. [3]James A. Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853.
The three-dimensional atomic structure of the single-stranded DNA canine parvovirus has been determined. Phases for the observed structure amplitudes were initially derived in part from a spherical shell model of the virus and eventually extended to 3.6 Å resolution. Icosahedral infectious virions contain 60 proteins that are VP2 or VP3 with some copies of VP1, while empty capsids contain primarily VP2 (the precursor of VP3) and VP1. The central structural motif of VP2 has the same topology (an eight-stranded antiparallel ß-barrel) as has been found in many other icosahedral RNA and DNA viruses. There is a 22Å long spike on the three-fold axes, a 15Å deep depression at the twofold axes on the viral surface. By analogy with rhinoviruses, the canyon may be the site of receptor attachment. Residues related to the antigenic properties of the virus are found on the threefold spikes. Electron density along the fivefold axes shows that some of the amino termini run to the exterior consistent with the observation that some VP2's in full particles can be cleaved by trypsin. The fivefold axes are surrounded by an unusual cylindrical structure formed by five ß-ribbons. A substantial volume of electron density is shown to correspond to at least an DNA nucleotides or about 12% of the total genome.

M 239 MINIMAL α-DOMAIN REQUIREMENTS FOR POLYOMAVIRUS EARLY TRANSCRIPTION, Kenneth Yoshimoto and Luis Villarreal, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717

Polyomavirus enhancer, required for viral replication and transcription, contains a small region,the α-domain, consisting of three cellular protein binding sites. Variants of the α-domain that were active for replication activation were tested for their ability to activate Py early gene transcription. The presence of a single binding site, PEA3, PEA1 or PEA2 was sufficient to activate replication of an origin-containing, enhancer-deleted construct in an FM3A-derived, T-antigen expressing cell line(FOP). Combining the PEA3 and the PEA1 binding sites synergistically activated replication to high levels. Combining PEA1 and PEA3 or PEA1 and PEA2 in the order opposite that found in Py resulted in repression of replication to levels below that of the monomers. To investigate the effect of these binding sites on transciption, a plasmid *containing* the luciferase coding sequence, and SV40 polyadenylation and small T-antigen splice sites, driven off the Py early promoter was constructed. Monomers, multimers and combinations of PEA3, PEA1 and PEA2 are being tested for transciption activation activity in FOP, 3T6 and aza myoblast cells. Early results suggest that a single combination of PEA3 and PEA1 is sufficient to activate transcription in FOP cells. The effect of reversing the order of the PEA3/PEA1 and PEA1/PEA2 combinations on transcription will be determined.

M 240 **TISSUE SPECIFIC EXPRESSION OF HPV-16 E6 AND E7 ONCOGENES LEADS TO DEVELOPMENTAL DEFECTS IN TRANSGENIC MICE. Anne E. Griep[1,2], Renee Herber[1], Jan Heideman[2] and Paul F. Lambert[1], Department of Oncology[1] and Biotechnology Center[2], University of Wisconsin, Madison, Wisconsin 53706.**

The human papillomavirus type-16 (HPV-16) is one of the papillomavirus genotypes which is frequently associated with cervical carcinoma. In cervical carcinoma tissue and in cell lines derived from cervical carcinoma, two translational open reading frames of the HPV-16 genome, E6 and E7, are found to be structurally intact and actively transcribed, implicating their gene products as agents in the development of this epithelial cancer. The E6 and E7 gene products have been characterized as immortalizing agents on the basis of *in vitro* assays in tissue culture cells. The E6 and E7 gene products are also known to interact with tumor suppressor gene products, p53 and retinoblastoma, respectively. To study the specific activities of the E6 and E7 gene products in a differentiating epithelial tissue *in vivo*, we created transgenic mice carrying a chimeric DNA fragment in which the E6 and E7 open reading frames are fused to the transcriptional regulatory signals for the αA crystallin gene. This promoter directs the expression of E6 and E7 specifically to the developing ocular lens, since it is activated before terminal differentiation of the lens epithelial cells. Transgenic mouse lineages have been established from three founder animals. All transgenic mice developed bilateral microphthalmia which was overtly apparent from late embryonic stages onward. Histological examination of lenses from transgenic mice indicated abnormalities in fiber cell differentiation, similar to those which developed when other immortalizing gene products were expressed in the developing lens. Biochemical analyses are in progress to correlate the observed histopathology with onset of transgene expression and to evaluate at the biochemical level the perturbation in lens cell differentiation. Preliminary results indicate that the transgenes are expressed specifically in the ocular lens. Our results suggest that the primary combined effect of the HPV-16 E6 and E7 gene products on differentiating epithelia is to inhibit normal differentiation. This property may relate to the etiologic role of these genes in cervical carcinoma.

Molecular Biology of Human Pathogenic Viruses

RNA Viruses and Hepadnaviruses

M 300 HUMAN T CELL MEMORY TO HUMAN PARAINFLUENZA VIRUS TYPE 1, Jane E. Allan and Karen S. Slobod, Departments of Immunology and Infectious Disease, St Jude Children's Research Hospital and Le Bonheur Children's Medical Center, Memphis, TN, 38101.

There is little information concerning the T cell response to human parainfluenza virus type 1 (hPIV-1), despite the intention to produce a vaccine for this childhood infection. We have analysed PBL from adults and find that memory $CD4^+$ and $CD8^+$ T cell responses are readily detected *in vitro*. hPIV-1-specific proliferative responses, with stimulation indices of 5-10 fold over background, and IL-2 production by $CD4^+$ T cells are readily demonstrable following incubation for 1 week with hPIV-1. Each of 18 adults and 2 children tested were found to respond. T cell lines cultured for long periods contained class 11 HLA-restricted, virus-specific cytotoxic activity, however, this is less evident in short-term cultures and appears to be dependent on the form of virus used for restimulation. CTL which are class 1 HLA-restricted were observed in short-term cultures, following 1-3 stimulations with virus, from each person tested and the frequency of circulating hPIV-1 specific precursor CTL was similar to that reported for other viral infections. We have investigated the specificty for viral proteins of the hPIV-1 specific, HLA-A2.1-restricted T cell response which from our initial studies appears to be a strong responder type and since this allele is common in the population.

M 301 THE ACTIVATION OF c-FOS and c-MYC PROTO-ONCOGENES BY HBV-X IS MEDIATED BY DIFFERENT TRANSCRIPTION FACTORS. ML Avantaggiati*, C Balsano*, G Natoli*, E De Marzio*, D Collepardo*, M Artini*, H Will#., E. Elfassi^, M Levrero*. *Fondazione A.Cesalpino - I Clinica Medica - Univ. of Rome - Italy. #Max Planck Institut - Martinsried bei Munchen -FRG. ^Institut Pasteur - Paris - France.

We have previously reported that full length and truncated versions of pHBx influence the transcriptional activity of both the endogenous c-myc and c-fos genes and extrachromosomal transfected c-myc and c-fos regulatory sequences, whereas c-Ha-ras transcription is not affected. The ability of pHBx to transactivate c-myc and c-fos regulatory sequences is restricted to some cell lines and is concentration dependent. Using a series of c-fos promoter deletion mutants we found that multiple sites in c-fos regulatory sequences the regulatory sequences are influenced by pHBx, including the serum response element, target for c-fos autoregulation, and two modified AP1 sites, positively regulated by the Jun-fos heterodimer. Cotransfection experiments using recombinants carrying the CAT gene under the control of the minimal TK promoter preceded or not by synthetic CRE, TRE, SRE or NFkB sites confirmed the ability of pHBx to stimulate both SRE and TRE as well as NFkB sequences. In the case of c-myc regulatory sequences, both deletion mutants studies and gel shift assays indicate that the E2F sites in the second promoter (P2) are the target for pHBx mediated stimulation. In conclusion pHBx influences c-myc and c-fos expression by affecting the activity of different cellular transcription factors acting, on multiple sites in the regulatory sequences.

M 302 IN VITRO TESTING OF PHYLLANTHUS NIRURI AQUEOUS EXTRACT AGAINST VIRAL DNA POLYMERASE ASSOCIATED WITH HEPATITIS B ANTIGEN : NON COMPETITIVE INHIBITION STUDY. Neha M Betawar, R A Deshmukh, S A Sengupta, Haffkine Institute, Parel, Bombay-12 and S A Hassarajani, BARC, Trombay, Bombay-85.

HBs Ag positive serum samples were collected from 45 patients suffering from acute viral hepatitis and were tested for e antigen by ELISA method. Hepatitis B antigen associated deoxyribonucleic acid(DNA) polymerase activity was detected in concentrated (high speed centrifuged), HBe positive serum samples by method described by Kaplan et al. DNA polymerase activity was determined by incorporation of [methyl 3H] thymidine 5'-triphosphate into DNA by serum. Aqueous extract of Phyllanthus niruri was shown to bring out invitro inactivation of DNA polymerase of virus. Kinetic studies using various concentrations of extract to dNTP substrate indicated that the inhibition mediated by the aqueous extract is of non-competitive type with respect to dNTP substrate.

M 303 *Abstract Withdrawn*

M 304 3' CIS-ACTING ELEMENT OF RUBELLA VIRUS GENOME HAS PROMOTER ACTIVITY.
Xi-Qing Cao, Darrell T. Liu, Hira L.Nakhasi, Division of Biochemistry and Biophysics, Center of Biologics Evaluation and Research,FDA, Bethesda, MD 20892
At the 3' end of rubella virus (+) stranded RNA there is a 12bp inverted repeat which is capable of forming a stem-loop structure. This inverted repeat is present in the region of the RNA which is implicated to be involved in the replication of the virus. Sequence analysis of the inverted repeat showed that there is a putative TATA box surrounded by GC rich sequences. To examine whether this element has promoter activity, a 158bp DNA fragment corresponding to the 3' end of the rubella virus RNA, which includes the 12bp inverted repeat, was inserted into a series of CAT (Chloramphenicol Acetyl Transferase) expression vectors. The results demonstrated that, this element has promoter activity which is dependent upon the presence of the SV40 enhancer sequence in the vector. In addition, only the cells which were transformed with SV40 virus could initiate the transcription from this element. Primer extension analysis of the CAT mRNA showed that the transcription initiates 21bp downstream from the TATA box. Experiments dealing with the deletions and point mutations of the promoter region are in progress to analyze the sequences which are necessary for the promoter function. DNA gel shift analysis of the promoter region is being performed to study the interaction of cellular and viral proteins which are involved in the initiation of transcription.

M 305 STUDY OF THE SECRETORY PATHWAY OF THE HEPATITIS B VIRUS e ANTIGENE.
Damien Carlier, Olivier Jean-Jean and Jean-Michel Rossignol. UPR 272, Institut de Recherches Scientifiques sur le Cancer; BP n°8, 94802 Villejuif, France.
The small (15.5 kDa) HBV e antigen (HBeAg), encoded by the C gene, is present in the serum of infected patients when viral replication occurs. Two steps of processing are required for its secretion. The first one eliminates the signal peptide located at the N-terminus of the 25 kDa precursor, giving rise to a 22 kDa protein (P22). The second processing event remove the arginine-rich domain located at the C-terminus of the P22 intermediate. This cleavage is due to a cellular aspartyl protease localized inside secretory vesicles. To study the molecular details of this step, we have introduced non-sense mutations in the 3' extremity of the HBV C gene. Adenovirus-based vectors containing the C gene or the mutated derivatives were transiently expressed in human cells and the C gene related products analyzed by immunoprecipitation. The results show that the HBeAg secretion is dependent of the presence of a sequence of 15 amino acids located in the arginine-rich domain. These results suggest that this sequence could contain a sorting signal allowing the entry of HBeAg in secretory vesicles. We have also studied the influence of this sequence on the secretion of normally non-secreted reporter gene products.

M 306 FUNCTIONAL STUDIES OF MEASLES VIRUS (MV) GENES BASED ON THE RECOVERY OF INFECTIOUS VIRUS FROM CLONED cDNA, R. Cattaneo, I. Ballart, M. Huber, K. Kaelin, A. Schmid, P. Spielhofer, and M.A. Billeter, *Institut für Molekularbiologie I, Universität Zürich, Hönggerberg, CH-8093 Zürich, Switzerland*

A procedure generating infectious MV from cloned MV cDNA (EMBO J. **9** (1990) 379-384) was applied to the functional test of MV matrix (M) genes cloned from brain cell RNA of patients who died of subacute sclerosing panencephalitis (SSPE). The functional M gene of the full length MV cDNA was exchanged with the M genes of four SSPE cases. One of the genes tested gave rise to infectious virus, whereas only the first halves of the other three genes were functional. These results demonstrate that M gene inactivation is not obligatorily correlated with SSPE development. We are currently applying the same experimental approach to the functional test of F protein intracellular domains, which are often altered in SSPE cases.

Three other lines of research are in progress. First, to define the sequence requirements for paramyxoviral RNA editing, full length MV cDNAs mutated in the canonical mRNA editing signal were constructed, and will be tested for production of infectious virus. Second, preliminary results indicate that two full length MV cDNAs containing the framework for an additional (seventh) gene in the form of a duplication of an MV intercistronic sequence and additional unique restriction sites, produce infectious virus. Third, we are trying to substitute the MV persistently infected cells used to provide helper function with cells transiently expressing only those MV proteins directly involved in replication or transcription.

M 307 MOLECULAR STUDIES OF COXSACKIEVIRUS CARDIOVIRULENCE: INFECTIOUS INTRATYPIC AND INTERTYPIC RECOMBINANT GENOMES. Nora M. Chapman, Steven Tracy, Beth-Ann Coller. Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha NE 68198.

Important sites of attenuation of polioviruses have been located in the 5' nontranslated region (NTR) and in the capsid coding region by the use of recombinants of virulent and avirulent strains generated by manipulations of cDNA genomes. To locate sites modifying virulence in the enterovirus coxsackievirus B3 (CVB3), we generated recombinants of the 5'NTR and capsid encoding regions of cardiovirulent and attenuated CVB3 strains. Inoculation of C3H/HeJ weanling mice has demonstrated that an important site of CVB3-induced murine cardiovirulence resides downstream of the 5'NTR. Intertypic recombinants with portions of the poliovirus 1 (PV1) 5'NTR substituted for that of CVB3 has shown that substitution of less than the entire 5'NTR can reduce the ability of the recombinant to replicate but that recombinants with the entire 5'NTR of PV1 are as viable as the parental CVB3 in HeLa cell culture. However, the replication of this PV1:CVB3 recombinant in C3H/HeJ mice is reduced compared the CVB3 progenitor. Substitution of a 3' region of PV1 (with part of the replicase encoding region and the 3'NTR) for the corresponding region of CVB3 severely attenuates replication of the recombinant in HeLa cell culture. Replication of this recombinant increases when 5'NTR sequences are from PV1 as well.

M 308 MOLECULAR ASSAY AS A POTENTIAL ALTERNATIVE TO THE MONKEY NEUROVIRULENCE TEST OF THE LIVE POLIOVIRUS VACCINE, Konstantin M. Chumakov, Laurie B. Powers, Igor B. Roninson[†], Kevin B. Noonan[†] and Inessa S. Levenbook, FDA Center for Biologics Evaluation and Research, Bethesda, MD 20892 and [†]University of Illinois at Chicago, Chicago, IL 60612.

The essential determinant of attenuation in the genome of Sabin live oral poliovirus vaccine (OPV) is the presence of uridine at position 472 rather than cytidine characteristic of wild type virus. Using a new highly sensitive method we have found that all lots of type 3 OPV contain some amount of C at position 472. The amount of 472-C was increased in lots which failed intraspinal neurovirulence test in monkeys. Passaging of type 3 OPV *in vitro* led to rapid accumulation of these mutants at a rate dependent on cell type and infection conditions. There were no changes at the position 2034 previously implicated in increased neurovirulence of some virus isolates or at several other positions which were reportedly changed in vaccine lots that failed monkey neurovirulence test. Our data emphasize the significance of the position 472 for the neurovirulence of type 3 OPV in the monkeys and suggest the use of this approach for evaluation of OPV lots and finding optimal conditions for manufacturing of safer vaccines.

M 309 POLIOVIRUS PROTEASE 3C DIRECTLY CLEAVES TFIIIC IN INFECTED CELLS, Melody E. Clark, Thomas Hammerle, Eckard Wimmer and Asim Dasgupta, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024 and Department of Microbiology SUNY, Stony Brook, NY 11794
Infection of HeLa cells with poliovirus leads to inhibition of pol I, II and III mediated transcription. In the pol III system, the activity of transcription factor TFIIIC is decreased after infection. Two TFIIIC-DNA complexes, complexes I and II, are seen in mock-infected cell extracts. Poliovirus-infected cell extracts contain complex II and a new complex called complex III. Complex I contains transcriptionally active TFIIIC while the other complexes contain inactive TFIIIC. We have infected HeLa cells with a poliovirus mutated at one amino acid in the 3C protease region (Se1-3C-02 provided by Bert Semler, UC Irvine) and found that no complex III was formed. When poliovirus protease 3C overexpressed and purified from bacteria was added to fractions containing complex I or complex II in a gel retardation reaction, complex III was formed. Since poliovirus protease 3C can convert a transcriptionally active form of TFIIIC into a transcriptionally inactive form, specific proteolysis of TFIIIC by poliovirus protease 3C is likely a mechanism by which poliovirus inhibits pol III transcription.

M 310 IDENTIFICATION OF ICAM-1 RESIDUES CRITICAL FOR ATTACHMENT OF HUMAN RHINOVIRUSES, Richard J. Colonno, R. Bruce Register, Donald W. Lineberger, and Carol R. Uncapher, Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
Human rhinoviruses (HRVs) are members of the picornavirus family and are the major causative agent of the common cold. Previous studies have demonstrated that 91 of the 102 known serotypes require a single cellular receptor, identified as the adhesion ligand ICAM-1, for attachment and subsequent infection. ICAM-1, which interacts with LFA-1 in the execution of immunological and inflammatory functions, is predicted to have 5 immunoglobulin-like domains. Using an *in vitro* transcription/translation system, we have previously mapped the binding site of three MAbs that block virus attachment to the first 82 amino acid residues (domain 1) of ICAM-1. However, no virus binding was detected using the *in vitro* synthesized ICAM-1 fragments. Glycosylation appears to play a role in virus binding since native deglycosylated receptor purified from HeLa cells also failed to bind virus. In order to study virus binding, we expressed modified ICAM-1 molecules in CEF cells using a RSV vector. Removal of each of the 4 glycosylation sites in domain 2 failed to compromise virus binding. To further map the region(s) of ICAM-1 required for virus binding, a synthetic gene was constructed that inserts 29 additional restriction sites into domains 1 & 2. Mutagenesis studies using the synthetic gene are in progress using the RSV expression system.

M 311 YEAST ARE INCAPABLE OF TRANSLATING RNAS CONTAINING THE POLIO-VIRUS 5'-UTR: EVIDENCE FOR A TRANSLATIONAL INHIBITOR, Peter Coward and Asim Dasgupta, Department of Microbiology and Immunology and Jonsson Comprehensive Cancer Center, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024

An infectious, full-length, poliovirus cDNA clone was placed under control of the GAL10 promoter and transformed into yeast. Expression of the cDNA by growth on galactose resulted in production of a poliovirus-specific mRNA, but did not result in altered growth characteristics of the yeast. In fact, we find that yeast are unable to translate poliovirus RNA and that this effect is a consequence of the poliovirus 5'-untranslated region (UTR). In cell-free translation assays, an activity present in yeast extracts inhibits the ability of HeLa extracts to translate RNAs containing the 5'-UTR. This activity is concentration dependent and can be fractionated over DEAE Sephacel columns. These results suggest that yeast contain a translational inhibitor which does not allow efficient internal initiation of translation as mediated by the 5'-UTR of poliovirus RNA.

M 312 AMELIORATION OF EMC VIRUS DISEASE BY DEPLETION OF CD4 LYMPHOCYTES, John E. Craighead and M. Barger, Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05405
The pancreas, parotid and lacrimal glands of mice undergo coagulation necrosis due to infection with picornavirus of several different types (EMC, Coxsackie B, and FMDV). Virus strains of the same serotype differ with regard to their ability to induce the lesions even though these tissues characteristically support luxuriant virus replication. Because the development of lesions in these zymogen organs is associated with the release of proteolytic enzyme autodigestion, the pathological process is triggered by an unidentified event. In our studies, CD8 and CD4 lymphocytes were depleted by the intraperitoneal inoculation of Mab before infection of adult male A/J mice with an encephalotropic strain of the encephalomyocarditis virus. Elimination of the CD4 cells prevented the development of lesions in the zymogen organs and reduced the severity of the associated encephalomyelopathy without dramatically affecting virus replication. Since disease in these animals develops 3-5 days after the inoculation of virus, traditional autoimmune processes would not appear to play an important role in the development of the disease. Accordingly, our research has focused on the question -- what role do CD4 cells play in the pathogenesis of the infection and the development of lesions in target organs? The possible mechanism involved will be considered in this presentation.

M 313 DETECTION OF HEPATITIS C VIRUS (HCV) AND IMMUNE RESPONSE TO HCV PROTEINS IN SERIAL BLEEDS FROM HEMOPHILIC PATIENTS, Stephen H. Dailey, Yves Laurian*, Suresh M. Desai, James M. Casey, J. P. Allain and Sushil G. Devare, Abbott Laboratories, Abbott Park, IL 60064, *Hemophilia Center, Kremlin-Bicetre, France

Hepatitis C Virus (HCV) is the major etiologic agent associated with non-A, non-B hepatitis (NANBH). Hemophilic patients receiving factor VIII preparations prior to 1985 (before introduction of HIV screening test and heat inactivation of factor VIII) were exposed to various infectious agents including viruses implicated in NANBH and AIDS. In the present study, 229 serial bleeds from 26 hemophilic patients were analyzed for the presence of HCV viral RNA sequences as detected by PCR after reverse transcription and antibodies to structural (CORE) and non-structural (C-100 and 33C) proteins were detected by specific enzyme linked immunoassays. The 26 hemophilic patients could be classifiied into five distinct categories based on the presence of HCV sequences. During the study period of 4 years, in 4 cases the viremia was cleared, 7 cases had persistent viremia, 7 cases which were initially negative but seropositive, became viremic subsequently, 6 cases had fluctuating viremia, whereas 2 seropositive cases did not show presence of HCV sequences. In the majority of cases, the immune response complemented the detection of viral sequences. Immune response to HCV CORE preceded the other markers in several cases. No correlation between alanine aminotransferase (ALT) levels and HCV viremia was found.

M 314 LCMV INFECTION AND GROWTH HORMONE: DISEASE IN THE ABSENCE OF CYTOPATHOLOGY, Juan C. de la Torre and Michael B.A. Oldstone, Division of Virology, Department of Neuropharmacology, The Research Institute of Scripps Clinic, La Jolla, CA 92037
C3H/ST mice persistently infected with lymphocytic choriomeningitis virus (LCMV) show abnormalities in growth and glucose metabolism but no evidence of cell injury. Experimental results showed that a defect in transcription was responsible for a growth hormone decrease. A detailed biochemical characterization of a pituitary cell line (PO) that expresses growth hormone (GH) and prolactin (PRL) was undertaken. The results obtained indicate: i) by Northern-hybridization experiments and transcript prevalence assays that the steady state level of GH-mRNA in the infected cells was three- to eight-fold lower than that in uninfected control cells. In contrast, levels of PRL or actin mRNA did not differ significantly between virally infected and uninfected cells; ii) the nucleotransport and half life of GH-mRNA were not affected in the infected cells; iii) by nuclear run-on experiments the rate of transcription of GH-mRNA was reduced about five-fold in the infected cells, whereas PRL and actin mRNA displayed similar rates of transcription in infected and uninfected cells; 4) transfection of PO cells with CAT under GH promotor control indicate that the virus effect is at the level of GH promotor.
Hence, a virus can selectively affect the transcriptional machinery of a specific cellular gene, thereby severely effect host physiology, in the absence of cytopathology, and cause disease. These observations are likely relevant to human disease.

M 315 IN VITRO ONCOGENICITY OF HEPATITIS B VIRUS; Wolfram H. Gerlich, Martin Höhne*, Stephan Schaefer, Maria Seifer Departments of Medical Microbiology and of Pharmacology*, University of Göttingen, D 3400 Germany. Transfection of SV40-immortalized non-tumorigenic fetal mouse hepatocytes (FMH202) with cloned dimeric HBV DNA results in highly tumorigenic hepatocyte clones while FMH cells transfected only by the selection marker remain non-tumorigenic (EMBO J 9:1137). Transfection of permanent mouse fibroblasts which do not contain SV40 T antigen (NIH 3T3 and LTK$^-$) by dimeric HBV DNA generates also clones with increased growth efficiency in soft agar. Transfection with an HBV DNA fragment containing enhancer I and X gene generates also FMH clones which grow in soft agar and induce nude mouse tumors, if the clones express much 17 kD X protein. Although the X clones express more X protein than the HBV clones, they do not grow as efficiently in soft agar and have a longer latency period before tumor development. X transfected 3T3 clones grow also significantly better than transfected control clones, but again not as good as the HBV transfectants. The data suggest that X protein of HBV is a viral oncogene -at least in vitro- but further factor(s) of HBV contribute probably to oncogenicity. The nude mouse tumors of HBV transfected FMH clones showed constitutive expression of c-fos, induction of endogenous retroviral elements IAP and MoMLV and rearrangements of the integrated HBV DNA. X protein was found to transactivate the serum response element of the c-fos promoter in these cells. Such a transcription element is also found in the LTR's of retroviral element IAP and of MoMLV. Possibly, X protein initiates increased transcription of cellular protooncogenes and furthermore rearrangements by endogenous retrotransposons.

M 316 INVOLVEMENT OF THE HYDROPHOBIC DOMAIN OF POLYPEPTIDE 3AB IN POLIOVIRUS RNA SYNTHESIS, Cristina Giachetti and Bert L. Semler, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717.

Polypeptide 3AB, the smallest precursor of the genome-linked protein VPg, is found associated with membranes of poliovirus-infected cells, probably due to the presence of a 22 amino acid hydrophobic domain at its carboxy terminus. Using a molecular genetic approach we have previously demonstrated that this domain participates in *in vivo* initiation of (+) strand RNA synthesis as well as in *in vitro* uridylylation of VPg.

To further study the structural requirements of the hydrophobic region of 3AB, we made a number of amino acid replacement, deletion, and insertion mutants aimed at decreasing its hydrophobic character. Changes of Thr-67 to Arg or Lys, change of Gly-74 to Glu, and mutants containing both amino acid changes resulted in non-viable clones, primarily due to an RNA synthesis defect. This defect could not be complemented by a polio-coxsackie recombinant virus, suggesting a *cis* acting function directed by this domain. More dramatic changes including the insertion of 9 hydrophobic amino acids between residues 80 and 81 and the deletion of residues 72 to 80 also resulted in non-viable cDNAs. These mutants have been further analyzed by *in vitro* translation of RNA transcripts of the mutated clones in the presence of microsomal membranes to evaluate the association of the mutated polypeptides with membranes. Our results suggest a correlation between the hydrophobic character of polypeptide 3AB and its role in poliovirus RNA synthesis.

M 317 THE POSITION OF AN IMMUNODOMINANT CTL EPITOPE WITHIN THE INFLUENZA VIRUS HA IS NOT CRITICAL FOR SENSITIZATION OF HA-SPECIFIC CTL CLONES.

Y. S. Hahn, V. J. Braciale and T. J. Braciale. Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Virus infected target cells express newly synthesized viral antigens which can associate with class I MHC molecules and sensitize these cells for lysis by specific cytolytic T lymphocytes (CTL). Antigens processed by this "endogenous" pathway appear to be generated by the cleavage of viral proteins into small peptides which can then associate with class I MHC molecules and be transported to the cell surface. In order to determine whether the position of an epitope within the native viral protein is important for antigen processing by the endogenous pathway, we have generated several hemagglutinin (HA) mutants of the influenza virus A/JAP/305/57 in which one immunodominant HA epitope (amino acid residues 202-221) has been repositioned within the HA protein. To generate these mutants, the epitope was removed from the HA gene by site-directed mutagenesis and synthetic oligonucleotides encoding the epitope were inserted at six locations throughout the HA protein. The mutant HA gene was then inserted into a vaccinia virus expression vector and recombinant viruses were produced. Recombinant vaccinia viruses expressing the altered HA protein were used to sensitize target cells for lysis by specific CTL clones in a standard ^{51}Cr release assay. We found that all six classes of mutant viruses were able to sensitize target cells as efficiently as wild type influenza virus for lysis by specific CTL clones. These data suggest that residues flanking antigenic site recognized by class I MHC restricted T cells do not influence the processing of the newly synthesized antigen and the presentation of the processed antigen fragment to T cells. We are also examining the role of individual amino acid residues for the presentation of the epitope to T cells by expressing the minigene encoding the antigenic moeity inside the cytoplasm.

M 318 CHARACTERIZATION OF THE TERMINAL REGIONS OF HEPATITIS C VIRAL RNA: IDENTIFICATION OF CONSERVED SEQUENCES IN THE 5'-UNTRANSLATED REGION AND POLY A TAILS AT THE 3'-END, Han, J.H., Shyamala, V., Richman, K.H., Brauer, M.J., Tekamp-Olson, P., Kuo, G., Choo, Q.-L. and Houghton, M., Chiron Corporation, 4560 Horton Street, Emeryville, California 94608, U.S.A.

HCV is a major etiological agent of non-A, non-B hepatitis worldwide. The molecular cloning of the virus and determination of the complete primary structure of the viral polyprotein revealed that HCV is related to human flavi- and animal pestiviruses and more distantly to plant poly- and carmoviruses. We have determined the nucleotide sequence at the extreme 5'- and 3'-termini of the HCV genome. Our analyses of these sequences show: 1) The nucleotide sequence in the 5'-untranslated region is highly conserved among HCV isolates collected from five continents. This suggests tat there is a strong evolutionary constrain operating on this sequence, indicating a likely role for this sequence in viral replication. 2) Within this region there are blocks of nt sequence homology with pestiviruses, but not with other viruses. 3) The relative position of short ORFs present the 5'-untranslated region of the HCV genome is similar to that of the pestiviral genome. 4) In vitro translation of viral RNA is inefficient unless these ORF's are removed, indicating that the 5'-unstranslation region is inhibitory for translation. 5) RNAs truncated at both the 5'- and 3'- ends are found suggesting the presence of both subgenomic mRNA and defective viruses. 6) Poly A tails are present at multiple sites on 3'-subgenomic RNAs. These data differentiate HCV from the flaviviruses and HCV appears to be substantially different from other known pestiviruses. These data are consistent with the assignment of HCV to a separate viral genus. Our data provide new insights into the organization of the HCV genome, that may have important ramifications regarding the replication strategy and evolution of the virus.

M 319 THE FLAVIVIRUS ENVELOPE PROTEIN E: ANTIGENIC STRUCTURE, FUNCTIONAL CHARACTERISTICS, AND CRYSTALLIZATION OF A MEMBRANE-ANCHOR-FREE FORM FROM TICK-BORNE ENCEPHALITIS VIRUS: Franz X. Heinz, Christian W. Mandl, Heidi Holzmann, Farshad Guirakhoo and Christian Kunz, Institute of Virology, Vienna, Austria, Barbara Harris, Felix Rey, and Stephen Harrison, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, U.S.A.

The flavivirus envelope protein E represents a typical amphiphilic membrane protein and is the major constituent of the virion surface. It is involved in early virus-cell interactions and induces a protective immune response in the infected and immunized host. Based on immunochemical analyses and comparative sequencing of mutants, a structural model was established which reveals a specific folding of the polypeptide chain into distinct protein domains (A,B,C). These seem to be involved in different functional activities. Domain A undergoes a conformational change upon exposure to acidic pH which activates the fusogenic potential of protein E in mature but not in immature virions, which contain an additional glycoprotein (prM). Sequence elements within domain B represent an important determinant of virulence. Single amino acid substitutions at specific sites of this domain lead to an almost complete loss of neurovirulence in the mouse model.
Limited trypsin digestion of purified virions liberates a membrane-anchor-free form of protein E. This soluble fragment exists as a dimer and yields needle-shaped crystals which allow its structure determination by X-ray diffraction analysis.

M 320 *Abstract Withdrawn*

M 321 MOLECULAR STUDIES ON THE VIRULENCE OF TICK-BORNE ENCEPHALITIS VIRUS

Heidemarie Holzmann, Franz X. Heinz, Christian W. Mandl, Farshad Guirakhoo and Christian Kunz. Institute of Virology, University of Vienna, Vienna, Austria

Seven variants of tick-borne encephalitis (TBE) virus were selected by the use of neutralizing monoclonal antibodies (MAbs). These mutants did not bind the selecting MAb and differed from the wild type sequence by only single amino acid substitutions, which were located at different sites in the envelope protein E of TBE virus.
The mutants were compared with respect to their virulence characteristics in the mouse model. One of the mutants, which had an amino acid substitution from tyrosine to histidine at residue number 384, revealed a strongly reduced pathogenicity after peripheral inoculation of adult mice, but retained its capacity to replicate in the mice and to induce a high titered antibody response. Infection with the attenuated mutant resulted in resistance to challenge with virulent virus. Comparison of non-conservative amino acid substitutions present in other attenuated flaviviruses provided evidence that a structural element including residue 384 is an important determinant of flavivirus virulence in general.

M 322 STUDY ON THE MECHANISM OF THE ANTI-INFLUENZA VIRUS ACTIVITY OF THE MURINE Mx PROTEIN

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The murine Mx1 gene is expressed after interferon induction and confers to animals selective resistance to influenza virus. The antiviral effect is also observed in tissue culture but the molecular basis for the virus inhibition is poorly understood. We have recently developed a completely artificial replication system for influenza virus using recombinant vaccinia vectors expressing influenza polypeptides along with reconstituted RNP molecules. This system has been used to analyze the antiviral actions of Mx1. Vaccinia viruses expressing the influenza virus polymerase can overcome the block in viral gene expression caused by Mx1 in a dose dependent manner. In addition, experiments utilizing various RNP constructs or subsets of the recombinant vaccinia vectors needed for replication has contributed to the elucidation of the antiviral mechanism of the murine Mx1 protein.

M 323 IDENTIFICATION OF PROTEOLYTIC ACTIVITY OF HEPATITIS A VIRUS 3C PROTEIN,

Xi-Yu Jia, Ellie Ehrenfeld and Donald F. Summers, Department of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City, UT 84132

All picornaviruses generate their functional proteins by proteolytic processing of a large polyprotein which represents the single primary translation product of the viral genome. The cleavage pattern of the HAV polyprotein has been predicted by computer-assisted amino acid sequence alignments, but no viral proteins other than capsid proteins have been observed in infected cells, most likely due to slow HAV replication cycles and failure to inhibit host cell protein synthesis. Thus, no viral protease activities or cleavage sites in viral precursor proteins have been identified. We have utilized cell-free translation of T7 RNA polymerase transcripts of HAV cDNAs representing various portions of the P3 region to study the protease activity of the 3C region. Comparison of the mobilities of the translation products on SDS-PAGE, pulse-chase analyses of the various products and immunoprecipitation of the translation products with antisera specific for 2C, 3C and 3D proteins demonstrated that a 2C3ABCD precursor is first cleaved at the 2C/3A junction, and this is most likely an autocatalytic, cis cleavage. Mutational analyses confirmed that 3C sequences were responsible for the observed cleavages, and showed that the C-terminal region of 3C is important for protease activity.

M 324 ANTIVIRAL DETERMINANTS OF RAT Mx2 PROTEIN MAP TO THE CARBOXYL TERMINUS, Ludger Johannes, Ellen Meier and Heinz Arnheiter, Laboratory of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, Maryland 20892
Mx proteins are intracellular, interferon-induced antiviral proteins found in vertebrates including man. They share sequence homologies predominantly in their amino terminal domains, not only among themselves but also with two constitutively synthesized proteins, rat dynamin (a microtubule-activated GTPase) and Vps1p (a component of the yeast protein sorting pathway). Rat cells synthesize two cytoplasmic Mx proteins, termed Mx2 and Mx3, each 659 amino acids long. Mx2 and Mx3 differ in only eight amino acid positions, three of which located in their amino terminal halves and five in their carboxyl terminal thirds. They also differ in their electrophoretic mobility. When constitutively expressed from cDNA clones in mouse 3T3 cells, Mx2 gives a granular immunofluorescent staining and protects cells against vesicular stomatitis virus (VSV), while Mx3 gives diffuse staining and does not protect against VSV. In order to delineate the determinants of anti-VSV activity of Mx2, cDNAs were constructed that code for chimeric proteins composed of either 431 amino acids from the amino terminal domain of Mx2 linked to 228 amino acids of the carboxyl terminus of Mx3, or vice versa. These proteins were called Mx2/3 and Mx3/2, respectively. SDS-PAGE of in vitro translated SP6 mRNAs showed Mx3/2 comigrating with Mx3, and Mx2/3 with Mx2, indicating that electrophoretic mobility is determined by the amino terminal halves of the molecules. However, anti-VSV activity appears to reside in the carboxyl terminal 228 amino acids, since Mx3/2 gave granular staining and conferred resistance against VSV, while Mx2/3 gave diffuse staining and did not protect.

M 325 THE GENERATION OF TYPE A INFLUENZA (H3N2) VIRUS VARIANTS IN VIVO AND HOST CELL SELECTION IN VITRO, Jacqueline M. Katz and Robert G. Webster, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, 38101.

When influenza virus in an original clinical sample is cultivated in embryonated chicken eggs, multiple distinct variants are isolated which possess amino acid substitutions in the hemagglutinin (HA) molecule. In contrast, a homogeneous population of viruses is isolated from the same clinical sample grown in mammalian cells. The polymerase chain reaction (PCR) technique has enabled the direct sequence analyses of the HA gene of influenza viruses present in original clinical samples from infected individuals. It has been determined that over the region of the H3N2 influenza virus HA gene encoding antigenic and receptor-binding sites, the predominant virus replicating in an infected individual is identical to that of viruses after growth in several different mammalian cell types tested but differs by one or two amino acids from the viruses grown in eggs. Molecular clones of DNA amplified from original patient samples or infected animals are being sequenced in order to detect minor variants which may exist within the infected individual. Such analyses will provide insight into the mechanisms of generation, selection and transmission of variants which may arise during replication of influenza viruses in the respiratory tract of the infected host.

M 326 STAGES OF VIRAL HEPATOCARCINOGENESIS. William Kaufmann[a], Chia Chiao[a] and John Cullen[b], Lineberger Comprehensive Cancer Research Center and Department of Pathology[a], UNC-Chapel Hill, NC 27514 and NCSU College of Veterinary Medicine[b], Raleigh, NC 27606
The American woodchuck is susceptible to infection by a hepatitis virus which resembles the hepatitis B virus (HBV) that infects humans. Chronic infection with HBV is associated with substantially increased risk of development of primary hepatocellular carcinoma (PHC) in both species. In other rodent models of hepatocarcinogenesis, cancer evolves from precursor lesions known as enzyme-altered foci. These pre-neoplastic foci may develop in far greater numbers than carcinomas and adenomas but share with neoplasia the features of hyperplasia and enhanced expression of onco-developmental markers, such as gammaglutamyl transpeptidase (GGT). Enzyme-altered foci also appear to share with carcinomas an ability to proliferate in cell culture under conditions in which normal cells senesce and terminally differentiate. We have searched for such precursors in woodchucks that are chronically infected with HBV. PHC were positive for expression of GGT while normal surrounding liver was generally negative. Many foci of GGT-positive hepatocytes also could be observed in tumor-bearing livers. When PHC were placed into cell culture, well-differentiated hepatocytes survived and grew. An epithelial-like cell with quite unusual morphology grew out of a culture isolated from liver surrounding the PHC. These preliminary results suggest that PHC in HBV-infected woodchucks may evolve from a precursor lesion. Supported in part by NIH grant CA42765.

M 327 THE INFLUENZA HEMAGGLUTINNIN ANCHORED BY A PHOSPHATIDYL-INOSITOL TAIL HAS STRUCTURAL SIMILARITIES TO THE WILD TYPE MOLECULE, George Kemble and Judith White, Dept. of Pharmacology, Univ. of California, San Francisco, San Francisco, CA 94143-0450.
A water soluble fragment of the influenza hemagglutinin (HA) can be released from the viral membrane by digestion with the protease Bromelain. The structure of BHA is known to high resolution. In addition BHA has been a very valuable tool for analyzing conformational changes and target bilayer interactions that occur during membrane fusion. Analogous ectodomain fragments of other viral membrane fusion proteins would be similarly useful in elucidating their fusion mechanisms. In this study we have tested a general method for generating such fragments by placing a cleavage site for phosphatidyl-inositol phospholipase C (PI-PLC) at the point of membrane insertion. We have generated such a recombinant HA molecule that is anchored into the lipid bilayer with a PI tail. HA-PI was expressed and transported to the cell surface as assessed by both immunofluorescence and cell surface labelling. Prelimary studies indicated that,like the wild type HA, PI-HA is a trimer. Moreover, HA-PI can be released from the cell by digestion with PI-PLC. We are currently assessing whether the released form of HA-PI undergoes the same low pH-induced conformational change and bilayer interactions that BHA does (steps essential for fusion). Furthermore, we are monitoring the fusogenic properties of HA-PI to ask whether it is sufficient for HA to be embedded in only one leaflet of the bilayer in order to promote fusion. This strategy for creating a water soluble ectodomain should be applicable to many other viral membrane fusion proteins and should therefore aid in elucidating both their biochemical properties and fusion requirements.

M 328 **MOLECULAR DETERMINANTS OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS VIRULENCE,** **Richard M. Kinney, Gwong-Jen Chang, Judith M. Sneider, Kiyotaka R. Tsuchiya, John T. Roehrig, and Dennis W. Trent, Molecular Biology Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, P.O. Box 2087, Fort Collins, CO, 80522.**
An epidemic VEE virus, strain Trinidad donkey (TRD), and its live attenuated vaccine derivative, TC-83 virus, have been sequenced. The positive-sense, 11.5-kb RNA genomes differ at 12 nucleotide positions: 1 in the 5'-noncoding region (5'-NC), 1 each in nsP2 and nsP3 nonstructural protein genes, 6 (one silent) and 2 (one silent) in the E2 and E1 envelope glycoprotein genes, respectively, and 1 in the 3'-noncoding region. We have constructed a fully-characterized, full-length cDNA clone of VEE TC-83 virus in the polylinker site of plasmid pUC18. Subsequently, TRD-specific cDNA regions were spliced into the TC-83 cDNA clone backbone to construct 18 TC-83/TRD recombinant clones. Recombinant viruses derived by transfection of BHK-21 cells with genomic RNA transcribed from linearized plasmid were tested for virulence in mice. The genetic virulence factor in the TRD/TC-83 virus pair is a constellation of genetic determinants dominated by the 5'-NC and a Thr-Val-Thr amino acid triad (TRD positions 120-192-296) in the E2 protein. E2 position 120 appears to be the most critical structural protein component of virulence. The TRD-specific 5'-NC appears to enhance the virulence potential of downstream TRD amino acid specificities.

M 329 TRANSGENIC MICE CARRYING THE HEPATITIS B X PROTEIN (pHBx)
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*Fondazione A.Cesalpino - I Clinica Medica - Università di Roma "La Sapienza" - Roma - Italia and #LBG - Hopital Necker - Paris - France
The pHBx is a 17 Kd nuclear protein that acts as a transcriptional activator on the HBV enhancer, the HIV1 and HIV2 LTRs, the beta-interferon gene, the class I and class II MHC genes and the c-fos and c-myc proto-oncogenes. The transgenic mouse provide an unique experimental system to study the molecular mechanisms of cellular transformation in vivo, from the earlier to the terminal stages of the disease and transgenic animal models for hepatic cancer have been developed. In order to study the pHBx protein function in vivo the HBV-X gene under the control of either the SV40 early promoter or the liver specific gene antithrombin III (ATIII) regulatory sequences have been introduced into mouse fertilized eggs. Four and two strains were obtained respectively. In these strains the transgene are integrated and trasmitted to the progeny as a Mendelian trait. Expression has been studied in the livers at various steps of development by Northern blot analysis and PCR. At this moment we can assume that the expression of pHBx at the levels achieved in these transgenics do not lead to evident proliferative effects in the liver after 12 months although nuclear changes can be observed in the livers of the animals of the ATIII strains as early as 1 month after birth. In order to study the capability of pHBx to cooperate with other proteins whose oncogenic potential has been already demonstrated in transgenic mouse models, we mate our animals with transgenic mice expressing SV40 T antigen or c-myc in their livers.

M 330 STRAIN-SPECIFIC POLYADENYLATION OF TICK-BORNE FLAVIVIRUSES
Christian W. Mandl, Franz X. Heinz and Christian Kunz,
Institute of Virology, University of Vienna, A-1095 Vienna, Austria
The genome of flaviviruses is a single positive-stranded RNA molecule with a length of approx. 11kb. The genomic 5'-terminus carries a Cap structure. The 3'-terminal non coding regions of all mosquito-borne flaviviruses that have been investigated so far are not polyadenylated but capable of forming a stable secondary structure. They further include several highly conserved sequence elements that were proposed to mediate functions of the viral replication cycle.In contrast, the genome analyses of tick-borne flaviviruses revealed the occurance of two different types of 3'-non coding regions: The first type was found in some strains of tick-borne encephalitis (TBE) virus and in Powassan virus. It is similar to that of mosquito-borne flaviviruses; it is not polyadenylated and may form a 3'-secondary structure resembling that predicted for other flavivirus genomes. However, there is no significant primary sequence homology with mosquito-borne flaviviruses in this part of the genome. The second type of 3'-non coding regions was found in certain strains of TBE virus. It is much shorter, lacks a 3'-secondary structure, but carries a poly-A tail. Currently we aim to identify common tick-borne-specific sequence elements. Furthermore, it is investigated, whether the two types of 3'-termini can be correlated with different biological properties of the respective virus isolates.

M 331 PERSISTENCE OF ENTEROVIRUSES IN DILATED CARDIOMYOPATHY: DETERMINATION BY PCR, Willem J.G. Melchers, Jan Zoll and Jochem M.D. Galama,
Department of Medical Microbiology, University of Nijmegen, POB 9101,
6500 HB Nijmegen, The Netherlands
Evidence is accumulating that enteroviruses can persist in chronic diseases as polymyositis, chronic myocarditis and end-stage dilated cardiomyopathy. Persistence may be caused by a defect in replication control.
To study the association between enterovirus persistence and dilated cardiomyopathy more directly, we have developed a PCR assay based on homologous sequences in the 5' NCR of the enterovirus group. A specific amplification could be obtained from 60 of 66 different enterovirus types. No amplification was obtained from ECHO 16, 22, 23 and Coxsackie A 11, 17 and 24. The enteroviral classification of ECHO 22 and 23 has recently been doubted. ECHO 16 is now being cloned for further characterization. No information is available for Coxsackie A 11, 17 and 24, but from their growth pattern and from the appearant lack of the conserved regions in the 5' NCR, the question raises whether they are properly classified as well.

With this broadly enterovirus specific PCR assay we were able to detect enteroviral sequences in 4 out of 7 cardiac biopsy specimens from patients with dilated cardiomyopathy. By sequencing the amplified products we are currently investigating which enteroviruses are involved.

M 332 PHOSPHORYLATION OF HEPATITIS B VIRUS PRECORE AND CORE PROTEINS, Jing-hsiung Ou and Chau-ting Yeh, Department of Microbiology, University of Southern California, Los Angeles, CA 90033
Hepatitis B virus precore and core proteins are two related proteins. The precore protein contains the entire sequence of the core protein plus an amino-terminal extension of twenty-nine amino acids. This amino-terminal extension contains a signal sequence for the secretion of the precore protein. Translocation of the precore protein across the endoplasmic reticulum (ER) membrane results in the cleavage of the signal sequence and the production of the precore protein derivative named P22. We now demonstrate that both P22 and the core protein are phosphoproteins. Microsomal fractionation and trypsin digestion experiments demonstrate that, although a fraction of phosphorylated P22 is located in the ER lumen, a substantial portion of it is trypsin-sensitive indicative of a cytosolic localization. Phosphorylation appears to occur in the carboxy terminus of P22, since the P22 derivative, P16, which lacks the carboxy terminus of P22, is not phosphorylated. Linking the carboxy terminus of the precore/core protein to heterologous cytosolic and secretory proteins led to the phosphorylation of the resulting chimeric proteins. This result suggests that phosphorylation of P22 and core protein is mediated by cellular kinases.

M 333 AN EXAMINATION OF THE INTRACELLULAR SITE OF CLEAVAGE/ACTIVATION OF THE SV5 FUSION PROTEIN, Reay G. Paterson and Robert A. Lamb, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston Illinois 60208-3500

The paramyxovirus fusion (F) protein is responsible for mediating the fusion of the viral membrane with the plasma membrane of a target cell thus delivering the virus ribonucleoprotein into the host-cell cytoplasm. In addition, the F protein mediates the fusion of adjacent cell plasma membranes which results in the formation of syncytia or polykaryons.

The F protein is synthesized as an inactive precursor F_0 which is cleaved by one or more host-cell proteases into the active form of the protein which consists of the disulfide-linked F_1 and F_2 subunits. The cleavage site of the SV5 F protein consists of five arginine residues and is cleaved by a ubiquitous protease (or proteases) that is present in all cell types examined so far. We have used a variety of approaches including the use of inhibitors of intracellular transport and cell fractionation, followed by analysis of the extent of post-translational modification of the F_0, F_1 and F_2 polypeptides, in order to determine the subcellular compartment in which cleavage of the SV5 F protein takes place. The data suggest that the cleavage event occurs in the trans-Golgi network.

M 334 TRANSCRIPTIONAL CONTROL IN VSV: PROTEIN KINASES SPECIFIC FOR NS1 AND NS2 AND EFFECTS ON TRANSCRIPTION, Jacques Perrault and J. David Beckes, Biology Department and Molecular Biology Institute, San Diego State University, San Diego, CA 92182

Many studies have pointed to the possible coupling of the vesicular stomatitis virus (VSV) transcription process to viral NS protein phosphorylation. We previously showed that at least two distinct protein kinase activities which remain bound to virion cores can phosphorylate the viral NS transcription factor in vitro (Beckes and Perrault, to be submitted). We show here that one of these (VSVK1) gives rise to the phosphorylated NS1 species while the second (VSVK2) converts NS1 to faster migrating species, collectively referred to here as NS2. Both kinases are active when virion cores transcribe their genome in vitro. However, NS1 to NS2 conversion is specifically inhibited by pre-treatment of virion cores with 5'-p-fluorosulfonylbenzoyl adenosine (FSBA) with no effect on the transcription process. Likewise, addition of cell extracts inhibits the appearance of NS2 without affecting transcription. The latter phenomenon is probably due to cellular phosphatases since extracts specifically remove phosphates from NS2 following in vitro phosphorylation. We conclude that NS1 to NS2 conversion is not required concomitantly with the transcription process.

M 335 THE MECHANISM OF ACTION OF 2'-CDG, A SPECIFIC INHIBITOR OF HBV REPLICATION, P.M.Price, R.Banerjee, A.M.Jeffrey, Depts of Biochem and Neoplastic Dis, Mt Sinai Med School, NY, NY 10029, and Inst of Cancer Res, Columbia Univ College of Physicians & Surgeons, NY, NY 10032.

The deoxyguanosine analogue, 2'-CDG, inhibits HBV replication by greater than 95% in the HBV-producing cell line, 2.2.15, as monitored by a decrease of secreted HBV DNA, HBV polymerase activity, and intracellular HBV DNA. Transcription of HBV RNA from chromosomally-integrated DNA was unaffected. The maximally effective concentration of 2-CDG was ≥ 20 ng/ml; 5 to 10 ng/ml produced ≈ 50% inhibition; ≤ 2 ng/ml produced little detectable inhibition. Guanine is incorporated into both DNA and RNA from deoxyguanosine (dGuo) using the "salvage" pathway. Although the incorporation of guanine into DNA from 2'-CDG was several times that from dGuo, there was no detectable incorporation of guanine into RNA from 2'CDG. There were similar rates of DNA incorporation of guanine from 2'CDG in 2.2.15 cells and in the parental cell line, HepG2, which does not contain HBV sequences. The DNA was analyzed to determine whether 2'-CDG acts as a chain terminator. Virtually all of the analogue was present at internal sites within the DNA, and there was no detectable exchange of radioactivity into other DNA nucleotides. The data indicated that although 2'-CDG could be incorporated into cellular DNA, this entry was not through the "salvage" pathway, but rather by *de novo* phosphorylation. The incorporation of 2'-CDG into DNA was not dependent on active HBV replication, or on the presence of HBV-specific proteins. The 2'-CDG did not act as a chain terminator of DNA synthesis. We are currently investigating whether 2'-CDG triphosphate can specifically inhibit the HBV polymerase.

We acknowledge a generous gift of 2'-CDG from J.A.Secrist and Y.F.Shealy of the Southern Research Institute. The work was supported by NIH grant CA34818.

M 336 THE ROLE OF N-LINKED OLIGOSACCHARIDES ON PROCESSING AND ANTIGENICITY OF RUBELLA VIRUS GLYCOPROTEINS E2 AND E1, Zhiyong Qiu, Tom Hobman and Shirley Gillam, Department of Pathology, University of British Columbia, Research Centre, 950 W28th Avenue, Vancouver, B.C., Canada V5Z 4H4
Rubella Virus(RV) is a simple enveloped RNA virus whose genome consists of a molecule of single-stranded positive-polarity RNA 10 kb in size. RV-infected cells in addition to containing the genomic RNA harbor a subgenomic RNA, identical to the 3' one-third of the genomic RNA which encodes the structural proteins. Translation of the subgenomic RNA produces a 110-kDa precursor polyprotein which is proteolytically processed to yield three structural proteins, capsid (C), E2 and E1. Both E2 and E1 proteins contained three potential N-linked oligosaccharides.
Oligonucleotide-directed mutagenesis was used to alter the N-linked glycosylation sites present on the E2 and E1 proteins, in order to analyze the role of glycosylation plays in the intracellular transport and antigenicity of E2 and E1. Brefeldin A and monensin (glycosylation inhibitors) have also been used to further characterize the E2 glycoprotein processing in COS cells. The E1 protein, containing both virus neutralization (VN) and hemagglutinin (HA) activities, is the dominant surface molecule of RV particles. Vaccinia recombinant viruses expressing E1 glycosylation mutant proteins were used to investigate the effect of N-linked glycosylation on the expression of VN and HA epitopes in E1.

M 337 A BORNA VIRUS cDNA ENCODING A PROTEIN RECOGNIZED BY ANTIBODIES IN HUMANS WITH BEHAVIORAL DISEASES, Juergen A. Richt, Susan VandeWoude, Rudolf Rott, Opendra Narayan and Janice E. Clements, Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205
Borna disease virus (BDV) causes a rare neurological disease in horses and sheep. The virus has not been classified since neither an infectious particle nor a specific nucleic acid had previously been identified. Recently, infection with a BDV-like agent has been associated with schizophrenia in humans. Antibodies to novel BDV-specific antigens were found in sera and CSF of such patients. To identify the genome of BDV a subtractive cDNA expression library was constructed with poly A selected RNA from a BDV infected MDCK cell line. A clone (B8) was isolated that specifically hybridized to RNA isolated from BDV-infected brain tissue and BDV infected cell lines. This clone hybridized to four BDV-specific positive strand RNAs (10.5, 3.6, 2.1 and 0.85 kb) and one negative strand RNA (10.5 kb) in BDV-infected rat brain. Nucleotide sequence analysis of the clone suggested it represented a full length mRNA which contained several open reading frames. In vitro transcription and translation of the clone resulted in the synthesis of the 14 and 24 kD BDV-specific proteins. The 24 kD protein, when translated in vitro from the clone, was recognized by antibodies in the sera of patients (3/7) with behavioral disorders. This BDV-specific clone will provide the means to isolate the other BDV-specific nucleic acids, and identify the virus responsible for Borna disease. In addition, the significance of BDV or a BDV related virus as a human pathogen can now be more directly examined.

M 338 TRANSMISSION OF VIRAL PERSISTENCE BY TRANSFECTION OF HUMAN CULTURED CELLS WITH RNA OF A PERSISTENT STRAIN OF ECHOVIRUS 6. V. Fay Righthand. Department of Immunology and Microbiology, Wayne State University, Detroit, MI 48201.
A cloned line of persistently infected (PI) cells by a strain of the normally lytic, echovirus 6 has been established. All of the cells contained nonlytic viral RNA and synthesized defective viral particles. The present study was undertaken to determine whether replication of nonlytic viral RNA occurred after transfection. Uninfected human WISH cells were transfected with RNA recovered from PI cell extracts and nonlytic viral particles produced by PI cells. Cytoplasmic RNA extracts were prepared at various times after transfection and examined for the presence of viral RNA. The viral RNA was detected by hybridization of Northern blots of cellular RNA extracts with cDNA probes of wild type, lytic echovirus 6. Increased concentrations of viral RNA were detectable in cellular extracts at 48 hours after transfection. Replicate transfected cultures, which underwent 6 division cycles during a 66 day growth period retained viral RNA. RNA extracts from the transfected cells did not produce cytopathology or lytic virus. These results indicated that a persistent viral infection can be established by transfection of uninfected cells with the genome of a persistent strain of echovirus 6

M 339 EXPRESSION, PURIFICATION AND CHARACTERIZATION OF ENCEPHALOMYOCARDITIS VIRUS (EMCV) RNA-DEPENDENT RNA POLYMERASE IN E.COLI. Sabita Sankar and Alan G. Porter, Institute of Molecular and Cell Biology, National University of Singapore, Kent Ridge Crescent, Singapore 0511.
The RNA-dependent RNA polymerase of EMC virus, a member of the Picornaviridae family, was expressed as a fusion protein with glutathione S-transferase (GST). This allowed facile purification of the fusion protein by affinity chromatography on immobilized glutathione. Inclusion of the thrombin cleavage site between the GST carrier and the enzyme facilitated the release of the purified mature EMC RNA polymerase from the GST carrier by proteolysis with thrombin. The purified recombinant enzyme has a molecular mass of 52 kDa and is recognized by immune sera raised against a peptide sequence corresponding to the carboxy terminus of the protein. The enzyme exhibits rifampicin-resistant poly(A)-dependent poly(U) polymerase activity as well as RNA polymerase activity which are both oligo(U) dependent. Template sized products are synthesized in in vitro reactions with EMC virus genomic RNA or globin mRNA. The availability of recombinant EMC virus RNA polymerase in a purified form will allow biochemical analysis of its role in the replication of the virus as well as structure-function studies of this unique class of enzyme.

M 340 DEVELOPMENT OF A CORONAVIRUS RNA VECTOR TO STUDY THE SIGNALS INVOLVED IN GENOME ENCAPSIDATION AND mRNA SYNTHESIS, Willy Spaan, Robbert van der Most and Peter Bredenbeek. Department of Virology, University of Leiden, the Netherlands
To study the signals involved in encapsidation, mRNA synthesis and genome replication of coronaviruses, we have developed an RNA vector based upon mouse hepatatis virus (MHV) defective interfering (DI) particles. DI particles of MHV strain A59 were generated on serial undiluted passaging; after 8 to 11 undiluted passages two RNA-species of 5.5 and 6.5 kb were observed, which were stable during further passage. Northern blot analysis of RNA isolated from purified virus revealed that both MHV-A59 DI-RNAs were very efficiently packaged, indicating that both RNAs contain a specific encapsidation signal. Restriction enzyme and sequence analysis of a 5.2 kb cDNA-clone containing DI-sequences, showed that the 5.5 kb DI RNA is composed of 3900 nucleotides from the 5' end of the MHV genome, 799 nucleotides from the 3' end of the second ORF of the polymerase gene and 805 nucleotides from the 3' end of the MHV genome. An internal 3.4 kb EcoRI-fragment of the cDNA-clone was used to reconstruct a cDNA-copy of a DI-genome. Genomic cDNA-clones were used to complete the 5' and 3' terminal sequences. Following transfection, T7-transcripts were efficiently amplified in the presence of helper virus. To identify an encapsidation signal several deletion mutants were constructed. Using RNA transfections a region corresponding to the 3' end of the second reading frame of the POL gene was identified to be involved in encapsidation. Currently the transcriptional activity of a 230 bp fragment containing the intergenic region involved in the synthesis of mRNA 7 is tested using the MHV DI vector.

M 341 EXPRESSION AND CHARACTERIZATION OF HCV STRUCTURAL PROTEINS USING *IN VITRO* TRANSLATION AND RECOMBINANT VACCINIA VIRUSES. K. Thudium, R. Spaete, K. Berger, Q.-L. Choo, M. Houghton & R. Ralston, Chiron Corporation, 4560 Horton Street, Emeryville, California 94608.
In order to characterize the HCV structural proteins, we constructed a vector for *in vitro* transcription containing the first 906 codons of the HCV polyprotein linked to a synthetic β-globin 5' UTR. Translation of RNA produced by this vector in rabbit reticulocyte lysate supplemented with canine microsomal membranes generated polypeptides of 11, 18, 35 and 72 kD. The gene order, proteolytic processing, glycosylation and membrane association of these polypeptides were analyzed by standard techniques. Based on these studies, the first 906 codons of the HCV genome encode three structural proteins which are integrated into microsomal membranes when expressed *in vitro*: an 18 kD pre-core (pre-C), a 35 kD envelope-1 glycoprotein (E1) and a 72 kD envelope-2 glycoprotein (E2). The 11kD polypeptide appears to represent a portion of the adjacent non-structural region. Expression of the HCV structural region using recombinant vaccinia viruses produced species of E1 and E2 which were essentially identical to those produced by *in vitro* translation. Pre-C was not detected in this system, although its expression was inferred from the cleavage of the polyprotein to generate E1 (and E2), and by truncation experiments. Analysis of the the subcellular localization of E1 and E2 by indirect immunofluorescent antibody staining and subcellular fractionation showed that both E1 and E2 were retained within the endoplasmic reticulum as core-glycosylated integral membrane proteins which lacked terminal sialic acid residues. Experiments with E2 mutants demonstrated that at least a portion of E2 could be processed within the golgi (sialated) and secreted if the C-terminal anchor region was removed. These experiments suggest that if the E1 and E2 species produced in the reticulocyte and vaccinia systems accurately reflect the processing of the HCV envelope proteins in a natural infection, then the hepatotropism of HCV may arise through binding of its envelope proteins to glycoprotein receptors such as the asialoglycoprotein receptor or mannose receptor.

M 342 CHARACTERIZATION AND MOLECULAR CLONING OF A COXSACKIE B VIRUS FROM A HUMAN MYOCARDITIC HEART. Steven Tracy, Nora M. Chapman, Mark A. Pallansch, Beth-Ann Coller, Melinda A. Beck, Peter Kolbeck, and Yvonne DeCory-Woronoff, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198 and Centers for Disease Control, Enterovirus Laboratory, Atlanta, GA 30333.
Enteroviruses can cause human myocarditis and can be molecularly detected in inflamed heart muscle. An enterovirus was isolated from both pre-mortem throat and post-mortem heart muscle of a 9-day old child with fulminant myocarditis. Anti-coxsackievirus B3 (CVB3) neutralizing antibody neutralized both viruses, but sequence analysis of the 5' non-translated region suggests identity with modern coxsackievirus B2 isolates. The heart isolate induced inflammatory heart disease in the mouse, whereas the throat isolate did not. Isolation of the enterovirus from myocarditic human heart, propagation in cell culture, demonstration of murine cardiovirulency, and virus re-isolation from murine heart is the first rigorous test of Koch's postulates for any enterovirus as an etiologic agent of human myocarditis, and validates the CVB3-induced murine myocarditis model for the human disease. Pending sequence analysis, this may be the first report of a naturally occurring genetic recombinant coxsackievirus. The genome of this virus is being cloned for sequencing and infectious chimeric genome analyses.

M 343 CHARACTERIZATION OF THE TRANSACTIVATING FUNCTIONS OF TRUNCATED PRE-S/S PROTEINS. G Natoli*, ML Avantaggiati*, C Balsano*, E De Marzio*, M Artini* D.Collepardo*, E. Elfassi#, A. Budkowska#, M. Levrero* *Fondazione A.Cesalpino - I Clinica Medica-Univ.of Rome-Italy. # Institut Pasteur - Paris - France
The mechanism by which HBV chronic infection increases the risk of developing primary hepatocellular carcinoma is not yet fully understood. Since the integration of viral regulatory sequences near cellular genes crucial for cell growth control has been described only in few cases of human PHC, insertional mutagenesis cannot be regarded as the main mechanism in HBV induced cellular transformation. Recently it has been demonstrated that COOH-truncated preS/S proteins encoded by fragment of HBV genome cloned from two hepatocellular carcinoma, can exert a significative transactivational activity on both viral and cellular promoters. In order to characterize this transactivating function we engineered two sets of plasmids carrying preS1/S2/S and preS2/S 3' deletion mutants respectively. We found that the deletion of 51 carboxi-terminal aminoacid did not generate the transactivating function, which instead appeared when 23 aminoacids more were removed, both in preS1/S2/S and in preS2/S proteins. The effect of truncated preS/S proteins on both c-myc regulatory sequences and the TPA responsive element (TRE) is concentration dependent and requires cellular protein Kinase C activity. Immunofluorescence and immunoprecipitation studies do not support the hypothesis that the acquisition of the new properties by the truncated preS/S proteins is due to a different subcellurar compartimentation.

M 344 ORIGINS AND EVOLUTION OF SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE) VIRUSES. Timothy C. Wong, Minoru Ayata, and Akiko Hirano, Department of Microbiology, University of Washington School of Medicine, Seattle, Washington 98195.

We have characterized a neurovirulent acute measles virus Nagahata strain, whose matrix (M) gene sequence differs considerably from the consensus M sequence of previously studied acute measles virus strains. The Nagahata M gene is strikingly similar to the M gene of Biken strain SSPE virus isolated in the same locale several years later. Biased hypermutation (U to C transitions) accounts for most (80%) of the changes between the Biken and Nagahata M genes. The Nagahata M gene is also related to the M genes of 5 other SSPE virus strains isolated in three different continents. This suggests previously unidentified neurotropic measles virus strains might cause SSPE world wide, and biased hypermutation might play a major role in evolution of SSPE viruses.

M 345 RESTRICTED V-SEGMENT USAGE IN T-CELL RECEPTORS FROM CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR A MAJOR EPITOPE OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS, Yusuke Yanagi, Ryuji Maekawa and Michael B.A. Oldstone, Division of Virology, Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Cytotoxic T lymphocytes (CTL) play an important role in recovery from a number of viral infections. They are also implicated in virus-induced immunopathology, as best demonstrated in lymphocytic choriomeningitis virus (LCMV) infection of adult immunocompetent mice. In order to study the structure-function relationship of T-cell recognition and the potential for manipulation and abrogation of the harmful immune response to viral infections, the structure of the T-cell receptor (TCR) in LCMV-specific CTL in C57BL/6 (B6) mice was investigated. Approximately 90% of LCMV-reactive CTL clones generated in H-2^b mice are specific for a short peptide fragment of the LCMV glycoprotein, residues 278-286, recognized in the context of the class I MHC molecule, D^b. Four CTL clones possessing this specificity were randomly selected from a collection of clones, and nucleotide sequences of their TCR genes were determined. All four clones were found to use V_α gene segments belonging to the $V_\alpha4$ subfamily. By RNA blot analysis, two more clones with the same specificity were also shown to express the $V_\alpha4$ mRNA. In contrast, three different V_β gene segments were used among the four clones examined. $J_\beta2.1$ was used by three of the clones. Restricted usage of V_α and possibly J_β segments in the CTL response to a major T-cell epitope of LCMV raises the possibility that immunopathology in LCMV infection can be treated with antibodies directed against such TCR segments.

M 346 **DIFFERENTIAL REGULATION OF THE TANDEM HEPATITIS B VIRUS SURFACE GENE PROMOTERS,** T.S.Benedict Yen & Dao-Xiu Zhou, Department of Pathology, UC San Francisco, CA 94143-0506

Two tandem promoters of the hepatitis B virus genome give rise to surface gene transcripts; the down-stream S promoter codes for mRNA species that are translated into the middle and small surface antigens, while the much weaker preS1 promoter codes for an mRNA that is mainly translated into the large surface antigen. All three forms of the surface antigen, which are co-linear in their carboxy-terminal portions, are found in the virion envelope, but over-expression of the large antigen can prevent secretion of all three forms and cause cellular injury. We have been investigating the cis-elements that differentially regulate the two promoters in hepatoma cells. Our results show that the preS1 promoter is activated by an up-stream HNF-1 site, but only in cooperation with an adjacent Oct-1 site. Neither of these sites affects transcription from the down-stream S promoter, which is activated by sequences within 70 bp up-stream of the major start sites. In addition, a region in the X gene acts as an enhancer that activates the S but not the preS1 promoter. The X gene product, a transcriptional trans-activator, has no signficant effect on either promoter. We are further defining the cis-elements in the S promoter and the enhancer, and characterizing the cellular factors that bind to them.

Herpesviruses, Poxviruses, Slow Viruses, Vaccines and Therapy

M 400 TRANSCRIPTIONAL REGULATION OF THE VACCINIA VIRUS LATE GENE TRANSACTIVATORS, Carl J. Baldick, Jr. and Bernard Moss, Laboratory of Viral Diseases, The National Institutes of Health, Bethesda, MD 20892

Vaccinia virus late gene transcription is dependent on DNA replication and the expression of three transactivator genes. Nuclease S1 analysis showed that expression of the transactivator genes is controlled in a manner distinct from that of either the early or late class of genes. In order to study the regulatory regions responsible for directing transcription of the transactivator genes, promoter fragments were ligated to a reporter gene. Quantitation of the reporter gene products were used to assay promoter strength in transfection experiments. These studies demonstrated that expression was dependent on vaccinia virus infection, but not DNA replication. This property is characteristic of the still poorly defined class of intermediate genes. 5' to 3' deletion analysis of these intermediate promoters indicated a minimum functional size of approximately 30 bp. An extensive series of single base substitutions within one of these promoters defined two regions critical for promoter function, one surrounding the RNA start site and a second just upstream.

M 401 CD4-*PSEUDOMONAS* EXOTOXIN HYBRID PROTEIN: ANTI-HIV ACTIVITIES IN CULTURED PRIMARY T-CELLS AND MACROPHAGES, AND ELIMINATION OF INFECTIOUS HIV BY SYNERGISTIC ACTIVITY WITH REVERSE TRANSCRIPTASE INHIBITORS, Edward A. Berger, Per Ashorn, and Bernard Moss, Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, MD 20892

CD4(178)-PE40 is a genetically engineered hybrid protein consisting of the HIV gp120-binding region of human CD4 linked to the translocation and ADP-ribosylation domains of *Pseudomonas aeruginosa* exotoxin A. In previous experiments with human T-cell lines, we showed that the hybrid toxin kills HIV-1 infected cells with high selectivity and potency, and inhibits HIV-1 spread. We now report that CD4(178)-PE40 selectively kills HIV-1 infected cell lines of the monocyte/macrophage lineage, and that it inhibits HIV-1 spread in primary T-cell and macrophage cultures. Furthermore, we observe strong synergistic activity between CD4(178)-PE40 and reverse transcriptase inhibitors (AZT or ddl) in blocking HIV-1 spread. When both types of drugs are present in cultures of T-cell lines infected with HIV-1, virus spread is completely blocked, as judged by protection of the target cell population from virus-mediated killing and complete inhibition of virus production. When drug administration is terminated after an initial treatment period of several weeks, no infectious virus remains; quantitative PCR analysis indicates complete elimination of HIV DNA. The surviving population is CD4-positive and readily killed upon re-exposure to fresh HIV, indicating that the drug treatment does not merely select for HIV-resistant cells. We conclude that the combination treatment completely eliminates infectious HIV-1 from the culture. Control experiments indicate that these synergistic effects are due to selective killing of the HIV-infected cells by CD4(178)-PE40, rather than to a simple neutraliztion effect by the CD4 moiety of the hybrid toxin. These results highlight the potential value of therapeutic regimens involving combination of a virostatic drug which prevents infection of healthy susceptible cells in the population, plus an agent capable of killing those cells that are already infected.

M 402 Hyperimmune sera against HIV-2 glycoprotein synthetic peptides mediate neutralization and ADCC activity.

E.Bjorling, K.Broliden, D.Bernardi, G.Utter, R.Thorstensson, F.Chiodi and E.Norrby.

Twentyfive 13 to 35 amino acids long peptides representing regions of human immunodeficiency virus type 2 (HIV-2), strain SBL6669, envelope proteins were evaluated for their immunogenic activity in guinea pigs. A number of the HIV-2 peptides were found to be capable of inducing strain SBL6669 neutralizing and antibody-dependent cellular cytotoxicity (ADCC) antibodies. Two overlapping peptides covering the amino acid sequence 311-337 representing the central and carboxy terminal part of the V3 region showed the most pronounced capacity to induce neutralizing antibodies. Two additional regions in the gp 125 containing linear sites reacting with neutralizing antibodies were identified, aa 119-137 and 472-509. The transmembrane protein, gp 36, pf HIV-2 harbored two regions of importance for induction of neutralizing antibodies, aa 595-614 and 714-729. These findings pare the way for development of synthetic vaccines against HIV-2 and possibly also SIV infections. The capacity of such a product to induce protective immunity can be evaluated in macaque monkeys.

M 403 IDENTIFICATION OF A NOVEL HUMAN NEUTRALIZING REGION OF HIV-1 gp41

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2. Dept. of Virology, National Bacteriological Laboratory, Stockholm

A novel neutralizing region of HIV-1 gp41 (a.a. 647-671) as well as the previously described V3 region of gp120 were identified and chartacterized with a panel of 80 HIV-1 antibody positive human sera. Levels of neutralizing antibodies against the HIV-1 strains IIIB, SF2 and RF were compared with reactivity in ELISA against peptides that correspond to certain regions of the HIV-1 envelope. A correlation between high neutralizing activity and strong seroreactivity against the peptides suggested that the corresponding regions might be involved in neutralization. Peptides representing these regions were also able to inhibit neutralization mediated by serum from HIV-1 antibody positive individuals. Neutralizing antibodies to the novel region of gp41 were found to be cross-reactive whereas they were found to be strain-specific for the V3 region of gp120. The results indicate that the conserved B-cell epitope of the HIV-1 gp41 (a.a. 647-671) elicits a virus neutralizing antibody response during natural infection in humans and may therefore be considered for inclusion in a vaccine against HIV-1.

M 404 SINGLE AMINO ACIDS IN THE V3 LOOP OF GP120 IMPORTANT FOR VIRUS NEUTRALIZATION BY HUMAN HIV-1 SPECIFIC ANTIBODIES

Per A. Broliden[1,2], K Broliden[2], L Åkerblom[3], J Rosen[4], E Norrby[2] and B Wahren[1].
1. Dept. of Virology, National Bacteriological Laboratory, 2. Dept. of Virology, Karolinska Institute, Stockholm, 4. Dept. of Veterinary Microbiology, Biomedical Center, Uppsala, Sweden, 5. RW Johnson Pharmaceutical Instititute, La Jolla, USA

The importance of the dependence on single amino acids in the V3 region of HIV-1 gp120 was evaluated for virus neutralization and antibody dependent cellular cytotoxicity (ADCC). Synthetic overlapping 15-mer peptides and a set of omission peptides covering a.a. 301-317 were used. Sera from 29 HIV-1 infected individuals at different stages of disease were tested for neutralization, ADCC and specific IgG reactivity. Six HIV-1 neutralizing monoclonal antibodies (Mabs) acted as controls. All MAbs reacted with a region (a.a. 304-318) of gp120, previously shown to induce neutralizing antibodies. The amino acids essential for reactivity were identified to be within the sequence GPGR (a.a. 312-315). The importance of this region for occurrence of neutralizing antibodies in infected humans was investigated using the same set of peptides. Out of 29 individuals, 21 were found to have neutralizing antibodies in titers between 100-1000. Among the neutralization-positive sera, 17/21 (81%) reacted with a.a. 304-318, compared with only one of eight sera (13%) negative in neutralization. When any of the four amino acids G, P, G or R were deleted, the seroreactivity of these neutralization-positive sera decreased considerably. The conserved sequence GPGR was therefore considered to be the most important for neutralization in this region also in human sera. One of the MAbs were able to mediate ADCC, indicating that the V3 region may be one important target for cytotoxicity. However, no correlation was found among the human sera between ADCC activity and IgG reactivity to a specific sequence within V3. Thus, the conserved sequence GPGR in the V3 region of gp120 is critical for virus neutralization by human HIV-1 specific antibodies.

M 405 B-CELL PHENOTYPE DEPENDENT CONTROL OF EBV ANTIGEN EXPRESSION. STUDIES WITH SOMATIC CELL HYBRIDS. Bertha Contreras-Brodin, George Klein and Maria G. Masucci. Department of Tumor Biology, Karolinska Institute. Stockholm Sweden.

Six nuclear antigens (EBNA-1 to 6) and three membrane proteins (LMP1, LMP-2A, and LMP-2B) are associated with the persistance of Epstein-Barr virus (EBV) in immortalized lymphoblastoid cell lines (LCLs).

The two EBV-associated human malignancies Burkitt lymphoma (BL) and Nasopharingeal carcinoma (NPC) have a restricted expression of EBNA 2-6 and LMP in comparison with LCLs that express all EBV antigens. Expression of EBV antigens correlates with the expression of B-blast markers.

In order to examine the phenotype dependent viral gene expression we have constructed a serie of somatic cell hybrids derived by the fusion of EBV positive BLs and LCLs with non B-cell lines. Analysis of EBV antigens in these hybrids showed that the expression of EBNA 2 to 6 is down regulated in the hybrids in parallel with the extinction of the B-cell specific markers CD 19, CD20, CD21, CD23 HLA class II antigens and Ig, suggesting that the expression of EBNA 2 to 6 is dependent on the B-cell phenotype.

EBNA-1 was the only EBV antigen regularly expressed in the hybrids indicating that EBNA-1 can be autonomously expressed.

Our results rise question weather B-cell specific transacting factors play a role in the regulation of EBNA 2 to 6 and weather EBNA 1 can be transcribed independent from the big primary transcript that generates all EBNAs after alternative splicing.

M 406 HERPES SIMPLEX VIRUS (HSV) GLYCOPROTEIN D-INDUCED BLOCK TO SUPERINFECTION OF HSV-INFECTED CELLS : MOLECULAR BASIS AND SIGNIFICANCE.

Gabriella Campadelli-Fiume[1], Sun Qi[1], Elisa Avitabile[1], Laura Foá-Tomasi[1], Renato Brandimarti[1], and Bernard Roizman[2]. The Section on Microbiology and Virology, University of Bologna, Italy,[1] and Viral Oncology Laboratories, The University of Chicago, Ill[2].

Earlier our laboratories reported that HSV-1 and -2 attach to but do not productively enter cell lines expressing constitutively gD (BJ cells). We designated the phenomenon as restriction to superinfection. A mutant virus [HSV-1(F)U10] unrestricted by BJ cells was selected. The mutation was mapped to the gD gene and consisted in a signle bp substitution that predicts a Leu_{25} to Pro substitution. Studies on HSV-1(F)U10, on the cell lines expressing mutant gD (gDU) and on immunoreactivity of gDU indicate that (i) gD expresses a specific function, determined by sequences around Leu_{25}, which blocks infectious entry of virus into cells synthesizing gD. The restriction domain of gD is distinct from the domain involved in virus entry, mapped previousvly. (ii) the target of gD restriction is the identical domain of the gD molecule contained in the envelope of superinfecting virus. (iii) the molecular basis of restriction does not involve competition for a host receptor involved in virus entry. The gD-mediated restriction may play a role in virion maturation. Since enveloped virions transit the cytoplasm within membrane-bound vesicles, in the absence of restriction virion envelopes would fuse with the vesicle membranes resulting in cytoplasmic de-envolpment of virions. Consistent with this prediction, late in infection cells infected with HSV-1(F)U10 show a large number of unenveloped nucleocapsids in the cytoplasm and a 50-fold reduction in accumulation of extracellular virus.

M 407 MURINE CYTOMEGALOVIRUS IE2 GENE ENCODES A TRANSACTIVATOR.

Rhonda D. Cardin and Edward S. Mocarski, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305.

Very little is understood about the genetic control or mechanisms involved in CMV pathogenesis. The large 230 kilobase pair DNA genomes of human and murine CMV encode over 200 gene products that regulate viral gene expression during viral growth as well as gene products presumably involved in virus/host interactions and latency. The murine CMV immediate-early gene 2 (ie2) encodes a 391 amino acid protein that is dispensable for growth in cell culture. In experimentally inoculated mice, ie2-deficient recombinant viruses do not exhibit significantly altered growth in target tissues such as the salivary gland, peritoneal cavity, liver, kidney, spleen, and lungs. We have recently demonstrated that the ie2 gene product can function in transient gene expression assays as a transcriptional activator of the major immediate-early ie1 promoter as well as its own promoter. The murine and human CMV enhancer regions share many similar sequence motifs that may be important in the regulation of immediate-early gene expression. Preliminary results demonstrate that IE2 can mediate transactivation through known human CMV enhancer repeat elements, suggesting that the murine CMV enhancer may be a target for IE2-mediated transactivation. Based on the predicted protein sequence, IE2 contains a putative leucine zipper and adjacent charged domains. A 4-3 repeat of hydrophobic residues occurs in this same region of the ie2 sequence that may be important for formation of a coiled-coil structure. Interestingly, IE2 shares an overall amino acid similarity of 44% to Epstein-Barr virus BZLF1, a known transactivator of the EBV immediate-early promoters. IE2 also demonstrates 49% amino acid similarity to the bovine papillomavirus E2 transactivator as well as 47-50% similarity to a HCMV protein family encoded for in the short (S) component of the viral genome, suggesting that these proteins may be functionally similar to MCMV IE2. Functional characterization of IE2 as a transactivating protein and murine and human CMV enhancer elements as targets for IE2-mediated transactivation should yield insights into murine CMV gene regulation and may be applicable to the overall understanding of human CMV pathogenesis.

M 408 NEW ANTIVIRAL TARGET ON HIV-1 REVERSE TRANSCRIPTASE REVEALED BY TIBO DERIVATIVES,

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Screening of pharmacologically acceptable prototype compounds has recently led to the discovery of a new series of ultraselective inhibitors of HIV-1 replication, the tetrahydro-imidazo [4,5,1-jk][1,4]-benzodiazepin-2(1H)-one and -thione (TIBO) derivatives. The TIBO compounds completely suppress the formation of proviral DNA in acutely infected cells, as revealed by polymerase chain reaction (PCR) analysis. TIBO derivatives are inhibitory to the reverse transcriptase (RT) of HIV-1, but not of HIV-2 or other retroviruses. The inhibition is most effective with poly(C).oligo(dG) as the template/primer and selectively directed against the RNA-dependent DNA polymerase activity and not the accompanying DNA-dependent DNA polymerase and ribonuclease H activity of HIV-1 RT. Kinetic studies point to an uncompetitive type of inhibition with regard to the template/primer. TIBO compounds are active against HIV-1 replication through a unique interaction with HIV-1 RT. The experimental data indicate the existence of a novel target on HIV-1 RT which is responsible for the inhibition of replication, and a mode of action unrelated to that of previously studied RT inhibitors.

M 409 AN ANTIGEN ENCODED BY THE LATENCY-ASSOCIATED TRANSCRIPT IN CULTURED PRIMARY NEURONS LATENTLY INFECTED WITH HSV-1.

Christian Doerig*, Lewis I. Pizer and Christine L. Wilcox
University of Colorado School of Medicine, Denver CO 80262

Wilcox and Johnson (1) have previously reported the infection *in vitro* of primary fetal rat neurons with Herpes simplex virus type 1 (HSV-1) under conditions that lead to viral latency, as defined by the following operational criteria : i) lack of expression of the viral gene products that are detectable during a productive infection, and ii) reactivation of the virus by specific stimuli. Transcriptional studies show that neurons harboring a latent infection *in vitro* express the latency-associated transcript (LAT); however these cells do not produce transcripts from other viral genes until the virus is reactivated by nerve growth factor (NGF) deprivation. Since viral expression restricted to LAT transcription is widely accepted as a marker for latency, these data suggest that our system not only mimics latency at an operational level, but is relevant for mechanistic studies of latent infection by HSV-1.

The LAT contains two open reading frames, but so far a corresponding protein encoded by LAT has not been detected. Experiments will be described, in which we demonstrate by immunocytochemical techniques that an antiserum directed against a bacterially expressed fusion protein containing a part of an LAT-encoded polypeptide recognizes an antigen specifically expressed in latently infected neurons cultured *in vitro*. This protein was not detected in mock-infected neurons nor in productively infected Vero cells. Western blot analysis revealed a 42 kd protein present in latently infected neurons. The significance of this novel latency-associated antigen, or LAA, will be discussed.

1) Wilcox, C.L. and Johnson. E. M. 1987. Nerve growth factor deprivation results in the reactivation of latent Herpes simplex virus *in vitro*. J. Virol. **61**: 2311-2315

M 410 ANALYSIS OF HCMV DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION, Hermann Einsele, Michael Steidle, Angelika Vallbracht, Gerhard Ehninger, Hans Dierck Waller, Claudia A. Müller, Department for Internal Medicine II, Medizinische Klinik, Institut für Epidemiologie und Klinik der Virus Krankheiten, Hygiene Institut, University of Tübingen, Germany.

60 patients who had undergone allogeneic bone marrow transplantation for different haemtological disorders were followed up for the development of HCMV infection using a highly specific and sensitive PCR technique in addition to conventional and rapid virus culture as well as hybridization and immunocytological analysis. 43 of the patients analysed were found to be HCMV positive by the PCR assay, in more than 80% of these patients the virus could be additionally detected by at least two of the other techniques described. PCR technique allowed detection of the virus more than two weeks prior to all the other techniques applied. A decrease in the leukocyte, lymphocyte count and particularly the absolute number of CD4+-T-cells after HCMV detection was associated with the development of symptomatic HCMV infection as observed in 26 patients. In contrast in 17 patients HCMV-positive in two assays no symptoms related to HCMV infection were observed. Organ involvement of HCMV as clinically suspected due to an increase in the serum levels of the transaminases or the development of interstitial pneumonia and proven by demonstration of the virus in the lung and liver by at least two of the techniques described was associated with marked immunohistological alterations on the cells infected by the virus, suggestive of an immunopathogenesis of HCMV disease after allogeneic bone marrow transplantation.

M 411 PROMOTER UTILIZATION AND METHYLATION STATUS IN EBV-CARRYING CELL LINES, Ingemar Ernberg, Hu LiFu, Ender Altiok, Janos Minarovits, Kerstin Falk, George Klein. Dept of Virology and Dept of Tumor Biology, Karolinska Institutet, Box 60 400, 10401 Stockholm, Sweden

Epstein-Barr virus (EBV) carrying, latently infected cell lines and tumors express 1-10 viral genes. The pattern of expression correlates to the cellular phenotype. Four known and at least one not yet identified promoter is involved in this expression. These promoters are partly cell type specific, so that Wp, Cp and the LMP-regulatory sequence (LRS) are utilized in different cell types. Cellular transcription factors are likely to be important for this cell type specificity. We have also found that methylation patterns are specific in the promoter/enhancer regions of highly methylated NPC- and BL-cells (type I). The LRS in NPC is an 800 bp unmethylated island, surrounded by extensive CpG methylation, in tumors where LMP-expression can be detected. It is completely methylated in LMP non-expressors. We are now investigating whether Wp also shows an expression related methylation pattern. The ori P region with the EBER-coding genes is always unmethylated (see Falk et al., this abstract volume). We propose that this specific methylation pattern has a role in the viral gene regulation.

M 412 Expression of HIV-1 antigenic determinants on chimaeric poliovirus particles

David Evans, P.D. Minor, J.McKeating, R.Weiss, C.Vella and J.W. Almond
Dept. of Microbiology, University of Reading, Chester Beatty Labs and NIBSC, UK.

The three dimensional and antigenic structure of poliovirus are well understood, and have allowed the construction of intertypic poliovirus antigen chimaeras exhibiting composite antigenic and immunogenic characteristics.[1] We have exploited this observed flexibility in the sequences that can be accomodated at certain positions of the poliovirus particle to develop the Sabin 1 vaccine strain of poliovirus as an epitope expression vector[2]. Known and predicted epitopes, of 12 to 18 residues in length, derived from the transmembrane and surface glycoproteins (gp41 and gp120) of HIV-1 have been engineered into antigenic site 1 of poliovirus, without abrogating virus viability[3]. The chimaeric virus particles have been characterised with regard to their antigenicity and their ability to induce neutralizing antibodies. Results obtained demonstrate that poliovirus chimaeras may have a role in HIV-1 epitope expression for diagnostic[4] and vaccine development purposes.

1. Burke et al. (1988) Nature 332, 81-82.
2. Burke et al. (1989) J.gen.Virol. 70, 2475-2479.
3. Evans et al. (1989) Nature 339, 385-388.
4. Vella et al. submitted for publication.

M 413 METHYLATION STATUS OF THE ORI P AND C-PROMOTER REGION IN A TYPE I BURKITT LYMPHOMA CELL LINE,

Kerstin Falk, Hu Li-Fu, Janos Minarovits, Georg Klein and Ingemar Ernberg.
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Burkitt's lymphoma (BL) cells carrying EBV only express EBNA-1, while in vitro transformed lymphoblastoid cell lines (LCL) express EBNA 1-6, as well as LMP 1 and 2. We analyzed two cell lines for methylation/ restriction enzyme sensitivity; Rael, a type I BL cell line and CBM1-Ral-Sto, an LCL established with EBV from Rael. Cellular and viral DNA's were cleaved with the methylation sensitive restriction enzyme (Hpa II) and with it's methylation insensitive isoschizomer (MspI) followed by Southern blotting analyses. Rael was found to be extensively methylated and CBM1-Ral-Sto unmethylated (J. gen. Virol. 1989, 70, 2989-3002). Seven probes covering EBER I and II, oriP and the Bam HI C promotor (coordinate 11305) regions were used. In Rael we found a distinct unmethylated island extending through the oriP region and the coding region for the EBER's. The lymfoblastoid cell line was hypomethylated in these regions, while the promotor region was unmethylated.

M 414 ENHANCEMENT OF RNA POLYMERASE II INITIATION BY A NOVEL DNA RECOGNITION DOMAIN DOWNSTREAM FROM THE CYTOMEGALOVIRUS ENHANCER/PROMOTER CAP SITE.

Peter Ghazal, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The promoter-proximal downstream sequences of genes transcribed by RNA polymerase II have recently been recognized as containing transcriptional regulatory elements. I have identified and characterized a novel regulatory domain downstream from the human cytomegalovirus that is recognized at the DNA level. This regulatory domain was shown to enhance the number of functional initiation complexes without significantly altering the apparent elongation rate by RNA polymerase II transcription. I have constructed and analyzed a series of mutations in the first exon by *in vivo* and *in vitro* transcription assays. Run-off *in vitro* transcription and DNA-binding experiments (DNase I-footprinting and mobility shift assays) identified two downstream elements that specify the interaction of cellular transcription factors. One of these elements contains a reiterated sequence motif, present twice within exon 1. The second element is an 18-bp sequence, located at approximately nucleotide position +33, that is conserved between species strains of CMV. The conserved element is recognized by two cellular nuclear proteins designated LTF A and B (for Leader Transcription Factor A and B) of 74 kDa and 50 kDa respectively as determined by photoactivated crosslinking. Screening a battery of different cell-type nuclear extracts for LTF A and B activity suggests that these proteins are ubiquitous transcription factors. This study of promoter-proximal downstream transcriptional elements describes a unique DNA target regio n for regulating latency and reactivation of HCMV.

M 415 SUPERINFECTION BY HSV CAN ALTER THE PATTERN OF HIV RNA EXPRESSION. Marjorie P. Golden, Sunyoung Kim, Scott M. Hammer, Elizabeth A. Ladd and Mary A. Albrecht, New England Deaconess Hospital and Harvard Medical School, Boston, MA 02215

Among potential cofactors, herpesviruses have received much attention because they are highly prevalent among HIV infected hosts. It was reported by us and others that HSV can activate HIV1 gene expression, as determined by HIV LTR CAT expression and superinfection of acutely and chronically HIV1 infected cell lines. The ACH2 cell line, derived from a chronically HIV1 infected T lymphocytic line, CEM, produces few progeny virions, but is inducible to increase virion production by treatment with PMA and GM-CSF. Resting ACH2 cells have an aberrant pattern of viral RNA expression which is altered by treatment with phorbol esters to reproduce the RNA patterns of actively replicating virions. We have found that HIV expression dramatically increased following superinfection of ACH2 cells with HSV, as determined by a 10-fold rise in reverse transcriptase activity and p24 antigen expression. The HIV RNAs expressed in resting ACH2 cells consist mainly of subgenomic RNA species, with little or no genomic RNA. We found that within 12 hours of HSV superinfection, there was a dramatic rise in the 2 kilobase mRNA (encoding viral regulatory proteins), followed by a marked increase in the genomic mRNA. Such a shift of transcriptional phases recapitulates the early-to-late transition of the productive infection observed during a single step growth cycle of HIV and supports our previous observation that upregulation of HIV1 expression occurs following HSV superinfection. These data also suggest that activation of HIV1 by certain heterologous viruses occurs at the level of RNA expression.

M 416 INTERFERON alpha VS INTERFERON alpha / INTERLEUKIN II IN PATIENTS WITH CHRONIC TYPE B HEPATITIS PREVIOUSLY UNRESPONSIVE TO INTERFERON alpha.
Georg Hess[1], Siegbert Rossol[1], Rita Rossol[1], Peter Gotzler[2], Karl-Hermann Meyer zum Büschenfelde[1]
[1]I. Medical Department, University of Mainz, 6500 Mainz, [2]Essex Pharma, 6500 Mainz.
Interferon alpha (IFN) results in loss of HBeAg and Hepatitis B Virus DNA in approximately 40 percent of treated patients with chronic hepatitis B (CAH-B). Twentyfour patients with CAH-B unresponsive to a first IFN course were randomized to receive 5 million units of IFN alpha s.c. (Schering/Essex) daily for four months or the same treatment combined with four 2 week courses of interleukin 2 (IFN / IL2) starting at week 3 of treatment. The interval between IL 2 courses was 2 weeks. IL 2 was given s.c. at 2.25 x 10^6 IU/m^2 2 x per day for 2 days followed by 1.8 x 10^6 IU/m^2/day for 12 days. The interval between the end of the 1. treatment and start of retreatment avaraged 13.4 months (range 6-25 months). Twelve patients with CAH-B each were randomized to each treatment arm. HBV-DNA became negative in 11 patients (7 on IFN, 4 on IFN/IL2), HBeAg became undectable in 4 patients (3 on IFN, 1 on IFN/IL2) at the end of treatment. All patients remained HBsAg positive. Side effects were frequent and reversible in both groups, however more severe in the IFN/IL2 group than in the IFN group. IL2 had be discontinued in 2 patients because of side effects. Severe local side effects (inflammatory reaction) occured only after IL2 application. The study shows that retreatment of patients with CAH-B previously unresponsive to IFN alpha was of low effectiveness. The addition of IL2 at the doses given and the schedule reported to IFN appeared to be of no benefit.

M 417 ISOLATION AND SEQUENCING OF THE LATENT MEMBRANE PROTEIN (LMP) GENE FROM A EBV STRAIN IN A CHINESE NASOPHARYNX CARCINOMA
Li-Fu Hu, E.R.Zabarovsky,Fu Chen,S.L.Cao,K.Falk,I.Ernberg,G.Klein and G.Winberg Dept.of Tumor Biology,Karolinska Institute,Stockholm,Sweden
The BamH-1 fragment containing the EBV LMP-gene was cloned from a genomic library of the nude mouse propagated Chinese NPC tumor CAO. Restriction polymorphism studies on DNA from NPC biopsies from China and Africa reveal that the present isolate represents a virus type prevalent in China but not in Africa. The sequence of the LMP-gene with its promoter and enhancer was determined. Comparison of the sequences for the B95-8, Raji and CAO isolates reveal differences unique to the CAO isolate in the promoter/enhancer as well as in the amino acid sequences of the protein. Structural differences in the protein were located chiefly in the 20 aminoterminal residues and in the array of repeated amino acids in the carboxy-terminal part of the protein, where the CAO isolate displays a cluster of seven perfect repeats of 11 a.a. Three of these repeats have no counterpart in the other virus strains. This,taken together with two unique deletions of 5 and 10 a.a.in the carboxy terminal part adds up to a protein of 405 a.a. compared to 392 a.a. for B95-8 and Raji. The larger protein size is demonstrated by Western blots on tumor material as well as after transfection of the cloned gene into cultured B-cells.

M 418 ULTRASENSITIVE NON-RADIOACTIVE DETECTION OF HERPES SIMPLEX VIRUS BY LCR, THE LIGASE CHAIN REACTION, Laurie Rinehardt, Hartmut Hampl, and Thomas G. Laffler, Abbott Laboratories, Abbott Park, IL 60302

We have developed a rapid, semiautomated amplified probe assay for HSV utilizing LCR technology. Traditional detection of HSV by virus culture and cytologic monitoring, which often take more than two days, has limited sensitivity, and depends on the presence of intact, culturable virus in test specimens. Our HSV LCR assay detects less than 100 HSV DNA molecules, and since the target region is short, the assay accommodates partially degraded specimens. A segment of the HSV genome approximately 50 bases in length is amplified by LCR and the product detected by an IMx® microparticle immunoassay. Our HSV LCR system eliminates blunt-end ligation background, resulting in an extremely sensitive non-radioactive assay with excellent specificity. Presently, a batch of 24 specimens can be analyzed in less than three hours. Sensitivity compares favorably with that of PCR, and the HSV LCR assay readily discriminates HSV1 from HSV2.

M 419 DOWNREGULATION OF CELL SURFACE CLASS I MHC ANTIGENS BY POXVIRUSES
Joanne L. Macen, Lynn K. Boshkov*, Andrea Opgenorth and Grant McFadden, Departments of Biochemistry and Medicine*, University of Alberta, Edmonton, T6G 2H7, Canada.

Poxviruses are a large group of complex DNA viruses that infect a variety of organisms and cause a wide range of disease pathologies. Poxviruses carry out their complete replicative cycles within the cytoplasm of infected cells and represent unique model systems for the study of numerous biological processes. Shope fibroma virus (SFV) is a tumorigenic leporipoxvirus which causes a localized benign fibroma in immunocompetent rabbits which regresses after two weeks due to a vigorous cell-mediated immune response. Malignant rabbit fibroma virus (MRV) and myxoma virus (MYX) are related tumorigenic leporipoxviruses which induce rapidly lethal systemic infections characterized by severe immunosuppression. Using a monoclonal antibody directed toward class I MHC proteins, we observe a rapid and specific decrease in the cell surface expression of class I MHC antigens in cell culture during infection with MRV or MYX. By 24 hours post infection, class I MHC antigens are nearly undetectable on the surface of infected cells. This downregulation requires late viral gene expression, and does not affect the cell surface levels of other glycoproteins, such as the transferrin receptor. Neither the benign SFV nor the orthopoxvirus vaccinia significantly decrease MHC antigen levels. An attenuated mutant of MYX, created by interuption of an ORF of unknown function designated as M-11, shows minimal downregulation of class I antigen expression. Therefore, decreases in cell surface class I MHC antigen expression during poxvirus infection correlate well with virus virulence. We are currently characterizing the nature of this downregulation at the genetic and molecular levels in order to assess the physiological relevance of these observations. Since many animal virus virulence factors act to attenuate host immune responses, these studies should further our understanding of the mechanisms underlying virus-induced immunopathology.

M 420 THE GLYCOPROTEIN GP116 OF HUMAN CYTOMEGALOVIRUS CONTAINS TWO ANTIBODY BINDING SITES IN CLOSE PROXIMITY.
Heidi Meyer and Michael Mach, Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, Loschgestraße 7, D-8520 Erlangen, FRG.

The glycoprotein gp116 of human cytomegalovirus (HCMV) is a target for neutralizing antibodies. It is a component of the gCI-complex consisting of gp58 and gp116. Like its homologue glycoprotein gB of HSV I it posseses a highly antigenic region at the very aminoterminal part of the molecule between aa 27-84. In Western blot analyses with a series of procaryotic expression clones and human sera as well as a monoclonal antibody, we have defined two antibody binding sites. Site I (aa68-77) contains a neutralizing epitope and was recognized by human sera and the human monoclonal antibody C23. This region is conserved among all HCMV strains tested so far. Site II corresponds to aa 50-54, which is not conserved among the two HCMV laboratory strains of known sequence (AD169 and Towne). Using affinity chromatography we purified antibodies against site II from pooled human sera and investigated the biological function in vitro. Antibodies against site II did not neutralize HCMV in tissue culture.

M 421 TRANSACTIVATION OF THE MAJOR CAPSID PROTEIN GENE OF HERPES SIMPLEX VIRUS TYPE 1 REQUIRES PROMOTER BINDING OF A NOVEL CELLULAR FACTOR, Robert Millette, Shin Chen, Ronald Wobig, Rosemary Lown, and Robyn Hauser, Department of Biology, Portland State University and Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, OR 97207

The $\beta\gamma$ or γ_1 class of late genes of herpes simplex virus type 1 (HSV-1), as exemplified by the major capsid protein (VP5) gene, are virtually silent in uninfected cells. However, these genes are effectively transactivated by superinfecting HSV-1 or by cotransfected immediate early (IE) HSV-1 genes. Using gel mobility shift and DNase I footprinting analyses, we have identified two major VP5 promoter complexes that are formed using nuclear extracts from either uninfected or infected HeLa cells. Both complexes involve a cellular factor that binds to a core sequence, GCCATCTTGAAT, located -63 to -74 base pairs relative to the cap site. We have demonstrated involvement of this sequence in transactivation of the VP5 gene by using deletions of the VP5 promoter coupled to the CAT gene in transfection assays. These studies showed that the above sequence is required for optimal transactivation of the VP5 promoter either by superinfecting HSV-1 or by cotransfected HSV-1 IE genes (ICP 0, 4, & 27). A computer search of GenBank revealed that close homologs of the VP5 binding sequence are found in a variety of viral and cellular promoters. These include the LTRs of HIV-1 and a number of other primate, murine, and avian retroviruses. Studies are in progress to isolate the cellular factor, to determine its function herpesvirus and retrovirus transcriptional regulation, and to clone its gene.

M 422 MOLECULAR MECHANISMS FOR CROSS-TRANSACTIVATION SYNERGIZES HIV AND HCMV GENE EXPRESSION. Jay A. Nelson[1], Joseph Garcia[2], Richard Stenberg[3], Richard Gaynor[2], and Peter Ghazal[1], [1]Research Institute of Scripps Clinic, La Jolla, CA 92037, [2]UCLA School of Medicine, Los Angeles, CA 90028, and [3]Eastern Virginia Medical School, Norfolk, VA 23501.

The HIV-1 Tat protein is a potent *trans*-activator of HIV expression. Tat strongly stimulates HIV promoter activity via a target response sequence (TAR) located downstream from the initiation site of transcription. Here, we present evidence that suggests the HIV-1 Tat protein can specifically *trans*-activate heterologous (HCMV major immediate-early promoter) and homologous promoters in a TAR-independent manner. Furthermore, the TAR-independent Tat *trans*-activation of promoters is synergistically enhanced in the presence of cytomegalovirus *trans*-activator proteins. The role of Tat in the Tat-TAR and TAR-independent systems is discussed. In particular, the role of opportunistic infections in AIDS is an important aspect of HIV pathogenesis. HIV and HCMV infect similar cell-types *in vivo* and in some instances co-exist within the same cell. Both viruses contain regulatory proteins which can reciprocally *trans*-activate and together positively enhance the level of expression. Therefore, the exacerbated growth of both HCMV and HIV in situations of coinfection may perhaps be explained at the molecular level by reciprocal *trans*-activation between the viruses.

M 423 **RECOMBINANT HERPESVIRUS VECTORS TO DEFINE PROTECTIVE IMMUNOGENS AGAINST RETROVIRUS INFECTION IN CATS.** Jack H. Nunberg*[1], Georgette E. Cole[1], Sandrina Stacy-Phipps[1], Annette L. Meyer[2], Pamela J. Berlinski[2], and Richard C. Wardley[2]. [1] Cetus Corporation, Emeryville, CA 94608, [2] The Upjohn Company, Kalamazoo, MI 49001, * present address: Merck, Sharp and Dohme Research Laboratories, West Point, PA 19486.

Retroviruses are known to cause a variety of infectious diseases of medical and veterinary importance. Despite an extensive literature in the area of retrovirus immunology, little is known about protective immunogens and protective immune responses in retroviral infection. To explore the role of retroviral envelope (*env*) and *gag* immunogens in vaccinal protection, we constructed recombinant feline herpesviruses (FHVs) expressing these proteins of the feline leukemia virus (FeLV). Expression cassettes, utilizing the human cytomegalovirus immediate early promoter, were inserted within the thymidine kinase gene of FHV. The FeLV *env* glycoprotein expressed by recombinant FHV was processed and transported to the cell surface much as in FeLV infection, with the exception that proteolytic processing to yield the mature gp70 and p15E proteins was less efficient in the context of herpesvirus infection. A recombinant FHV containing the FeLV *gag* and protease genes expressed both *gag* and *gag*-protease precursor proteins. Functional protease was produced which mediated the proteolytic maturation of the FeLV *gag* proteins as in authentic FeLV infection. Preliminary studies in cats indicated that the FeLV *env* protein expressed during vaccinal infection was immunogenic. Additional studies, including those utilizing *gag* immunogens, are planned. Use of these recombinant FHVs as live virus vaccines may provide insight as to the role of specific retroviral proteins in protective immunity.

M 424 OPEN READING FRAMES, PROMOTERS AND ENHANCERS IN THE IMMEDIATE-EARLY REGION OF PSEUDORABIES VIRUS: COMPARISON WITH HSV1, Václav Pačes[1,2], Čestmír Vlček[2], Zbyněk Kozmík[2] and Martin Schwyzer[3]; [1]Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510; [2]Inst. Molec. Genetics, Czechoslovak Acad. Sci., CS-16637 Prague, Czechoslovakia; [3]Inst. of Virology, Univ. Zurich, CH-8057 Zurich, Switzerland

A segment (15 kbp) of the Pseudorabies virus (PRV) genome, comprising and encompassing its immediate-early (IE) gene, was sequenced. Several open reading frames (ORF) were found in it. One of them (ORF3) overlaps with the IE gene in the opposite orientation. It is in the same region where latency-related transcripts in opposite polarity to the IE gene were reported. There is a potential promoter (P2) upstream of ORF3 containing binding motifs for several transcription factors. Specific binding of nuclear proteins to four Sp1 sites was detected. Promoter P1 is upstream of the IE gene and it contains a TATA-box, CCAAT motif, and Sp1 and NF-μE1 binding sites. Four closely associated NF-μE1 and oct-1 motifs were found further upstream from P1. These upstream elements were essential for efficient expression of PRV IE gene *in vivo*. It also increases efficiency of the HSV1 tk promoter. Two potential enhancer elements (E3, E4) were identified in the sequenced region by an enhancer trap experiment. Linked to P1, E3 acted as an enhancer and E4 as a silencer. The PRV IE gene product repressed transcription from its own promoter and activated the SV40 early promoter. The transactivating virion protein Vmw65 of HSV1 had the opposite effect on these promoters. The regulatory elements of PRV will be compared with those of HIV1.

M 425 **A 38-kDa PROTEIN OF COWPOX VIRUS (BRIGHTON) INHIBITS THE SYNTHESIS OF A 15-LIPOXYGENASE DiHETE.** **[1]Palumbo, GJ., [2]Glasgow, W., [2]Eling, T., and [1]RML. Buller. [1]Laboratory of Viral Disease, NIAID, NIH, Bethesda, MD 20892, and [2]Laboratory of Molecular Biophysics, NIEHS, Research Triangle Park, NC 27709**

The Brighton strain of cowpox virus (CPV-BR) induces flat, bright red, well demarcated lesions on the chorioallantoic membrane (CAM) of the 12-day old chick embryo, while the deletion of a gene which encodes a 38-kDa protein (mutant virus CPV-BR.D1) results in the generation of a raised, white, and opaque lesion due to a massive inflammatory cell influx into the site of virus infection. Upon investigation of different chemoattractant generating systems, we recently found that inhibitors of arachidonate metabolism, and specifically the lipoxygenase (LO) pathway, block the morphogenesis of poxviruses in vivo and in vitro. To further investigate the influence of poxvirus infection on arachidonic acid metabolism, B-SC-1 cells were infected with either CPV-BR or CPV-BR.D1, and then stimulated with the calcium ionophore A23187 in the presence of [^{14}C]-linoleic acid. Analysis of the arachidonate metabolites in infected cells by reverse-phase HPLC demonstrated a similar spectrum of cyclooxygenase and LO pathway products, except for the synthesis of a chemoattractant <15-LO series DiHETE> by cells infected with the 38-kDa minus mutant virus. We propose that the function of the 38-kDa protein is to prevent the synthesis of 15-LO series DiHETE by host enzymes from precursors needed for virus replication.

M 426 DETECTION OF "ZEBRA" (BZLF-1) EXPRESSION IN MALIGNANT AND NON MALIGNANT EBV-CONTAINING HUMAN TISSUES, Patton, D.F., Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE 68198.

Defective EBV (hetDNA), first identified in a subclone of the P3HR-1 cell line, was shown to be capable of disrupting EBV latency *in vitro*, and to consist of a deleted and rearranged genome. The specific rearrangement which appears to be responsible for activation of replication is the abnormal juxtaposition of *BamHI* W and Z fragments which results in upregulation of ZEBRA protein (EB1, Zta, BZLF1). ZEBRA is an immmediate early gene product which binds the AP-1 binding site and transactivates the EBV productive cycle. Detection of the *Bam* W-Z rearrangement in clinical samples can be done using polymerase chain reaction (PCR) to frame the abnormal W-Z junction. We have detected this rearrangement in 2 tissues containing replicating EBV; a) oral hairy leukoplakia, a nonmalignant lesion, and b) a thymic carcinoma. Identification of the *Bam* W-Z rearrangement *in vivo* suggested that hetDNA may play a biologically significant role similar to that shown *in vitro* of replication activation. Western blot analysis using monospecific anti-ZEBRA serum has been performed on tissues containing EBV. We have found evidence of ZEBRA expression in two tissues thus far. Correlation with presence of hetDNA and with disease process will be discussed.

M 427 ANTI-HIV ACTIVITY OF PROTEASE INHIBITORS *in vitro*. Deborah Paul, Mark Knigge, David Mack, Dale Kempf*, Dan Norbeck*, John Erickson*, Hepatitis/AIDS Research & *Anti-Infective Research, Abbott Laboratories Diagnostics & *Pharmaceutical Products Divisions, North Chicago, IL 60064.

HIV requires the activity of a virus-encoded aspartic protease for processing of p55*gag* and p160*gag-pol* polyproteins essential for assembly and maturation of infectious virions. Thus HIV protease is a good candidate for inhibition by antiviral drugs. Several classes of protease inhibitors were developed using computer-aided drug design techniques. The *in vitro* toxicity and anti-HIV activity of different classes of inhibitors were assessed in an acute infection using 100 ifu of 6 different strains of HIV in T-cell lines and primary PBL's. The 50% inhibitory concentration (IC50) of the compounds ranged between 8uM & 6nM, with comparable IC50's obtained for each inhibitor against all strains and in all cell lines tested. Verification of mechanism of action of the inhibitors was obtained by Western blot of chronically infected HIV/CEM treated with drug. Inhibition of p55 processing was seen as early as 12 hrs after drug was added, and occurred in a dose-dependent fashion. Minimal or no infectious virus was found after 7 days of drug treatment. Though virus levels did decrease, protease inhibitors did not totally eliminate HIV from chronically infected cells. However, they did cure chronically infected cells co-cultivated with uninfected cells. Studies addressing questions of reversibility and post-exposure treatment indicated long-lasting, potentially irreversible inhibition, as well as positive indications for use up to 3 days post-exposure. Thus these classes of protease inhibitors appear efficacious *in vitro* as potential drug candidates for use against HIV infection.

M 428 SIGNAL AMPLIFICATION BY MAJOR CYTOMEGALOVIRUS IMMEDIATE EARLY (CMV-IE) GENES OF HIV PROMOTER (LTR) TRANSACTIVATION. Carlos V Paya, Susan Michelson, Fernando Arenzana-Seisdedos, Jean L Virelizier. Institut Pasteur, 75724 Paris Cx 15. France.

HIV-LTR transactivation occurs following cell activation via PKC but not PKA-dependent pathways and by herpesvirus infection. We tested if CMV-IE could: 1) influence HIV-LTR transactivation by PKA-dependent stimuli, and 2) further amplify transactivation induced by PKC-dependent signals.

HIV-LTR linked to a luciferase reporter gene was transiently transfected in a CD4+ T cell lymphoblastoid line (Jurkat clone) with or without CMV-IE 1-2 genes. CMV-IE alone transactivated the HIV-LTR(10-fold). Stimulation of PKA by Forskolin or a cAMP analog (8bromo-cAMP) did not transactivate HIV-LTR, but did so in the presence of CMV-IE (20-fold). Stimulation of PKC-dependent pathways by TNF and PMA increased HIV-LTR activity 20 and 100-fold, respectively in the presence of CMV-IE compared to 3 and 4 fold in its absence. Similar results were observed upon stimulation by cross-linked CD3 and CD28 antibodies. CMV-IE mediated amplification was independent of the NFkb binding motif within the HIV LTR. Furthermore, all the above stimuli were shown to transactivate the CMV promoter and to increase CMV-IE gene transcription.

CMV-IE can mediate HIV-LTR transactivation by PKA-dependent stimuli and amplify PKC-dependent stimuli in T-lymphocytes.

M 429 ATTENUATION OF VACCINIA VIRUS;ANIMAL CHARACTERIZATION OF MUTANTS Lendon G. Payne, Moon Soo Lee, Michael Roos, Laura McGuigan, Kenneth A. Smith, Nancy Attardo-Cormier, Lawrence C. Cohen and Bryan E. Roberts. Applied BioTechnology, Inc., 80 Rogers St., Cambridge, MA 02142, USA.

Eight mutants were constructed in a plaque purified isolate of the NYCBH strain of vaccinia virus. Individual genes were altered by either deletion or insertion mutagenesis. Single gene mutations were generated in the hemagglutinin (HA-), thymidine kinase (TK-), small subunit of the ribonucleotide reductase (sRR-), Bam HI site in Hind III F (BamF-), host range (HR-) and both of the growth factor (GF-) genes. Two large deletion mutants, one 20 Kb to the left and one 12 Kb to the right of the host range gene, were generated through condensation reactions between vaccinia promoters ectopically inserted at the 3' end of the host range gene. A TK- deletion mutant in the WR strain was also constructed. All of the mutants were characterized in a mouse model with regard to intracranial and intranasal LD-50, replication in the brain, dissemination after a respiratory infection, growth and pock formation in the skin, immunogenicity and transmission to naive cage mates. The results from these experiments have provided unique insight into vaccinia pathogenesis and attenuation.

M 430 A SITE-SPECIFIC MUTATION IN THE EXTRACELLULAR DOMAIN OF HERPES SIMPLEX VIRUS 1 (HSV-1) GLYCOPROTEIN B (gB) AFFECTS ENTRY AND CELL-TO-CELL SPREAD. Lenore Pereira, David Navarro, Pedro Paz, and Ishtiaq Qadri. Division of Oral Biology, School of Dentistry, University of California San Francisco, San Francisco, CA 94143.
HSV-1 gB is an envelope glycoprotein that is required for infectivity and promotes virion penetration into cells. To map the functional domains of this molecule, we constructed a mutant virus by cotransfecting intact viral DNA with plasmid DNA containing a gB gene with a site-directed mutation at cysteine 382. We selected a recombinant, RVCys-5, that failed to react with several monoclonal antibodies having virus-neutralizing activity. Analysis of this mutant showed that RVCys-5 forms plaques at the permissive temperature (37ºC) but not at the nonpermissive temperature (39ºC). This defect was overcome by treating the mutant-infected cells with polyethylene glycol. Analysis of the viral proteins made at 39ºC in cells infected with a high multiplicity of RVCys-5 showed that synthesis of the major HSV-1 regulatory protein, α4, and the viral glycoproteins was comparable to that of the parental virus, with the exception of gB. Although the mutant gB formed stable dimers, they were partially glycosylated and remained sensitive to endoglycosidase H. Our results showed that mutant RVCys-5 specifies an underglycosylated form of gB with conformation-dependent changes and suggest that these altered properties affect entry and cell-to-cell spread of virus.

M 431 INTERFERON INHIBITS THE NF-κB-MEDIATED ACTIVATION OF HIV-1, **Paula M. Pitha and Waldemar Popik, The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD 21205**
We have been studying the effect of interferon (IFN) on the HSV-1-mediated activation of HIV transcription, using HeLa cell lines containing an integrated tat defective HIV (dt4) and a cell line which contains both tat defective HIV and a plasmid in which the expression of human IFN-α_2 is directed by HIV-LTR (α_1). In dt4 cells, HIV viral transcripts were detected as early as 2 hr post-induction. However, no HIV transcripts could be detected in α_1 cells infected with HSV-1; infection, however, led to IFN synthesis (50 to 100 U) in α_1 cells. Using isolated nuclei from dt4 and α_1 cells, we found that the IFN-induced inhibition occured at the transcriptional level. We have previously shown that IE175 and IE110 gene products of HSV-1 were essential for the activation of HIV-LTR. The detailed analysis of the IE175 and IE110 mRNAs and proteins in dt4 and α_1 cells showed only marginal inhibition of IE175 and IE110 proteins in the α_1 cells. We found, however, a difference in the binding pattern of nuclear proteins from dt4 and α_1 cells to the enhancer region of HIV-LTR. While the extracts from HSV-1-infected dt4 cells formed the NF-κB complex, the extracts from α_1 cells formed several complexes with different mobilities. Thus IFN, possibly through induction of new transcriptional factors, seems to interfere with the formation of a functional NF-κB complex.

M 432 **DELETION OF THE HSV-1 ICP22 GENE AFFECTS VIRAL HOST RANGE,** Kimber L. Poffenberger, Pat Raichlen and Ronald C. Herman, SYNTEX RESEARCH, 3401 Hillview Ave., Palo Alto, CA 94304.
The five immediate early (IE) proteins of HSV-1 are responsible for the progression to early and late gene expression during productive infection. Proteins ICP4 and ICP27 are essential; ICP0 synergizes with ICP4; ICP47 is nonessential in tissue culture. Little is known about the function of the fifth IE protein, ICP22. Previous studies, including one with a virus having a deletion encompassing part of both ICP22 and the adjacent Us2 ORF, as well as another using an antisense oligonucleotide to ICP22, suggest that viral growth is inhibited in certain cell types when ICP22 expression is affected. We describe here the construction and characterization of an HSV-1 mutant (del22Z) from which only the coding region of the ICP22 gene has been removed and replaced by the bacterial LacZ gene. This virus synthesized no detectable ICP22 transcript or protein product upon infection of tissue culture cells. The loss of ICP22 caused a decreased yield of progeny virus in all cell types tested, especially those of human origin. The normal cascade of HSV-1 gene expression was altered in del22Z-infected cells. There was prolonged expression of some early gene products and delayed appearance of some late gene products. Although the absence of ICP22 was partially overcome in some host cells, our results demonstrate that ICP22 enhanced virus production both *in vitro* and *in vivo*.

M 433 HERPES SIMPLEX VIRUS DNA SYNTHESIS AT A PREFORMED REPLICATION FORK IN VITRO, Samuel D. Rabkin, Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Preformed replication forks have been constructed in vitro consisting of double-stranded circular DNA with a 5' single-stranded tail that is not complementary to the template. Proteins involved in the assembly and movement of a replication fork should be able to assemble on this substrate and synthesize DNA via a rolling circle type mechanism.

Using this substrate we have reconstituted rolling circle DNA synthesis in vitro with proteins isolated from HSV-1 infected cells. The products of DNA synthesis have been analyzed by alkaline agarose gel electrophoresis and electron microscopy. The proteins from the nuclei of infected cells have been fractionated by column chromatography. Rolling circle DNA synthesis was dependent upon HSV DNA polymerase and ATP and was stimulated by ICP8 and proteins present in uninfected cells. A complex between the preformed fork substrate and infected cell proteins can be isolated by gel filtration. This in vitro DNA replication system should prove useful in the identification and characterization of the enzymatic activities required at the HSV replication fork.

M 434 CD4 POLIOVIRUS CHIMAERAS.

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Intertypic chimaeric polioviruses previously constructed were shown to possess composite antigenicity and dual immunogenicity[1]. This prompted the investigation of Sabin type 1 as a presentation vector for neutralizing epitopes of other pathogens including HIV-1 and human papilloma virus HPV16[2,3]. We have extended this work for the expression of defined epitopes of human CD4, the receptor for HIV. The ability of antisera to these chimaeras to block HIV infection and syncytia formation will be discussed. Those chimaeras containing CD4 residues which are believed to be involved in binding HIV are being assessed for their ability to bind gp120.

1. Burke et al. (1988) Nature 332, 81-82.
2. Evans et al. (1989) Nature 339, 385-388.
3. Jenkins et al. (1990) J. Virology 64, 1201-1206.

M 435 THE MAJOR HERPES SIMPLEX VIRUS TYPE-1 DNA-BINDING PROTEIN, ICP8, IS A ZINC METALLOPROTEIN, William T. Ruyechan and Sharmila S. Gupte, Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

The primary amino acid sequence of ICP8 deduced from the DNA sequence of the UL29 ORF contains a potential metal binding domain of the form Cys-X_{2-5}-Cys-X_{2-15}-A-X_{2-4}-A where A may be either histidine or cysteine and X is any amino acid. The putative metal-binding sequence in ICP8 encompasses residues 499-512 as follows: C-N-L-C-T-F-D-T-R-H-A-C-V-H.

Atomic absorption analysis of several preparations of ICP8 indicates the presence of one mole of zinc per mole of protein. The zinc is resistant to removal by dialysis against concentrations of EDTA which deplete zinc from alcohol dehydrogenase. The bound zinc can be removed by the action of the reversible sulfhydryl reagent p-hydroxymercurimethyl-sulphonate and the zinc depleted protein transiently retains DNA-binding activity. Digestion of both native and zinc-depleted ICP8 with V8 protease indicates that the bound zinc is required for the structural integrity of the protein. Site specific mutagenesis of relevant amino acids within the putative zinc finger is currently underway.

M 436 EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN-1 DNA INTERACTION: DISTORTION OF DNA BY SITE SPECIFIC BINDING. Waris A. Shah, Richard F. Ambinder, Diane S. Hayward and Gary S. Hayward, Department of Pharmacology and Molecular Sciences, The Johns Hopkins Medical School, Baltimore, MD 21205
Epstein-Barr virus DNA replication during latency requires a cis-acting Ori-P (origin of plasmid replication) and a trans-acting viral nuclear protein EBNA-1 of 641 amino acids encoded by the BamH1-K fragment. A bacterial expressed C-terminal fragment of EBNA-1 protein binds as a dimer to 24 high-affinity DNA binding sites in Ori-P and to two low-affinity sites in the BamH1-Q fragment in the EBV genome. Bal31 nuclease deletion analysis defined the minimal DNA-binding domain between amino acid 493 to amino acid 584. A potential DNA recognition motif from amino acid 513 to amino acid 533, and a protein dimerization function from amino acid 554 to amino acid 584 have been dissected out using site-directed mutagenesis. EBNA-1 binding to a consensus DNA binding site bends the DNA and induces structural changes in the protein that causes it to become resistent to proteinase-K digestion. These physical interactions of EBNA-1 with the DNA may play a direct role in DNA replication.

M 437 BIOSYNTHESIS AND STRUCTURE OF CHIMERIC HIV *Gag* PROTEINS CONTAINING AN AMINO-TERMINAL SIGNAL/ANCHOR PEPTIDE, Vicki H. Slone and Lawrence A. Hunt, Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, KY 40292.
The *gag* proteins of HIV-1 are synthesized as a cytoplasmic polyprotein that is membrane-anchored by an amino-terminal myristic acid and subsequently cleaved by a viral specific protease. To express HIV *gag* proteins as non-cytoplasmic (possibly, cell-surface) membrane-anchored antigens, we have constructed chimeric viral genes encoding the amino-terminal signal/anchor peptide of respiratory syncytial virus G (RSVG) followed by HIV *gag* polypeptides lacking the first 14 amino acids and the amino-terminal myristylation signal. Cell-free transcription/translation studies indicated that chimeric proteins of the expected size were produced. These proteins expressed antigenic determinants of the native HIV *gag*. Although the amino-terminal RSVG domain did function as a membrane anchor in the presence of canine pancreatic microsomal membranes, the single potential asparaginyl glycosylation site in an immunologically important domain of p17 was not utilized. Expression of a chimeric RSVG-HIV *gag-pol* fusion gene encoding HIV protease resulted in products consistent in size and antigenic determinants with HIV specific proteolytic cleavage. Mammalian cell expression studies are currently being conducted to determine if these chimeric genes can encode membrane-anchored, cell-surface immunogens.

M 438 ANALYSIS OF HERPES SIMPLEX mRNA DESTABILIZING ACTIVITY IN A CELL-FREE SYSTEM, Sorenson, C. M.[1], Hart, P. A.[2], Kwong, A. D.[3], and Ross, J.[1,4], McArdle Laboratory for Cancer Research, [2]School of Pharmacy, [4]Department of Pathology, University of Wisconsin, Madison, WI, [3]Memorial Sloan-Kettering Institute, New York, NY

The regulation of mRNA turnover plays an important role in mammalian gene expression, and mRNA decay rates can fluctuate in response to exogenous factors. For example, infection with herpes simplex virus type 1 (HSV-1) induces rapid degradation of host mRNAs. HSV-1 induced destabilizing activity is attributed to a virion component, the virion host shutoff (*vhs*) polypeptide. HSV-1 mutants, *vhs1* and *vhs* Δ Sma, are defective in virion-associated host shutoff function(s) and do not induce destabilization of cellular mRNAs during the early stages of infection (Kwong and Frenkel, 1987, PNAS, 84: 1926-1930; S. Read, personal communication).

We have used an *in vitro* mRNA decay system (Ross and Kobs, 1986, J. Mol. Biol., 188: 579-593) to analyze the mechanism by which HSV-1 destabilizes mRNAs. Polysomes from uninfected human erythroleukemia cells were used as a source of mRNA. These polysomes were mixed 1:1 in cell-free reactions with either polysomes or post-polysomal supernatant (S130) from HSV-1- or mock-infected murine erythroleukemia cells. Normally stable mRNAs such as γ-globin were degraded rapidly with extracts from infected cells. S130 from cells exposed for only 15 min to wild-type virus caused a four-fold decrease in γ-globin mRNA stability *in vitro*. In contrast, S130 from *vhs1*-, *vhs* ΔSma-, or mock-infected cells did not destabilize γ-globin mRNA. The stability of normally unstable mRNAs, such as histone and c-*myc*, was not accelerated by S130 from infected cells. No mRNA destabilizing activity was detected in polysomes from infected cells. Experiments are in progress to characterize how the *vhs* protein and any other required factors induce mRNA decay and to delineate the pathway of host mRNA decay following infection.

M 439 PRELIMINARY CHARACTERIZATION OF A NOVEL BASE SUBSTITUTION IDENTIFIED DURING RNA SEQUENCE ANALYSIS OF THE SABIN 3 POLIOVIRUS VACCINE STRAIN, Joanne M. Tatem, Carolyn Weeks-Levy, Willard Waterfield, Susan J. DiMichele, Steven J. Mento, Lederle-Praxis Biologicals, Pearl River, NY 10965

The authentic RNA sequence of Sabin 3 used in vaccine in the United States has been determined. The sequence of the Sabin 3 vaccine strain, called LED 3, is unique. A novel base discovered in LED 3 is contained in one of the viral capsid protein coding regions and predicts a new Sabin 3-specific amino acid change as compared to published sequences (Stanway et al., 1983; Toyoda et al., 1984). The reversion is observed to occur after replication of vaccine virus in the gut of primary vaccinees and after replication in nervous tissues of neurovirulence test monkeys. Passage conditions have been identified that lead to the reversion of the LED 3 specific base in the capsid region as well as the reversion of the attenuated base to the wild type base at 472. The observation that these two base positions are found to revert to wild type bases during passage suggests that there is a selective advantage for virus containing the wild type bases at these positions.

M 440 CONSERVATION OF GENE ORGANIZATION OF HUMAN HERPESVIRUS-6 (HHV-6) AND HUMAN CYTOMEGALOVIRUS AND IDENTIFICATION OF A CANDIDATE IMMEDIATE EARLY GENE LOCUS IN HHV-6. B.J. Thomson, M.E.D. Martin, J. Nicholas, S. Efstathiou[1] and R.W. Honess. Division of Virology, National Institute for Medical Research, London, NW7 1AA, and [1]Division of Virology, Cambridge University, Cambridge, CB2 2QH, UK.

HHV-6 is a recently isolated herpesvirus which is highly prevalent in adult populations. The virus is tropic for CD4 positive lymphocytes and persists in the peripheral blood of a majority of seropositive individuals. We have recently determined the restriction endonuclease map of a Ugandan isolate of HHV-6 (U1102). The genome consists of a largely unique sequence of 141 kb flanked by direct repeat units of 10 kb. This structure differs from that determined for the other human herpesviruses, including human cytomegalovirus (HCMV). Previous sequencing studies of HHV-6 U1102 have, however, demonstrated that both the arrangement of genes and the predicted amino acid sequence of their products closely resembles that of HCMV. We now present evidence, based on sequencing a further 34 kbp of selected regions of the genome, that the organization of coding and non-coding features of HHV-6 U1102 is collinear with that of the unique long component of HCMV. In particular, a single region of HHV-6 contains a local deficiency of CpG dinucleotides which identifies the major transactivating (immediate early, IE) genes in HCMV. The sequence of this region in HHV-6 contains a tandem array of repeats, each containing binding sites for cellular transcription factors NFkB and AP-2, which is likely to constitute the major IE enhancer. This region has been amplified using the polymerase chain reaction and the product shown to transactivate a range of heterologous promoters, including the HIV LTR.

M 441 DERIVATIVES OF PLANT POLYHYDROXYALKALOIDS: ANTIVIRAL ACTIVITY AND MODE OF ACTION, +A Stanley Tyms, +Debra L Taylor, +S Parvin Ahmed, Prasad S Sunkara, Mohinder S Kang, +MRC Collaborative Centre, London, Marion Merrell Dow Research Institute, Cincinnati, USA

The octahydroindolizine castanospermine (CAST) and the piperidine deoxynojirimycin (DNJ) isolated from tropical plants have been shown to have potent antiviral activity against HIV-1 and HIV-2. Both lead compounds have been derivatised with the aim of enhancing antiviral effects. We have investigated a series of acyl analogues of CAST, which represents the most potent inhibitor of α-glucosidase 1 of the glycoprotein processing enzyme (IC_{50} 0.1μM). Evidence for the importance of this enzyme as a target in infected cells was demonstrated by glycan analysis of virion glycoproteins which showed the presence of high glucose glycopeptides. 6-O-butanoyl CAST (BUCAST) completely inhibited HIV glycoprotein synthesis after acute infection and caused a major restriction in the cleavage of gp120 from gp160 in treated chronically infected cells. This α-glucosidase 1 inhibitor had potent effect on the growth of human cytomegalovirus which was also related to the reduced expression of a virion associated glycoprotein. The critical dependence of both viruses on α-glucosidase 1 for the correct expression of glycoproteins suggests that this enzyme is a favourable antiviral target.

M 442 SIGNAL AMPLIFICATION BY MAJOR CYTOMEGALOVIRUS IMMEDIATE EARLY (CMV-IE) GENES OF HIV PROMOTER (LTR) TRANSACTIVATION. Carlos V Paya, Susan Michelson, Fernando Arenzana-Seisdedos, Jean L Virelizier. Institut Pasteur, 75724 Paris Cx 15. France.

HIV-LTR transactivation occurs following cell activation via PKC but not PKA-dependent pathways and by herpesvirus infection. We tested if CMV-IE could: 1) influence HIV-LTR transactivation by PKA-dependent stimuli, and 2) further amplify transactivation induced by PKC-dependent signals.

HIV-LTR linked to a luciferase reporter gene was transiently transfected in a CD4+ T cell lymphoblastoid line (Jurkat clone) with or without CMV-IE 1-2 genes. CMV-IE alone transactivated the HIV-LTR(10-fold). Stimulation of PKA by Forskolin or a cAMP analog (8bromo-cAMP) did not transactivate HIV-LTR, but did so in the presence of CMV-IE (20-fold). Stimulation of PKC-dependent pathways by TNF and PMA increased HIV-LTR activity 20 and 100-fold, respectively in the presence of CMV-IE compared to 3 and 4 fold in its absence. Similar results were observed upon stimulation by cross-linked CD3 and CD28 antibodies. CMV-IE mediated amplification was independent of the NFkb binding motif within the HIV LTR. Furthermore, all the above stimuli were shown to transactivate the CMV promoter and to increase CMV-IE gene transcription.

CMV-IE can mediate HIV-LTR transactivation by PKA-dependent stimuli and amplify PKC-dependent stimuli in T-lymphocytes.

M 443 TRANSFORMING ACTIVITY OF DNA SEQUENCES OF HHV-6 (GD STRAIN) ISOLATED FROM A PATIENT WITH CHRONIC FATIGUE SYNDROME

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The oncogenic activity of Human Herpesvirus-6 (HHV-6) DNA (GS strain) in NIH 3T3 cells has been reported previously (Razzaque, Oncogene 5, 1365, 1990). In the current study, we confirm and extend this observation to include another strain, HHV-6 GD, which was isolated by Dr. J. Luka from peripheral blood lymphocytes of a patient with chronic fatigue syndrome. Restriction enzyme analysis and monoclonal antibody (to GS strain) reaction did not show any difference between GS and GD strains of HHV-6. Like GS strain, the genomic DNA from GD strain could also morphologically transform NIH 3T3 cells. Two focus-derived cell lines established from this DNA transfection produced tumors in nude mice when injected subcutaneously. The transforming activity of this strain was identical to that of HHV-6 GS strain. With polymerase chain reaction using primers corresponding to the major capsid protein gene of HHV-6, viral DNA sequences were detected in both the focus-derived and tumor-derived cell lines. Further analysis on the DNA sequences in these transformants and their role in transformation will be discussed.

M 444 THE EBV LATENT MEMBRANE PROTEIN (LMP1) MIMICS THE ACTION OF PHORBOL ESTER IN INDUCING HOMOTYPIC ADHESION MEDIATED BY CD11a/CD18 IN A MURINE B-CELL LINE:

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The effect of the EBV BNLF-1 gene product (latent membrane protein 1, LMP1) on homotypic adhesion of a murine B-cell, A/J-95 was compared to that of 12,13-phorbol dibutyrate (PDBu). Three independent clones of A/J-95 cells, expressing high levels of the LMP1 protein after retroviral transfer of the BNLF1 gene under the HSV-1 TK-gene promoter, displayed a 5-7-fold increase in homotypic adhesion compared to controls. The addition of PDBu to the parental A/J-95 cells resulted in a similar increase in homotypic adhesion. The increased adhesion of the cells could in both cases be inhibited almost completely with a monoclonal antibody to the CD11a subunit of the adhesion molecule CD11a/CD18 (Leu-CAMa, LFA-1) but not with a monoclonal antibody to CD54 (ICAM-1). The high level constitutive cell surface expression of CD11a, CD18 and CD54 was not significantly altered in cells expressing LMP-1, suggesting that the increased homotypic adhesion observed was caused by functional activation rather than by a quantitative increase in the adhesion molecules.

The present experiments validate previous observations of LMP1-mediated aggregation of human B-cells in a murine system, demonstrate that LMP1, like phorbol esters, activate the CD11a/CD18 dependent homotypic adhesion between B-cells to a comparable degree, and suggest that LMP1, like phorbol esters, participates in intracellular signalling pathways to accomplish this end.

M 445 DIFFERENTIAL ANTIVIRAL EFFECTS OF DEXTRAN SULFATE MOLECULAR WEIGHT 1,000 AS COMPARED TO DEXTRAN SULFATE SAMPLES OF HIGHER MOLECULAR WEIGHT, M. Witvrouw, D. Schols, G. Andrei, R. Snoeck, R. Pauwels, J. Balzarini and E. De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Dextran sulfates [prepared from dextran fractions with molecular weights (m.w.) ranging from 1,000 to 500,000] are potent inhibitors of enveloped (i.e. retro-, herpes-, toga-, arena-, and rhabdo-) viruses. They are not active against non-enveloped viruses such as polio, Coxsackie or reovirus. Within the m.w. range of 3,000 to 500,000, no much variation is observed in the antiviral effects of the different dextran sulfate samples. However, the 1,000 m.w. sample behaves quite differently. In contrast with the higher m.w. samples, the 1,000 m.w. sample is virtually inactive against herpes simplex virus, vesicular stomatitis virus and togaviruses (Sindbis, Semliki Forest). Yet, it is almost as active as the higher m.w. samples against cytomegalovirus and arenaviruses (Junin, Tacaribe). Most remarkable are the differences in the inhibitory effects of the 1,000 m.w. sample on different human immunodeficiency virus (HIV) strains: against HTLV-III_B, HTLV-III_{RF} and HIV-2_{EHO} it is less active, but against HIV-1_{HE} and LAV-2_{ROD} it is more active than the higher m.w. samples, whether based on inhibition of viral cytopathicity or syncytium formation.

Late Abstract

A RECOMBINANT VACCINIA VIRUS (rvv) EXPRESSING HIV-gp120-VESICULAR STOMATITIS VIRUS (VSV) GLYCOPROTEIN (G) TRANSMEMBRANE (TM) AND CYTOPLASMIC TAIL (CT) REGIONS INDUCES SUPERIOR IMMUNE RESPONSE COMPARED TO rvv EXPRESSED gp160

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The transmembrane region of retoviruses is immunosuppressive. In order to produce a vaccine against HIV-1, with a superior immune response, the transmembrane region of HIV-1 was replaced with that of the VSV G TM and CT regions. This rvv, vvE13 was compared to a rvv expressing gp160,vvE1. The level of expression of gp120-VSV G TM CT was lower than that of gp160 by immunoblot analysis and immunoprecipitation with anti gp160 sera for both proteins and anti VSV G CT for gp120-VSV G TM CT and anti gp41 sera for gp160. vvE13 did not induce syncytia, whereas vvE1 did induce syncytia in CD4+ cells. Both rvvs produced a cell surface protein recognized by antibody to gp120. To test the immune response of the two rvvs mice were immunized. Antibody response and lympocyte proliferation were superior with vvE13 as compared to vvE1. These results indicate that replacement of the HIV-1 transmembrane region gp41, by the VSV G TM CT regions can improve the immunogenicity of HIV-1 rvvs, which may be of importance in vaccine development.