HUMAN RETROVIRUSES

Organizers: Jerome E. Groopman, Irvin Chen, Myron Essex and Robin Weiss
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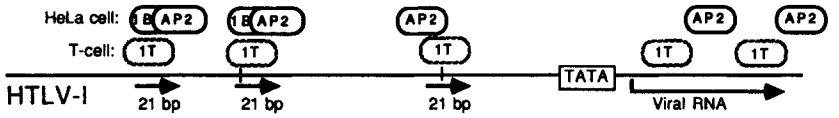
Discussion: HTLV Molecular Biology

G 001 MULTIPLE SEQUENCE MOTIFS IN THE HTLV-I LTR ARE TRANS-ACTIVATED BY HTLV-I TAX_1.
John Brady, Imre Boros, Susan Marriott, Janet Duvall, Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Our laboratory has identified several DNA sequences within the HTLV-I LTR that can be trans-activated by tax_1. Oligomers of the HTLV-I 21 bp repeats cloned upstream of either a homologous or heterologous promoter induce transcription up to 50-fold in the presence of tax_1. In the natural setting of the LTR, however, the 21 bp sequences are not arranged in tandem repeats, but are separated by either 30 or 80 nucleotides. We have determined by cotransfection assays that these spacer DNA sequences are in fact important for tax_1 trans-activation. First, the insertion of non-specific DNA sequences between a tandem copy of the HTLV-I 21 bp repeats results in a decrease in transcriptional activity. Secondly, in contrast to the results obtained with non-specific DNA, the insertion of sequences between -117 to -160 and -197 to -232 increase transcription in the presence of tax_1 by 10- to 25-fold. Using a DNA gel-shift analysis, we have observed specific protein interactions with these tax_1 responsive sequences. Interestingly, though there is limited sequence homology between the two DNA fragments, in vitro competition analysis suggests that the same protein(s) interacts with both regulatory sequences. The importance of these sequences and DNA-protein interactions in context of tax_1 trans-activation of the HTLV-I LTR will be discussed.

G 002 ISOLATION AND CHARACTERIZATION OF CELLULAR PROTEINS THAT BIND TO THE HTLV-I AND HTLV-II TRANSCRIPTIONAL CONTROL REGIONS.
Jennifer K. Nyborg, William T. Golde, and William S. Dynan, Dept. of Chem. and Biochem., Campus Box 215, University of Colorado, Boulder, CO 80309.
Efficient transcription of HTLV-I RNA requires specific DNA sequences within the proviral LTR, including three imperfect 21-bp repeats. We have previously shown that cellular proteins bind to these repeats (1). We have now used a combination of techniques to demonstrate that the repeats are recognized by three distinct proteins, two of which appear to be cell-type specific. HeLa cell extracts contain both the transcription factor AP-2, which binds to sites adjacent to and overlapping each repeat, and a second protein, HEF-1B, which binds cooperatively with AP-2 to sequences within the repeats. Transformed T-cell extracts do not contain AP-2 activity, and although HEF-1B is present, most of the binding to the 21-bp repeats is attributable to a third activity, HEF-1T. The HEF-1T protein is readily distinguished from the others by its position of elution in ion exchange chromatography and by its relative affinity for different binding sites.

HEF-1T has been purified to near-homogeneity by DNA-affinity chromatography. The purified protein binds to a total of five sites in the HTLV-I LTR, including the three 21-bp repeats and two additional sites located downstream of the initiation site for HTLV-I RNA synthesis (see diagram). It also binds to the three 21-bp repeat sequences in the LTR of the related virus, HTLV-II. In contrast, binding was not detected to the LTR of HIV-1. The occurrence of HEF-1T in the type of cell normally infected by HTLV-I and HTLV-II, the presence of at least eight sites in the two viruses, and the association of sites with known transcriptional control elements in the 21-bp repeats strongly suggest a role for HEF-1T in viral RNA synthesis. Further characterization of the functional properties of HEF-1T is in progress.



(1) Nyborg, J. K., Dynan, W. S., Chen, I.S.Y., and Wachsman, W. Proc. Natl. Acad. Sci 85:1457-1461 (1988).

G 003 REGULATORY MECHANISM OF HTLV-II GENE EXPRESSION,Kunitada Shimotohno,Masataka Ohta and Tsuyoshi Akagi,Virology Division, National Cancer Center Research Institute,Tokyo 104,Japan. Human T-cell leukemia viruses have common features of having regulatory genes, called tax and rex, between env and 3'long terminal repeat (LTR). In case of HTLV-II, three protein, p38 for tax_{tax}^{tax}, p26 and/or p24 for rex proteins, are expressed from this region. $p38^{tax}$ is responsible for the gene specific enhancement of viral RNA transcription from LTR and p26 and/or p24 regulate the primarly transcript at post transcriptional manner.

To further clarify the function of rex proteins, the plasmid in which 5'half of the viral DNA including 1st splice donor and acceptor site was linked to the downstream of cytomegalovirus promoter (CMV) was constructed and the level of primary and spliced transcripts from the plasmid in presence or absence of rex protein was measured. Although tax and rex gene products do not affect the efficiency of transcription for CMV promoter, the total level of the viral RNAs (unspliced plus spliced form) present in cells which express rex protein was more than four times higher than the transcripts in cells not expressing rex protein. However, amounts of spliced form of the transcript was decreased in cells expressing rex. This suggests that rex proteins control the level of unspliced and spliced RNAs by a concerted manner. A cis-regulatory element for rex protein in the transcript was determined. This sequence is present in downstream from the first splice donor site of the viral genome RNA.

To know the function of tax and/or rex to cellular gene expression in HTLV infected cells, retroviruses which express tax and/or rex was constructed. These viruses were efficiently infected to various human cells as well as murine lymphoid cells.

Epidemiology and Clinical Aspects of HTLV

G 004 EFFECT OF IMMUNIZATION ON HTLV-I INFECTION IN RABBITS, I. Miyoshi, S. Kotani, N. Takehara, Y. Iwahara, and Y. Ohtsuki, Departments of Medicine and Pathology, Kochi Medical School, Kochi 781-51, Japan

We have established a rabbit model of HTLV-I infection in which the virus was transmitted by blood transfusion and from mother to offspring. Foster-nursing experiments provided evidence for milk-borne transmission of HTLV-I. The present experiments were undertaken to investigate whether active or passive immunization will confer protection against challenge infection of HTLV-I in rabbits. Six rabbits were inoculated intravenously three times with 2-4 x 10^7 Ra-1 cells at two-week intervals. Ra-1 is an HTLV-I-producing rabbit lymphoid cell line transformed by coculture with MT-2 cells. The cells were heat-inactivated at 56°C for 30 min prior to inoculation. This heat-treatment was shown to be lethal to HTLV-I and HTLV-I-producing cells. All immunized animals seroconverted for HTLV-I with antibody titers of 1:160 to 1:640 as tested by immunofluorescence. Radioimmunoprecipitation using ^{3}H-leucine-labeled MT-2 cell lysate showed the presence of serum antibodies to most major HTLV-I proteins including gp68 and gp46. At two weeks after the last immunization, the rabbits were challenged with transfusion of 20 ml of blood from HTLV-I-infected rabbits. Virus infection in the immunized rabbits was assayed by the following two methods. First, peripheral lymphocytes were collected before and after blood transfusion and cultured in the presence of human recombinant IL-2. Only post-transfusion cultures from all six rabbits yielded lymphoid cell lines positive for HTLV-I antigens and virus particles. Second, six normal rabbits were each transfused with 20 ml of blood from the immunized rabbits three months after challenge infection. All normal recipients became seropositive for HTLV-I 2-4 weeks after blood transfusion. Furthermore, two groups of rabbits were given synthetic HTLV-I env peptide or partially purified rabbit anti-HTLV-I IgG and then similarly challenged with HTLV-I infection by blood transfusion. If confirmed by these experiments, our findings suggest that vaccines will not be protective against HTLV-I infection.

HIV Genome: Structure and Function

G 005 PACKAGING SIGNALS FOR HIV-1, Andrew Lever, Heinrich Gottlinger, William Haseltine, Joseph Sodroski, Dana-Farber Cancer Institute, 44 Binney Street, Boston MA USA

The exact site of packaging signals in the HIV genome is as yet unknown although, analogous to other retroviruses, it is supposed that the area 3' to the splice donor and 5' to the gag ATG is a likely candidate. We have constructed mutations in this region and produced viruses which are highly replication defective yet produce abundant viral protein. This replication defect appears to be cis-acting and we have investigated the ability of the mutants to produce virions which contain no RNA and their ability to package other defective viral genomes containing marker genes. These results will be discussed.

G 006 **REGULATION OF HIV-1 GENE EXPRESSION BY *tat* AND *rev*.**
George N. Pavlakis, Barbara K. Felber, Connie M. Wright-Drysdale & A. Athanassopoulos, BRI-Basic Research Program, NCI-FCRF, Frederick, MD 21701

Two viral regulatory proteins of HIV-1, tat and rev, are necessary for viral expression in human cells. These proteins interact directly or indirectly with unique cis-acting elements existing within the viral nucleic acid. tat interacts with the tat-responsive element (TAR) which is localized in the R region of the LTR. rev interacts with the rev-responsive element (RRE) localized within the env region. tat increases the steady-state levels of all viral mRNAs. rev increases the levels of the unspliced viral mRNAs which contain RRE at the expense of the multiply spliced mRNAs not containing RRE. rev increases the stability and affects the sequestration of the RRE containing mRNAs. rev affects the stability of the RRE containing mRNAs independent of splicing, demonstrating that splicing is not necessary for rev function.

We have proposed that rev acts in the nucleus before the primary transcript is spliced. The expression of tat and rev after infection of human cells results in the establishment of a steady state and the production of low levels of the regulatory proteins tat, rev, and possibly nef, and the production of high levels of structural proteins and infectious virions. Feed-back regulation by the rev protein on the levels of the mRNAs producing tat, rev and nef is an essential regulatory circuit of HIV-1 allowing the balanced expression of the viral proteins and the generation of infectious virus. Intracellular and extracellular conditions disturbing this regulatory balance may have profound effects on the outcome of viral infection.

G 007 TRANSCRIPTIONAL REGULATION OF HIV, B. Matija Peterlin, Shaw-Yi Kao, Paul A. Luciw*, Mark J. Selby, and Sandra E. Tong-Starksen, Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, CA 94143 and *Department of Medical Pathology, University of California, Davis, CA 95616

Activated and proliferating T cells are required for optimal HIV replication and gene expression. Signals emanating from Ti/CD3 and accessory molecules (CD2, CD4, CD5, CD28) result in increases in intracellular free Ca^{++} and translocation of protein kinase C. Binding of nuclear factors of activated T cells (NFAT-1) and immunoglobulin κ B (NFκB) to the U3 region of the HIV-1 LTR result. NFAT-1 and NFκB act synergistically to increase transcription from the 5' LTR. Their effects can be blocked by cyclosporin A and H7, respectively. In B cells, which constitutively express NFκB, high levels of HIV-1 gene expression and viral replication are seen. *Trans*-acting factors from EBV and HBV also affect HIV-1 LTR gene expression. The effects of T cell activation signals and HIV encoded *trans*-activator (*tat*) are multiplicative. *Tat*, which is required for HIV induced cytopathology, acts on sequences from positions -17 to +80 in the HIV-1 LTR called TAR (*trans*-acting responsive region). *Tat* affects transcriptional elongation through the HIV-1 LTR, as evidenced by nuclear run-on experiments and the recovery of prematurely terminated and full-length transcripts in the cytoplasm of transfected cells in the absence and presence of *tat*, respectively. Using clustered and compensatory mutations in TAR, the minimal *cis*-acting sequences and the stem-loop in TAR required for *trans*-activation by *tat* were defined. Similar data were generated with the HIV-2 LTR.

PNAS 83: 9734-9738 (1986)
PNAS 84: 6845-6849 (1987)
Nature 330: 489-493 (1987)
AIDS 2: 185-193 (1988)

G 008 PROGRESSIVE GENETIC CHANGES IN HIV-1 GENES COINCIDENT WITH DISEASE PROGRESSION, Andreas Meyerhans (1), Rémi Cheynier (1), William Saurin (2), Shirley Kwok (3), John Sninsky (3), Jan Alberts (4), Birgitta Asjo (4) and Simon Wain-Hobson (1), Laboratoire de Biologie et Immunologie Moléculaires des Rétrovirus (1) and Unité de Programmation Moléculaire et Toxicologie Génétique (2), Institut Pasteur, Paris ; Cetus Corporation, Emeryville, CA (3) ; Dept. of Virology, Karolinska Institute, Stockholm (4).

We have analysed the HIV-1 proviral population of four virus isolated taken from the same individual as he progressed towards AIDS (healthy carriers - PGL - ARC - AIDS). Tat gene sequences were amplified by PCR and cloned into an expression vector. Twenty plasmid clones of each isolate were sequenced. The data show that there is a significant shift in the viral populations with the course of infection. The population of clones increased in complexity as the disease progressed.
Functional studies complementing the structural analysis will allow us to interpret better these data.

Discussion: HIV Genome

G 009 TRANS-REGULATION OF HIV-1 GENE EXPRESSION, Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center., Durham, NC 27710.

The HIV-1 genome encodes two trans-acting gene non-structural products, termed tat and rev, which are required for viral replication in vitro. The tat gene product greatly enhances the expression of sequences linked to the HIV-1 LTR and acts, at least in part, by increasing the rate of HIV-1 LTR specific transcription. This effect is mediated by an LTR sequence, termed the trans-activation response (TAR) element, which is located predominantly 3' to the transcription start site. The TAR element overlaps the DNA binding site for a constitutively expressed cellular protein, however the role of this factor in transcriptional activation by the nuclear tat protein currently remains uncertain.

The rev protein is also predominantly located in the nucleus of expressing cells and appears to act post-transcriptionally to regulate the expression of the viral structural gene products. The unspliced or singly spliced transcripts which encode the viral gag and env structural proteins are observed at only very low levels in the cytoplasm of cells transfected with rev defective proviruses, while the doubly spliced mRNAs which encode the viral non-structural proteins are present at elevated levels. In contrast, the pattern of spliced versus unspliced viral mRNA in the cell nucleus appears unaffected by rev co-expression. This suggests a role for rev in the regulation of either the splicing or the nuclear export of viral transcripts. Of particular interest is the observation that expression of rev can result in a shift in the expression of the viral tat protein from a full length form, encoded by a fully spliced tat mRNA, to a truncated protein encoded by a singly spliced form of tat mRNA. This observation suggests the intriguing possibility that the regulation of HIV-1 gene expression may involve a degree of communication between these two viral trans-activators to establish the optimum level and quality of viral protein synthesis.

Immunopathogenesis of HIV

G 010 IMMUNOPATHOGENIC MECHANISMS OF HIV INFECTION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

The central pathogenic event in HIV infection is the high affinity binding of the HIV envelope glycoprotein (gp120) to the CD4 molecule that is present on the surface of T4 helper/inducer lymphocytes and monocyte/macrophages. HIV infection in vivo is characterized by a massive depletion of T4 cells resulting in immunosuppression and opportunistic diseases. In vitro, T4 cells are killed following exposure to HIV. Several mechanisms for a direct cytopathic effect of HIV on T4 cells include: a massive increase in the permeability of the cell membrane as a result of the budding of many virus particles from the surface of the cell; accumulation of unintegrated viral DNA in the cell cytoplasm; and intracellular complexing of CD4 molecules and HIV gp120. Potential indirect mechanisms of cell killing include: syncytia formation between HIV infected and uninfected T4 cells; immune-mediated destruction of uninfected T4 cells that have either processed and presented gp120 or have bound gp120 on their surface; and the development of cross-reacting antibodies to gp120 and the major histocompatibility complex. In addition to HIV-induced cytopathic effects, we have shown that HIV can cause functional impairments of antigen-responsive T4 cells. Recently, we have also shown that cells of the monocyte/macrophage lineage can be infected with HIV and appear to be resistant to the cytopathic effects of HIV. Viral replication in monocyte lineage cells occurs predominantly intracellularly into cytoplasmic vacuoles. In addition, we have demonstrated that purified progenitor bone marrow cells can be infected with HIV. As seen in monocyte/macrophages, HIV-infected bone marrow cells are resistant to HIV-induced cell killing and support intracellular virus replication. Thus, both bone marrow cells and monocyte/macrophages can serve as reservoirs of virus infection in the body. Following infection with HIV, a small fraction of cells survive which harbor the virus in a latent form. We have shown that HIV can be induced from latently or chronically infected cells following exposure to mitogens, antigens, or transfected heterologous viral DNAs. To further characterize the mechanisms of conversion from a latent or chronic infection to active virus expression, we have developed an in vitro model system of chronically HIV-infected promonocytes and T cells. We have shown that cytokines, including tumor necrosis factor alpha, can upregulate the expression of HIV in these chronically infected cell lines and that induction of HIV expression occurs via a transactivating mechanism on the HIV promotor.

G 011 BIOLOGIC HETEROGENEITY OF HIV AND ITS RELATIONSHIP TO PATHOGENESIS

J. A. Levy, C. Cheng-Mayer, M. Tateno, L. A. Evans and J. Homsy, Cancer Research Institute and Department of Medicine, University of California, School of Medicine, San Francisco, CA 94143

The human immunodeficiency viruses HIV-1 and HIV-2 are associated with a wide spectrum of clinical presentations ranging from Pneumocystis carinii pneumonia to asymptomatic infection. These differences in pathologic expression may be related to particular HIV strains. In examining several HIV isolated from individuals with varied clinical symptoms, biologic and serologic heterogeneity among them was recognized. Differences were noted in cell tropism, kinetics of virus replication, degree of cytopathology, effect on CD4 antigen expression and the sensitivity of the viruses to neutralization and/or enhancement by serum antibodies. These studies led to the characterization of variants recovered from the brain that had features different from those recovered from the blood. In particular, the brain isolates were not very cytopathic, less sensitive to serum neutralization, did not grow well in established T cell lines but grew very well in peripheral blood macrophages. In addition, the brain isolates did not modulate substantially the amount of CD4 antigen on the surface of infected CD4+ lymphocytes. Similar studies identified changes in a particular HIV isolate over time in the same individual. Viruses isolated at the time that the disease has progressed had characteristics of increased "virulence". These viruses had a wide cellular host range, replicated rapidly in infected cells, produced extensive cytopathic effects and killed cells efficiently. These HIV-induced plaques in MT4 cells and also could infect human fibroblasts. Thus, progression of disease appeared to correlate with the emergence of a more virulent strain of HIV.

Using serologic studies, HIV could be placed into potential serotypes that reflected their sensitivity to neutralization (high or low). Moreover, some viruses were found to be neutralized by some sera, while others were enhanced in their infection of lymphocytes and macrophages. In some cases, a virus was neutralized or enhanced depending on the serum used. Thus, pathogenesis reflects the interaction of particular HIV with the host response. The virus can become more pathogenic if the host cannot suppress virus replication. Moreover, the production of enhancing antibodies by the infected individual might increase disease progression. These parameters of HIV infection need to be further pursued in antiviral approaches.

G 012 HIV PATHOGENESIS IN PATIENTS WITH HEMOPHILIA, John L. Sullivan, Departments of Pediatrics, Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655

Over the past five years we have prospectively studied HIV pathogenesis in a cohort of 164 individuals with hemophilia. While the majority (136 individuals) of HIV seropositive hemophiliacs were infected during or prior to 1983, 24 individuals seroconverted between 1983 and 1984 and 28 individuals remain seronegative. HIV has been isolated from approximately 30% of infected individuals and 18% have had detectable (>30 pg/ml) p24 core antigen in serum. Absolute numbers of CD4 cells have progressively declined ($<900/mm^3$) in 84% of those infected while 16% have stable absolute CD4 cells ($\geq 900/mm^3$). Over the first five years of observation 16 individuals (12%) have developed AIDS or ARC. Studies of HIV specific immune responses have shown 88% of HIV seropositive individuals to have detectable circulating cytotoxic T cells directed against HIV gag and envelope proteins. In addition HIV seroconversion is rapidly followed by the appearance of ADCC antibodies (titers ranging from 1:1000 to 1:1,000,000) directed against only HIV envelope proteins. Neutralizing antibodies are slow to appear and persist in extremely low titer (ranging from <1:5 to 1:80). These results suggest that persistence of active CTL and ADCC responses in the absence of a strong neutralizing antibody response may result in progressive immune attrition.

Virus-Cell Interactions

G 014 RECOMBINANT CD4-PSEUDOMONAS EXOTOXIN HYBRID PROTEIN SELECTIVELY KILLS HIV-INFECTED CELLS, Edward A. Berger*, Vijay K. Chaudhary**, Tamio Mizukami*, Thomas R. Fuerst*, David J. FitzGerald**, Bernard Moss*, & Ira Pastan**, *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, & **Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
A potential approach to AIDS therapy involves the development of cytotoxic agents targeted to selectively kill cells infected with HIV. We have constructed chimeric genes encoding novel proteins with the gp120-binding regions of human CD4 linked to active portions of Pseudomonas exotoxin A. One such recombinant protein, designated CD4(178)-PE40, has been expressed in large quantities in E. coli and purified to near homogeneity. The protein displays ADP-ribosylation activity and specifically binds to both surface-localized and soluble gp120. It is highly cytotoxic for human T-cell lines chronically infected with HIV, but is inactive against the uninfected parental cells. Control experiments indicate that this specificity results from the presence of the CD4 moiety on the toxin and the expression of the HIV envelope on the susceptible cells. The hybrid toxin is active against cells expressing envelope proteins from divergent HIV isolates and from SIV. No significant cytotoxicity is observed with cells expressing high surface levels of MHC Class II antigens. CD4-Pseudomonas exotoxin hybrid proteins are thus promising new agents for AIDS therapy.

G 015 DESIGNING SOLUBLE CD4 ANALOGS FOR AIDS THERAPY

Daniel J. Capon, [1]Steven M. Chamow, [2]Joyce Mordenti, Scot A. Marsters, [1]Timothy Gregory, [3]Hiroaki Mitsuya, [4]Randal A. Byrn, [5]Catherine Lucas, [6]Florian M. Wurm, [4]Jerome E. Groopman, [3]Samuel Broder and Douglas H. Smith.

Departments of Molecular Biology, [1]Recovery Process Research and Development, [2]Pharmacological Sciences, [5]Medicinal and Analytical Chemistry and [6]Cell Culture Research and Development, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA, 94080. [3]The Clinical Oncology Program, National Cancer Insitute, National Insitutes of Health, Bethesda, MD, 20892. [4]Division of Hematology-Oncology, Harvard Medical School, New England Deaconess Hospital, Boston, MA, 02215.

We have used parts of the sequence of CD4, the cellular receptor for HIV, to construct a novel type of antibody-like molecule, termed immunoadhesin. In these molecules the antigen-binding region (Fab) of antibody has been replaced by the gp120-binding domain of CD4. Our immunoadhesins bind gp120 and block HIV-1 infection of T cells and monocytes in a manner indistinguishable from soluble rCD4. They also possess some properties of antibodies, including long plasma half-life and high-affinity binding to Fc receptors. The improved plasma half-life should allow increased plasma levels, blocking virus more effectively than rCD4, and Fc receptor binding may recruit mechanisms for the elimination of virus and virally-infected cells. These constructs offer hope for an active defense against HIV-1.

G 016 INHIBITION OF THE HIV/SIV VIRUSES BY SOLUBLE DERIVATIVES OF T4.

R.Sweet, J. Arthos[+], K. Deen, J. Fornwald, M. Chaikin, A. Truneh, J.S. McDougal[#], Q. Sattentau^, P. Clapham^, R. Weiss^, P. Maddon* and R. Axel*. Smith Kline & French Labs, King of Prussia, Pa. 19406, [+]Univ. of Penn., Phila., Pa. 19002, [#]Centers for Disease Control, Atlanta, Ga. 30333 ^MRC- London, UK and *Columbia Univ., NY, NY 10032. CD4 is a non-polymorphic protein on the surface of T-lymphocytes which plays a role in antigen-dependent T-cell activation. The human CD4 antigen, T4, has been subverted to serve as a receptor for the HIV viruses through recognition of the viral envelope protein, gp120. We have described a recombinant, soluble form of T4, consisting of only the external domain of the native receptor, and demonstrated that this protein, sT4, efficiently inhibits HIV infection and virus-induced cell fusion in vitro. sT4 is now in development as a potential therapeutic for AIDS.

We have exploited the recognition of virus by sT4 to map the gp120 binding site on T4. The external domain of T4 is composed of four tandem subdomains which share homology with immunoglobulin light chain variable regions (V_1 - V_4). Soluble derivatives of sT4 containing only the amino-terminal V1 or V1+V2 domains were expressed in E. coli and mammalian cells. In quantitative assays, the V1 protein was shown to retain high affinity binding to gp120 and to inhibit virus infection and virus-mediated cell fusion. To fine map the binding site, divergent amino acids between murine and human CD4 were mutated to the sequence in the murine receptor, which does not recognize HIV, and were expressed in soluble CD4 proteins. Critical residues were located within the region 41-55, a region which is particulary divergent between human and mouse. Mutations which disrupted binding also abrogated the inhibition of viral infection and cell fusion. In the context of sequence and structural homology, the gp120 recognition site encompasses the CDR 2 region in the variable domain of immunoglobulin light chains.

Vaccine Strategies

G 017 ANALYSIS OF THE PRINCIPAL HIV NEUTRALIZING EPITOPE , Scott D. Putney[1], Kashi Javaherian[1], James R. Rusche[1], Albert T. Profy[1], Kent J. Weinhold[2], Alphonse J. Langlois[2], Thomas J. Matthews[2] and Dani Bolognesi[2], [1]Repligen Corporation, One Kendall Square, Building 700, Cambridge, MA 02139 and [2]Department of Surgery, Duke University Medical School, Durham, NC 27710. The principal HIV neutralizing epitope is located in C-terminal half of gp120 and is flanked by cysteines which are joined by disulfide cross bridges. This epitope is a loop structure on the surface of virus infected cells. Using peptide immunogens consisting of approximately 12 amino acids from the tip of this loop structure, antibodies were elicited that neutralize the corresponding virus isolate and prevent fusion of virus infected cells. In addition, these peptides were able to bind all neutalizing and fusion inhibiting antibodies elicited by larger envelope immunogens. These data localize the principal neutralizing epitope to this part of gp120, and a large majority of HIV-1 isolates have a conserved gly-pro-gly-arg amino acid sequences as part of this epitope. We have constructed peptides containing this domain from several HIV-1 isolates, and these peptides elicit antisera that neutralize multiple isolates.

In addition to being a target of neutralizing antibody, this epitope serves as a T-cell target. We have generated CD4+ T-cell clones from seronegative donors that respond to peptides containing this sequence. These are currently being assayed for cytolytic activity.

G 018 *ANTIBODY HETEROCONJUGATES AND ANTIBODY-ANTIVIRAL CONJUGATES REACTIVE WITH HIV-1 INFECTED CELLS*, Joyce M. Zarling[1], Patricia A. Moran[1], Joan Sias[1], Jan McClure[1], Jeffrey A. Ledbetter[1], and Fatih Uckun[2], [1]Oncogen, Seattle, WA 98121; [2]Department of Therapeutic Radiology, University of Minnesota, Minneapolis, MN. 55455

We have carried out studies with monoclonal antibody (Mab) heteroconjugates to focus PBL to kill HIV-1 infected cells and studies with Mab conjugated to pokeweed antiviral protein (PAP) to inhibit HIV-1 replication. A Mab (110.4), directed against HIV-1 gp120, was conjugated to an Mab against CD3 on T cells or with an Mab against the CD16 Fc receptor on large granular lymphocytes (LGL). PBL from both HIV seronegative and seropositive humans lysed HIV infected CEM cells in the presence of either the CD3X 110.4 or the CD16X 110.4 heteroconjugate but not in the presence of non-conjugated antibodies. The CD3X 110.4 and the CD16X 110.4 heteroconjugates focus $CD8^+$ T cells and LGL, respectively, to kill HIV infected CEM cells. When asking whether these Mab heteroconjugates could also focus PBL to lyse autologous or allogeneic HIV infected $CD4^+$ blasts, we found, surprisingly, that PBL from most HIV-1 infected, but not uninfected, homosexuals, have PBL lytic for $CD4^+$ T cell blasts, including those from uninfected donors. Lysis of the $CD4^+$ T cells blasts is blocked by Mab to CD3 and is diminished by eliminating $CD8^+$ PBL, thus indicating that lysis is mediated by cytotoxic T cells (CTL). CTL from HIV-1 infected humans also lysed chimpanzee T cell blasts but not human B cell lines or rat or mouse cells. In some experiments, $CD8^+$ cells from some HIV infected humans also lysed autologous $CD4^+$ blasts not expressing HIV antigens. In contrast, PBL from HIV-1 infected chimpanzees, which do not develop disease, did not lyse autologous or allogeneic chimpanzee or human $CD4^+$ T cell blasts. Thus, $CD4^+$ cell depletion which occurs in humans may, in part, be due to CTL lytic for $CD4^+$ cells. Since monocytes serve as reservoirs for HIV infection, we asked whether inhibition of HIV replication in a monocyte line (U937) would occur in cells treated with an Mab-antiviral conjugate consisting of an Mab to CD14 expressed on monocytes, conjugated to pokeweed antiviral protein (PAP), previously shown to inhibit replication of other viruses. Treatment of U937 cells with concentrations as low as .005 μg/ml of the CD16X PAP conjugate inhibited production of HIV, whereas concentrations of more than 10 μg/ml of nonconjugated PAP were required to inhibit HIV replication. Thus, conjugates consisting of Mab to cell surface antigens or to HIV antigens expressed on the cell surface conjugated to anti-viral agents may be of therapeutic value in HIV infections.

Animal Retrovirus Models

G 019 EVALUATION OF PROTOTYPE HIV VACCINES IN CHIMPANZERS, Larry O. Arthur,[1] Stephen W. Pyle,[1] Bror Morein,[2] Julian W. Bess Jr.,[1] Peter L. Nara,[3] and Peter J. Fischinger.[3] [1]Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility (NCI-FCRF), Frederick, MD; [2]Department of Virology, The Royal Veterinary College Biomedical Center, S-751 23 Uppsala, Sweden; [3]NCI-Office of the Director, Virus Control Unit, NCI-FCRF
The external envelope glycoprotein (gp120) of HIV is an ideal candidate for an AIDS vaccine because it has been shown to specifically bind to the CD4 molecule on the surface of T4-positive cells to initiate infection; antisera to gp120 neutralizes in vitro HIV infection; and gp120 is a target for T-cell cytotoxicity in HIV-infected cells. We have purified gp120 from HTLV-IIIB and evaluated the glycoprotein as a potential vaccine against HIV infection. Chimpanzees which we previously immunized with alum-precipitated gp120 gave a moderate, short-lived, type-specific immune response which failed to protect the chimpanzees when challenge with a titered stock of HTLV-IIIB. To enhance the immunogenicity of gp120, a number of adjuvants were examined and gp120 immunostimulatory complexes (iscoms) amplified the immunity to gp120. Rhesus monkeys inoculated with gp120 iscoms developed precipitation titers of 1:36,450 and HTLV-IIIB neutralization titers of 1:256. Neutralization titers of 1:8 and 1:4 were detected against the HIV variants, HTLV-IIIRF and HTLV-IIIMN, respectively, indicating a broadening of the neutralization immune response. In addition, chimpanzees immunized with gp120 iscoms developed 1:72,900 precipitating and 1:128 neutralizing titers. These responses are comparable to antibody titers of chimpanzees experimentally infected with HIV. Research sponsored, at least in part, by the National Cancer Institute, DHHS, under contract NO1-CO-74102 with Program Resources, Inc.

G 020 SIV INFECTED MACAQUES: A MODEL FOR IMMUNOPREVENTION AND IMMUNOTHERAPY, Gardner, M., Luciw, P., Carlson, J., McGraw, T., Jennings, M., Lerche, N., Marx, P., and Pedersen, N., Department of Medical Pathology and California Primate Research Center, University of California, Davis 95616
SIV-infected macaques represent an animal model with many similarities to HIV-infected humans. The advantages and potential drawbacks of this animal model of AIDS will be summarized. The humoral and cellular immune response and pathology of rhesus monkeys infected with SIV will be presented and studies now in progress will be updated. These include: (1) attempts to protect rhesus monkeys against challenge with live SIV by immunization using inactivated whole SIV-adjuvant formulations, and (2) attempts to boost SIV immunity in pre-infected rhesus by inoculation with an inactivated SIV-adjuvant immunogen. In addition, the SIV system provides a unique opportunity to test genetic engineering approaches aimed at defining viral determinants that induce protective immunity. Viral antigens are being produced in recombinant expression vectors and "attenuated" live viruses are being constructed by site-directed mutagenesis procedures.

G 021 THE IMMUNE RESPONSE TO SIV/DELTA INFECTION IN THE RHESUS MONKEY, M. Murphey-Corb, L. Martin, G. Baskin, J. Blanchard, B. Davison-Fairburn, Delta Regional Primate Research Center, Tulane University, Covington, LA. 70433. Analysis of the humoral immune response, changes in lymphocyte subsets, and virus-specific antigenemia in macaques experimentally infected with SIV/Delta has revealed that these responses parallel in many respects those observed in AIDS and also provide reliable markers for disease progression in the infected monkey. Monkeys infected with an attenuated SIV strain remained healthy, show no lymphocyte subset changes, and produce antibody to virion proteins that persist throughout the infection, even though the virus may become refractory to isolation. These findings correspond to a lack of detectable virus-specific antigenemia. Some macaques that develop immunodeficiency disease initially make strong antibody responses. Thereafter, changes in lymphocyte subsets, particularly a decline in the helper-inducer subset of T lymphocytes, occurs which precedes a decline in antibody to core proteins. Antibody often fluctuates inversely with recurrent antigenemic episodes. These antigenemic episodes are reminescent of persistent equine infectious anemia virus (EIAV) infection in the horse wherein antigenic variants emerge after evading host protective immune responses. In SIV infected rhesus monkeys, antigenemic episodes may occur months before outward signs of clinical disease and are indicative of disease progression. Several months prior to death, a selective loss of antibody to gag, but not env, determinants was most frequently observed which corresponds to progressive antigenemia. Reciprocal fluctuations in antigenemia and antibody to p14, the putative gene product of X ORF, are also observed in monkeys infected with pathogenic virus. These changes suggest that this protein may be involved in more extensive virus replication in vivo. An apparent defect in the ability to mount an antibody response to SIV is consistently observed in immunosuppressed monkeys that develop retroviral encephalitis. The association of encephalitis with a particular isolate of SIV/Delta over a wide range of inoculating doses suggests that specific viral factor(s) may be responsible for interference with antibody production and subsequent viral entry into the central nervous system.

These observations confirm the the value of this primate model system in both elucidating the pathogenic mechanisms employed by this class of retroviruses and developing effective immunoprophylactic and chemotherapeutic strategies.

Therapeutic Intervention

G 022 EXPRESSION AND STRUCTURE-FUNCTION CHARACTERIZATION OF THE HIV-1 PROTEASE AND ITS GAG-POL SUBSTRATE. C. Debouck, I. Deckman, J. Malinowski, J. Gorniak and J. Strickler, Smith Kline and French Laboratories, King of Prussia, PA 19406.
The gag, pol and env coding regions of HIV are translated by the host machinery into large polyprotein precursors that are subsequently cleaved by a trypsin-like cellular protease (env) or by a protease encoded by the virus itself. The retroviral protease is unique and essential suggesting that specific inhibitors of this enzyme will block the maturation and infectivity of the virus without interfering with the host cell physiology. In an effort to identify inhibitors of the retroviral protease, we have constructed several vectors for the expression of the enzyme in bacteria. In some constructs, we observed that the protease processed itself from a larger precursor by specific cleavage at Ser/Thr.X.X.Phe*Pro sites. This mature, 11-kDa protease was purified to homogeneity and shown to be identical to the virion-associated enzyme. We have used recombinant forms of Pr55gag and Prpol to demonstrate that the recombinant protease accurately cleaved these polyproteins when coexpressed in bacteria. We are using this system to assess the proteolytic activity of mutated forms of the protease and to identify residues critical for its catalytic activity or dimer-forming ability.

G 023 RECENT PROGRESS IN DEVELOPMENT OF ANTIVIRAL DRUGS AGAINST HUMAN IMMUNODEFICIENCY VIRUS INFECTION. Hiroaki Mitsuya, Robert Yarchoan, Makoto Matsukura, Seiji Hayashi, and Samuel Broder The Clinical Oncology Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The acquired immunodeficiency syndrome (AIDS) remains a significant and worsening medical problem since it was first described as a clinical entity more than six years ago. However, some progress has been made in the treatment of the disease. As with any virus, the different stages in the life cycle of HIV present a variety of potential targets for antiviral agents. Reverse transcriptase is one of the most attractive targets, and there have been successes at a clinical level using this as a target for new therapies, notably with 3'-azido-2',3'-dideoxythymidine (AZT), a member of the 2',3'-dideoxynucleoside family.

These years, a great deal of knowledge in terms of structure/activity relationships has emerged. For example, members of 2',3'-dideoxynucleosides as a triphosphate form could compete with the binding of normal nucleotides to DNA polymerases or could be incorporated into the viral DNA at the stage of reverse transcription and bring about retroviral DNA chain termination because normal 5'→3' phosphodiester linkages cannot be completed. Clinical trials on another 2',3'-dideoxynucleosides, 2',3'-dideoxycytidine (ddC) and 2',3'-dideoxyadenosine (ddA)/2',3'-dideoxyinosine (ddI) are underway in the National Cancer Institute.

It is also worth stressing that effective therapy for HIV infections may well depend on a combination of therapeutic strategies without relying on any one single agent, in part because the emergence of drug-resistant strains might be less likely.

AZT represents only a first step in developing practical chemotherapy against pathogenic human retroviruses, and it is certainly not a cure. In the long term, its true value may be as a validation of the key assumptions underlying antiviral strategies for intervening against established AIDS. From a practical point of view, the development of AZT has already stimulated the research for identifying other even more effective antiviral drugs for therapy of AIDS. It is worth noting that a number of antiviral agents including recombinant soluble CD4, anionic polysaccharides, acyclic nucleoside analogues, and "antisense" oligodeoxynucleotides. As we learn more about the structure/activity relationships of these drugs, we should be able to improve our options for therapy, and one could be cautiously optimistic in that more effective therapies will be developed in the not too distant future.

G 024 THE *IN VITRO* ASSESSMENT OF ANTIVIRAL DRUGS FOR HIV

Douglas D. Richman, Departments of Pathology and Medicine, University of California San Diego and San Diego Veterans Administration Medical Center, San Diego, California, 92161.

The *in vitro* assessment of the antiviral activity of candidate drugs against HIV has almost exclusively utilized standard laboratory strains like LAV-1 and HTLV-III_B in continuous cell lines. Results from multiple laboratories have in general yielded consistent conclusions about the relative activities of drugs. These assays do not, however, provide information about the activity of different antiviral drugs against multiple isolates or about the activities of drugs in different cell types relevant to the pathogenesis of HIV infection, especially primary macrophages.

The problems of determining antiviral activity against fresh human isolates of HIV with the standard assays utilizing inhibition of cytopathology or p24 antigen production will be reviewed. Resolution of this problem with a plaque assay with CD4-HeLa cells will be presented with results using multiple isolates (see Larder, this meeting).

The macrophage has been increasingly appreciated as a reservoir and a critical cell in the pathogenesis of HIV. Marked differences exist in the replication in macrophages by macrophage tropic and standard laboratory strains of HIV. Endotoxin contamination of commonly utilized reagents can significantly inhibit the replication of HIV in macrophage cultures. The effects of interferons, other cytokines and several antiviral drugs on the replication of HIV in primary human monocyte derived macrophages will be reviewed.

Immunology

G 100 METHODS FOR DETECTING PROVIRAL DNA AMPLIFIED IN VITRO AND THEIR USE FOR ANALYSIS OF RETROVIRAL VARIANTS, M.A. Abbott*, B.J. Poiesz*, B.C.Byrne*, S. Kwok+, J.J. Sninsky+ and G. D. Ehrlich*, *Department of Medicine, SUNY HSC at Syracuse, 750 E. Adams St., Syracuse NY 13210 and +Cetus Corporation, 1400 Fifty-third Street, Emeryville, CA 94608. We analyzed several detection methods used to characterize amplified sequences following TAQ directed polymerase chain reaction (PCR) for human retroviruses. The use of a combination of hybridization formats and direct incorporation assays uncovered specific HTLV-I homologous regions in several HTLV-I seronegative T-cell lymphomas and a patient with an atypical progressive myelopathy. This multiphasic approach also enabled us to identify variants of HTLV-I and HIV-1 in patients with prototype disease. For a diagnostic assay designed to detect a particular retrovirus it was necessary to include a hybridization step because sequences homologous to certain primers were present in most human DNA preparations and yielded discrete products after amplification. These products could be discriminated by hybridization from amplified prototype proviral sequences in most cases. The simultaneous detection of multiple retroviruses in a single sample was accomplished by using several primer pairs and probes in the amplification reaction and liquid hybridization, respectively.

G 101 HIV-1-tat TRANSGENIC MICE AND TRANS-ACTIVATABLE VECTORS FOR TRANSFER OF GENES INTO THE MOUSE EMBRYO. Marc ALIZON, Ted CHOI, and Rudolf JAENISCH. Whitehead Institute for Biomedical Research. 9 Cambridge Center, Cambridge MA02142, USA.

We want to use the HIV-1 trans-activating protein (tat) to obtain conditional and/or tissue-specific expression of genes introduced into the mouse embryo. For that, we have created transgenic mice strains expressing tat, by micro-injection of fertilized eggs with this viral gene placed under control of the human histone H4 promoter / enhancer region. A high level of expression is seen in the testis of these mice strains, which can be used as recipients of reporter genes placed under control of a promoter activatable by tat.

Two types of experiments are possible. One is the mating of tat mice with other transgenic mice carrying a gene of interest placed under control of the HIV-1 LTR, and studying the effects of its expression in the progeny. Since the level of activity of the HIV-1 LTR in the absence of tat is very low, this system is particularly promising for cell ablation experiments, or to study the effect of lethal genes, for which it would prove difficult to obtain transgenic animals if a constitutively active promoter was used.

The second type of application is the targetting of reporter genes to specific tissues by infection of embryos from the tat mice with MLV-derived retroviral vectors, in which the HIV-1 LTR is used as an internal promoter. Alternatively, we have constructed different chimeric MLV/HIV LTRs, by inserting the tat-target sequence at various locations in the MLV LTR. Expression of the CAT gene driven by these chimeric LTRs is increased in NIH3T3 cells expressing tat, and we have therefore constructed retroviral vectors from these LTRs. Given the low background seen in the absence of tat, the use of these vectors to express reporter gene, such as lacZ, would considerably ease cell lineage experiments, the tissue or cellular subset expressing tat being tagged in the recipient mice.

G 102 COMPETITION ANALYSIS OF IN VITRO TRANSCRIPTION FROM THE HIV LTR IN HELA CELL NUCLEAR EXTRACTS. James C. Alwine and Steven L. Zeichner. Department of Microbiology, School of Medicine, University of Pennsylvania , Philadelphia, PA, 19104-6076.

Competition analyses of in vitro transcription from the human immunodeficiency virus (HIV) long terminal repeat (LTR) using HeLa cell nuclear extracts indicated that an effective competitor must include large amounts of intact LTR sequence, spanning virtually its entire length. Subdivision of the LTR by restriction enzyme cleavage produced competitor fragments that competed much less efficiently than the intact LTR. This suggests that the formation of a stabilized complex of transcription factors capable of initiating accurate transcription involves a much larger region of the LTR than predicted from other analyses. Sequences at the 5' end of the LTR make an important contribution to transcription complex formation and stabilization. In addition, cellular sequences on the 5' flanking side of the specific LTR studied (isolated from an infectious provirus) improved the competitive ability of some LTR fragments, suggesting that the cellular sequence environment surrounding the integrated HIV provirus may influence transcription complex formation and gene expression from the provirus.

G 103 ANTIBODIES TO THE NEF PROTEIN AND TO NEF PEPTIDES IN HIV1 SERONEGATIVE INDIVIDUALS, Jean Claude Ameisen, Bruno Guy, Bernard Mach, André Tartar, Sophie Chamaret, Yves Mouton, Jamal Khalife, Luc Montagnier, Jean Pierre Lecocq, André Capron. CIBP, Institut Pasteur, 59019 Lille, France

The silent period that follows infection by the human immunodeficiency virus (HIV1) and precedes seroconversion represents a problem for the screening of blood supply. Using a radioimmunoassay and a Western blot technique with purified nef recombinant protein and 6 nef synthetic peptides, we detected antibodies to the product of an HIV1 regulatory gene, nef, that appears involved in maintenance of proviral latency, in 8 HIV1 seronegative, viral antigen-negative and virus culture-negative individuals at risk for HIV1 infection. The anti-nef response was correlated with the detection of HIV1 DNA sequences, following enzymatic amplification of HIV1 DNA in peripheral blood mononuclear cells. Such latent HIV1 infection have lasted to date up to 8 months. HIV1 DNA negative at risk individuals and controls had no antibodies to nef protein or peptides. Kinetic studies of HIV1-seropositive individuals also showed 1) antibodies to nef preceding seroconversion; 2) the persistence of antibodies to nef and of HIV1 DNA in a case of spontaneous complete HIV1 seronegativation.
Our findings suggest the potential value of a diagnostic strategy based on the screening for anti-nef antibodies, followed by confirmation by HIV DNA amplification. They also suggest that HIV regulatory gene should be considered as candidates for the design of alternative vaccines against AIDS.

G 104 ANTI-T-LYMPHOCYTE ANTIBODIES : DETECTION ON CELLS FROM HIV-1-INFECTED INDIVIDUALS, *Blair Ardman,*John Bristol,**Eleanor Levy, ***Mark Kowalski, *Mark Ryan and ***Joseph Sodroski, *Department of Medicine, New England Medical Center Hospitals, **Department of Microbiology, Boston University Medical School, ***Department of Pathology, Dana Farber Cancer Institute, Boston MA 02111.

In order to determine if anti-T-lymphocyte antibodies could be important in the pathogenesis of HIV-1 infection, T lymphocytes were obtained from individuals seropositive for HIV-1 infection and examined for the presence of surface-bound immunoglobulin. 65% of HIV-1-infected individuals (n=40) were found to have antibodies that bound to the surface of their own T lymphocytes. In contrast, anti-T-lymphocyte antibodies were not detected on the surface of lymphocytes obtained from seronegative homosexual (n=20) or heterosexual (n=20) subjects. In positive individuals, the percentage of T lymphocytes with bound antibody ranged from 2% to 78% (mean = 17%). The binding of the antibodies to the T lymphocytes in positive individuals appeared to be specific because the percentage of antibody-bound T lymphocytes did not correspond to the degree of a patient's hypergammaglobulinemia. The anti-T-cell antibodies were found on both CD4+ and CD8+ cells in the majority of positive individuals. However, the lymphocyte subset distribution of the anti-T-cell antibodies did not correlate inversely to the absolute CD4 or CD8 counts. Experiments to determine the antigen specificity of the anti-T-lymphocyte antibodies were performed and the results will be presented. Thus far, our data suggest that the anti-T-cell antibodies which bind to a patient's own T cells are different from those antibodies in patient sera that bind only to allogeneic lymphocytes. These reactivities are important to distinguish in order to understand the etiology and pathogenic significance of such antibodies.

G 105 INTERNALISATION KINETICS AND INTRACELLULAR SORTING OF CD4; COMPARISON OF DIVERSE CELL TYPES, J. Armes, A. Pelchen-Matthews, M. Marsh and R. Weiss, Institute of Cancer Research, London, England.

The CD4 molecule is involved in the generation of T-cell immunity and is also the receptor for HIV. CD4 has been shown to downregulate from the cell surface following incubation with a number of T-cell mitogens and by incubation with phorbol ester.

We have developed a novel technique of surface labelling CD4, in order to follow the constitutive modulation of CD4 in different cell types. This modulation is being compared to that following binding of iodinated univalent and divalent ligands. An initial comparison between these different modes of modulation has shown different kinetics of internalisation and also increased degredation of CD4 following binding to divalent ligand. These results are being compared to those obtained with non-human cell lines transfected with CD4 and which are resistant to HIV infection. It may then be possible to predict the level at which the block to HIV infection, in these non-human cell lines, occurs.

The intracellular transport of CD4 through endosome and lyososome compartments is being studied in various cell types, including polarised epithelial cell lines, expressing transfected CD4. We are particularly interested in the role of CD4 phosphorylation as a mechanism for intracellular signalling.

G 106 TRANS-ACTIVATION OF THE HTLV-I LTR BY p40^{tax}, Benjamin Berkhout, Oliver J. Semmes and Kuan-Teh Jeang, Laboratory of Molecular Microbiology, NIAID, Bethesda, MD 20892. We have investigated the mechanism of trans-activation of the HTLV-I LTR by viral protein p40tax. Salient observations from our experiments indicate that i) p40tax has no detectable direct DNA-binding properties; ii) a set of cellular factors bind to the p40tax-responsive element within the HTLV-I LTR; and iii) a cellular signal transduction pathway is likely involved in the activation of the HTLV-I LTR promoter. We will present evidence addressing these points and characterizing the role of p40tax in the activation mechanism.

G 107 TRANSCRIPTIONAL INDUCTION DUE TO tat PROTEIN, Kathleen Boris[1], Eva Adam[2], Ajit Kumar[1,2], (1) Program in Genetics (2) Department of Biochemistry, George Washington University Medical Center, Washington, DC 20037.

Expression of the HIV tat gene is known to be essential for viral replication in culture. The tat protein has been shown to cause increased expression of genes under control of the viral LTR. At the transcriptional level, tat has been shown to effect the rate of transcription from the HIV LTR. Okamoto and Wong-Staal addressed the effect of tat on transcription in vitro using extracts of HIV-infected cells. Although they demonstrated a 12-fold activation of transcription, it is not clear if this effect was due to the presence of functional tat protein. In order to study the activity of the tat gene product distinct from other HIV proteins, we have cloned the tat gene in the baculovirus eukaryotic expression system. We show the tat producing cells support efficient transactivation in vivo. In order to address the extent of transcriptional induction due to tat protein, we have used a cell-free in vitro transcription system. tat protein was supplemented from baculovirus infected cells and compared to supplementation from tat producing HeLa and simian CV1 cells.

G 108 **SEVERAL ANTIGENIC DETERMINANTS EXPOSED ON THE GP120 MOIETY OF HIV-1 GP160 ARE HIDDEN ON THE FREE GP120 ENTITY, Clotilde Thiriart*, Myriam Francotte*, Joe Cohen*, Catherine Collignon*, Anne Delers*, Suzy Kummert*, Chantal Molitor*, Daniel Gilles+, Piet Roelants+, Frans Van Wijnendaele+, Michel De Wilde* and Claudine Bruck*, *Department of Molecular and Cellular Biology, +Department of Human Vaccine Development, Smith Kline Biologicals, Smith Kline-RIT, rue de l'Institut 89, B-1330 Rixensart, Belgium.** Mouse monoclonal antibodies reactive to the HIV-1 envelope glycoprotein precursor gp160 of the HTLVIII$_B$ isolate were characterized in radioimmunoprecipitation and Western Blot tests using HTLVIII$_B$ as antigen. The reactivities of those monoclonal antibodies were also measured in a capture EIA using III$_B$, RF and ARV$_2$ isolates as well as recombinant gp160 and gp120 (BH10 clone) produced in mammalian cells. Striking differences in exposure of specific epitopes were noted between the gp120 component of the gp160 precursor and the mature, isolated gp120 released in the culture supernatant of CV1 cells infected with a gp160 vaccinia recombinant. These conformational rearrangements affecting the gp120 moiety of the HIV-1 envelope glycoprotein might have important implications on its immunogenicity and on vaccine development.

G 109 CHARACTERIZATION OF POLYMERASE ACTIVITY ASSOCIATED WITH CULTURED PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH KAWASAKI DISEASE,Jane C. Burns, Alice S. Huang, Jane W. Newburger, Donald YM. Leung, Depts of Pediatrics and Microbiology,Harvard Medical School,Boston,MA 02115. Kawasaki disease(KD) is an acute systemic vasculitis of unknown etiology. Affected children have a particle-associated reverse transcriptase activity(RTA) in supernatants of cultured peripheral blood mononuclear cells(PBMC). To further characterize this RTA, we established 77 cultures from the PBMC of 39 KD pts.and compared them to 29 control cultures. The median peak picomoles of dTMP incorporated was significantly higher in cultures from KD pts. vs. controls (4.7 vs. 2.5 pmoles,$p<.001$). KD PBMC cultured between the 3rd and 9th weeks after onset of fever were most likely to be associated with RTA. Peak RTA was positively associated with older age ($r=0.64,p=0.02$), greater magnitude of the serum IgA response ($r=0.64,p=0.08$) and IgM response($r=0.75,p=0.006$). The appearance of RTA was not associated with a decrease in overall cell viability. Pooled RTA-positive supernatants concentrated by density gradient centrifugation(banded at 1.15-1.20gms/ml sucrose)showed radiolabel incorporation only with poly(rC): oligo (dG) and not with poly(dA):oligo(dT), oligo(dT), or absence of template/primer. These data suggest that PBMC from KD pts. may harbor a polymerase-associated agent which is most readily detected in the early convalescent phase of KD in older pts. who mount a marked humoral immune response.

G 110 ANALYSIS OF HTLV-I ENVELOPE USING SYNTHETIC PEPTIDES AND SITE-SPECIFIC ANTISERA, Wendell T.W. Ching, John K.S. Chia, Irvin S.Y. Chen, David D. Ho, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA 90048

Sixteen peptides were synthesized according to the sequence of HTLV-I envelope (gp46 and p21). Sera from 28 patients with adult T-cell leukemia (ATL), 20 with HTLV-I-associated myelopathy (HAM), and 33 normal controls were tested for reactivity with the synthetic peptides by an enzyme immunoassay. Partial analysis to date shows that one region in the C-terminus of gp46 and another in the N-terminus of p21 are recognized by certain ATL and HAM sera. A complete study to define the most immunogenic domains of HTLV-I envelope in HTLV-I-infected persons will be presented. The synthetic peptides have also been conjugated to keyhole limpet hemocyanin and subsequently used to raise site-specific antisera in rabbits. The resultant antisera have high-titer anti-peptide antibodies as determined by enzyme immunoassays. The reactivity of these antisera with gp46 or p21 is being determined by radioimmunoprecipitation. In addition, the activity of these antisera in blocking HTLV-I lymphocyte transformation and HTLV-I mitogenic effect will also be assessed.

G 111 DETECTION AND CHARACTERIZATION OF ANTIBODIES THAT RECOGNIZE RECOMBINANT SOLUBLE CD4 IN SERA OF HIV-POSITIVE SUBJECTS, Tyler J. Curiel*, Robert T. Schooley*, Beatrice Burrus**,Richard A. Fisher**, and Theresa Liu**, *Infectious Disease Unit, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, and **Depts. of Cell Biology & Immunology and Molecular Biology, Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142.

We examined the sera of 44 persons seropositive for human immunodeficiency virus (HIV) to assess the prevalence of antibody (Ab) to a recombinant soluble form of the T lymphocyte surface marker CD4 (α-rsT4). Sera were screened with an enzyme-linked immunosorbent assay employing a 375 amino acid recombinant soluble CD4 (rsT4). Five sera were α-rsT4 positive in contrast to none among the HIV-seronegative controls. The presence of α-rsT4 was confirmed by immunoprecipitation of rsT4 with these sera. Protein A-Sepharose 4B column fractionation of sera yielded the α-rsT4 activity in the immunoglobulin fraction. The immunoglobulin fractions from some sera prevented the formation of syncytia between chronically HIV-infected H9 cells and the $CD4^+$ cell line C8166 *in vitro*. The role of these Abs in HIV-related disease pathogenesis is currently undefined. We are continuing to screen sera, and to undertake further study of these Abs, to characterize their relationship to the clinical expression of HIV infection.

G 112 HIV SPECIFIC CYTOTOXIC T LYMPHOCYTES IN LUNGS Patrice Debré, Brigitte Autran, Laboratory of Cellular and Tissular Immunology, CHU Pitié Salpétrière, Paris, France

Cell-mediated cytotoxicity play a major role in host cellular defenses against viruses. Up to now, distinct-effector cells against HIV have been reported but their relative importance is still unknown. We have described HIV-specific, HLA-restricted cytotoxic T lymphocytes in broncho-alveolar lavage (BAL) fluids of HIV-infected patients. We have now analysed 68 BAL fluids from 47 cases without detectable opportunistic lung infections or tumors and 21 cases with lung infections, some of them sequentially. All had an alveolar lymphocytosis mainly composed of CD8+ D44+ cells. In a direct cytotoxicity assay, we could distinguish two types of alveolar cytolytic cells. Firstly, we could detect an HLA-class I restricted cytolysis of autologous HIV-infected alveolar macrophages (AM). The alveolar CTL could lyse HIV+ allogeneic AM only if they share at least one HLA antigen and their activity was totally inhibited by addition of anti-HLA class I monoclonal antibody. Additionnal experiments carried out with HLA-A2 alveolar lymphocytes, showed a specific cytolysis of P815 murine cells doubly transfected with the HLA-A2 and the HIV-enveloppe genes. The detection of an HLA-restricted specific cytolysis was related to the presence of CD8+ cells reacting with the D44 moAb. Secondly, in some patients we could detect a Natural-Killer activity against K562 target cells related to the presence of CD16+ lymphocytes in their BAL fluid. The NK cells had no cytolytic activity against autologous or allogeneic AM whether they were matched for HLA-antigens or not. Finally, we could never detect an Antibody-Dependant-Cellular-Cytotoxicity-like activity in the BAL fluid cells of our patients. Both HLA-restricted cytotoxicity and NK activity could coexist ; however those activities evolved independantly : NK cytolysis could be detected at all stages of the disease ; in contrast, the HLA-restricted HIV-specific cytotoxicity decreased with the outcome of opportunistic infections, whereas a CD8 lymphocytic alveolitis persisted. The lung model provides then an interesting model for the study of the cellular immune defenses against HIV and their clinical relevance.

G 113 MONOCLONAL ANTIBODIES AGAINST HIV ANTIGENS, Claude Desgranges(1), Véronique Boyer(1), Sylvie Souche(1), Pascal Madaule(2) and Daniel Zagury(3). (1) Anticorps Monoclonaux Humains INSERM U 271, 151 Cours A. Thomas 69424 Lyon Cedex 03. CNRS GIF-sur-Yvette 91198 et (3) Université P. et M. Curie Paris FRANCE.

Human monoclonal antibodies against HIV antigens were obtained after immortalisation with Epstein-Barr virus of B lymphocytes from different HIV seropositive individuals. B lymphocytes were isolated from peripheral blood of normal individual immunized with a recombinant HIV vaccinia virus (gp160), or from asymptomatic, ARC or AIDS patients. The different human monoclonal antibodies will be presented with their biological (neutralization activities) and immunological characteristics. They were analyzed for the different epitopes recognized with synthetic peptides or proteins expressed in yeast model. These human monoclonal antibodies will be used : for variant selection from different patients, for passive immune therapy development and for production of anti-idiotypes for vaccination.

G 114 INTRACELLULAR EXPRESSION OF CD4 RECEPTORS BY BLOOD MONOCYTES FROM NORMAL AND HIV INFECTED PATIENTS. **Lionel G. Filion, Carlos A. Izaguirre, G.E Garber, Lothar Huebsch, Departments of Microbiology & Immunology and Medicine, University of Ottawa, Ottawa, Canada, K1H 8M5.**

Monocytes are surface CD4+, susceptible to HIV infection and may play an important role in maintaining the chronicity of the disease. From published studies it is unclear if CD4 is present in all or a subset of monocytes and if there is intracellular CD4 receptor. We have done an immunofluorescence (Flow cytometry, microscopy) analysis of monocytes fron normal donors and HIV infected patients using anti-CD4 (Leu-3a) and the monocyte specific marker anti-CD14 (Leu-M3) monoclonal antibodies. We found: 1) By flow cytometry 85% of monocytes show dual positivity for CD14 and CD4 (10 experiments). 2) Two color immunofluorescence microscopy (IFM) of surface markers confirmed that most CD14+ monocytes are also CD4+ (76%). 3) Intracellular CD4 receptors (with blocking of surface CD4)): two-color IFM revealed that essentially all CD14+ monocytes are also CD4+. A few monocytes are CD14-4+. The staining intensity of CD4 was 3+ to 4+. T cells were negative for intracellular CD4. 4) HIV infected patients: in four patients the frequency and intensity of surface and intracellular staining for CD4 was not different from that of normal monocytes. We conclude that the monocyte CD4 receptor content is mainly intracellular and that it may play an important role in the retention of HIV virions reported by other investigators and thus play a role in the chronicity of the disease. The physiologic role of intracellular CD4 in monocytes remains to be determined.

G 115 **UNIQUE ENHANCER ELEMENT FOR THE TRANS-ACTIVATOR ($p40^{tax}$) OF HTLV-1 DISTINCT FROM cAMP- and TPA-RESPONSIVE ELEMENTS**, Jun-ichi Fujisawa and Mitsuaki Yoshida, Department of viral Oncology, Cancer Institute, Toshima-ku, Tokyo 170, JAPAN.
The trans-activator ($p40^{tax}$) of Human T cell leukemia virus type 1 (HTLV-1) is a transcriptional factor that activates the long terminal repeat (LTR) of HTLV-1 and a cellular gene for interleukin-2 receptor α (IL-2Rα). A sequence responsible for tax-mediated trans-activation of the LTR has been mapped into the transcriptional enhancer consisted of three direct repeats of 21 base pairs (bp) in the U3 region of the LTR. Mutagenesis of the 21 bp in a construct of LTR-CAT and assay of the CAT activity identified a sequence (A/T)(G/C)(G/C)CNNTGACG (T/A) as a responsible element (TRE). With the same strategy, sequences required for cAMP and TPA activations were also analyzed. These three responsible sequences shared the middle region of the 21 bp as essential sequence, but differ from each other. It has recently been reported that an NF-kB binding site in the IL-2Rα gene and HIV-LTR is responsible for tax-meidated trans-activation. However, the TRE in the HTLV-1 LTR appeared to be different from the NF-kB binding site by sequence comparison and competition in a protein binding assay. These findings suggested that the tax gene product can interact with multiple cellular factors that recognize each different enhancer, thus resulting in transcriptional activation of various cellular genes.

G 116 **CELLULAR PROTEINS BIND TO THE RNA-TAR REGION OF HIV-1**, Anne Gatignol[1], Kuan-Teh Jeang[2] and Ajit Kumar[1], (1) Department of Biochemistry, George Washington University Medical Center, Washington, DC 20037 and (2) Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Human Immunodeficiency Virus HIV-1 encodes for a trans-activator protein (tat) using a doubly spliced mRNA generated through the joining of two separate coding exons. The tat protein acts through the tat responsive element (TAR) contained in the 5' untranslated region of the virus, probably at both a transcriptional and a translational level. The mechanism of this action is unclear but it may require cellular factors. We have investigated the binding of cellular proteins to the TAR RNA using nuclear extracts from tat producing and non-producing cell lines derived from CV1 and HeLa cells using a gel retardation assay, we found several probe migration differences with these various extracts. In RNA UV crosslinking experiments, we determined the approximate sizes of the proteins bound to the TAR RNA probe. We will correlate these in vitro findings with in vivo biological function (Supported by NIH Grant A125531)

G 117 HOMOLOGY BETWEEN HIV I GP41 AND HLA CLASS II MOLECULES LEADS TO GENERATION OF IMMUNOSUPPRESSIVE ANTIBODIES IN EARLY STAGES OF HIV I INFECTION. Hana Golding, Gene M. Shearer, Kathleen Hillman, Robert A. Zajac, Mario M. Clerici, and Basil Golding. Div. of virology and Div. of Blood and Blood Products, FDA, and Experimental Immunol. Branch, NCI, NIH. Bethesda MD 20892.
HIV I infected individuals are often presented with reduced immune responses early in the infection prior to the drastic reduction in $CD4^+$ T cells number. Sera from such HIV I infected individuals (stage 1-2) were found to contain antibodies (Ab) against a conserved region in gp41 (aa 837-844) which crossreact with an homologous sequence present in all HLA class II beta chains (aa 19-25). These Ab bound also to native class II molecules and were shown to be potent inhibitors of $CD4^+$ T cell responses against tetanus toxoid and allogeneic stimulators in vitro. The crossreactive Ab could also eliminate class II-bearing cells (such as EBV lines and activated T cells) by antibody dependent cellular cytotoxicity (ADCC). Affinity chromatography was used to purify these autoantibodies from patients' sera. The eluted IgG retained the immunosuppressive activities of the original sera. Therefore, this virally-triggered autoimmune mechanism may contribute to early immune impairment in HIV-infection.

G 118 RETROVIRUS INTEGRASE PROTEINS: CHARACTERIZATION BY CIRCULAR DICHROISM AND EMPIRICAL PREDICTION METHODS, Duane Grandgenett and Thy-Hou Lin, Institute for Molecular Virology, St. Louis University, St. Louis, MO 63110. The retrovirus integrase (IN) protein is essential for integration of viral DNA into host DNA. The secondary structure of the purified IN protein from an avian retrovirus was investigated by both circular dichroism (CD) spectroscopy and five empirical prediciton methods; namely, the Chou-Fasman, Garnier-Osguthorpe-Robson, Nishikawa-Ooi (NO), and a joint preparation scheme which combined all three of these methods, plus a pure a priori one, the Ptitsyn-Finkelstein method. Among all the methods used, the NO prediction gave the closest match in the composition of secondary structure to the CD result, although the other methods each correctly predicted one or more secondary structural group. Most of the α-helix and β-sheet states predicted by the Ptitsyn-Finkelstein method were in accord with the NO method. Secondary structural predictions by the NO method were extended further to include IN proteins from five phylogenetic distinct retroviruses, as well as HIV-1, HIV-2, SIVagm, SIVmac, HTLV-1, and HTLV-2. The secondary structure predicted for each IN protein was aligned based on the alignment of sequences for these IN proteins. A generalized structure was deduced for the IN proteins from the most conserved His-His-Cys-Cys block downstream to amino acid residue 220. Correlation of the biological function derived from the study of site-directed mutagenesis of the IN proteins with the predicted secondary structures for both wild-type and mutant IN proteins will be presented.

G 119 DIAGNOSTIC UTILITY OF SELECTED EPITOPES ON HIV ENV AND GAG PROTEINS DEFINED BY MOUSE MONOCLONAL ANTIBODIES, J.C. Hunt, S. Mehta, and S.G. Devare, Human Retroviruses Department, Abbott Laboratories, Abbott Park, IL 60064.

Unique epitopes on HIV gag and env proteins have been identified using mouse monoclonal antibodies. These antibodies can be effectively employed for a variety of diagnostic purposes which include: (1) the detection of HIV-1 seropositive individuals based on the presence of antibody to gp41, (2) the detection and monitoring of HIV-2 p24 levels in infected individuals, (3) discrimination between individuals infected with HIV-1 vs. HIV-2 based on seropositivity against the HIV transmembrane proteins, and (4) discrimination between HIV-1 and HIV-2 env and gag proteins by mapping of conserved and non-conserved epitopes. A competitive EIA has been developed which utilizes a specific monoclonal antibody to HIV-1 gp41 which can readily detect HIV-1 seropositive individuals. Monoclonal antibodies which bind to HIV-1 p24 enable the detection of 1-2 pg of HIV-1 p24/ml of serum or plasma from infected individuals. Discrimination of HIV-1 vs. HIV-2 seropositive individuals can be exploited by employing a non-crossreactive monoclonal to HIV-2 transmembrane protein. Finally, HIV-1 Proteins can be differentiated from HIV-2 proteins based on non cross-reactive epitopes they display.

G 120 DRUG-INDUCED IN VITRO REVERSAL OF THE DEFICIENCY OF CD4+ T-CELLS AND T-CELL PROGENITORS IN HIV INFECTED PATIENTS. Carlos A. Izaguirre, Jeanne Drouin and Lothar Huebsch, Department of Medicine, University of Ottawa, and Ottawa General Hospital, Ottawa, Canada, K1H 8M5.

HIV-infected patients have a depressed blastogenic response and in our lab. we have found that T cell colony formation is also severely depressed (no colony formation in 8 of 10 patients). It is not known if the inability to generate new cells is due to destruction or suppression of T cell precursors. Our objective was to determine if the anti-HIV drugs castanospermine (CSP) and dextran sulfate-8000 (DS) have an effect on the regeneration of CD4+ T cells and their precursors. We used a T cell colony assay, PHA stimulated T cell proliferation, and mixed lymphocyte response cultures (MLR). Cells were phenotyped for CD4 and CD8 markers. Virus production was measured using both cocultures and the reverse transcriptase assay. We found: 1) Both CSP (50 µg/ml) and DS (50 µg/ml) have a remarkable effect on T cell colony formation with colony numbers going from around zero to near normal numbers (12 experiments (exp.)). 2) In the presence of CSP and DS, the PHA induced proliferation becomes normal, the number of CD4+ cells increases to normal levels, and the CD4:8 ratio increases to ≥1 (8 exp.). 3) CSP and DS do not affect the proliferative response (PHA-induced and MLR) of normal lymphocytes (12 exp.). 4) Virus production appears not to be affected by CSP (2 exp.). We conclude that besides the destruction of CD4+ T cells by HIV, there is inhibition of their precursors which prevents repopulation of this T cell subset. CSP and DS overcome this inhibition thus allowing the rapid growth and development of T cells and its precursors in vitro.

G 121 FORMATION AND RELEASE OF HIV-1 Pr55^{gag} "VIRUS-LIKE" PARTICLES FROM BACULOVIRUS INFECTED INSECT CELLS, Dirk Gheysen, Eric Jacobs, Françoise de Foresta, Myriam Francotte, Denise Thines* and Michel De Wilde, Molecular and Cellular Biology Department, Smith Kline-RIT, 89 rue de l'Institut, B-1330 Rixensart, Belgium, *Department of Cellular Biology, UCL, Louvain-La-Neuve, Belgium.

Using a baculovirus expression system, the HIV-1 gag precursor Pr55^{gag} was produced in Spodoptera frugiperda cells, in the absence of the viral protease. A p55 gag product was detected in the cell extracts by immunoblot analyses, as well as faster migrating gag products probably generated by cellular proteases. In addition, p55 antigen was found in the culture medium in a "particulate" form. The p55 gag precursor has the same mobility and antigenic properties as compared to the HIV-Molt cell lysate gag 55Kd band, and is also efficiently myristoylated. We have demonstrated by electron microscopy experiments that this myristoylated precursor gag protein was released in large amounts from the insect cells as budding Pr55^{gag} particles. In contrast, no myristoylation and no particle formation was observed with a mutant Pr55^{gag} construct in which the N-terminal glycine codon was deleted. In that case, non-myristoylated p55 gag protein was produced intracellularly at comparable levels that the wild type Pr55^{gag}. This demonstrates that the Pr55^{gag} produced in these baculo infected insect cells has the intrinsic property to form budding particles, provided that the protein is efficiently myristoylated. Supprisingly, in cells producing the non-myristoylated mutant Pr55^{gag}, important amounts of intracellular particles were found in the cytoplasm but also into the nucleus. Two putative nuclear location signals were found in the gag coding sequence. The possible meaning of such a targeting process will be discussed.

G 122 REPLICATION OF THE HUMAN IMMUNODEFICIENCY VIRUS-1 AND IMPAIRED DIFFERENTIATION OF T-CELLS FOLLOWING IN VITRO INFECTION OF BONE MARROW IMMATURE T-CELLS. C. Jasmin[1], Y. Lunardi-Iskandar[1], M.T. Nuygere[2], V. Georgoulias[1], F. Barre-Sinoussi[2], J.C. Cherman[2]. [1] INSERM U 268, 14-16, av. Paul Vaillant Couturier, 94804 Villejuif ; [2] Department of Virology, Inst. Pasteur, 25, rue du Dr. Roux, 75015 Paris, France.

HIV-1 infection in vitro of normal BMMC (bone marrow mononuclear cells) depleted of mature T cells was studied. BMMC depleted either of CD3, or of CD2 as well as of both CD2 and CD3 cells could replicate HIV-1, irrespectively of the presence of macrophages/monocytes. Infected bone marrow cells were shown to differentiate during the culture into $CD3^+$, $CD4^+$, $CD8^+$ and $CD1^+$ cells. Moreover, 9-14% of the cells also expressed the viral proteins p24 and gp120 on their surface. Double staining studies revealed that 72% and 83% of the $CD4^+$ cells expressed the gp120 and p24 ; moreover, CD4 cell-depletion of in vitro differentiating cells early after in vitro infection completely prevented virus replication. These findings strongly suggest that virus replication occured in $CD4^+$ cells. T-cell colony growth from infected BMMC, either unfractionated or depleted of mature T-cells, was impaired in a time-dependent manner, and the differentiation capacity of T cell precursors was abnormal. Colony cells displayed an immature cell phenotype ($CD1^+$ cells) and the viral proteins gp120 and/or p24 could be also detected on $CD1^+$ cells. In addition, pooled colony cells derived from infected CD_2 and CD_3-depleted BMMC could infect normal mitogen-activated lymphocytes in co-culture experiments. These findings, seem to indicate that HIV-1 can infect immature bone marrow T cells and be transmitted to the progeny but the massive viral replication occurs only when the cells differentiate towards $CD4^+$ cells.

G 123 ANALYSIS OF RETROVIRUSES BY DETECTION OF AMPLIFIED VIRAL RNA SEQUENCES ISOLATED FROM VIRAL PARTICLES, Ernest S. Kawasaki and John J. Sninsky, Departments of Molecular Biology and Infectious Diseases, Cetus Corporation, Emeryville, CA 94608

For obvious reasons, methods for the rapid detection and identification of retroviruses have become increasingly important. At the nucleic acid level, the use of Polymerase Chain Reaction or PCR has greatly increased the sensitivity of detecting viral sequences. With retroviruses, analyses may be carried out on integrated proviral DNA, on intracellular RNA transcripts, or on RNA genomes from viral particles. For diagnostic and clinical purposes, detection at the level of viral particles is of importance since a positive result would signify the presence of a productive infection. We have developed simplified protocols for detecting RNA from viral particles using an extension of the PCR technique. Viral pellets or viruses in serum or tissue culture medium are first digested in the presence of NP-40 and Proteinase K. After heat denaturation of the protease, an aliquot of the virus preparation can be used directly in a reverse transcriptase reaction, followed by PCR amplification of the viral cDNA sequences. This method is rapid and sensitive, with ~100 viral particles being easily detectable. The protocol can be used for any RNA virus, and would even be less complex for analyzing DNA viruses, since the reverse transcriptase step would be eliminated.

G 124 NEUTRALIZING MONOCLONAL ANTIBODIES TO HIV AND THEIR USE IN ANALYZING NEUTRALIZATION-RESISTANT MUTANTS GENERATED IN CHIMPANZEES

Elaine Kinney Thomas[+], Mitra C. Singhal[+], James Blake[+], Edna S. Dickinson[++], Joyce Chinn[+], Shiu-Lok Hu[+], Patricia N. Fultz[+++], M. Kathleen Shriver[++], [+]Oncogen, Seattle, WA 98121, [++]Genetic Systems Corporation, Seattle, WA 98121, [+++]Yerkes Primate Center, Atlanta, GA 30322.

We have previously described four monoclonal antibodies (MAbs), 110.3-110.6, which recognize the envelope protein, GP120 of human immunodeficiency virus (HIV), and neutralize virus infectivity of strains HIV-Bru and HIV-IIIB (E. Kinney Thomas et al, 1988 AIDS 2:25-29). Using a synthetic peptide, we have identified the recognition sequence of the MAbs in a region encompassing amino acid (aa) numbers 308-328 (numbered according to the Bru isolate). Virus neutralization and sycytium inhibition by MAb 110.4 can be abrogated by this peptide. Overlapping, truncated peptides synthesized from this region were used in ELISA-based, direct and competitive binding assays to further delineate the recognition epitope of each of the MAbs. The data indicate that the antibodies bind to the following residues within peptide 308-328: MAb 110.3 (aa313-319), MAbs 110.4 and 110.5 (aa313-328), and MAb 110.6 (316-328). These MAbs which recognize slightly different epitopes within this region have been used as probes to identify and study neutralization-resistant mutants of HIV generated during serial passages in chimpanzees. Two chimpanzees were infected with strain Bru which had been serially passaged twice through other chimpanzees. Virus reisolated from one animal was resistant to neutralization by any of the four MAbs, while virus isolated from the other animal was weakly neutralized by only MAb 110.4 and 110.5. The basis for differential neutralization by these MAbs is under investigation.

G 125 GROUP-SPECIFIC NATURE OF HIV ENVELOPE-SPECIFIC ADCC RESPONSES IN ANTIBODY-POSITIVE SERA, Richard A. Koup, Cheryl A. Pikora, Peter H. Levine, Sarah McKenzie, Gail Mazzara, Dennis Panicali and John L. Sullivan, Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA 01655 and Applied Biotechnology, Inc., Cambridge, MA

The strain-specificity of HIV neutralizing antibody activity has previously been demonstrated by the inability of certain human sera to consistently cross-neutralize HIV strains with widely divergent envelope genes (IIIB and RF). To determine if the antibody-dependent cell-mediated cytotoxic (ADCC) responses of human sera also exhibit strain-specificity, sera from recent seroconverters with strain-specific neutralizing antibody against IIIB and RF were tested for their ability to mediate ADCC against target cells infected with recombinant vaccinia vectors expressing envelope genes of IIIB or RF. Thirty-five sera were also screened for their ability to preferentially mediate ADCC against target cells expressing IIIB or RF envelope gene products. All HIV-seropositive sera tested to date, even those with absent neutralizing activity against IIIB or RF strains of HIV, have been able to mediate ADCC against both IIIB and RF envelope-expressing targets. In addition, the ADCC titers against those divergent targets were similar in all sera tested (range 1:1000 to 1:1,000,000). The group-specific nature of HIV-specific ADCC responses may be important for cell-associated virus protection when considering vaccine development.

G 126 RETROVIRAL DNA IN THE PLASMA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND ITS POSSIBLE PATHOGENETIC RELEVANCE, Werner Leitmann, Martin Herrmann, Friedel Krapf and Joachim R. Kalden, Institute of Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, FRG

The production of autoantibodies, concomitant in immunodeficiency disorders like AIDS, is a major pathogenetic mechanism in most rheumatic diseases, including systemic lupus erythematosus (SLE). As recently shown, reactivity towards autoimmune targets (snRNP, DNA etc.) might mimic an initial immune response against an endogeneous or foreign retroviral epitope. The role of DNA and RNA from SLE patients' plasma in the pathogenesis, which was suggested by their biochemical and immunogenic properties, was substantiated by sequencing of plasma DNA and analysis of *in vitro* functions of total plasma nucleic acids (PNA). Plasma DNA clone E6 had a striking homology to the gag-pol sequence of HIV 1 (85% in 150 bp), as well as to some human endogeneous retroviruses. Hybridization showed homologous sequences in all SLE-derived plasma specimen, but not in mixed cryoglobulinemia patients, who also carry relevant amounts of nucleic acids in their plasma. A human EBV-immortalized B cell line, established from a healthy donor (B62), was then transfected by SLE patients' PNA and screened for PNA-induced alterations. In morphology, transfected cells (B62/SLE) were caused to form syncytia and individual cell vacuolization, although no viral particles were found extracellularly. Overall IgM synthesis was reduced, and, reflecting more specific viral interaction, some cells expressed retroviral epitopes detected by an antiserum against FeLV. B62/SLE cells produced myristoylated polypeptides absent in controls (p15, p27, p38, p65), which were similar to those of cells infected by human or animal retroviruses. B62/SLE cells were found to induce an RNA of approx. 10 kb by uridine incorporation; using plasma DNA clone E6 or an incomplete HIV 1-probe for RNA or DNA hybridization, we showed retroviral mRNA synthesis and low-level episomal DNA cross-reactive to E6, but no integrated proviral sequences. An altered reactivity in Western Blots using SLE patients'sera and similar results in Northern blotting in 2 out of 7 patients' PBL lines, as compared to B62/SLE experiments, indicated that the observed phenomena correlate to SLE pathogenesis and suggest the activation of a complete or partially defective viral genome rather than expression of one single epitope.

G 127 QUANTITATIVE DETERMINATION OF PROVIRAL DNA SEQUENCES BY THE POLYMERASE CHAIN REACTION (PCR) S. Kwok, D. Kellogg and J. Sninsky, Department of Infectious Diseases, Cetus Corporation, Emeryville, CA 94608 In retroviral infections there exist a dynamic equilibrium among cell-free virus particles, uninfected cells and infected cells harboring either transcriptionally active or dormant proviruses. By determining the level of viral DNA and/or RNA relative to the total number of cells in the peripheral blood, one can assess the effect of antiviral agents on the status of the infection equilibrium. As an intial step, we demonstrate that the DNA amplification procedure, polymerase chain reaction (PCR), can be used to quantitate the amount of viral DNA present in peripheral blood. In our model studies we chose to amplify two sequences; a cellular sequence encoding a region of the histocompatibility loci (HLA-DQα) and a viral sequence encoding a portion of the gag gene of HIV-1. Amplification of the HLA sequence serves two purposes. First, by selecting appropriate conditions, one can determine from the extent of HLA amplification, the total number of cells present in a given sample. Second, since the primers amplify across a polymorphic region of the HLA-DQα locus, HLA typing can be performed with allele-specific probes to monitor sample disorder. We demonstrate that both the HLA and HIV sequences can be quantitatively co-amplified in a single reaction. Furthermore, we have developed a detection strategy that enables quantitative detection of both target sequences simultaneously.

G 128 ANTIBODIES MEDIATING CELLULAR CYTOTOXICITY (ADCC) AND NEUTRALISATION IN CHILDREN BORN TO HIV-INFECTED MOTHERS.

Kristina Ljunggren[1], V. Moschese[1,3], PA.Broliden[1,2], B. Wahren[2], P. Rossi[1,3] and M. Jondal[1]
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OBJECTIVE: To relate the ADCC and neutralising capacity in sera from children born to HIV-infected mothers with stage of disease.
METHOD: Seroreactivity to HIV was determined by ELISA and Western Blot analysis. Antibodies mediating ADCC were detected in an 3 hr ^{51}Cr-release assay with normal lymphocytes as effectorcells and HTLV-IIIB-infected U937-2 cells as targets. In the neutralisation assay, sera and virus (HTLV-IIIB) were preincubated and then added to peripheral blood mononuclear cells. On day 4 and 8, p24 antigen-release was measured.
RESULTS: The HIV-infected children in the non-AIDS group had a higher frequency (69%, n=16) of ADCC-mediating antibodies than the children in the AIDS-group (42%, n=12). Also, the mean ADCC titer was higher, 4500 and 1950, respectively. An even more striking difference was seen when comparing the frequency of neutralising antibodies for the non-AIDS and AIDS-groups, 69% and 8%, respectively. Further data on these immune-parameters will be presented concerning paired samples from mother and child.
CONCLUSION: A higher frequency of HIV-specific antibodies mediating ADCC and neutralisation was correlated to a better clinical stage of HIV-infection in children born to HIV-infected mothers.

G 129 FUNCTION OF HIV-1 INFECTED MACROPHAGE/MONOCYTES. Dean L. Mann, Frances LeSane, Susan Gartner, and Mikulas Popovic, National Cancer Institute, NIH, Bethesda, MD 20892. Cells of the monocyte/macrophage (m/m) lineage regulate many aspects of the immune response and are the most likely cell reservoir for HIV-1 in the infected host. We have undertaken studies to determine the capacity of HIV-1 infected m/m to present antigen, a function of monocytes in the initiation of the immune response. M/m were isolated from peripheral blood lymphocytes (PBL) obtained from HIV-1 seronegative individuals, infected with HIV-1, and pulsed with tetanus toxoid (TT). The ability to present TT to autologous T cells was assessed by ^{3}H thymidine incorporation and infection monitored by RT activity. Controls consisted of uninfected m/m with and without TT and infected and uninfected m/m without TT. Antigen (TT) presentation to autologous T cells by HIV-1 infected m/m was found to be comparable to uninfected m/m. HIV-1 infected m/m without TT stimulated and infected autologous T cells. The T cell response and HIV-1 infection increased by 2-3 fold when TT was presented by HIV-1 m/m. All antigen induced T cell response and HIV-1 infection was abrogated by monoclonal antibodies to MHC class II antigen HLA-DR,DP and by anti CD4 but not with antibodies to MHC class I antigen or MHC class II HLA-DQ. These results show that HIV-1 infected m/m continue to function as antigen presenting cells. Furthermore exogenous antigen presentation enhances infection of T lymphocytes which can be blocked by antibodies reacting with m/m cell surface antigens that regulate the monocyte T cell interaction in the immune response.

G 130 IDENTIFICATION OF HELPER T CELL EPITOPES FROM GP 160 OF HIV THROUGH THE USE OF PEPTIDE-PHOSPHOLIPID CONJUGATES. G. Goodman-Snitkoff and Raphael J. Mannino, Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208
Our laboratory has developed a new, powerful technique for investigating the immune response to peptide epitopes, involving the covalent coupling of peptide to phosphatidyl-ethanolamine, followed by complexing with additional lipids and phospholipids to form a peptide-phospholipid complex. These complexes can be used to immunize mice in the absence of protein carriers or adjuvants, thus facilitating the study of the immune response to a small chemically defined antigen. Use of this technology has allowed us to identify two T helper cell epitopes in conserved regions of HIV gp 160 not previously identified by computer algorithims, defined by amino acids 485-518 and 585-615. Immunization with these peptides in peptide-phospholipid complexes results in the production IgG_1 and IgG_2 antibodies, which cross react with cloned fragments of the whole protein. Using this technology we have begun to characterize the immune response to individual peptide antigens. The response of H2-k mice to amino acids 494-518 of gp 160 of HIV, has been analyzed. The optimal dose of a peptide containing both B and T_h cell epitopes was found to be 15-30 ug, depending on the route of administration. IM immunization required less antigen for optimum antibody response than did IP. Anchorage in the phospholipid complex is a strict requirement for an antibody response. Additional variables, such as phospholipid composition and method of cross-linking have been studied and will be discussed. We believe that the use of this peptide-phospholipid complex technology will be significant both for studying the immune response to single epitopes and for vaccine development.

G 131 CHARACTERIZATION OF A HIGHLY CYTOPATHIC SUBGROUP OF IMMUNODEFICIENCY VIRUS TYPE 2, Jan McClure, Genetic Systems Corporation/Oncogen, Seattle, Washington, 98121.
We have compared and analyzed seven HIV-2 isolates and one SIV isolate for polypeptide composition, antigenic variance, pathogenicity, and growth characteristics. From this comparative analysis we can identify two subgroups of HIV-2. Subgroup A, represented by the HIV-2 prototype ROD, demonstrates a moderate cytopathogenicity, a particular glycoprotein profile, and reactivity to our entire panel of HIV-2 specific monoclonal antibodies. Subgroup B demonstrates an extreme cytopathogenicity, a different glycoprotein profile, no cross reactivity with some of our HIV-2 monoclonal antibodies, and the capacity to readily infect macaque cells in vitro. Animal studies are in progress to evaluate the in vivo pathogenicity of this HIV-2 subgroup B.

G 132 ROLE OF MONONUCLEAR PHAGOCYTES AS HIV RESERVOIRS, M. Juliana McElrath and Zanvil A. Cohn, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021.

Human immunodeficiency virus (HIV) production was determined in mononuclear cells from 36 seropositive homosexual males. We detected p24 antigen production in blood mononuclear cells in 54% of asymptomatic, 71% of ARC, and 100% of AIDS patients. The CD4+ T cell fraction was preferentially infected in the 3 clinical stages. Production of HIV from blood monocyte-derived macrophage cultures was similar in the 3 clinical stages (24-33%). Bone marrow and blood mononuclear cells cultured simultaneously in 14 individuals yielded virus from both sources. Virus from bone marrow aspirates was primarily found in the mononuclear nonadherent cells, not the adherent monocyte-enriched fraction. We conclude that the mononuclear phagocyte is not an exclusive source of productive HIV infection in seropositive individuals.

G 133 POST-BINDING NEUTRALIZATION OF HIV-1 IS MEDIATED THROUGH THE IMMUNODOMINANT, TYPE-SPECIFIC EPITOPE, Peter Nara,[1], Nancy Dunlop,[1], Scott Putney,[2], Stephen Pyle,[3], Larry Arthur,[3], Peter Fischinger,[1], and Robert Gallo.[4] [1]Office of the Director, Virus Control Unit, NCI-Frederick Cancer Research Facility, Frederick, MD; [2]Repligen Corporation, One Kendall Square, Cambridge, MA; [3]Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD; [4]National Institutes of Health, Bethesda, MD.
If neutralizing antibody is to limit cell-free viral spread in the infected host it must be capable of rapidly inactivating the virus either before, during, or after adsorption to the target cell. We analyzed sera in a quantitative, HIV-1 induced, syncytial-plaque assay for their ability to neutralize both cell-free and preadsorbed HIV-1 virions. Sera from HIV-1 infected humans and chimpanzees, as well as antisera against immunoaffinity purified gp120, PB-1, and RP135 (the immunodominant, type-specific neutralizing epitope, amino acid 307-321) vaccinated animals were capable of neutralizing HIV-1 during and after adsorption to the target cell. Post-binding neutralization could be demonstrated at both 37 and 4C. Inability to remove adsorbed virus by washing or trypsin treatment indicated that neutralization did not involve CD4-gp120 binding. If vaccination with HIV subunits can induce high titered broadly reactive neutralizing antibody which can function in pre- and post-HIV binding to the cell, protection from cell-free virus challenge may be feasible.

G 134 APPLICATION OF SYNTHETIC PEPTIDES FOR DETERMINATION OF TYPE SPECIFIC ANTIBODIES TO THREE HUMAN RETROVIRUSES, Ale Närvänen, Mirja Korkolainen, Sari Kontio and Marja-Liisa Huhtala, Labsystems Research Laboratories, Pulttitie 8, SF-00880 Helsinki, Finland
We have screened, by using synthetic peptides, native antigenic determinants of the transmembrane proteins of HIV-1 and HIV-2 and the env polypeptide of HTLV-I. There are three native antigenic regions in the transmembrane protein of HIV-1, nine in the transmembrane protein of HIV-2 and three in the env polypeptide of HTLV-I. The amino acid sequences of the highly immunoreactive native antigenic determinants of HIV-1 and HIV-2 differ from each other. Interestingly these epitopes are located in hydrophobic regions of the corresponding polypeptides according to Eisenberg's hydropathy index. We show that both in HIV-1 and HIV-2 there are highly immunoreactive epitopes which remain undetectable with conventional immunochemical method e.g. in immunoblotting, EIA or RIPA.
Synthetic peptides derived from three membrane proteins give better sensitivity and specificity in the differentiation of HIV-1, HIV-2 and HTLV-I infection.

G 135 CROSS PROTECTIVE CYTOTOXIC T CELL IMMUNITY BETWEEN HIV-1 AND HIV-2, Douglas F. Nixon, Charles R. Rizza, Jonathan Rothbard and Andrew J. McMichael, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU and I.C.R.F., London, U.K.
Failure of current vaccines to protect against challenge with HIV in the presence of antibody responses underlines the importance of studying cell mediated immunity to HIV and defining the HIV epitopes that stimulate cytotoxic T lymphocytes (CTL). We have studied HIV-1 seropositive patients for CTL responses to HIV-1 gag, or synthetic peptides from the HIV-1 gag sequence. CTL were elicited by stimulation in vitro of peripheral blood mononuclear cells by autologous mitogen activated T cells. In one patient with a gag specific response we have identified a peptide epitope that is recognised by CTL in association with HLA-B27. In order to test whether this peptide could induce cross protective immunity against HIV-2, analogue peptides from the HIV-1 variant HIV-Eli, HIV-2 and SIV sequences were made and tested in the lysis assay. At an E:T ratio of 30:1 the index peptide gave 69% lysis, 15% with no peptide. HIV-2 ROD/NIH2, HIV-Eli and SIVMM142 gave lysis in the range 56-60% but SIVK6W78 gave only 39% lysis. Thus, CTL specific for a peptide sequence from HIV-1 can recognise the HIV-2 peptide from the corresponding sequence. Peptide sequences which stimulate CTL immunity against HIV-1 may thus also protect against HIV-2; such peptide sequences could be included in vaccines to induce cell mediated immunity against HIV-1 and HIV-2.

G 136 **EXPRESSION OF HIV-1 GENES BY MONOVALENT AND MULTIVALENT VACCINIA VECTORS**, Dennis L. Panicali, Gail Mazzara, Sara McKenzie, Eric Day, Sandra Katz and Janet Lyons, Applied bioTechnology Inc., 80 Rogers St., Cambridge, MA 02142. Recombinant vaccinia viruses expressing foreign proteins have been proposed for use as recombinant vaccines for a number of infectious diseases including AIDS. Vaccinia vectors can also serve as very useful tools for the assessment of specific immunological responses elicited by viral infection or vaccination, such as antigen specific cytotoxic T lymphocyte and antibody-dependent cell-mediated cytotoxic responses.

We have developed a series of both monovalent and multivalent vaccinia vectors expressing the env, gag, and pol genes of HIV-1. These can be used both as reagents for determining the nature of the immune responses specific to these antigens and for use as potential vaccine candidates. Vectors have been constructed to explore how differences in antigens, viral promoters and viral backgrounds affect expression levels and antigen presentation. We have found that expression levels of env can be highly variable depending on the promoter used while gag levels are more consistent. Additionally the strain of vaccinia used can affect immune responses in vivo.

G 137 HTLV-I REGULATION OF LYMPHOTOXIN PRODUCTION. N. L. Paul and N. H. Ruddle, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510. HTLV-I infection is associated with the production of many cytokines. Here we analyze its association with lymphotoxin (LT; TNF-β). To determine the specificity of the association between HTLV-I and LT, we have studied LT production by Northern blot and biologic assay of HTLV-I$^+$ (MT-2 and HUT-102) and HTLV-I$^-$ (H9 and Jurkat) cell lines. MT-2 and HUT-102 produce LT mRNA and protein (>1,000 U cytotoxic activity for WEHI-164 cells which is blocked by anti-LT antibody) in the absence of any exogenous stimulation. H9 and Jurkat do not produce LT constitutively, though LT mRNA and biologic activity can be induced in H9 (8 U LT cytotoxic activity) by PHA. Thus, two HTLV-I-infected T cell lines produce high amounts of LT without activation, while two uninfected human T cell lines do not.

The association of HTLV-I infection with LT production was analyzed at the molecular level by transfecting 5'-LT gene-CAT constructs into MT-2 and H9 cell lines. High amounts of CAT activity were seen in transfected MT-2 cells while in transfected H9 only small amounts were seen. This parallels the LT mRNA and protein production by these cells and suggests viral activation of LT at the transcriptional level.

We propose that LT is activated by HTLV-I, perhaps by the trans-activator gene, *tax-I*. LT has many biological activities, including B cell and osteoclast activation. HTLV-I induced activation of LT may explain manifestations of infection such as polyclonal B cell activation and hypercalcemia in adult T cell leukemia/lymphoma patients.

Supported by NIH grant RO1 CA 16885 and Training grant T32 AI 07019.

G 138 **RELATIONSHIP BETWEEN ANTIBODY DIRECTED AGAINST THE CARBOXYL-TERMINAL REGION OF HIV GP120 AND MARKERS OF HIV SPECIFIC IMMUNO-REGULATION**, V.Polonis, A. Shafferman, T. Lee, G. Smith, D. Burke and R. Redfield, WRAIR, Washington D.C. 20307. Prior studies showed an inverse relationship between viral isolation from PBMC and stage of HIV infection. This relationship could be a consequence of variations in HIV specific immunoregulation. In general, antibodies directed against envelope proteins are important in viral immunity. Although total antibody directed against gp120 does not correlate with stage of infection, antibody directed against the carboxyl-terminal region inversely correlated with stage of HIV infection. Here we evaluated the relationship between anti- 448C antibody, stage of infection and in vivo antigen load (as determined by the ability to isolate HIV from PBMC). Employing a quantitative protein dot blot assay, a panel of 135 HIV positive sera from sequential patients who underwent clinical staging and PBMC retroviral cultures were analyzed for reactivity against 448C and against B-galactosidase fusion proteins containing epitopes within 448C. The results demonstrate that in patients with late stage HIV infection (Walter Reed stage 5 and 6), the presence of antibody directed against 448C correlates with the inability to isolate HIV from PBMC. These data suggest that 448C contains an epitope (or epitopes) which correlate with both earlier stage of HIV infection and markers of effective viral immune regulation. Epitope mapping within 448C is ongoing to further define these observations.

G 139 CHARACTERIZATION OF AN HIV-2 RELATED VIRUS WITH A SMALLER SIZED ENVELOPE PRECURSOR.

Marie-Anne REY, Bernard KRUST, Anne G. LAURENT, Denise GUETARD, Yves RIVIERE, Luc MONTAGNIER and Ara G. HOVANESSIAN

Unité d'Oncologie Virale, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris 15, France.

A new cytopathogenic isolate of the human immunodeficiency virus related to HIV-2 strain was isolated from peripheral blood lymphocytes of an Ivory Coast patient with AIDS. This isolate referred to as HIV-2 MAN could be differentiated by its envelope precursor and the extracellular glycoprotein which are 20kd molecular size smaller than those of HIV-2 ROD isolate. Furthermore, the apparent molecular weight of the major core protein of HIV-2 MAN is 28kd instead of 26kd of HIV-2 ROD. In addition, the product of the vpx gene which is a characteristic feature of the HIV-2 strain, is 14kd in HIV-2 MAN compared with 16kd in HIV-2 ROD. In contrast to these, the envelope precursor of HIV-2 MAN forms a transient dimer during its maturation as it is the case for HIV-2 ROD.

Deglycosylated precursors of HIV-2 ROD and MAN have an apparent molecular weight of 80 and 60kd, respectively. Partial proteolysis of the envelope precursors from the two isolates with Staphylococcus aureus V8 protease gave a distinct pattern of polypeptides. Thus suggesting that the differences between the envelope proteins of the two HIV-2 isolates are due to their amino-acid composition. Accordingly,murine polyclonal antibodies raised against HIV-2 ROD envelope do not recognize the corresponding envelope proteins of HIV-2 MAN in immunoblotting and immunoprecipitation assays. These data emphasize the utility of analysis of HIV proteins as a rapid method to characterize new viral isolates and show the heterogeneity of HIV-2 isolates in West Africa.

G 140 COMPLEMENT-MEDIATED, ANTIBODY-DEPENDENT ENHANCEMENT OF HIV-1 INFECTION *IN VITRO*, W. Edward Robinson, Jr., David C. Montefiori, and William M. Mitchell, Department of Pathology, Vanderbilt University, Nashville, TN 37232. Utilizing an MT-2 cell *in vitro* infectivity assay, we demonstrate that HIV-1 seropositive serum can, in some cases, enhance infection by HIV-1. The two components of this phenomenon are shown to be an IgG to HIV-1 and the alternative complement pathway. Enhancing activity has been found in >90% of HIV-1 seropositive sera tested (N=90). Enhanced infections are characterized by accelerated viral protein and RNA accumulation and infectious virus release. Evidence suggests that the envelope glycoproteins of HIV-1 are important components of this antibody dependent enhancement (ADE) of HIV-1 infection. Protein N-glycosylation influences complement fixation as some inhibitors of glycoprotein processing enzymes (swainsonine and deoxymannojirimycin) cause an accelerated rate of HIV-1 infection in the presence of complement. Complement receptors present on MT-2 cells may act as the mediator of enhanced infections. ADE of HIV-1 infection is also demonstrated in sera from HIV-1 antibody-positive chimpanzees and appears to occur through a complement-mediated mechanism analogous to ADE of HIV-1 infection mediated by human serum. These findings are of potential importance in light of current HIV-1 vaccination protocols using envelope-derived vaccines.

G 141 INHIBITION OF LYMPHPROLIFERATION BY A SYNTHETIC PEPTIDE WITH SEQUENCE IDENTITY TO HIV gp41, C.L. Ruegg, C.R. Monell and Meryl Strand, Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore MD 21205

We tested the hypothesis that the transmembrane protein gp41 of HIV mediates an immunosuppressive effect analogous to that previously reported for the transmembrane proteins of other human and animal retroviruses. Peptides were synthesized containing sequences from two regions of the HIV transmembrane protein gp41, HIVenvaa581-597 and aa655-671. These sequences were selected on the basis of their homology to the previously identified immunosuppressive sequence of the transmembrane proteins p15E and gp21 of animal retroviruses and HTLV-I/II, respectively. When the peptides were tested for immunosuppressive activity, peptide aa581-597 was found to specifically inhibit human and murine lymphoproliferation to control levels, whereas peptide aa655-671 had no immunosuppressive activity. It is of interest that immunodominant B and Th cell epitopes have been mapped to the same region, aa581-597, that we show here to be immunosuppressive. Furthermore, it has been suggested that a humoral immune response to the aa581-597 region may prevent the immunosuppressive effects of gp41 in vivo. The balance between the immunosuppressive and immunogenic activities localized to this region may be critical for immunoprophylactic and therapeutic intervention and therefore warrants further investigation.

G 142 IDIOTPYE VACCINES FOR HIV BASED ON CD4 ANTIBODIES: RECENT PROGRESS
Sattentau, Q.J.*, Truneh, A.**, Arthos, J.**, Healey, D.*, Broadhurst, K.*, Rothbard, J.R.***, Beverley, P.C.L.***, Sweet, R**.
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The human immunodeficiency virus, HIV, binds to the V1 domain of the CD4 antigen via a conserved region of the envelope glycoprotein, gp120. Monoclonal antibodies (mAbs) reactive with the V1 domain of CD4 which are highly effective at blocking the CD4/gp120 interaction may mimic the binding region of gp120. Thus anti-idiotype antibodies raised against CD4 mAbs may resemble CD4 and bind to gp120, inhibiting virus binding to and infection of CD4 bearing cells. We have mapped the epitopes of the CD4 V1 domain with which a panel of CD4 mAbs reacts using a series of CD4 molecules containing site-directed mutations. This data combined with cross-inhibition studies between CD4 mAbs and their anti-idiotypes has allowed modelling of the CD4 V1 region based on an immunoglobulin light chain variable domain. We conclude that none of the CD4 mAbs that we have tested mimic the gp120 antigen in binding to CD4. This is in accordance with the weak or undertectable reactivity of the anti-idiotypic reagents with gp120. We are currently raising new mAbs against CD4 V1, which will be used to prepare further anti-idiotypic reagents.

G 143 HIV-1 INFECTION DOWNREGULATES EXPRESSION OF MHC-I GENES AT THE LEVEL OF TRANSCRIPTION, Judith A. Scheppler[1,3], Janet K. A. Nicholson[1], David C. Swan[2], A. Ahmed-Ansari[3] and J. Steven McDougal[1], Immunology Branch[1] and Cellular Biology and Biochemistry Branch[2], Centers for Disease Control, and Department of Pathology and Laboratory Medicine[3], Emory University School of Medicine, Atlanta, GA 30333.

In vitro human immunodeficiency virus type 1 (HIV-1) infection of CD4+ peripheral blood lymphocytes, and the CD4+ cell lines, CEM-E5, HT, and U937, results in decreased expression of major histocompatibility complex class I (MHC-I) molecules on the cell surface. Downregulation of cell surface class I molecules results from decreased total MHC class I protein and decreased mRNA levels for MHC-I. Downregulation is specific for class I molecules and is not due to selection of a subpopulation of cells with preexisting low level class I expression because the downregulation is apparent before significant numbers of infected cells are lost. HIV-1 infected cells are less susceptible to lysis by MHC-I-specific cytolytic T cells. Transcriptional downregulation of MHC-I and relative resistance to MHC-I-specific cytolysis may contribute to the difficulty in detecting HIV-I-specific, MHC-restricted cytolytic responses in HIV-1 infection.

G 144 ENZYME IMMUNOASSAY FOR HTLV-I ANTIBODIES USING RECOMBINANT *ENV* ANTIGEN, Jonathan Seals, Chung-ho Hung, Joanne Recchia, Richard Thorn, Virginia Braman, Annelie Wilde, Gerald Beltz, and Dante Marciani, Cambridge BioScience Corp., Worcester, MA 01605.
The increasing frequency of HTLV-I infection in the U.S. has led to recommendations for screening of donated blood for antibodies to this virus. A number of tests have been described that will utilize viral lysate in an enzyme immunoassay (EIA) format. However, tests based on recombinant viral antigens will ultimately allow greater specificity, sensitivity, manufacturing safety, and adaptability to other assay formats. For this reason, we have developed an EIA using a recombinant *env* antigen, available for research purposes, to help generate the data needed to better understand this virus. This recombinant *env* antigen contains 132 amino acids, derived from the C-terminus of gp46 and the N-terminal region of gp22. It was expressed in *E. coli* using a vector containing the bacteriophage lambda pL promoter and C_{II} gene leader sequence. The protein (14 kd) was purified to greater than 99% by a variety of chemical and chromatographic methods, and was shown to be free of *E. coli* contamination. The recombinant envelope antigen was employed in a microtitre plate EIA format using HRP-conjugated second antibody. This assay yielded positive results for 116 of 120 samples that were positive by viral lysate EIA. The specificity of the assay with 800 random sera was 99.5%. The analysis of discrepant samples by a variety of confirmatory assays has produced conflicting results concerning the nature of the samples. These samples are being further studied to characterize the viral lysate and recombinant EIA tests.

G 145 MOLECULAR ANALYSIS OF CD4 INTERACTION WITH THE LYMPHOCYTE SPECIFIC TYROSINE KINASE: ROLE IN T CELL ACTIVATION AND HIV INFECTION, R.P. Sekaly, D. Lamarre, T.W. Mak* and S. Meloche, Laboratory of Molecular Immunology, IRCM, Montreal, Canada; *Ontario Cancer Institute, Canada. It has been proposed that CD4 functions as an adhesion molecule that binds to non polymorphic regions of MHC class II antigens (Ags) and thereby stabilizes the MHC-class II T cell receptor interaction. Other data however suggest that CD4 may play a signalling role in T cell function. Hence stimulation of T cells with cross-linked or immobilized anti-CD3 or anti-TcR antibodies in the absence of APC or any source of class II MHC, can be inhibited by anti-CD4 antibodies. More recently B66, a CD4 specific antibody, has been shown to induce proliferation and differentiation of resting $CD4^+$ T cells confirming that CD4 can transmit a positive signal. It has been recently shown that CD4 is possibly associated intracellularly with the lymphocyte specific tyrosine kinase (lck). We are introducing CD4 or truncated forms of CD4 together with or without a cDNA encoding lck into T cells which are $CD4^-lck^-$. We have already established that these cells express a T cell receptor and can be activated by anti-TcR antibodies to produce various lymphokines. Our experiments will enable us to determine if the signalling function of CD4 (activation with CD4 mAb) operates via an intracellular association with lck. Moreover since we have introduced lck and CD4 into various type of cells, we will determine the specific phosphorylation substrates of this tyrosine kinase. Since CD4 is also the receptor for HIV, we will determine if the expression of lck is required for HIV infection.

G 146 CONTROL OF HIV REPLICATION IN CD4 LYMPHOCYTES: "LATENCY" AND INDUCTION OF ACTIVE VIRAL REPLICATION. Celsa A. Spina, John Guatelli and Douglas D. Richman, VA Medical Center and UCSD School of Medicine, San Diego, CA 92161.

Although it is believed that HIV can establish a latent or persistent infection in CD4 lymphocytes, it is not understood how the virus is maintained in this quiescent state, or which components of cell activation and replication initiate a productive virus cycle. The delineation of these mechanisms is essential to the understanding of HIV-induced pathology and to the design of rational, effective intervention therapies. To address these questions, we have chosen an experimental system using isolated, purified CD4 lymphocytes from healthy HIV seronegative donors, *in vitro* infection with the LAV-1 strain of HIV, and sequential biochemical signals of T cell activation. Our studies show that HIV can infect and establish a persistent state in unstimulated, non-replicating CD4 cells isolated from peripheral blood. Low levels of soluble p24 antigen are detectable as early as 24 hrs. post-infection and gradually increase over an 8-10 day period; viral RNA is also detectable by hybridization with cDNA probes. Binding of PHA to the $CD3/T_i$ receptor complex of these persistently infected CD4 cells induces increased transcription of viral nucleic acids and a burst of p24 production. Cycles of active virus replication can be induced in such cultured cells by the addition of PHA at varying times after HIV infection, up to 10 days. Using various combinations of biochemical cell activation signals, addition of calcium ionophore (A-23187) plus PMA induces a productive cycle of HIV replication which consistently surpasses the levels achieved with PHA stimulation. The addition of A-23187 or PMA alone does not induce virus replication. Further experiments in this system have determined that: 1) full induction of HIV replication by PHA activation is dependent on the addition of exogenous IL-2; induction by PMA + A-23187, is not; and 2) although PHA induces similar levels of IL-2 receptor expression on HIV-infected and uninfected CD4 cells, stimulation of cellular DNA synthesis in the presence of IL-2 is greatly diminished in the HIV-infected cells. Studies are now in progress to determine the presence and relative quantity of specific viral mRNA transcripts in these CD4 cells, persistently infected with HIV and during the inductive phases of active virus replication.

G 147 ANTIBODIES TO sCD4 IN SERA FROM HIV-1 INFECTED INDIVIDUALS, Clotilde Thiriart, Jaap Goudsmit2, Peter Schellekens3, Francis Barin4, Daniel Zagury5, Michel De Wilde and Claudine Bruck, Molecular and Cellular Biology Department, Smith Kline-RIT, 89 rue de l'Institut, B-1330 Rixensart, Belgium, 2Human Retrovirus Laboratory, AMC, Amsterdam, The Netherlands, 3Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, 4Centre Hospitalier Régional et Universitaire Bretonneau, Laboratoire de Virologie, 37044 Tours, France, 5Institut Jean Godinot, Université P. et M. Curie, 4 place Jussieu, Paris, France.

A direct enzyme immunoassay (EIA) using the recombinant soluble form of CD4 (sCD4) produced in rodent cells as antigen was applied to detect antibodies to CD4 in sera from HIV-1 and HIV-2 infected patients. High titers of antibodies to sCD4 were found in sera from 12.6 % of the 253 HIV-1 infected persons included in this study, but not in 120 normal human sera. The reactivity of these antibodies with sCD4 was confirmed by a Western Blot analysis. Sera from HIV-2 infected persons (9 sera analyzed), sera from HIV-1 infected chimpanzees (10 sera analyzed) and sera from humans immunized with a recombinant vaccinia virus exprssing gp160 (10 sera analyzed) scored negative for antibodies to sCD4. Attempts to correlate the evolution of the disease with the presence or absence of serum antibodies to sCD4 in a panel of well-documented HIV-1 seropositive cases did not reveal any clear correlation. The occurrence of CD4 anti-CD4 complexes, either soluble or at the surface of patient lymphocytes, will have to be assessed before a possible role for anti-CD4 reactivity in AIDS can be excluded.

G 148 DETECTION OF HIV ANTIBODIES USING A SINGLE-STEP IMMUNOBLOT, WILLIAM J. TODD, D. GENE LUTHER, NELL G. MORRIS, PAUL A. BARSTAD[1] AND LARRY O. ARTHUR[2]. VETERINARY SCIENCE, MICROBIOLOGY AND PARASITOLOGY, LSU AND LAES, BATON ROUGE, LA 70803; [1]NIAID VACCINE DEVELOPMENT SECTION, [2]NCI BIOLOGICAL PRODUCTS LABORATORY, [1,2]PROGRAM RESOURCES, INC., NCI-FREDERICK CANCER RESEARCH FACILITY, FREDERICK, MD 21701.

WE DEMONSTRATE THAT PARTICLE MARKERS, SUCH AS PROTEIN A COATED COLLOIDAL GOLD, CAN BE USED AS PROBES TO DETECT ANTIBODIES OF TEST INTEREST IN THE PRESENCE OF ALL OTHER ANTIBODIES. THIS OBSERVATION PERMITS USE OF THE SENSITIVE ANTIBODY LABELING METHODS IN SIMPLIFIED TEST FORMATS. HERE WE DESCRIBE A SINGLE-STEP IMMUNOBLOT TO DETECT THE PRESENCE OF HIV SPECIFIC ANTIBODIES IN HUMAN SERA. THE ASSAY IS DONE BY ADDING MICROLITER AMOUNTS OF SERUM OR WHOLE BLOOD TO A TEST VIAL CONTAINING THE HIV TARGET ANTIGEN ATTACHED TO A NITROCELLULOSE STRIP, AND ONE ML OF PROTEIN A COATED COLLODIAL GOLD. IF HIV SPECIFIC ANTIBODIES ARE PRESENT IN THE TEST SAMPLE, A RED SPOT WILL BE FORMED AT THE ANTIGEN SITE, INDICATING A POSITIVE TEST. IF HIV ANTIBODIES ARE NOT PRESENT, THE SITE OF THE ANTIGEN WILL REMAIN UNCOLORED. DETERGENT LYSATES OF PURIFIED HIV-1 VIRIONS AND HPLC PURIFIED CORE ANTIGEN, P24, WERE BOTH SUCCESSFULLY USED AS ANTIGENS FOR HIV SEROLOGY USING THIS ONE-STEP METHOD. THE RESULTS ARE COMPARED TO DATA OBTAINED BY RADIO-IMMUNE PRECIPITATION.

G 149 COMPARISON OF REGULATION OF HIV-1 AND HIV-2 GENE EXPRESSION BY T-CELL ACTIVATION SIGNALS, Sandra E. Tong-Starksen, Paul A. Luciw and B. Matija Peterlin, Department of Medicine, Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143

A second human retrovirus, HIV-2, has been described in West Africa as the etiologic agent of a clinical syndrome identical to the AIDS of HIV-1 but perhaps associated with a longer asymptomatic phase. The LTR's of HIV-1 and HIV-2 respond to the mitogenic T-cell activation signals, phytohemagglutinin (PHA) and phorbol myristate acetate (PMA). The responsive *cis*-acting element in the HIV-1LTR is the trancriptional enhancer that includes two repeated sequences with functional and structural similarity to nuclear factor κ B(NFκB) binding sites. The HIV-2 enhancer contains only one such site (C box, positions -108 to -98); the more proximal site (D box, positions -94 to -84) is divergent. Although the C box conferred inducibility by T-cell activation signals and bound NFκB, this element did not account for the full response of the HIV-2LTR to PHA and PMA. The D box did not confer inducibility and did not bind NFκB, but displayed basal enhancer function. In contrast to HIV-1, regulation of HIV-2 gene expression by PHA and PMA involves an interaction between the HIV-2 enhancer, upstream elements, and the region responsive to the *trans*-activator, *tat*. These observations suggest that T-cells infected with HIV-2 may require stronger antigenic stimulation to allow high level expression and productive infection.

G 150 A MONOCLONAL ANTIBODY TO SIV_{MAC} ALLOWS THE AMINO TERMINAL SEQUENCE OF GP32 AND THE IDENTIFICATION OF A HYPERVARIABLE REGION, Fulvia di Marzo Veronese, Toshiaki Kodama, Ronald C. Desrosiers, Robert C. Gallo and Mangalasseril Sarngadharan, Bionetics Research, Inc., Rockville, MD 20850, New England Regional Primate Research Center, Harvard Medical School, Southboro, MA 01772, and Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20892. A monoclonal antibody recognizing an antigenic determinant on the env transmembrane protein, gp32 of SIV_{MAC} has been developed and designated SF8/5E11. Radiolabel sequencing of the amino terminus of both gp160 and gp32 confirmed the position predicted for both cleavage sites. The cleavage site between the signal peptide and the external env glycoprotein resides between the cysteine residue at position 21 and the threonine residue at position 22 starting from the first residue after the env gene initiator methionine. The env precursor polyprotein gp160 is cleaved between arginine 526 and glycine 527 to give rise to the external glycoprotein and the transmembrane of SIV_{MAC}. The reactivity of SF8/5E11 was found to be type specific, since it did not crossreact with neither SIV_{SMM} or SIV_{MNe} transmembrane proteins. Futhermore, this monoclonal did crossreact with a number of molecular clones from individual macaque isolates other than the original SIV_{MAC}, but failed to crossreact with some others. Thus, it appears that the epitope recognized by this antibody is situated in a hypervariable region of gp32. Studies aimed at identifying this region will be discussed.

G 151 COMMON AND UNIQUE T CELL EPITOPES OF HIV-1, Britta Wahren, Jonathan Rosen, Tiit Mathiesen and Hans Wigzell, Department of Virology, National Bacteriological laboratory, S-105 21 Stockholm, Johnson & Johnson Biotechnology Center, La Jolla, California, Department of Immunology, Karolinska Institute, S-104 01 Stockholm

The aim of this study was to delineate HIV peptides to which patients react during a natural infection. Peptides covering the gag and part of envelope regions of HTLV-III$_B$ were synthesized as overlapping linear sequences. Representing the envelope, the peptides causing cellular proliferation were a) from a hyper-variable region which also induced neutralization, b) from a constant region with both immunogenic and proliferation-inducing properties and c) from the CD4-binding/T1 region. IgG reactivity was concentrated in the aminoterminal peptides of the known immunodominant region of gp41. Gag peptides gave the highest and most frequent T cell proliferative responses, whereas gp41-derived peptides displayed the best Ig reactivity. These informations on immune responses in natural infection are important for attempts of inducing protective T cell responses.

G 152 Group and Type-Specific Cytotoxic T Lymphocytes (CTL) in HIV-1 Infection. Bruce D. Walker*, Karen Birch-Limberger*, Silvia Marli**, Bernard Moss**, Robert T. Schooley*. *Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; **Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD.

Freshly isolated peripheral blood mononuclear cells from HIV-1 infected individuals demonstrate equivalent lysis of target cells expressing the recombinant HTLV-IIIB envelope and the HTLV-IIIRF envelope. In contrast, an HIV-1 envelope specific CTL clone, designated 63D45 (obtained by limiting dilution in the presence of IL-2 and a non-specific stimulus [anti-CD3]), lyses the envelope IIIB-expressing target cell up to 70% above control at an effector: target ratio (E:T) of 2.5:1, and does not lyse the evelope IIIRF-expressing target cell. The effector cell is a CD8+ lymphocyte, and lysis is restricted by the HLA class I antigen B8. In contrast, RT-specific CTL obtained from this same patient are restricted by the class I antigen Bw62. After 65 days in culture, with periodic restimulation with feeder cells and anti CD3 monoclonal antibody, there has been no loss of CTL activity with this clone. Using this technique, we have generated a number of HIV-specific CTL clones. This approach should prove useful in mapping the epitopes of HIV recognized by CTL, and may facilitate rational subunit vaccine design.

G 153 SIV INFECTS A MINOR SUBSET OF CIRCULATING CD4+ LYMPHOCYTES IDENTIFIED BY CELL SURFACE MARKERS LINKED TO ACTIVATION.

Dennis M. Willerford*, Mike Gale#, Edward A. Clark# and W. Michael Gallatin*
Fred Hutchinson Cancer Research Center *, and the Washington Regional Primate Research Center#, Seattle, Washington.

Using the Polymerase Chain Reaction primed with oligonucleotides from the gag region of SIV, we were able to localize viral sequences to a minor subset of activated CD4+ lymphocytes from viremic macaques infected with SIV/mne. Using dual-color flow cytometry on pre-selected CD4+ lymphocytes we identified subpopulations by surface expression of a 95kd mw heterotypic adhesion receptor (HAR) and CD45R. These markers identify at least 4 subpopulations representing distinct maturational stages in the pathway of T-cell activation: differing in cell cycle position, signalling requirements for proliferation to CD3 crosslinking, migratory behavior *in vivo*, and effector function, including immunologic memory. When these subpopulations were isolated, SIV was found exclusively in one of these subsets. We conclude that SIV infection *in vivo* depends on a discrete step in the activation of CD4+ T-cells. Approaches to defining cellular requirements for SIV infection will be discussed.

HTLV and HIV-I

G 200 ANALYSIS OF GENETIC DETERMINANTS FOR MONOCYTE/MACROPHAGE TROPISM OF HIV-1,
Mikulas Popovic, Barbara Beaver, Suzanne Gartner, Elizabeth Read-Connole, Robert C. Gallo, Marvin Reitz, Jr., Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, MD 20892
Different HIV-1 isolates exhibit heterogeneity with respect to their preferred natural host cell type. We have studied an HIV-1 isolate designated HTLV-III$_{Ba-L}$, which is able to grow on monocyte/macrophages (M/M). We have cloned a 4.2 kb fragment of HTLV-III$_{Ba-L}$ proviral DNA from infected M/M which contains parts of the vif and nef genes and the entire tat, rev and env genes.
Various biologically-active clones of hybrid viruses were constructed by substitution of analogous regions of HTLV-III$_{HXB2D}$ with HTLV-III$_{Ba-L}$ and transfected into cos-1 cells. Several such constructs were either not infectious or could only be transmitted by co-cultivation. One, however, which contained a 3' portion of gp120 coding region and a 5' portion of the gp41 from HTLV-III$_{Ba-L}$ was infectious for both normal T-cells and M/M, even under cell-free infection conditions. In addition, 50- to 100-fold less of this hybrid was required for infection than was required of HTLV-III$_{HXB2D}$. This suggests that at least some of the genetic determinants for growth on M/M lie within the env gene.

G 201 CHARACTERIZATION OF MONOCYTOID CELL CLONES DEFECTIVE FOR HIV-1 REPLICATION,
François Boulerice, Romas Geluziunas, Alla Lvovich and Mark A. Wainberg, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada, H3T 1E2.
We infected the monocytoid cell line U-937 with HIV-1 and established a persistently-infected population of such cells. From this, single clones of cells were derived by a limiting dilution technique. As judged by immunofluorescence, all clones obtained were postive for p24. While a majority of these clones produced high titers of viral reverse transcriptase, some of them (27%) were negative in this regard. No infectious particles were found in the cell supernatant of these clones or in respective cell lysates obtained by freeze-thawing. These cells were negative by immunofluorescence for both p51/66 and p.15. Southern blotting revealed the presence of proviral DNA. However, mRNA expression analysis revealed differences corresponding to these altered protein profiles, in comparison with viral mRNA expression in replication-competent clones. These findings suggest that an abortive viral replication cycle may take place in some monocytes/macrophages. Such a phenomenon may have implications in terms of the role of such cells in both viral latency and in persistent viral infection.

G 202 HIV LTR ACTIVATION IN XENOPUS OOCYTES : TAT ACTIVATES POST-TRANSCRIPTIONALLLY BUT IN THE NUCLEUS,
Martin Braddock, Alistair Chambers, Wilma Wilson, Martin Baron, Sally E. Adams, Keith Gull, M. Peter Esnouf, Alan J. Kingsman and Susan M. Kingsman, Department of Biochemistry, University of Oxford, British Biotechnology, Cowley, Oxford,
The tat gene product of Human Immunodeficiency Virus (HIV-1) stimulates gene expression via the TAR element in the HIV-1 LTR and at the 5' ends of HIV mRNAs. The present mechanism of action of tat is, at present unclear. We have developed an expression system using particulate Ty-VLPs (Adams et al; Nature 1987, and Cell; 1988) recently modified which produces free, soluble tat protein by cleavage of the Ty-tat fusion protein with factor Xa protease (Braddock et al; Cell submitted). We show that the LTR is activated by tat in Xenopus oocytes, that tat must be localised in the nucleus for activation and using -amanitin, that tat acts post-transcriptionally. The role of a putative nuclear localisation sequence is under investigation. This expression strategy is applicable to probing the localisation of any cellular or viral protein.

G 203 MUTATIONAL ANALYSIS OF HIV-1 TAT AND TRANS-ACTIVATING RESPONSIVE (TAR) ELEMENT. D. Brake, M. Rosenberg and C. Debouck, Dept. of Molecular Genetics, Smith Kline and French Laboratories, King of Prussia, PA 19406.
Experiments were conducted to determine the subcellular localization of tat wild-type and deletion mutants in COS-1 cells transiently transfected with tat-expression plasmids. Indirect immunofluorescence of wild-type tat revealed a predominant nucleolar localization. Surprisingly, functionally inactive tat deletion mutants lacking either the cysteins-rich or lysine/arginine-rich region retained nuclear/nucleolar localization. Electron microscopy studies are in progress to more clearly define tat subcellular compartmental localization.
We have shown that when the trans-activating responsive (TAR) element is positioned at the 5' end of an mRNA, the translation of this message is substantially reduced. In an effort to understand this phenomenon, single base alterations in the putative TAR stem-loop structure were tested for their inhibitory effect on message translation in rabbit reticulocyte extracts as well as in Xenopus oocytes. Correlations between relative translational efficiency and ability to confer tat-dependent trans-activation will be presented.

G 204 HETEROGENITY IN INDEPENDENT HIV 1 ISOLATES, R. W. Buckheit, Jr.[1], M.W. Cloyd[2], and Ronald Swanstrom[1] [1]Department of Biochemistry, University of North Carolina at Chapel Hill; [2]Department of Microbiology, University of Texas Medical Branch Galveston, Texas
Infection of CEM cells with independent isolates of the human immunodeficiency virus (HIV) results in cytopathicity, cell killing, and the release of infectious progeny virus. We have quantitated the results of infection with eleven independent isolates of HIV and found that significant heterogeneity exists between isolates in each of these parameters. (1) Infection with different isolates results in the induction of a range of cytopathic effects, from large giant cell formation to cell clumping. (2) Different isolates induce cell killing at different rates but the relative ordering of viruses is dependent on the method chosen to standardize the amount of virus used to initiate infection. (3) Even when infections are initiated with equivalent amounts of virus, each isolate has distinctive growth properties in CEM cells. In addition, each isolate associates to varying degrees with a cellular nuclease which masks RT activity, leading to an apparent absence of RT activity with certain isolates.

G 205 POLYMERASE CHAIN REACTION AS A MEASURE OF VIROLOGIC LOAD IN HIV-INFECTED PATIENT SAMPLES, B.C. Byrne*, J.J. Li*, J.J. Sninsky+ and B.J. Poiesz*, *Department of Medicine, SUNY HSC at Syracuse, 750 E. Adams St., Syracuse NY 13210 and +Cetus Corporation, 1400 Fifty-third Street, Emeryville, CA 94608. Polymerase chain reaction (PCR) increases the concentration of a specific nucleotide sequence by many orders of magnitude in vitro, thus rendering rare sequences detectable by hybridization. Used alone, the DNA polymerase from Thermus aquaticus (Taq) recognizes only DNA; in conjunction with reverse transcriptase a population of cDNA molecules mirrors a sequence-specific population of RNA in a preparation and PCR can amplify the cDNA. Thus PCR and RT/PCR hold the potential to identify latent infection by DNA-based amplification and active infection by RNA-based amplification. We employed PCR to follow changes in proviral copy concentration among 160 samples from 42 patients involved in antiviral drug trials and compare variations in this parameter with other measures of viremia. Further, we show optimization studies for the development of RT/PCR, demonstrate its sensitivity and reliability, and compare PCR and RT/PCR as measures of HIV status among fifty seropositive individuals and an equal number of individuals from control populations.

G 206 SEROLOGICAL REACTIVITY OF RECOMBINANT HTLV-I PROTEINS, S. Coates, A. Harris, E. Keitelman, D. Parkes, C. Smith, M. Ferrer, H. Liu, S. Wallingford, Y. Teramoto, J. Brandis and M. Sliwkowski. Triton Biosciences Inc., 1501 Harbor Bay Parkway, Alameda, CA 94501. Three proteins (*env*, *gag*, and *tax*) encoded by the HTLV-I genome were cloned and expressed in *E. coli*. The envelope protein contained a substantial part of gp46 and a majority of the p21e domain. The *gag* construct encoded all of p24 and portions of p19 and p15. In addition to these two structural proteins, a full-length *tax* (p40X or *tat*) construct was also obtained. All three recombinant proteins were purified to near homogeneity. These purified proteins were then evaluated for their utility in detecting antibodies in individuals infected with HTLV-I. Recombinant *env*, *gag* and *tax* proteins were used in an immunoblot assay to test for reactivity with human sera. Fifty sera known to have antibody to HTLV-I viral lysate by ELISA, and fifty normal individuals were evaluated. Antibodies to *r-env*, *r-gag*, and *r-tax* were detected in 90%, 98%, and 84%, respectively, of patients known to be infected with HTLV-I. No false reactives were detected with normal samples tested. Two thousand human sera, from a population known to be at risk for HTLV-I infection, were tested by ELISA for antibodies to recombinant *tax*. Antibodies to recombinant *tax* were found in 155 (7.8%) of these sera. Almost half of these sera (71 of 155) scored negative when tested with commercially available viral lysate ELISA. These 71 sera were retested by radioimmune precipitation assays and by immunoblotting. Some of these sera (41 of 71) were found to be reactive with *env* and *gag*, in addition to *tax*. Furthermore, some serum samples appeared to have a humoral immune response limited only to the *tax* protein. These studies demonstrate the utility of these recombinant proteins in detecting antibody responses to HTLV-I infection.

G 207 *cis*- AND *trans*-ACTING COMPONENTS OF THE HIV *rev* REGULATORY PATHWAY. Alan Cochrane, Ann Perkins, Steven Ruben, Chein-Hwa Chen and Craig A. Rosen, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110.
Expression of the HIV *rev* protein is required for expression of virion structural genes. Previous studies have shown that the *rev* protein may facilitate virus replication by the relief of an inhibitory effect exerted by sequences located within the *env* gene. We demonstrate that the existence of such an inhibitory sequence is not unique to this gene. Rather, using a heterologous assay, the existence of functionally similar inhibitory sequences was detected within both the HIV *gag* and *pol* genes. In addition, the inhibitory effect exerted on gene expression by these sequences was alleviated by addition, in *cis*, of the CAR (*cis*-acting *rev* responsive element) sequence and expression, in *trans*, of the *rev* protein. The definition of these new inhibitory sequences has facilitated preliminary characterization of the minimal sequence elements required for *rev* regulation.

In parallel with the above studies, site-directed mutagenesis of Rev was used to define those features, including both primary sequence and post-translational modification, which are essential for Rev function. Our results demonstrate that Rev is a nucleolar phosphoprotein. The requirement of this modification to the protein's capacity to function as a transactivator *in vivo* will be presented.

G 208 HIV INFECTION OF PRIMARY HUMAN MONOCYTE-DERIVED MACROPHAGES, R. Collman, H. Freidman, S. Douglas, R. Walker, J. Hastings, N. Hassan, F. Gonzalez, N. Nathanson, University of Pennsylvania Medical Center, Philadelphia, PA 19104-6076

We are studying the natural variation in cellular tropism of HIV isolates. We have refined standard methods for the culture of human monocyte-derived macrophages and compared them with peripheral blood lymphocytes, a T cell line (SUP-T1), and a monocytoid line (U937). Using well characterized reference viruses which are T lymphocyte-tropic (strain 3B) or monocyte-tropic (strains SF162; AD; DV) and several assays for virus replication (p24 antigen production, ID50, RT, and immunofluorescence) we have observed a reproducible pattern of tropism. (i) all reference strains replicate freely in PBLs; (ii) monocyte-tropic but not lymphocyte-tropic strains replicate in macrophages but to lower titer than in PBLs; (iii) lymphocyte-tropic and some monocyte-tropic strains replicate freely in SUP-T1 and U937 cells (U937 cells resemble the host range of SUP-T1 cells but show no cytopathic effect). This descriptive matrix is being used to characterize the tropism range of fresh isolates from peripheral blood and neural sites. Studies are under way to (a) clone selected isolates; (b) examine the role of CD4 in viral entry; (c) compare neutralizing epitopes for macrophage- and lymphocyte-tropic viruses. Recent results will be reported.

G 209 **Targeting AIDS DNA With Sequence Reading Ligands.** James C. Dabrowiak, Gregory M. Raner, Frank Guiliano, Hiroko Kishikawa and Ying-Ping Jiang, Department of Chemistry, Syracuse University, Syracuse, New York 13244-1200.

In an effort to identify new types of agents for treatment of AIDS at the DNA sequence level, we have carried out footprinting and strand scission studies on a segment of the LTR of HIV. Two DNA molecules, a 65-mer and a 71-mer possessing sequences derived from ~-108 to ~-43 of HIV were chemically synthesized and purified using gel electrophoresis. The compounds were single end-labeled and subjected to chemical cleavage with a probe which identifies low melting regions of DNA. Cleavage occurred within the κB elements and two of the three SP1 binding sites closest to the start site. The 71-mer possesses terminal non-identical restriction sites which allows for its incorporation into a pUC-13 derived plasmid, Δ-71-c-fos-CAT, to yield a new plasmid HIV-CAT, having the chloramphenical acetyltransferase (CAT) gene under the control of the sequences present in the 71-mer. Progress on both footprinting studies involving small ligands capable of binding to AIDS sequences and the development of an assay system to test the ability of the ligands to influence gene expression will be reported. Research supported by the American Foundation for AIDS Research.

G 210 ENZYMATIC AMPLIFICATION OF EXOGENOUS AND ENDOGENOUS RETROVIRAL SEQUENCES FROM DNA OF PATIENTS WITH TROPICAL SPASTIC PARAPARESIS, Susan Daenke, Charles R.M. Bangham, Rodney E. Phillips and John I. Bell, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom.

Using oligonucleotide primers that hybridize to conserved sequences in the reverse transcriptase (RT) gene, we have amplified by the polymerase chain reaction three sequence variants of HTLV-I from the genomic DNA of five patients with tropical spastic paraparesis (TSP), and a fourth variant from a healthy carrier of HTLV-I. This strategy, combining the sensitivity of the polymerase chain reaction with cross-reactive primers, may be useful in the search for known or novel retroviruses in other diseases of possible retroviral aetiology. We have also used non-cross-reactive primers to amplify other regions of the HTLV-I genome which may be concerned in determining the tissue tropism of the virus.

G 211 VPR IS DISPENSABLE FOR HIV INFECTIVITY, REPLICATION, & CYTOPATHICITY Douglas Dedera and Lee Ratner, Department of Medicine, Washington University, St. Louis, MO 63110

Objective: The vpr gene is found in HIV-1, HIV-2, and SIV-MAC and antibodies to VPR are found in HIV-1 infected individuals. The goal of this study was to define the activity of VPR.

Methods: A functional proviral clone of HIV-1, X, was used to construct proviral clones with frameshift or site-directed mutations which truncate VPR from 78 to 40, 31, 22, or 2 amino acids, respectively. Each clone was transfected into COS-1 cells, and then cocultivated with H9 cells to produce virus preparations.

Results: Viruses produced from each vpr mutant provirus were compared to that produced by the parental provirus, X. No significant differences were noted in their infectivity, replication properties, or cytopathicity for different lymphoid cell lines (Jurkat, H9, Molt 3, CEM, Sup T1), monocytoid cell line (U937), or peripheral blood lymphocytes. Southern blot hybridization confirmed the presence of the mutation in DNA from the infected cells, and demonstrated a similar level of DNA seqeunces in cells infected with the X virus and those infected with the vpr mutants.

Conclusions: VPR is dispensable for virus replication and cytopathicity in tissue culture. Experiments are currently in progress to define the role of vpr in animal model systems.

G 212 GENES OF HUMAN IMMUNODEFICIENCY VIRUSES, THEIR EXPRESSION IN ESCHERICHIA COLI, AND THEIR UTILITY IN DIAGNOSIS OF VIRAL INFECTION. S.M. Desai, K.R. Rupprecht, J.M. Casey, R.A. Aneja, D.E. Kramer, D.M. Braun, R.A. Gutierrez, K. Boardway, S. Stramer, R.G. Allen, H. Hampl, G.J. Dawson, and S.G. Devare, Abbott Laboratories, Abbott Park, IL 60064.

Human immunodeficiency viruses type I and type 2 (HIV-I and HIV-2) have been implicated in acquired immunodeficiency syndrome (AIDS). The structural genes of these viruses encoding antigens are useful in developing diagnostic assay procedures to detect viral specific immune response in the infected individuals. The presence of specific antibodies to viral antigens thus provides a means to diagnose virus infection. The recombinant DNA derived HIV-I and HIV-2 antigens were compared with tissue culture derived viral proteins in an attempt to evaluate their utility to replace viral antigens in diagnostic assays. Structural as well as nonstructural proteins were molecularly cloned and expressed in Escherichia coli. The data on comparative evaluation indicates that 1. There is equivalency in rDNA proteins and tissue culture derived antigen for the detection of antibodies to HIV. 2. The rDNA derived antigens can be effectively used for screening as well as confirmation of diagnosis of viral infection. 3. The rDNA derived antigens also can be used for discriminating infection of HIV-1 from that of HIV-2.

G 213 ANALYSIS OF THE C-TERMINAL DOMAIN OF THE HIV gp41 BY INSERTION OF PREMATURE TERMINATION CODONS: EFFECTS ON INFECTIVITY AND PROTEIN SYNTHESIS. John W. Dubay[1], Lilly Kong[2], John Kappes[2], Beatrice Hahn[2], Eric Hunter[1]. [1]Department of Microbiology, [2]Department of Medicine, University of Alabama, Birmingham, Alabama

The TM glycoprotein (gp41) of the human immunodeficiency virus type 1(HIV-1) contains an unusually long cytoplasmic domain with several regions highly conserved among various isolates. To determine the functional role of the cytoplasmic domain a series of nine point mutations, each resulting in premature termination codons, were inserted into the carboxy-terminal coding region. The termination codons resulted in the deletion of 18 to 191 amino acids from the C-terminus of gp41 without affecting *tat* or *rev* reading frames. These mutated envelope genes were inserted into an infectious molecular HIV-1 clone (pHXB2Dgpt) and the effects of the mutations on syncitia formation and protein synthesis were determined by transfection of the clones into Cos-1 and SupT-1 cells. Mutations that resulted in termination prior to the hydrophobic anchor domain resulted in a secreted glycoprotein complex and as expected the virus was non-infectious. Surprisingly the deletion of just 18 amino acids from the C-terminus of gp41 yielded virus greatly reduced in its ability to form syncitia. Further deletions resulted in a partial restoration of syncitia formation up to a deletion of 108 amino acids in which near normal syncitium formation was observed. Deletions greater than this result in virus unable to replicate or form syncitia. None of the mutations prevented virion assembly or release. We conclude from these results that the cytoplasmic domain contains at least two regions required for glycoprotein function. The first of these is region at the C-terminus that has been predicted to form two amphipathic helices, which when disrupted by the above mutations yields a virus incapable of forming syncitia. The second region coincides with a conserved hydrophilic domain within the first 50 amino-acids of the cytoplasmic domain of HIV-1 which when mutated renders the virus non-infectious. Experiments aimed at further characterizing the molecular basis of these defects will be discussed.

G 214 EFFECT OF HUMAN T CELL LEUKEMIA VIRUS, TYPE I tax PROTEIN ON THE ACTIVATION OF THE HUMAN VIMENTIN GENE. Madeleine Duc Dodon, Alain Lilienbaum, Denise Paulin & Louis Gazzolo. Immuno-Virologie Mol. Cell. UMR5 CNRS-UCBL Fac Médecine A. Carrel 69372 Lyon; Labo. Biol. Mol. Paris 7-Institut Pasteur, Paris- FRANCE

Vimentin is a cytoskeletal protein that belongs to the family of intermediate filaments. The gene coding for this protein behaves as a growth regulated gene in many cells, including fibroblasts and human peripheral blood mononuclear cells. The steady state levels of vimentin mRNA are cell-cycle dependent. In human lymphoid cells, the level of vimentin expression varies in different lineages. In fibroblastic cells, this level can be increased by addition of serum. We have found that HTLV-I transformed T cells contain a higher amount of vimentin RNA (four times) than HTLV-I negative lymphoblastoid T cell. A similar increase in vimentin mRNA expression was observed in a HTLV-I transformed non-producer T cell line, expressing only the viral trans-activating protein "tax" coded by the pX gene. These findings suggest that vimentin mRNA induction is linked to the expression of the tax protein. To investigate the effect of the tax protein on the activation of vimentin gene, the promoter region of this gene has been isolated, sequenced and linked to the reporter "chloramphenicol acetyl transferase" gene. The activity of this recombinant gene was analysed for transient expression by cotransfection with a tax protein expression vector into Vero, HeLa and U937 cells. Our results indicate that the tax protein is able to activate the human vimentin gene in these cells. Furthermore, experiments performed with deletion mutants of the vimentin promoter showed that the 5' flanking region of the human vimentin gene which is required for activation by the tax protein was mapped within 241 bp upstream from the transcription initiation site. This specific sequence responsive to tax induction is different to the known sequence responsive to serum induction.

G 215 RECOMBINANT ELISAS FOR THE DIFFERENTIAL DIAGNOSIS OF ANTIBODIES TO HIV-1 AND HIV-2, S.R. Earle, T.A. Harper, J.M. Casey, T.J. Bolling, W. Mandecki, S. Desai, F. Barin*, and J.P. Allain, Abbott Labs, Abbott Park, IL 60064 and *Virology Lab, CHRU Brettonneau, Tours, France.

The etiological agents of AIDS, HIV-1 and HIV-2, cause identical clinical symptoms in infected individuals. The current screening tests for HIV-1 are not type-specific but show extensive cross-reactivity with anti-HIV-2. Due to this, it was of interest to develop tests that could accurately distinguish between antibodies to HIV-1 and HIV-2. Synthetic DNA constructs coding for the N-terminal portion of the transmembrane envelope proteins of HIV-1 and HIV-2 were prepared in cloning vectors. Both recombinant HIV proteins were expressed as fusion proteins in *E. coli* at levels up to 20% of total cell mass. Separate ELISAs were made using the purified recombinant proteins of either HIV-1 or HIV-2. Each was coated on a solid phase for capture of antibodies and coupled to HRPO for use as a probe for detection of bound antibodies. Sera or plasma from 198 HIV-1 and 56 HIV-2 infected individuals were evaluated. Samples were documented by specific western blot. 100% of the HIV-1 samples were positive in the HIV-1 ELISA, while 11% reacted in the HIV-2 ELISA. 98% of the HIV-2 specimens were positive in the HIV-2 ELISA, while 4% were reactive in the HIV-1 test. By plotting sample to cutoff ratios for HIV-1 and HIV-2 reactivity, 253/254 samples could be accurately diagnosed. These results demonstrate that the N-terminal portion of the transmembrane envelope proteins of HIV-1 and HIV-2 can be used to develop type-specific immunoassays.

G 216 **Detection of Anti-HTLV-I Tax Antibodies in HTLV-I ELISA Negative Individuals and TSP/HAM Patients: Confirmation Infection by PCR**

Garth D. Ehrlich[1], M.A. Abbott[1], S. Bhagavati[2], J.B. Glaser[3], M. Sliwkowski[4], J.J. Sninsky[5], B.J. Poiesz[1], Departments of Medicine, SUNY HSC at Syracuse 13210[1] and Brooklyn 11203[2], Richmond Memorial Hospital, Staten Island, NY 10304[3], Triton Biosciences, Alameda, CA 94501[4], Cetus Corporation, Emeryville, CA 94608[5].

The HTLV-I tax gene's cognate protein product Tax is a nuclear protein and as such is not packaged in the mature virion. Therefore, serological assays based on purified viral proteins as the antigen source will not detect anti-Tax antibodies. Using whole cell lysates prepared from HTLV-I cell lines we developed a radioimmunoprecipitation assay (RIPA) to overcome this potential window in detection. Within a cohort of parenteral drug abusers 50% of the HTLV-I infected individuals were ELISA negative, but possessed antibodies to the HTLV-I Tax protein. This reactivity was confirmed using specific Tax ELISAs and western blots prepared from recombinant Tax protein. PCR was used to confirm the presence of the provirus in these individuals. These assays also demonstrated the presence of a functional tax gene in (20/20) 100% of the TSP/HAM patients examined.

G 217 HUMAN MONOCYTE/MACROPHAGES ARE THE MAJOR RESERVOIR OF HIV IN VIVO.

Suzanne M. Crowe, John Mills, Tarek Elbeik, Jean Kirihara, Nancy McManus, Patricia Lekas, and Michael S. McGrath. AIDS Immunobiology Research Lab and University of California, San Francisco, Department of Medicine, San Francisco General Hospital, San Francisco, CA 94110.

Recent data have shown that monocyte/macrophages (mo) can be infected with HIV in vitro. We wished to examine the relative contributions of mo and lymphocytes to the in vivo viral reservoir. Peripheral blood (PB) mo (33 subjects, AIDS, ARC, ASx), splenic mo (7 subjects undergoing splenectomy for HIV-related thrombocytopenia), peritoneal mo (1 subject, HIV-related renal failure, fluid examined on several occasions) were compared with lymphocytes from the same donor and source in the same assay for HIV p24 antigen expression (by immunocytofluorographic analysis) and by in situ hybridization with a full length HIV gene probe. 0-12.6% of PBmo and 0-15.2% of splenic mo expressed HIV p24 at the time of isolation. After 4 days of in vitro culture, 14.4% of PB mo expressed HIV p24 (range 2.4 to 49%; standard error:2.4%) with RNA dot blot analysis showing accumulations of HIV RNA with no concomitant increase in HIV DNA. Linear regression analysis of results comparing p24 staining with in situ hybridization showed a 0.8 coefficient of correlation. These data suggest HIV infected mo provide a major reservoir of HIV in vivo.

G 218 MOLECULAR INTERACTION EXISTS BETWEEN HHV-6 AND HIV-1 IN CELL SUSCEPTIBLE TO DUAL INFECTION AND LEADS TO INCREASED HIV EXPRESSION. Barbara Ensoli*, Paolo Lusso*; Francoise Schachter*, Steven Josephs*, Jay Rappaport*, Franco Negri*, Robert C. Gallo*, and Flossie Wong-Staal*. *Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892. AIDS patients are frequently coinfected with HIV-1 and HHV-6 viruses and we have shown that HHV-6 productively infects HIV-1 infected CD4+ T-lymphocytes, leading to premature cell death (P. Lusso, et al). Here we show that HHV-6 infected cells stimulate expression of HIV-1 LTR-linked CAT gene. Sequences necessary for this activation do not include the tat responsive region (TAR) and additive effects are seen when the HHV-6 infected cells are co-transfected with tat gene. By use of deletion mutants, we localized a 55 bp region (positions -103 to -48) of the HIV-LTR that responds to HHV-6 activation. HHV-6 infected cells also induce the HIV-2 and the SIV_{mac} LTR/CAT gene, but not the HTLV-1 LTR. Increased levels of HIV RNA are seen by in situ hybridization in HHV-6/HIV doubly infected cells compared to those infected with HIV alone. RNase protection assays specific for the HIV-1 LTR revealed that in HHV-6 infected cells the level of steady state RNA parallels the stimulation of CAT activity. These data suggest that coinfection of CD4+ T-cells by HIV and HHV-6 viruses can stimulate expression of HIV and could enhance the consequences of HIV infection in individuals with concomitant HHV-6 infection (i.e., CD4+ T-cell depletion).

G 219 TISSUE SPECIFIC MUTATIONS IN THE HIV-1 EXTERNAL ENVELOPE V3 DOMAIN, Leon G. Epstein'*, Willy J.A. Krone*, Jacques de Jong*, Marjon Clement*, Leroy R. Sharer', Jaap Goudsmit*; 'Department of Neurosciences, Pediatrics and Pathology, UMD-New Jersey, Medical School, USA; *Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands.
The V3 domain (aa307-321) of the HIV-1 external envelope (gp120) is the binding site for viral strain specific neutralizing antibodies and has a conserved β-turn structure (GPGR) flanked on either side by 5 amino acid residues which are divergent between sequenced HIV-1 isolates. Due to this combination of conserved and variable regions the V3 domain was choosen to study in-vivo mutation in post-mortem brain and spleen tissue from 4 HIV-1 infected children. HIV-1 DNA was amplified using the polymerase chain reaction method with primers which bracket the complete V3 region of gp120. The amplified DNA was sequenced directly and in addition amplified DNA was cloned. Nucleotide sequences derived from cloned DNA confirmed the sequences obtained directly from the amplified DNA and revealed additional point mutations in the amino acids flanking the GPGR tetrad. Divergence in these flanking amino acids was found between patients and between brain and spleen tissue from the same patient.

Patient 1, brain	RSINIGPGRALYTT	Patient 2, brain	KSIHIGPGRAFYTT
spleen	RSINMGPGRVLYTT	spleen	KGINIGPGRAFFTT

G 220 EXPRESSION OF THE tax GENE OF HTLV-I AND HTLV-II IN TRANSGENIC MICE, **Barbara K. Felber, M. P. Rosenberg, N. Grammatikakis, J. Ewald, D. A. Swing, N. Jenkins, N. Copeland & G. N. Pavlakis,** BRI-Basic Research Program, NCI-FCRF, Frederick, MD 21701
The genome of the HTLV-I family of retroviruses encodes three nuclear regulatory proteins from overlapping reading frames. One of these proteins, tax, is a transcriptional activator of the viral LTR promoter. tax acts via a cis-acting element of approximately 10 bp found in three copies upstream of the TATA box. This element acts as an enhancer of promoter activity in the presence of tax. The element shares homology with the cyclic AMP responsive element found in many cellular promoters. The core element also exists within the c-fos promoter. We have shown that c-fos promoter constructs are activated by tax after transfections in mouse cells in tissue culture. In addition, we have generated transgenic mouse lines producing tax1 or tax2 from different promoters. Some of the transgenic lines display an abnormal tail phenotype consisting of an acanthotic and parakeratotic epidermis, hyperplasia of the dermis with vascular ectasia and accumulation of pycnotic cells and neutrophils. tax mRNA was detected in several tissues of these animals, including tail, brain, lung, liver and muscle. Examination of the c-fos expression in the tissues of the transgenic animals revealed high levels of c-fos expression in some tissues, especially the tail and brain. One transgenic line developed low incidence of tumors in the tail. Different tax expression constructs resulted in transgenic animals developing either mesenchymal or multiple Schwann cell tumors. These results indicate that tax may participate in tumorigenesis via the transcriptional activation of cellular oncogenes. We have also shown that tax can activate in trans the expression of an LTR-CAT construct introduced into mice. By mating the LTR-CAT animals to tax-producing animals, we were able to demonstrate transactivation in specific tissues depending on the promoter used for tax expression. This system provides a convenient expression switch allowing the controlled expression of genes in some mouse tissues.

G 221 TRANSCRIPTIONAL MAPPING OF THE HUMAN FOAMY RETROVIRUS GENOME, Rolf M. Flügel and Walter Muranyi, Institute of Virus Research, German Cancer Research Center, 6900 Heidelberg, Federal Republic of Germany

The human foamy virus (HFV) that belongs to the nononcogenic subfamily of retroviruses was isolated from the lymphoblastoid cells of a nasopharynx carcinoma patient by Achong et al. in 1971. The HSV genome was molecularly cloned and its complete primary sequence determined by nucleotide sequencing. To gain more insight into the expression of the HFV genome, viral RNAs obtained after productive infection were mapped. Region-specific molecular clones were constructed and used as hybridization probes specific for the 5' LTR, the leader, gag, pol, env and the three bel genes. Northern blot hybridization revealed that HFV genomic RNA and subgenomic transcripts were detectable in infected human embryonic lung cells, but not in uninfected cells. Several HFV RNAs of distinct sizes specifically hybridized to genomic regions that correspond to the gag-pol, env and to the novel bel genes. S1 nuclease protection analysis was used to study the splicing events that led to the synthesis of subgenomic RNAs. The results indicate that spumaviruses appear to have a complex pattern of transcription comparable to that found in AIDS and other lentiviruses.

G 222 ACTIVATION OF HUMAN T LYMPHOCYTES BY HTLV-I: A CD2-DEPENDENT EVENT.Louis Gazzolo, Madeleine Duc Dodon & Alain Bernard. Immuno-Virologie Mol. Cell. UMR5 CNRS-UCBL Fac. Médecine A. Carrel 69372 Lyon; Labo d'Immunologie Tumeurs de l'Enfant, IGR, Villejuif, FRANCE.

Human lymphocytes can be activated by a variety of stimuli including plant lectins as well as monoclonal antibodies (mAb) directed against certain cell surface molecules, such as CD3, Ti, CD2. We have previously shown that Human T-cell Leukemia Virus, type 1 (HTLV-I) can induce the early proliferation of human quiescent T cells through the expression of IL2 receptors and the synthesis of IL2. Non infectious virions are still able to activate these cells, suggesting that envelope glycoproteins are involved in this process. This activation process might be considered as the first step of the T cell proliferation linked to HTLV-I.
Blocking experiments with a variety of mAb were then performed to determine which activation pathway may be implicated in the HTLV-I induced proliferation. Cell surface molecules, such as CD3/TCR, CD4, CD8 did not participate to the the HTLV-I induced mitogenic event. Conversely, the availability of the CD2 molecule appears to be a prerequisite for the activation of T lymphocytes by this human retrovirus. Indeed, mAb recognizing specific epitopes of the CD2 molecule are inhibiting the proliferative response to either phytohemagglutinin or HTLV-I. These results are consistent with our observations showing that human thymocytes are induced to proliferate when incubated with HTLV-I particles. The data suggest that viral receptors are closely linked to the CD2 molecule on the surface of T cells. Experiments are now in progress to characterize the CD2-viral receptor complex and to define the interplay of viral glycoproteins with this complex on the membrane of T lymphocytes.

G 223 **UNINTEGRATED VIRAL DNA IN HIV-1 INFECTION IN VITRO AND IN VIVO,** **Jan L.M.C. Geelen*, Suzanne Jurriaans*, Willy J.A. Krone*, Marjon Clement*, Lia Smit*, Nicole Back*, Leon G. Epstein**, Jaap Goudsmit*; *Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands; *Department of Neurosciences, Pediatrics, UMD New-Jersey Medical School, USA.**

In a number of retroviral infections unintegrated viral DNA (UVD) may persist in infected cells and may be related to cytopathicity. Brain and spleen tissue of HIV-1 infected children were analyzed for the presence of UVD. In addition the kinetics of UVD was studied in HTLV-IIIB infection of Molt-3 cells in vitro. Covalently closed circular viral DNA was amplified by the polymerase chain reaction utilizing primers which bracket the LTR. Specificity of the amplified sequences was determined by Southern blot analysis, using an LTR-specific probe. In vitro HIV-1 specific UVD was detected as early as 1 day post-infection and persisted thereafter, sometimes spaced by a short period of undetectable levels of UVD depending on the infecting virus titer. High levels of UVD were detected in both the brains and spleens of children with HIV-1 associated progressive encephalopathy indicating active infection at both sites.

G 224 THE EFFECT OF HIV-1 ON CD4 ANTIGEN EXPRESSION IN THE U-937 MONOCYTIC CELL LINE, Romas Geleziunas, François Boulerice and Mark A. Wainberg, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada, H3T 1E2. FACS analysis revealed that over 90% of U-937 cells express CD4 antigen at their surface. Following infection of this cell line with HIV or induction of differentiation with phorbol esters (PMA), expression of CD4 antigen was almost completely lost, without any loss of viability. Consequently, we were unable to infect PMA-treated U-937 cells. Northern blot analysis showed that there was no significant decrease of T4 mRNA levels in infected or PMA-treated monocytes. In contrast, $T4^+$ lymphocyte controls, infected with HIV, showed markedly decreased levels of T4 mRNA and surface CD4. Furthermore, treatment of chronically infected U-937 cells with PMA lead to a shut-down in production of progeny virus. Our results suggest that HIV causes a post-transcriptional inhibitory effect on CD4 expression in monocytes. In addition, U-937 differentiation leads to a decrease in cell surface CD4, thus possibly renders the resultant differentiated cells non-susceptible to HIV infection.

G 225 MUTATIONAL ANALYSIS OF THE HIV-1 CAPSID PRECURSOR MATURATION: EFFECTS ON VIRAL MORPHOGENESIS, Heinrich Gottlinger and William Haseltine, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA, USA

The primary translation product of the HIV-1 gag region undergoes myristylation and proteolytic processing. To determine their role in the viral morphogenesis we have selectively blocked these two modification steps in proviral clones using site-directed mutagenesis. The myristylation of the capsid precursor was prevented by a substitution of its N-terminal glycine residue. A proviral clone bearing this mutation directed the synthesis of normal amounts of viral proteins upon transfection into COS7 cells; however, no viral particles were released into the culture supernatant. The processing of the capsid precursor into the mature capsid proteins was not significantly affected by the absence of myristylation, in contrast to results obtained by others in other retroviral systems (Rein et al., PNAS 83:7246, 1986; Rhee and Hunter, J. Virol. 61:1045, 1987). Point mutations in two conserved regions of the HIV-1 protease gene completely blocked the capsid maturation cleavage. The virions released by these mutants from COS7 cells were non-infectious and had a morphology similar to immature wild type particles. Despite the absence of a processed reverse transcriptase (RT) the virions contained significant amounts of RT activity. The selective blockade of the N-terminal p24 cleavage site yielded non-infectious virions that showed a core-like condensation of the ribonucleoprotein complex but did not form the conical cores typical for lentiviruses. The alteration of the C-terminal p24 cleavage site led to the formation of highly unusual budding structures. We conclude that drugs which block myristilation and proteolytic cleavage should prevent viral replication and be used in the treatment of HIV-1 infections. The accumulation of viral coat at the membrane of transfected COS7 cells apparently stalled in the process of budding by a defect in sphere formation.

G 226 ISOLATION AND MOLECULAR CHARACTERIZATION OF HTLV-I AND HTLV-II FROM THE PERIPHERAL BLOOD OF INTRAVENOUS DRUG USERS, Ira Gore, W. Don Decker, Tracey L. Snyder, Stanley H. Weiss*, William A. Blattner*, Beatrice H. Hahn, and George M. Shaw, Departments of Medicine, Biochemistry, and Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294; *National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Populations of intravenous drug users (IVDU) with high (up to 50%) prevalence of HTLV-I/II seropositivity have been identified. Because HTLV-I and HTLV-II are difficult to distinguish serologically, this study was undertaken to characterize the retroviral infections of eleven HTLV-I/II seropositive IVDU by DNA analysis. Viably frozen peripheral blood mononuclear cells from each subject were used to establish cultures and to prepare high molecular weight DNA for amplification in the polymerase chain reaction (PCR). One IVDU from Newark was shown to be infected with a unique HTLV-II provirus which differs from previously described isolates by several restriction sites. Ten IVDU from New Orleans are currently under investigation: seven of them have evidence of HTLV-I or HTLV-II infection by PCR amplification of uncultured cell DNA. Of the seven PCR positive IVDU, five have positive cultures as well. Differential PCR amplification and Southern blot-hybridization with HTLV-I and -II specific probes are being performed to evaluate the relative contributions of HTLV-I and HTLV-II to infection of IVDU and to enhance the understanding of disease mediated by these human retroviruses.

G 227 MUTATIONS IN THE HIV-1 EXTERNAL ENVELOPE DOMAIN V3 PARALLELED BY A PHENOTYPIC SWITCH TO SYNCYTIUM INDUCTION, Jaap Goudsmit*, Matthijs Tersmette*, Jacques de Jong*, Willy Krone*, Marjon Clement*, Han Huisman*, Frank Miedema*; *Human Retrovirus Laboratory, Academic Medical Center, *Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands.

Antibody capacity to inhibit cell fusion is elicited by the third variable domain of the external envelope protein gp120 of the human immunodeficiency virus type 1 (HIV-1) during natural infection. In two individuals, during 2 years of follow-up, a transition from non syncytium inducing to syncytium inducing isolates was observed. Using the polymerase chain reaction DNA was amplified from these isolates and the nucleic acid sequence was determined, using primers which bracket the complete V3 region of gp120. While the C and N terminal end of this domain bordering two cysteines as well as a GPGR quartet in the middle portion of this domain were highly conserved, distinct amino acid changes were observed paralleling the phenotypic switch to syncytium formation. These amino acid changes occurred consistently at the same distance from the β-turn sequence GPGR. The impact of these changes on the binding capacity of natural antibodies of these patients was studied by expressing these sequences in a prokaryotic expression system and using them as antigen for antibody specificity and affinity studies.

G 228 MUTATIONAL ANALYSIS OF VPX, A PROTEIN SPECIFIC TO HIV-2 AND SIV, Mireille Guyader, Keith Peden, Luc Montagnier and Michael Emerman, Unité d'Oncologie Virale, Institut Pasteur, Paris, France.

Nucleotide sequence comparison between HIV-1, HIV-2 and SIV has revealed the presence of an open reading frame in the central region of the genomes of HIV-2 and SIV that has no counterpart in HIV-1. This new open reading frame, called vpx, has the potential to code for a protein of 112 amino acids that is highly conserved between HIV-2_{ROD} and SIVmac. To determine whether or not this open reading frame is expressed, antisera to peptides corresponding to the N-terminus and C-terminus of the predicted product of HIV-2 were raised in rabbits. These antisera immunoprecipitated a 16-kd protein from the cell extract and medium of cells infected with HIV-2, and a 14-kd protein from SIV-infected cells. Mutations in the vpx open reading frame eliminated the synthesis of the 16-kd protein in HIV-2-infected cells, confirming that this protein is the product of this gene. Full-length clones of HIV-2 containing these mutations are infectious in two established lymphocyte lines and a monocyte cell line. Results of studies on the infectivity and replication rate in several cell lines and primary cells will be presented.

G 229 THE HUMAN IMMUNODEFICIENCY VIRUS rev GENE PRODUCT AND A CIS-ACTING ELEMENT REGULATE THE LEVELS OF CYTOPLASMIC env mRNA, Marie-Louise Hammarskjold, Jessica Heimer, Bjorn Hammarskjold and David Rekosh, Departments of Microbiology and Biochemistry, State University of New York at Buffalo, Buffalo, NY 14214

A single SV40 late replacement vector which expresses both the rev and envelope genes of HIV was used to examine the mechanism underlying the dependence of env gene expression on the rev protein. When rev was deleted from the vector, levels of cytoplasmic env mRNA in transfected cells were dramatically decreased, compared to when rev was present. In contrast, the levels of env RNA in preparations of total RNA were not significantly different with or without rev co-expression. It was possible to restore env expression from the vector lacking rev, by supplying rev in trans, providing that a cis-acting sequence was also present. This sequence was mapped to a 755bp region within the env open reading frame and it was shown that the sequence could be moved, but that it worked only in its original orientation. The results suggest that rev functions to regulate nuclear export of env mRNA. Results of experiments to further map the cis-acting regions within the env mRNA will be presented.

G 230 HIV TRANSACTIVATION AND VIRUS PRODUCTION REQUIRE A SPECIFIC HUMAN CHROMOSOME COMPLEMENT IN HUMAN-HAMSTER HYBRID CELLS. C.E. Hart*, C.Y. Ou*, L.T. Bacheler+, S.R. Petteway+, J.W. Wasmuth^, G. Schochetman*. *AIDS Program, Centers for Disease Control, Atlanta, GA. +E.I. DuPont Nemours, Medical Products Department, Wilmington, DE. ^Department of Biochemistry, University of California School of Medicine, Irivne CA. In this study a series of human/Chinese hamster ovary (CHO) hybrid cell clones were used to probe for a human chromosome complement necessary for high level tat 3-directed transactivation and HIV production. CHO and hybrid clone cultures transfected with pLTR-CAT alone had similar basal CAT activities. CAT activities in the 18 hybrid clones cotransfected with pLTR-CAT and pSV-tat 3 were high in 3 clones (9-10 fold versus CHO), moderate in 2 clones (1.7-2 fold versus CHO), and the remaining 13 clones had CAT activities equal to or less than that of CHO. Transfection of the hybrid clones with an infectious whole proviral DNA clone of HIV showed that the 3 clones with a high level of transactivation support a high level of virus production; one of the two clones transactivating at a moderate level support significant levels of virus production. The parent CHO and other hybrid cell lines with basal transactivation levels also had basal virus production. Karyotype analysis showed a direct concordance of a specific human chromosome content in hybrid clones to the ability to transactivate and produce virus.

G 231 MUTATIONAL ANALYSIS OF THE CONSERVED BASIC DOMAIN OF THE HIV-1 TAT PROTEIN,
Joachim Hauber, Michael H. Malim and Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, N.C. 27710.
The pathogenic human retrovirus Human Immunodeficiency Virus Type 1 (HIV-1) encodes a non-structural protein, termed tat, which is able to activate viral gene expression when present in trans. The tat protein is localized within the nucleus of expressing cells and acts, at least in part, by enhancing the rate of HIV-1 long terminal repeat (LTR) specific transcription. The tat gene consists of two coding exons which together define a small protein of 86 amino acids. Inspection of the amino acid composition of the tat protein and comparison of this sequence with sequences of other known nuclear proteins revealed the presence of a 15 amino acid stretch within the tat protein which may constitute a nuclear localization signal. This sequence, which extends from aa 22 to 37 in the HXB-3 isolate of HIV-1, is highly conserved between isolates of HIV-1 and the related primate immunodeficiency viruses HIV-2 and SIV. Indirect immunoflourescence and cellular fractionation experiments demonstrate that the site directed mutagenesis of this conserved domain of the HIV-1 tat protein abrogates the targeting of this protein to its nuclear site within expressing cells. In addition, it will be shown that these mutations affect the in vivo stability of the tat protein. Furthermore, we will demonstrate that the mutagenesis of this sequence in the tat gene directly affects the phenotype of this protein by reducing its potential to trans-activate HIV-1 specific gene expression. These experiments allow the more precise determination of essential amino acid sequence requirements for the in vivo action of tat.

G 232 EVALUATION OF THE HIV I TAR-SEQUENCE IN GENE EXPRESSION
Hansjörg Hauser and Jianhong Cao
Genetics and Cell Biology Department, GBF, Mascheroder Weg 1, D-3300 Braunschweig, FRG

The cis-acting sequences responsive to the tat gene product, the trans-acting responsive (TAR) region of HIV I, are located immediately adjacent to the site of transcriptional initiation. Because the TAR-sequence is transcribed, the mechanistic level of transactivation has to be determined. In order to test wether posttranscriptional transactivation is involved, the TAR sequence has been transferred on a heterologous transcription unit by integration in between the SV40 promoter and the firefly luciferase gene. Transfection or microinjection into fibroblastoid and lymphoid cells demonstrate that i) the TAR sequence reduces expression of the reporter gene considerably, ii) this reduction can at least be partially restored by coexpression of tat gene, iii) the effect of transactivation is less compared to constructs harboring the complete HIV I LTR plus the TAR sequence. Ongoing experiments on microinjection of in vitro transcribed RNA in mammalian cells will clarify whether TAT acts on the mRNA level.

G 233 PURIFICATION AND IN VITRO CHARACTERIZATION OF HIV REVERSE TRANSCRIPTASE AND INTEGRASE, Dag E. Helland, Chris Farnet, Allan Hey and William A. Haseltine, Division of Human Retroviruses, Harvard Medical School, Dana-Farber Cancer Institute, Boston MA 02115

HIV reverse transcriptase and integrase have been expressed as recombinant proteins in Eicheridia coli. The reverse transcriptase is expressed and purified to homogenicity as a TrpE fusion protein. The enzyme is present in two forms, a 103kD and a 87kD form. By activity gel analysis we have shown that both forms have enzymatic activity. The Trp E fusion partner has been removed from the fusion protein by HIV protease after purification. The purified enzyme is stable and has been used for in vitro studies. The properties of this enzyme is very similar to the enzyme present in virions and is therefore well suited for screening for inhibitors.

The integrase is expressed as a protein with an apparent molecular mass of 31kD. The protein is soluble in E. coli extracts and has been purified to near homogenicity. The purification of this protein is followed by using a specific rabbit antiserum against the integrase.

In vitro characterization and properties of the purified integrase protein will be presented.

G 234 DETECTION OF HIV-1 SPECIFIC DNA IN SERUM OF HEMOPHILIACS POST-TRANSFUSION, Indira K. Hewlett C. Ann Hawthorne, Martin Ruta, Robert A. Gregg, Jean-Pierre Allain and Jay S. Epstein, Division of Blood and Blood Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, Abbott Laboratories, Chicago, Illinois

Sera from fifteen hemophiliacs were analyzed pre and post-transfusion for the presence of viral DNA by tne polymerase chain reaction (PCR). Serum was deproteinized and nucleic acid precipitated was amplified with primer pairs to the gag and env region of the HIV-1 genome. We were able to detect viral DNA in all 15 samples post-transfusion, while no reactivity was observed in samples prior to transfusion. The use of serum PCR vs. antigen or antibody for early detection in hemophiliacs that are transfused with HIV-1 positive blood is being further studied by analyzing sera collected at various time intervals post-transfusion.

G 235 DEFINING THE INTERVAL BETWEEN ACQUISITION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION AND DETECTION OF ANTIBODY, C. Robert Horsburgh Jr., Chin-Yih Ou, Janine Jason, Alan R. Lifson, Kenneth H. Mayer, Ira M. Longini Jr., Scott D. Holmberg, Gerald Schochetman, Harold W. Jaffe, Centers for Disease Control, Atlanta GA; Fenway Community Health Center, Boston MA; and San Francisco Department of Health, San Francisco CA

A prolonged period of HIV infection without development of detectable antibody has been reported. Such a phenomenon poses risk for transmission of HIV through blood or sexual contact by persons who are unaware that they are infected. To estimate the duration of the virus-positive, antibody-negative period we investigated 26 homosexual men and 11 men with hemophilia for whom samples were available before the time of seroconversion. Seroconversion was established by detection of antibody to HIV by whole virus enzyme immunoassay (EIA) and confirmed by Western blot or immunofluoresent assay; preseroconversion samples were EIA negative. Peripheral blood mononuclear cells were assayed for HIV DNA by the polymerase chain reaction technique using gag region primers; serum was tested for the presence of p24 antigen using antigen-capture EIA. Six persons had HIV DNA detected before seroconversion; all had HIV DNA detected only on the seronegative date closest to the time of seroconversion. Three persons had antigen detected prior to seroconversion; in each case HIV DNA was also detected. Using a Markov model, we estimated the median time from first detection of HIV DNA by PCR to first detection of HIV antibody to be 3.2±3.7 months. Prolonged periods of latent HIV infection without detectable antibody appear unlikely.

G 236 ANALYSIS OF VPX FUNCTION IN HIV-2 INFECTED CELLS
Wen Hu, Nancy Vander Heyden, and Lee Ratner, Department of Medicine, Washington University, St. Louis, MO 63110
Objective: A novel gene, designated vpx, is present in the genomes of HIV-2, SIVs, but not HIV-1. Our goal was to define the role of VPX in HIV-2 replication and cytopathicity.
Methods: A functional proviral HIV-2 plasmid clone (pHIV-2) was made and used to construct site directed mutants which eliminate the AUG initiator codon of vpx (MX1), which convert the 22nd amino acid to a termination codon, or which simultaneously eliminate the first and second AUG codons and frameshift the gene introducing a termination codon at codon position 70.
Results: Transfection of pHIV-2 and MX1 into COS-1 cells gave rise to virus which could be passaged in H9 or CEM cells or in peripheral blood monocytes. Hybridization data with DNA from the infected cells demonstrated the presence of the mutation in MX1 infected cells, and equivalent levels of DNA sequences in pHIV-2 and MX1 infected cells. Immunoprecipitation analysis demonstrated a 16 kd protein in cells infected with the pHIV-2 virus but not the MX1 virus, and equivalent levels of GAG and ENV proteins.
Conclusions: These data suggest that VPX is dispensable for virus replication. Further analysis of the MX1 mutant as well as the other two mutants are underway to define whether VPX has quantitative effects on infectivity, virus replication, or cytopathicity.

G 237 EFFECTS OF HIV REGULATORY PROTEINS, TAT AND REV, ON IN VITRO GENE EXPRESSION,
Joseph V. Hughes, Kathleen LeGrow, Jon H. Condra, Corrille M. DeWitt, Mary-Ellen Davies, Donald Lineberger, Richard J. Colonno, and Robert L. LaFemina, Department of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
The TAT and REV gene products are the two most important and essential regulatory proteins for HIV gene expression. We have established an expression system based on the reporter gene human growth hormone (HGH) driven by the HIV-LTR to measure TAT induction. Co-transfection of TAT and HIV-LTR-HGH in U373 glioma or 143 osteosarcoma cell lines results in a 10-40 fold stimulation, while HeLa and SW480 cells demonstrated a high basal HGH expression (without TAT) and lower levels of stimulation. The stimulation of the HIV-LTR-HGH construct is being further examined in selected stable cell lines co-transfected with HGH and TAT. To examine the essential nature of the REV gene, a mutant REV virus was constructed with two site-directed mutations made in the first exon of REV. The mutant HIV was able to replicate only in stable REV-expressing cells (U373 or 143), although some protein expression did occur in SW480 cells. To measure REV activity, a number of plasmids were constructed linking various ENV fragments to the HGH gene. The ENV sequences greatly reduced the HGH expression (80 to 95%), which was relieved by addition of TAT, but maximal expression required both REV and TAT. Titration of REV demonstrated a dose dependent stimulation with inhibition at higher concentrations. Antivirals directed against these essential regulatory proteins should prove useful for the therapeutic intervention in AIDS.

G 238 CELL SURFACE EXPRESSION, GLYCOSYLATION AND ENDOCYTOSIS OF WILD TYPE AND MUTANT CD4 MOLECULE, A RECEPTOR FOR HUMAN IMMUNODEFICIENCY VIRUS IN MAMMALIAN CELLS,
M. Abdul Jabbar, Debi P. Nayak, Jonsson Comprehensive Cancer Center and Department of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024
The cytoplasmic domain of CD4, a receptor for HIV is conserved among species suggesting that the domain perhaps plays a central role in intracellular targeting mechanism. I have investigated the requirement of the domain in exocytic and endocytic pathway of mammalian cells, by generating nested set of deletions in the region of the molecule. The gene encoding the wild type and the mutant proteins are introduced into monkey kidney cells and transactivated by a recombinant vaccinia virus synthesizing T7 RNA polymerase. The data show that the deletion of the domain does not appear to affect the intracellular movement of the molecule and its targeting to cell surface. The glycosidic modification of the CD4 was also analyzed and shown that one of the two glycosylation sites is not processed to complex carbohydrate. Furthermore, the role of the domain in endocytosis of the receptor was also studied. Supported by NIAID First Award to MAJ (R29 AI29831).

G 239 *TRANS*-ACTIVATION OF HIV-1 LTR-DIRECTED GENE EXPRESSION BY *tat* REQUIRES PROTEIN KINASE C. Aya Jakobovits, Arnon Rosenthal and Daniel J. Capon, Department of Molecular Biology, Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080

An understanding of how the dormant provirus of human immunodeficiency virus (HIV-1) is triggered to begin viral replication may help us to understand the progressive nature of HIV-1 disease. The activation of latent HIV-1 requires both mitogenic stimulation of the infected cell and the protein product of the HIV-1 *trans*-activator (*tat*) gene. Because protein kinase C (pkC) is essential to the induction of T cell proliferation by certain mitogens, we have investigated the involvement of pkC in HIV-1 activation using cells depleted of functional pkC by prolonged exposure to the mitogen PMA and cells treated with the pkC inhibitor H-7. As predicted from the previous demonstration of a PMA-responsive enhancer element in the HIV-1 LTR, the down-regulation or inhibition of pkC decreases the basal level of LTR-directed expression. Unexpectedly, however, both treatments also abolish *trans*-activation of the HIV-1 LTR by *tat*, although synthesis of the *tat* protein is not affected. HIV-1 LTR mutants with deleted or inactivated PMA-responsive enhancer elements still require pkC for *trans*-activation. Transfection of a pkC cDNA clone fully restores *trans*-activation in cells pretreated with PMA, whereas an inactive pkC cDNA mutated at the ATP binding site does not restore *tat* activation. Thus our results indicate that pkC mediates the regulation of HIV-1 *trans*-activation by mitogenic stimuli, independently of its effect on PMA-responsive enhancers in the HIV-1 LTR, and that pkC inhibitors may be able to suppress the activation of latent HIV-1 and its ongoing replication.

G 240 RECOMBINANT PROTEINS OF HIV IN IMMUNOBLOTS AS DIAGNOSTICS FOR DISCRIMINATION OF HIV-1 AND HIV-2 INFECTION. Gerhard Jahn, Watoky M. Nkya*, Isidore Zohoun**, Sylvia Ellinger, Stephan Stenglein, and Andreas Baur. Institut für Klinische und Molekulare Virologie der Universität Erlangen-Nürnberg, Loschgestraße 7, D-8520 Erlangen, F.R.G.; *KCMC, Moshi, Tanzania; **Banque de Sang, Université Cotonou, Benin.

For the detection of antibodies against HIV-1 or HIV-2 we expressed proteins representing **gag** and **env** specific epitopes of either virus. A highly efficient prokaryotic expression vector was taken, which carries the **E. coli** lac-promotor and aa 1-375 of the **E. coli** lac Z gene. The expressed recombinant proteins served as antigens in the immunoblot test system. Sera of ELISA positive individuals from Germany, Tanzania (East-Africa) and Benin (West-Africa) were analysed. The immunoblots with recombinant proteins proved to be a reliable test system for serodiagnosis of HIV infection and was suitable in the discrimination between HIV-1 and HIV-2 infection.

G 241 EXPRESSION OF HIV RNA IN EPSTEIN-BARR VIRUS IMMORTALIZED CELLS PRODUCTIVELY AND NON-PRODUCTIVELY INFECTED BY HIV, Frank E. Jones, Karen Dahl and George Miller, Yale University School of Medicine, New Haven, CT 06510. HIV strains show three different types of behavior in an EBV immortalized human cell line called X50-7. The RF2 strain rapidly induces severe cytopathic effects including syncytial formation and pyknosis and eventually kills the culture. Strains MN and SB establish persistent non-cytopathic infection and yield extra-cellular virus. In X50-7 cells carrying the MN and SB strains there is abundant expression of three species of HIV RNA of 9.5 Kb, 4.1Kb and 1.9Kb. X50-7 cells infected with the HTLVIIIB strain become non-productive after an initial period of virus production. They do not release virus, but virus can be recovered from intact cells by co-cultivation. This transition from productive to non-productive behavior has occurred in two successive generations of cellular subclones of X50-7 cells initially infected with the HTLVIIIB strain. Among second generation subclones, 2 of 10 studied expressed levels of the 3 HIV RNAs which were considerably lower than those found in producer cell lines such as X50-7/MN. We were unable to detect HIV RNA in 8 cell subclones. Thus in X50-7 cells infected with the HTLVIIIB HIV strain there is either suppression of expression of the HIV genome (ie "latency") or there is loss of the HIV genome from some of the population (ie "cure"), or there is segregation of a non-susceptible population (ie "escape"). Experiments are underway to distinguish among these possibilities.

G 242 EPIDEMIOLOGY AND BIOLOGY OF HIV-2

Phyllis Kanki*, Richard Marlink*, Souleymane M'Boup** and Max Essex*, *Harvard AIDS Institute, Boston,Mass., **University of Dakar, Dakar, SENEGAL.

A cohort of 1165 Senegalese prostitutes have been followed for 3 years. Serodiagnosis of HIV-2 and HIV-1 were performed by immunoblot on 2363 samples. On women seen more than once, 892 person-years of observations have been collected with a mean of 21 months observation. The present seroprevalence of this cohort is 10.8% HIV-2, 0.9% HIV-1 and 0.2% HIV-1/HIV-2. Over the 3 years, 2.6% seroconversion to HIV-2 was seen and 0.25% seroconversion to HIV-1.

A subset of this cohort has been followed in a clinical-immunologic prospective study. HIV-2+ women were matched to HIV-2- women, by age, nationality, and years of prostitution. We have failed to find signs of lymphoadenopathy, oral candidiasis, ARC or AIDS in either the seropositive or seronegative women followed. Our results indicate distinct differences in the epidemiology and biology of HIV-2 as compared to HIV-1.

G 243 STUDIES ON THE FUNCTION OF VPX IN HIV-2, John C. Kappes, W. Don Decker, Shei-Wen Lee, Jinseu Park, Casey D. Morrow, Shiawhwa Su, Lilly I. Kong, Ron C. Desrosiers*, George M. Shaw, and Beatrice H. Hahn, Departments of Medicine, Microbiology, and Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294 and *Department of Microbiology, New England Regional Primate Research Center, Southborough, MA 01772.

The genomic organization of HIV-1, HIV-2 and SIV is very similar, represented by LTR-gag-pol-vif-central region-env-nef-LTR. HIV-2, SIV_{MAC} and SIV_{AGM}, however, contain an additional open reading frame, designated vpx, that is situated in the central region and is not found in HIV-1. We recently demonstrated that vpx of HIV-2 and SIV_{MAC} is expressed *in vitro* and *in vivo*, identified its putative gene product, expressed it in a prokaryotic and eukaryotic system, and showed that vpx is packaged into mature virions. To further study the role and biological significance of vpx we most recently constructed a transfection-competent plasmid clone of HIV-2/ROD and utilized it for site-directed mutagenesis of the vpx open reading frame. An internal HindIII (vpx containing fragment) was subcloned into M13 and mutagenized so as to abolish vpx translation. Nucleotide sequence analysis confirmed the mutation and Western blots demonstrated the complete absence of vpx in progeny virus of the transfected mutants. Comparison of the phenotype of vpx-deficient virus with that of HIV-2/ROD wild type demonstrates that vpx deficient HIV-2 is fully transfection competent. Vpx-deficient virions are cell-free and cell-to-cell transmissible, their syncytia-forming ability is equal to that of vpx-containing HIV-2 virions, and the absence of vpx does not influence the extensive *in vitro* cytopathic potential of the virus in SupT1 and CEMx 174 T-cell lines. Finally, there is no difference in the amount of progeny virus produced over time in cultures infected with wild type or mutant virus. Taken together, these data suggest that vpx is not essential for viral replication, infectivity, or cytopathicity in certain immortalized T-cell lines *in vitro*. Studies are underway to delineate whether vpx facilitates early steps in the viral life cycle and whether it is important in infection of non-immortalized cells.

G 244 TEMPORAL ASPECTS OF DNA AND RNA SYNTHESIS DURING A ONE-STEP GROWTH CYCLE OF HIV. Sunyoung Kim*, Randal Byrn+, Jerome Groopman+ and David Baltimore*. * Whitehead Institute, and Department of Biology, MIT, Cambridge, MA 02142; +New England Deaconess Hospital, Harvard Medical School, Boston, MA 02115.

We have developed a procedure for producing sufficiently high-titer HIV1 stocks from a homogeneous, cloned viral DNA to allow synchronous infection of 10-20% of H9 or primary T cells. By limiting observations to the first 32 hours following infection, we can then approximate one-step growth conditions. Linear DNA was first evident at 3 to 4 hours postinfection. Viral RNA was first found at 12-24 hours postinfection. The earliest RNA was enriched in subgenomic species, suggesting that the virus has early and late transcriptional phases. Linear DNA first appeared in the cytoplasm and later was also recovered from the nucleus. Circular DNA could be detected in the nucleus at 8-12 hours postinfection.The copy number of linear DNA was not exceptionally high compared to other retroviruses. At later times, when virus spreads to all cells, there was still a low amount of linear and circular DNA. Chronically infected H9 cells have very little free viral DNA.

G 245 BIOLOGICAL AND BIOCHEMICAL STUDIES OF THE HIV-1 PROTEASE, Nancy E. Kohl, Paul L. Darke, Lenora J. Davis, Jill C. Heimbach, Chih-Tai Leu and Irving S. Sigal, Department of Molecular Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Retroviral proteins are synthesized as polyprotein precursors which are posttranslationally processed to yield the mature viral proteins. A viral-encoded protease is responsible for proteolytic maturation of the gag and gag-pol precursor proteins. In HIV-1, the protease is a 99 amino acid polypeptide encoded by the 5' end of the pol open reading frame. We have shown that the HIV-1 protease is essential for viral replication (Kohl et al, PNAS 85, 4686, 1988). An HIV-1 provirus in which the putative active site Asp residue was replaced with an Asn residue gave rise to virions that contained unprocessed gag polyprotein and were not infectious. To further study this enzyme, it has been expressed in E. coli in an enzymatically active form and purified to homogeneity. Current efforts are focused on determining the relationship between the biological activity of the enzyme and its biochemical properties.

G 246 GENETIC ANALYSIS OF SEQUENTIAL HIV-1 ISOLATES FROM AN INFECTED LAB WORKER, L.I.Kong, M.E.Taylor, W.D.Decker, S-W.Lee, D.Waters*, W.A.Blattner[a], B.H.Hahn, and G.M.Shaw, Depts. of Medicine, Microbiology and Biochemistry, Univ. of Alabama at Birmingham, B'ham AL 35294; *Program Resources, Inc, Frederick, MD 21701; [a]Natl. Cancer Inst., NIH, Bethesda MD 20892

To better understand the nature of genetic and antigenic variation of HIV-1 in humans, virus was sequentially isolated and characterized from a previously reported (Science 239:68, 1988) lab worker (LW) infected by the H9/HTLV-IIIb strain of virus. Between 9/4/85 and 5/18/88, 7 HIV-1 isolates were established in 3 independent laboratories from the peripheral blood mononuclear cells (PBMC) of LW by co-cultivation with PBMC of normal donors. Southern blot hybridization patterns of all 7 LW isolates were indistinguishable from each other and from a component of the H9/HTLV-IIIb isolate after digestion with 7 restriction endonucleases (SstI, PvuII, BglII, HindIII, EcoRI, CvnI, KpnI). Recombinant λ phage libraries from LW virus isolates from 9/4/85 (λOF001), 11/30/87 (λFD651), and 5/18/88 (λLW5A) were prepared and 10, 8, and 10 full-length HIV-1 clones, respectively, were analyzed. All 28 clones were identical to each other and to the BH10 clone of H9/HTLV-IIIb in 7 of 7 restriction enzyme patterns; except for 2 clones from the LW5A isolate that differed in single PvuII polymorphisms. The nucleotide and deduced amino acid sequences of the gp120 immunodominant loop region (aa302-337) of 3 λOF001 clones, 3 λFD651 clones, and 8 λLW5A clones were determined as follows:

```
BH10   C T R P N N N T R K S I  R I  Q R G P G R A F V T I  G K I  G N M R Q A H C
λOF001 - - - - - - - - - - R- - - - - - - - - - - - - - - - - - - - - - - - - - -
λFD651 - - - - - - - - G- R- - - - - - - - - T- - - - - - - - - - - - - - - - - -
λLW5A  - - K- - - - - G- R- - - - - - - - - T- - - - - - - - - - - - - - - - - -
```

Clones from within each of the 3 isolates were identical to each other but between isolates showed cumulative nucleotide point mutations that resulted in the amino acid changes shown. Transfection-derived virus strains from these LW HIV-1 clones have been generated for analysis of antigenic and biologic properties. Results of these studies will provide insight into the nature and significance of host-viral interactions mediated by the viral envelope protein.

G 247 ROLE OF MEMBRANE FUSION IN HIV-1 REPLICATION, SYNCYTIUM FORMATION AND SINGLE CELL KILLING, Mark Kowalski, Tatyana Dorfman, William Haseltine, Joseph Sodroski, Dana-Farber Cancer Institute, Division of Human Retrovirology, 44 Binney Street, Boston, MA

The envelope glycoprotein of human immunodeficiency virus (HIV-1) is synthesized as a 160kD precursor glycoprotein (gp160) that is processed to the external 120kD glycoprotein (gp120) and the transmembrane 41kD glycoprotein (gp41). Binding of gp120 to the CD4 receptor leads to fusion of the viral envelope with the target cell membrane and allows for viral entry and replication. Virus replication in T lymphocytes is accompanied by cytopathic effects, including the formation of syncytia and single cell lysis. Previous studies in our lab employing mutagenesis of the HIV envelope glycoprotein have identified the hydrophobic amino terminus of the gp41 transmembrane protein as an important determinant of membrane fusion. The effect of mutations in this region on viral replication and cytopathicity has been assessed. Different mutations in the amino terminus of gp41 can differentially affect viral replication, virus-induced syncytium formation, and single cell lysis. These results demonstrate the importance of cell fusion in viral replication, and illustrate how changes in this region can alter viral pathogenicity.

HTLV and HIV-II

G 300 MOLECULAR CHARACTERIZATION AND COMPLETE NUCLEOTIDE SEQUENCE ANALYSIS OF AN ATTENUATED ISOLATE OF HIV-2, Prasanna Kumar, Huxiong Hui, John C. Kappes, Jeffrey S. Parkin, George M. Shaw, and Beatrice H. Hahn, Departments of Medicine, Biochemistry, and Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294.

HIV-1 and HIV-2 are two distinct classes of human immunodeficiency viruses known to cause AIDS in the U.S., Europe, and Africa. A particular isolate of HIV-2, termed HIV_{ST}, was recently obtained from a healthy prostitute from Senegal, West Africa, and was found to be less cytopathic *in vitro* when compared to prototype $HIV\text{-}2_{ROD}$ and HIV-1, as well as SIV_{MAC} viruses (Kong *et al.*, Science 240:1525-1529, 1988). A recombinant λ phage library was prepared from a cell line producing $HIV\text{-}2_{ST}$. A full-length proviral clone designated λ JSP4-27 was isolated, shown to be transfection competent, and subsequently sequenced in its entirety. The data showed that the genomic organization of $HIV\text{-}2_{ST}$ was very similar to that of $HIV\text{-}2_{ROD}$ and SIV_{MAC} with the exception of an in-frame TAA stop codon in the vpr open reading frame. When compared to the prototype $HIV\text{-}2_{ROD}$ sequence, an overall nucleotide sequence homology of 90% was observed. The amino acid homology between different genes were 93% for gag, 92% for pol, 88% for vif, 86% for vpx, 85% for rev, 82% for env, 78% for nef, 75% for tat, and 80% for vpr. While the overall structure of the $HIV\text{-}2_{ST}$ envelope gene was very similar to that of $HIV\text{-}2_{ROD}$, 18% amino acid sequence differences, including changes in putative fusion sequence, were observed. One of the most conserved regions was the LTR with only 4% nucleotide sequence differences. From these analyses we conclude that: (a) $HIV\text{-}2_{ST}$ differs from $HIV\text{-}2_{ROD}$ to the same extent as do independent HIV-1 isolates from each other; (b) vpr appears not to be required for *in vitro* replication; and (c) changes in the $HIV\text{-}2_{ST}$ envelope glycoprotein might be responsible for its *in vitro* phenotype. Studies are under way to extend these findings and to further define molecular determinants for the attenuated pathogenicity of $HIV\text{-}2_{ST}$.

G 301 USE OF HIV TRANS-ACTING ELEMENTS IN RETROVIRUS VECTORS FOR HIGH EXPRESSION OF GENES IN LYMPHOID CELLS. C. LaMonica I. Shapiro*, C. Meier, V. Vlach, M. Stevenson. *Karlinska Institute, Stockholm, Sweden. Molecular Biology Laboratory, Department of Pathology and Microbiology UNMC, Omaha NE 68105. The Human Immunodeficiency Virus Type I contains powerful regulatory proteins which control the rate of replication and subsequent processing of HIV genes during the viral life cycle. We have used this powerful regulatory mechanism to drive expression of foreign genes inserted in retroviral vectors. In one approach, HIV enhancer and transacting response element sequences were placed downstream of the 5'MLV long terminal repeat(LTR) and immediately 3' of the viral enhancer tar elements were placed either the human IL2 cDNA or the chloramphenicol acetyltransferase gene. In this case, transactivation is provided by coinfection with another retrovirus vector containing the viral tat and art coding regions. In the second approach, the first exon of tat1 was inserted between HIV enhancer tar sequences and IL2 coding sequences. Infection of human lymphoid cells with both vectors results in high production of biologically active interleukin 2 as determined in a sensitive CTLL assay. The first approach is useful in that expression of insert DNA is attained only on coinfection with tat expressing vector, and thus offers an inducible property to the expression system. The second approach provides all of the features necessary for transactivation and expression on the one retrovirus vector, thus negating coinfection. Both vectors provide a level of expression far in excess of that obtained with retroviral long terminal repeats or with powerful constitutive promoters. In addition, due to selective tropism and replication of HIV-1 for lymphoid cells, these vectors allow efficient expression of genes in these cell types.

G 302 PURIFICATION AND CHARACTERIZATION OF FULL LENGTH RECOMBINANT HIV-1 REVERSE TRANSCRIPTASE, Gary M. Lazarus, Christine Debouck, Chester A. Meyers, and Valerie Mizrahi, Smith Kline and French Laboratories, King of Prussia, PA, 19406-0939.

Full length, enzymatically active HIV-1 reverse transcriptase (RT) was produced in milligram quantities from a novel E. coli expression system. The enzyme was purified from cell lysates by phosphocellulose and Q- Sepharose column chromatography. The purified product consisted of approximately equal amounts of two peptides of molecular weight 66 and 51 kDa. The product was judged to be 95% pure by SDS- PAGE. The amino acid composition analysis agreed with that predicted from the gene sequence. NH_2- terminal sequence analysis indicated that both peptides had the same amino acid sequence in the first 16 cycles that could be identified. This sequence was identical to that reported for RT isolated from virions. Carboxy- terminal sequencing studies will also be presented. The recombinant product displayed both reverse transcriptase and ribonuclease (RNase) H activity. Only the 66- kDa peptide activity, suggesting that the domain responsible for this function is mostly, if not entirely, in the COOH- terminal region of the molecule.

G 303 PACKAGING SIGNALS FOR HIV-1, Andrew Lever, Heinrich Gottlinger, William Haseltine, Joseph Sodroski, Dana-Farber Cancer Institute, 44 Binney Street, Boston MA

The exact site of packaging signals in the HIV genome is as yet unknown although, analogous to other retroviruses, it is supposed that the area 3' to the splice donor and 5' to the gag ATG is a likely candidate. We have constructed mutations in this region and produced viruses which are highly replication defective yet produce abundant viral protein. This replication defect appears to be cis-acting and we have investigated the ability of the mutants to produce virions which contain no RNA and their ability to package other defective viral genomes containing marker genes. These results will be discussed.

G 304 DELETION MUTANTS IN U3 REGION OF HIV-1 HAVE POSITIVE OR NEGATIVE EFFECTS ON THE VIRAL REPLICATION AND THE PROMOTER ACTIVITY, Yichen Lu and William A. Haseltine, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA USA

The human immunodeficiency virus type 1 (HIV-1)is the primary etiological agent of the acquired immune deficiency syndrome (AIDS) and associated diseases. An important regulatory mechanism of the viral replication is the control of the viral gene transcription, which mainly results from the interactions between the regulatory factors and the cis-acting regulatory sequences within the long terminal repeats (LTR) of the virus. We have studied the effects of different deletion mutants in the LTR, including the NRE, the enhancer, the TATA box, and some other possible protein binding sequences, on the viral replication as well as their promoter activity. We demonstrate that there are indeed direct relations between the specific sequence blocks and the viral replication rates. We also can show that a regulatory factor, which is not the nef nor the vpu can down regulate the viral growth.

G 305 THE REV GENE OF HIV-1: A MODULATOR OF VIRAL STRUCTURAL AND REGULATORY GENE EXPRESSION, Michael H. Malim, Joachim Hauber and Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, N.C. 27710.

HIV-1 encodes two predominantly nuclear transactivators, tat and rev, that are required for viral replication in transfected cells. Each protein is expressed from an overlapping yet distinct gene consisting of two exons, thus these genes share the same intron. The tat transactivator greatly enhances the expression of sequences linked in cis to the viral LTR, whilst rev is required for the expression of the viral structural proteins, gag, gag-pol and env. Using a variety of genomic and copy DNA expression plasmids in transient transfection studies, we have previously demonstrated that rev acts to increase the levels of unspliced mRNAs within the cytoplasm. This permits structural gene expression. In terms of transactivator expression this has differing consequences. The non-expression of the second exon of tat by the inhibition of splicing across its intron and the presence of a stop codon adjacent to the splice donor, truncates the gene product by 14 aa at the carboxyl terminus (86 aa to 72 aa); to date this foreshortened tat appears to be fully functional. In contrast, the first exon form of rev is completely inactive. Thus the expression of rev results in the down regulation of its own synthesis. We will present data that addresses the precise mechanism of action of rev. This is currently believed to be largely post-transcriptional and can be potentially at the level of RNA splicing, stability, nuclear export or even translation. In addition, we will discuss experiments aimed at defining the target sequences for rev action within the HIV genome; and the possible identification of cellular factor(s) involved in these processes.

G 306 **Disparate Effects of Two Herpesvirus Immediate-Early Gene Trans-activators on the HIV-1 LTR**, David Markovitz, Shannon Kenney, James Kamine, Marilyn Smith, Randy Fenrick, Eng-Shang Huang, Craig Rosen and Joseph S. Pagano. Lineberger Cancer Research Center, University of North Carolina at Chapel Hill 27599-7295 and Dana-Farber Cancer Institute, Boston, MA 02115.

The BMLF1 region of the Epstein-Barr virus (EBV) genome and the immediate-early (IE) region of human cytomegalovirus (HCMV) both encode proteins which can trans-activate heterologous promoter/chloramphenicol acetyl transferase (CAT) constructs, including a human immunodeficiency virus type-1 promoter/CAT construct (S. Kenney, J. Kamine, D. Markovitz, R. Fenrick, and J. Pagano, PNAS, 85:1652-1656, 1987; M. Davis, S. Kenney, J. Kamine, J. Pagano, and E. S. Huang, PNAS, 84:8642-8646, 1987). We here demonstrate that this trans-activation by the EBV BMLF1 gene product, which we have previously shown to be largely post-transcriptional, is reporter gene dependent. In contrast, trans-activation by the HCMV-IE gene product(s), previously shown to be mediated at the RNA level, is reporter gene independent and requires the presence of HIV promoter sequences.

G 307 DYE-MEDIATED PHOTOLYSIS OF HIV-1 AND HIV-INFECTED CELLS, Linda S. Martin*, Gregory J. Krueger**, Sherry L. Orloff*, Fritz Sieber**. *Centers for Disease Control, Atlanta, GA 30333, **Midwest Children's Cancer Center, Milwaukee, WI 53201 We evaluated the anti-viral effect of the lipophilic photosensitizer, merocyanin 540 (MC540) on cell-free human immunodeficiency virus, type 1 (HIV-1) and HIV-infected cells. This dye, which has low toxicity for humans, has previously been shown to inactivate cytomegalovirus, herpes simplex virus, and HTLV-1. It also binds selectively to immature hemopoietic cells, and some leukemia and tumor cells. Cell-free HIV, HIV-infected phytohemagglutinin (PHA)-stimulated lymphoblasts, or H-9 HIV-infected cells, were treated with MC540 and then exposed to white light for specified time intervals. After treatment, the ID_{50} titers of the preparations were determined. Treatment of cell-free HIV with dye and a 15 min exposure to light decreased the ID_{50} titers, ranging from 10^2-10^6, to undetectable in most cases. When H-9 cells were assayed, the cellular ID_{50} titers ranged from 1-60. This number increased with increased exposure to light. After treatment for 60-90 min, little or no virus could be detected following co-cultivation of treated H-9 cells with PHA lymphoblasts for 7 days. Similar results were obtained when 3-7 day HIV-infected PHA lymphoblasts were treated with MC540 and light. Increasing the cell concentration 10 fold resulted in decreased efficacy of treatment. Treatment of virus or cells with light or dye alone did not reduce the ID_{50} titer more than 1 log. Treatment of blood products with MC540 and light may significantly reduce the amount of HIV, both cell free and cell associated.

G 308 EXPRESSION, AND PURIFICATION OF RECOMBINANT HIV-1 p24 AND EVALUATION OF IMMUNOLOGICAL REACTIVITY BY SPECIFIC ANTIGEN ASSAY, Bruce J. McCreedy, *Sylvia Crush-Stanton, *Bonnie Swerdlow, *Thomas M. Venetta, *Bryan T. Butman, and Pierce R. Youngbar, Organon Teknika Corporation, Durham, NC 27704 and *Bionetics Research Institute, Rockville, MD 20850. A full length recombinant clone of p24 was generated from a cDNA clone of $HIV\text{-}1_{RF}$[1]. The stable recombinant p24 protein was expressed in E. coli and purified from the cell mass as a soluble protein. The purified protein was shown to be authentic p24 by western blotting and probing with anti-p24 monoclonal antibodies and by N-terminal amino acid sequencing. The purified recombinant protein represents amino acids 133-363 of the gag open reading frame and contains all of the antigenic determinants previously identified by epitope mapping experiments using a panel of anti-p24 monoclonal antibodies and several p24 peptides of varying lengths derived from a subgenomic library of $HIV\text{-}1_{RF}$ gag DNA. The immunological reactivity of the purified recombinant p24 protein was determined by a p24 specific antigen assay. These results were quantitated and compared with data obtained using native p24 antigen purified from HIV-1 viral lysate. The results indicate that the recombinant p24 antigen may prove to be useful for in vitro diagnostics.

[1] Kindly provided by Beatrice Hahn, Flossie Wong-Staal and R.C. Gallo.

G 309 MOLECULAR HETEROGENEITY OF THE AIDS ASSOCIATED LYMPHOMAS (AANH-L). M.S. McGrath*, L. Kaplan*, E. Feigal*, F. Khayam-Bashi*, B.Herndier+, C. Grimaldi#, T. Meeker#. *AIDS Activities Division, Department of Medicine, UCSF San Francisco General Hospital, San Francisco, CA 94110: +Department of Pathology, UCSF, San Francisco; #Department of Medicine, UCSF, Ft. Miley VA Hospital, San Francisco, CA 94121. AIDS associated non-Hodgkins lymphoma is increasing in frequency in individuals infected with HIV. In order to test whether a specific etiology or risk factor could be identified in this disease, we performed an in depth molecular analysis on 24 biopsy specimens obtained at time of diagnosis from patients with AANH-L. Immunoglobulin gene rearrangement analysis on DNA extracted from these specimens identified 14 as monoclonal and 10 as polyclonal B cell proliferations. Eight of ten of the polyclonal lymphomas had an immunoblastic phenotype; the monoclonal lymphomas included Burkitt's and large cell histologies with all six primary CNS lymphoma having an immunoblastic phenotype. EBV DNA was present in one third of all tumors. C-myc rearrangements were detected in 6 out of 14 of the clonal lymphomas. Patients with polyclonal lymphomas lived longer than patients with monoclonal disease (p =.003) and had higher total CD4 cell numbers at the time of diagnosis (369/120). We conclude that at least two characteristic disease processes occur in individuals with AANH-L, with non-EBV associated polyclonal B cell lymphomas occurring in up to one-third of patients presenting with a peripheral, non-CNS lymphoma.

G 310 BIOLOGICAL SIGNIFICANCE OF HIV-1 ENVELOPE HETEROGENEITY. Terry McNearney, Benjamin Thielan, Robert Whittier, David Trowbridge, & Lee Ratner, Dept. of Medicine, Washington University, St. Louis, MO 63110. Objective: This study examines the nature and biological significance of naturally occuring mutations in HIV-1's derived from blood, brain, and lung, of 4 patients involved in a common source outbreak. Methods and Results: Isolates were obtained by brief cultures on peripheral blood mononuclear cells from tissues of an HIV-1 positive blood donor (Pt 1) and 3 transfusion recipients who developed AIDS (Pts 2-4). Isolates from the lung and brain of Pt 2 replicated to 100-fold higher levels on monocytes compared to the blood isolates from the same patient; no differences in growth on lymphocytes were noted. The 3' portion of the genome was cloned from each isolate, and env sequenced. The predicted amino acid sequence from the lung isolate of Pt 2 shows a divergence of 17% compared to clone HXB2. The positions of Cys residues are conserved in 8U and 2 amino acid differences in the CD4 binding region are found. The sequence of a blood isolate from Pt 3 shows only 0.6% amino acid differences from that of the lung isolate of Pt 2. Conclusions: There are few sequence differences between isolates of 2 different individuals infected from the same source. Profound differences in the ability of isolates to grow on monocytes may therefore be determined by one or a few amino acid sequence changes. Analysis of functional clones derived from these isolates will better define the molecular basis for monocyte-tropism.

G 311 INHIBITION OF HIV MEDIATED CYTOPATHICITY BY POLY (L-LYSINE) CONJUGATED SYNTHETIC ANTISENSE OLIGODEOXYRIBONUCLEOTIDES, C. Meier, P.L. Iverson*, M. Stevensen, Molecular Biology, Department of Pathology and Microbiology, *Department of Pharmacology, UNMC, Omaha, NE 68105 The ability of Poly(L-lysine)-conjugated and methylphosphonate conjugated synthetic HIV antisense oligodeoxyribonucleotides to protect susceptible host cells from the cytopathic effects of HIV infection was studied. Following infection of a cytolysis sensitive CD4+ cell line MT4, cells were cultured in the presence or absence of oligomer and viral infectivity and cytopathicity monitored. By day 2 post-infection the abundance of viral antigens in all infected cultures indicated that the oligomers did not significantly affect viral infectivity. Similarly, no significant effects on relative viral RNA loads, as evidenced by a ribonuclease protection assay, were apparent although presence of poly(L-lysine) modified oligomer complimentary to the HIV splice donor resulted in 60-70% reduction in virus production from infected cells. In addition, cells cultured with poly(L-lysine) modified splice donor oligomer were protected from HIV mediated cytopathic effects while the other cultures rapidly succumbed to the cytotoxic effects of HIV infection. The presence of poly(L-lysine) conjugated oligomer resulted in the establishment of a persistent HIV infection characterized by a high level virus release in the absence of cell death while treatment of persistently infected cells with phorbol exter resulted in renewed cytotoxicity. These results demonstrate the ability of synthetic antisense oligonucleotides to confer cytopathicity resistance to susceptible host cells.

G 312 EFFECTS OF p40 (TAX) OF HTLV-I ON GENE EXPRESSION AND ON CELL GROWTH,
Masataka Nakamura, Kiyoshi Ohtani, Kinya Nagata, Noboru Numata, Masaru Niki and Kazuo Sugamura, Department of Bacteriology, Tohoku University School of Medicine, Sendai 980, Japan
Human T-cell leukemia virus type I (HTLV-I) is an oncogenic retrovirus although no typical oncogene is present. At the 3' end of the viral genome a novel open reading frame which encodes three distinct proteins has been identified. One of them, p40, has been established as a trans-acting transcriptional activation factor which transactivates cellular and viral genes, and is thought to be involved in leukemogenesis. We previously demonstrated that forskolin and TPA that are activators of adenylcyclase and protein kinase C respectively is able to induce enhancer function of HTLV-I, and that the SV40 enhancer is activated by p40 in a limited repertorie of cells. The mechanism by which p40 transactivates cognate and non-cognate genes was studied. We found that (1) p40 and forskolin mediated activation required a sequence within a 21 bp core enhancer, which contains a core sequence of a cyclic-AMP responsive element. (2) Nuclear extract from p40-expressing cells, however, gave the same pattern of the gel shift with the 21 bp sequence as that attained by nuclear extract from p40-unexpressing cells. (3) Expression of a protooncogene, c-fos, was enhanced by p40. (4) A subfragment of the SV40 enhancer, which contains an element for NF-kB, was a target element for transactivation by p40. These results indicate that p40 induces at least two independent pathways for transactivation, which are mediated by distinct cellular factors. Experiments for evaluating the ability of p40 to stimulate cells to grow will be presented.

G 313 THE HIV tat GENE INDUCES BOTH DERMAL LESIONS RESEMBLING KAPOSI'S SARCOMA AND HEPATOCELLULAR CARCINOMA IN TRANSGENIC MICE,
Laura A. Napolitano[1,3], Jonathan Vogel[1], Steven H. Hinrichs[2], R. Kay Reynolds[1], Paul A. Luciw[2], and Gilbert Jay[1], [1]Laboratory of Molecular Virology, National Cancer Institute, Building 41, Bethesda, MD 20892, [2]Department of Human Pathology, University of California School of Medicine, Davis, CA 95616, [3]Howard Hughes Medical Institute, Bethesda, MD 20814
The tat gene of HIV is a potent transactivator of viral gene expression and is essential for viral replication. When the tat gene, under control of its own regulatory region, is placed in the germline of mice, skin lesions that resemble Kaposi's Sarcoma in AIDS are induced. In addition, hepatocellular carcinoma occurs at high frequency in older (>17mos.) male mice. This animal model should allow us to investigate the role of HIV, and specifically the tat gene, in cancers associated with HIV infection.

G 314 PURIFICATION OF BIOLOGICALLY ACTIVE *tat* PROTEIN: ASSAY FOR TRANSACTIVATION AND *tat*:HOST-PROTEIN INTERACTIONS. Peter Nelbock, Reiner Gentz, Chein-Hwa Chen and Craig A. Rosen. Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110. Expression of the human immunodeficiency virus *tat* protein is required for virus replication. A genetic approach was used to facilitate the purification of biologically active Tat. A recombinant *tat* protein containing a stretch of six histidine residues and a protease cleavage site was engineered and purified to greater than 95% homogeneity in a single step using an immobilized metal ion chromatographic procedure with selectivity for proteins with neighboring histidine residues. A method for introduction of protein into cell monolayers was used to demonstrate that the purified Tat retained biological activity. Tat function was completely blocked in the presence of transcription inhibitors, therefore demonstrating the requirement of ongoing mRNA synthesis for transactivation. These studies indicate that the mechanism of transactivation is unlikely to involve a direct action of Tat on mRNA stability, transport or translation and provides the basis for a rapid assay that can be used to identify inhibitors of transactivation. The purified Tat:RNA protein was also used to examine Tat RNA interactions as well as interactions with host cell factors. The results of these studies will be presented.

G 315 NEF RESPONSIVE SEQUENCES (NRS) OF THE HIV-1 LTR MEDIATE TRANSCRIPTIONAL SUPPRESSION. Thomas Niederman and Lee Ratner, Department of Medicine, Washington University, St. Louis, MO 63110.
Objective: The nef gene of HIV-1 encodes a 206 amino acid, 27 kd product that down regulates virus replication. This effect is mediated by NEF suppression of transcriptional initiation. This study identified a cis acting element in the HIV-1 LTR which is responsive to NEF. Methods and Results: Two proviral HIV-1 clones were constructed, pHIV F+ which bears an intact nef gene, and pHIV F- with a frameshift at codon 34. These plasmids were transfected into COS-1 cells. Nuclear run-off assays and viral RNA and protein analysis demonstrate that NEF suppresses RNA initiation and viral RNA and protein levels 2-10-fold. A NEF expression vector, pSVF, was made using an SV40 promoter. Co-transfection of pSVF in 4-fold excess with HIV-LTR-CAT resulted in an 8-fold depression of CAT activity. NEF-mediated suppression was dose-dependent and was specific for the HIV-1-LTR in that the SV40 promoter, and the LTRs of HTLV-I, MPMV, RSV were unaffected. HIV-1-LTR CAT deletion mutants were used to map the NRS. These constructs were co-transfected into COS-1 cells with or without pSVF or TAT. NRS was mapped between nucleotides -156 and +80 relative to the RNA inititiation site. Conclusions: NEF is a specific transcriptional silencer of the HIV-1 LTR acting through sequences between -156 and +80. Precise definition of NRS and the mechanism of transcriptional down-regulation are being analyzed.

G 316 PURIFICATION OF A T CELL FACTOR THAT BINDS TO THE CONSERVED 21-BP REPEATS IN THE HTLV-I AND HTLV-II PROVIRAL LTR'S, Jennifer K. Nyborg and William S. Dynan, University of Colorado, Boulder, CO 80309.

Efficient expression of Human T Cell Leukemia Virus type I (HTLV-I) requires three imperfect 21-bp repeats, located in the proviral long terminal repeats (LTR), upstream of the viral RNA initiation site. We have used DNase I footprinting to show that these repeats are recognition sites for three distinct cellular proteins, and that the presence of these factors varies between cell types. One of these proteins, present in HeLa cells but not T lymphocytes, is identical to the previously described transcription factor AP-2. AP-2 binds winthin and immediately adjacent to each of the three 21-bp repeats in HTLV-I, and to two sites downstream of the RNA initiation site. A second factor, HEF-IB, is present in all cell types tested. HEF-IB binds independently to the promoter distal 21-bp repeat, and cooperatively, with AP-2 to the middle repeat. A third factor, HEF-IT, is present in T cells and binds directly to each of the 21-bp repeats in both HTLV-I, and the related virus, HTLV-II. HEF-IT was not detected in HeLa cell extracts. We have purified HEF-IT to apparent homogeneity, and have begun biochemical characterization of the protein. We have also initiated functional studies to identify the role of HEF-IT, together with other cellular and viral factors, in HTLV-I gene expression.

G 317 HIV INFECTION OF CD4$^+$ T CELLS DOES NOT REQUIRE ENDOCYTOSIS OF THE CD4 MOLECULE, Gregg M. Orloff[1,2], J. Steven McDougal[1], [1]Centers for Disease Control, Immunology Branch, and [2]Emory University School of Medicine, Department of Microbiology and Immunology, Atlanta, GA 30333.

The infection of CD4$^+$ T cells by HIV was examined to determine if internalization of the CD4 molecule is a requisite step in the infection process. Virus was bound to cells under conditions that do not permit penetration and subsequently placed in a permissive environment. Cell surface CD4 densities were measured by immunofluorescence at various times after infection. No alteration in CD4 density was detected at any time after infection using this technique.

G 318 INACTIVATION OF HIV USING COMMERCIAL LYSING REAGENTS FOR FLOW CYTOMETRY ANALYSIS, Sherry L. Orloff, Centers for Disease Control, Atlanta, GA 30333.

Many clinical and research laboratories routinely perform flow cytometric analysis on white blood cells in blood which is potentially contaminated with HIV. Cell surface immunofluorescence staining is performed with whole blood preparations, in which the red blood cells are lysed using a commercial preparation. Two of these products, FACS lysing solution and COULTER CLONE immunolyse and fixative, were evaluated for their ability to inactivate cell-free HIV (LAV prototype strain) and H-9 cells infected with HTLV-III. The FACS lysing solution inactivated both cell-free HIV and infected H-9 cells as determined using a sensitive ID_{50} bioassay and an antigen ELISA. ID_{50} titers of test preparations were rendered undetectable following treatment for 10 minutes. COULTER CLONE immunolyse was also shown to reduce ID_{50} titers of HIV. Studies are currently underway to assess the efficacy of inactivation of HIV by the COULTER CLONE fixative which contains formaldehyde and methanol, agents which have already been reported to inactivate HIV.

G 319 PEPTIDE INHIBITORS OF HIV-INDUCED CELL FUSION, Randall J. Owens, Ranga V. Srinivas, G.M. Anantharamaiah, Jere P. Segrest and Richard W. Compans, Departments of Microbiology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294.
The envelope glycoprotein of human immunodeficiency virus (HIV) is essential for virus attachment and entry into host cells. Additionally, when expressed on the plasma membrane of infected cells, the envelope protein is responsible for mediating cell fusion which leads to the formation of multinucleated giant cells, one of the major cytopathic effects of HIV infections. We have synthesized two different classes of oligopeptides that inhibit HIV-induced cell fusion. The first class represents short peptides with various degrees of homology to the N-terminus of gp41. These peptides are effective inhibitors of cell fusion *in vitro* at concentrations ranging between 0.5 mM and 8 mM. The greatest inhibition was observed with a peptide that was 100% homologous to the first six amino acids of gp41, while slight changes in sequence reduced the peptide's inhibitory activity. The second class of peptides represent amphipathic alpha-helixes of the type found in the plasma apolipoproteins; they have both strong affinities for phospholipid membranes and a strong disruptive effect on bilayer organization. We tested the possibility that amphipathic helix-containing peptides and proteins may inhibit those steps of HIV infection involving membrane fusion. Apolipoprotein A-I, the major protein component of high density lipoprotein, and its amphipathic peptide analogs proved to be potent inhibitors of HIV-induced cell fusion at concentrations below 100 micro-molar. Neither class of peptides had any effect on envelope protein synthesis, processing or transport; however, they did promote dissociation of gp120 from gp41 at the cell surface. These results indicate that two classes of structurally different oligopeptides inhibit HIV-induced cytopathology by what appears to be a similar mechanism, and that they should be evaluated futher as potential antiviral agents.

G 320 SUBSTRATE DETERMINANTS OF THE HIV PROTEINASE, Kathryn Partin, Hans-George Kräusslich, Eckard Wimmer, and Carol Carter. Dept. of Microbiology, SUNY at Stony Brook, Stony Brook, NY, 11794. We are using site-directed mutagenesis to determine the minimum requirements for processing by the HIV proteinase at the cleavage site which generates the amino terminus of CA (p24). Single amino acid substitutions have been made at and around the Y/P site at amino acid 132-133, including "conservative" and "radical" substitutions at each position. The mutated precursor proteins were incubated with partially purified HIV proteinase generated in *E. coli*, which permitted us to distinguish between sites which partially inactivated the substrate versus those which completely inhibited it. By this assay we see three classes of mutants: 1) those in which processing was completed generating WT CA, 2) those in which processing was completed generating variant CA, and 3) those in which processing was incomplete, generating a resistant 42kD intermediate. These results suggest that both sequence and conformation are essential for accurate processing by the HIV proteinase.

G 321 DISCRIMINATORY ANTIBODY ASSAYS FOR HIV-1 AND HIV-2. Deborah A. Paul, M. Knigge, J. Hunt, R. Allen, G. Dawson, Abbott Laboratories, North Chicago, IL 60064. An assay that will distinguish between HIV-1 and HIV-2 would be useful for epidemiologic studies and differential diagnosis of HIV infection. There is significant cross-reactivity of antibodies to core and polymerase proteins of the two viruses, but envelope proteins have less amino acid homology (35%) and therefore offer the potential ability to discriminate between antibodies to HIV-1 or HIV-2. Monoclonal antibodies to the transmembrane env glycoprotein of HIV-1 and HIV-2 were generated and used in competition assays. The competitive assay format offered much better discrimination than direct assay formats even when env enriched fractions were used on the solid phase. Sample antibody competed with monoclonal antibody for binding to either native purified HIV-1 or HIV-2 coated on a solid phase. A labeled second antibody was used for the enzyme reaction. Antibody was detected in sera with titers of 1:150,000 HIV-2 and 1:6500 HIV-1. All HIV-1 or HIV-2 antibody positive sera were detected as such in their respective assay. Less than 5% cross-reactivity was seen, due to inherent similarities of HIV-1 and HIV-2 at gp41 shown on Western blot and at gp160 (but not gp120) on RIPA. 20% of sera which tested positive for HIV-2 antibody showed evidence of "dual infection", reacting as positive in both competitive assays and giving banding patterns on Western blot against HIV-1 and HIV-2, including reactivity at gp120, consistent with infection with both agents. Whether these are truly dual infections or just high degrees of immunologic cross-reactivity remains to be determined. Infection with HIV-1 and/or HIV-2 can be discriminated using native virus, with specificity derived from monoclonal antibodies to envelope proteins.

G 322 Seroprevalence of HTLV-I in Various Patient Populations. Bernard J. Poiesz[1], Garth Ehrlich[1], Lawrence Papsidero[2], Richard Montagna[2] and the HTLV-I Working Group. [1]Department of Medicine, SUNY Health Science Center, Syracuse, NY 13210 and [2]Cellular Products, Inc., Buffalo, NY 14202

Over a six month period sera and peripheral blood mononuclear cells (if available) were collected from the patients listed below. Sera was analyzed for anti-HTLV-I antibodies using an ELISA assay and confirmed by a Western blot and RIPA assay. A designation of positive required three repeats on ELISA and reactivity to at least 3 HTLV-I gene products on Western blot and RIPA.

Diagnosis	No. of Specimens	No. Positive	%	P value
Volunteer blood donors	10,000	2	(0.02)	—
Paid blood donors	6,010	7	(0.12)	0.02
Intravenous drug abusers (Caucasian)	223	10	(4.50)	<0.0001
Homosexuals, hemophiliacs	148	1	(0.67)	0.04
CML,AML,ALL,CLL, Rheumatologic disorders	700	0	(0.00)	NSD
Lymphoma	860	8	(0.95)	<0.00001
A) Hodgkin's	300	0	(0.00)	NSD
B) Non-Hodgkin's	500	8	(1.60)	<0.00001
1) Low grade	360	0	(0.00)	NSD
2) Other than low grade	200	8	(4.00)	<0.00001

Distinct differences in HTLV-I seroprevalence existed amongst the populations. At time of presentation, analyses for HTLV-I and HTLV-II DNA via PCR will also be available.

G 323 THE RESPONSE OF THE HTLV 1 LTR TO CYCLIC AMP, Harry T. Poteat, Paula Kadison, Kathleen McGuire, Lisa Park, Joseph G. Sodroski, and William A. Haseltine, Harvard School of Public Health and Dana Farber Cancer Institute, Boston, MA. 02115 The human T cell leukemia virus type 1 long terminal repeat contains a heptanucleotide sequence similar to the cyclic AMP responsive element (CRE) consensus sequence within each of its 21 nucleotide repeat sequences (TAR 21s). These sequences, which control transcriptional initiation of the HTLV 1 provirus, are responsive to intracellular levels of cyclic AMP. Analysis of deletion and site directed mutations of the HTLV 1 LTR show that the CRE like sequence is required for the cyclic AMP mediated increase in transcription. Although the CRE like sequences are contained within the TAR 21 sequences which confer tax protein responsiveness to the HTLV 1 LTR, several lines of evidence show that the mechanisms of promotor induction by cyclic AMP and the tax protein are to a degree independent. Measurement of cyclic AMP in tax producing cell lines and matched controls shows that tax does not increase cellular cyclic AMP levels; furthermore elevated levels of cyclic AMP are not necessary for transactivation by the tax protein. The results suggest that a signal which inhibits proliferation and activation in normal T lymphocytes may trigger a mitogenic response in HTLV 1 infected cells. The data have implications for viral persistence, control of viral gene expression, and viral pathogenesis.

G 324 IDENTIFICATION AND SYNTHESIS OF THE EPITOPE FOR A HUMAN MONOCLONAL ANTIBODY WHICH CAN NEUTRALIZE HTLV-I, S. Ralston, P. Hoeprich, and R. Akita, Triton Biosciences Inc., Alameda, CA 94501. A monoclonal antibody (mAb), designated 0.5α, derived from a patient with adult T-cell leukemia was found previously to neutralize the HTLV-I virus in *in vitro* assays. We designed experiments to determine the epitope for this mAb. Using simultaneous multiple peptide synthesis, we synthesized 481 overlapping octapeptides which corresponded to the sequence of the HTLV-I envelope glycoprotein (gp46). We mapped the epitope for mAb 0.5α to lie between residues 186-195 of gp46. This result was confirmed by independently synthesizing a peptide, [Tyr^{175}, Cys^{176}]*env*175-196, which bound specifically to mAb 0.5α with an approximate K_a = 4 X 10^7 M^{-1}. In addition, this peptide inhibited mAb 0.5α binding to gp46 derived from T-cells infected with HTLV-I. This epitope containing peptide may facilitate understanding HTLV-I infection of T cells.

G 325 Mutation of the C-Terminal Arg Residue of the HIV-1 gp120 Blocks Envelope Protein Cleavage.M.S.Reitz,H.-G. Guo, F. diMarzo Veronese, R. Pal, V.S.Kalyanaraman, and R.C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Inst., NIH, Bethesda, Md. and Dept. of Cell Biology, Bionetics Research Laboratory, Inc., Rockville, Md

The env proteins of HIV-1 are synthesized as a precursor polypeptide (gp160) which is cleaved to an external gp120 and a transmembrane gp41. The cleavage site for retroviral envelope polypeptides is immediately to the carboxy side of a cluster of basic amino acids, which in HIV-1 terminates at arg511. We changed this arg residue to a thr. The mutant, thr511, when transfected into cos-1 cells produced normal levels of viral structural proteins, but the gp160 was not detectably cleaved, and no infectious virus was produced. Release of gp160 or gp 120 into the media is not detectable, and the transfected cells lack the ability to form syncytia when cocultured with CD4+ cells. The data suggest that HIV-1 envelope cleavage is required for transmission both by release of infectious particles and by cell to cell fusion.

G 326 PREVALENCE OF HTLV-1 ANTIBODIES AND HTLV-1 DNA AMONG RELATIVES OF UK TROPICAL SPASTIC PARAPARESIS PATIENTS. J.H. Richardson, J.K. Cruickshank[1], A.L. Newell[1], P. Rudge[1], A.G. Dalgleish[1], O. Morgan[2]. 1. Clinical Research Centre, Harrow, HA1 3UJ, UK, 2. University of the West Indies, Kingston 7, Jamaica.

17 UK TSP patients of Jamaican origin all have antibodies to HTLV-1. We have examined HTLV-1 antibody prevalence in first degree relatives of these patients to investigate factors associated with transmission (breast feeding, blood transfusion, age, length of time spent in an endemic area). 60 out of 69 living relatives were tested and antibodies were detected in 3 of 25 (12%) relatives born and resident in Jamaica (mean age 52 yrs), 5 of 21 (24%) Jamaican-born relatives now resident in the UK (mean age 46 yr) but none of the 14 offspring born and resident in the UK (mean age 20 yr). All the relatives had been breast fed and none had had blood transfusions. The data do not support a pattern of mother to child transmission. Increasing age and/or having lived in the Caribbean appear to increase the risk of sero-conversion. HTLV-1 antibody titres were significantly higher in the TSP patients than in their sero-positive but asymptomatic family members. Using a DNA amplification technique (PCR), we have detected HTLV-1 gag and X region sequences in peripheral lymphocytes of TSP patients and sero-positive relatives. Sero-negative relatives are now being examined.

G 327 EFFECT OF 3'AZIDO-2'3'DIDEOXYTHYMIDINE (3'AZT) ON HIV-1 REPLICATION AND ON CELLULAR GENE EXPRESSION IN INFECTED CELLS, Ronald Rooke and Mark A. Wainberg, Department of microbiology and immunology, McGill University, Montreal, Canada, H3T 1E2.

The drug 3'AZT, used clinically in AIDS patients, acts specifically as a viral chain terminator of nascent HIV-1 DNA, because of its high affinity for the RT enzyme. Our studies have shown that 3'AZT inhibits accumulation of the episomal form of HIV-1 DNA during productive infection. Such drug-treated cells nevertheless progress toward death showing that accumulation of viral DNA in infected cells is not the main cause of cytopathy of HIV-1. Exposure of chronically infected cells to various concentrations of 3'AZT (0-50 lg/ml) for up to 180 days did not cause any noticeable variation in the affinity of the RT enzyme for AZT nor did it cause any variation at the genomic level. However, it resulted in significantly diminished production of both progeny virus and RT activity. We postulate that 3'AZT may be able to complex with newly-formed RT (p51/66) and to interfere in some way with assembly of progeny HIV-1 at the cell membrane. Furthermore, northern blot and FACS analyses done on HIV-1-infected PBL show that 3'AZT reestablishes the cellular virus receptor (CD4) protein expression, decreased by HIV-1 infection at the cell surface, by augmenting CD4 mRNA levels. Other data show that 3'AZT acts as a general transcription enhancer similar to 5'azacytidine.

G 328 **RIBOZYMES TARGETED TO HIV-1 RNAs**, John J. Rossi and Piroj Chang, Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, Calif. 91010. **The self-catalyzed cleavage of certain plant viruses and virusoids as well as newt satellite RNAs appear to share a universal requirement for a set of conserved nucleotides which have been configured into a so-called "hammerhead structure". The basic elements of this structure are the nucleotides 5'- $GAAACX_nGUN^V$------CUGANGA_3'. When these sequences come together in the correct conformation, an autocatalytic cleavage event takes place at the nucleotide indicated above. We have taken advantage of this system to develop ribozymes which hydrolyze HIV-1 RNAs in vitro. Two strategies have been utilized. The first was to locate $GAAACX_nGU$ sequences in HIV, and then design synthetic RNAs complementary to flanking sequences while simultaneously supplying the missing conserved sequences, which in effect supplies one half of the ribozyme in trans. The second strategy involves incorporating all of the conserved elements except the GU(N) in a ribozyme targeted to any GU(N) in HIV-1. This strategy, first proposed and demonstrated to work on a CAT message by J. Haseloff and W. Gerlach (Nature:334, 585, 1988) supplies a complete, precisely targeted ribozyme in trans. We will report on the in vitro cleavage results, which demonstrate the variability of efficiency with each cleavage site. In vivo experiments are also planned, and the results, if any, from these will also be described.**

G 329 FUNCTIONAL ANALYSIS OF THE HUMAN T-CELL LEUKEMIA VIRUS *tax* PROTEIN. Steven Ruben, Richard Kramer[1], Ann Perkins and Craig A. Rosen, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110; [1]Department of Molecular Genetics, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Gene expression directed by the HTLV-1 LTR and IL-2 receptor (IL-2-R) regulatory sequences is transcriptionally activated by the *tax* protein encoded by the 3' end of the viral genome. The LTR and IL-2-R *tax* responsive sequences are dissimilar. Moreover, *tax* does not appear to bind to these sequences directly; rather at least two distinct host cell proteins have been shown to interact with each of the individual *tax* responsive elements. These studies have lead to the hypothesis that *tax* mediates transactivation through an interaction or activation of pre-existing host transcriptional regulatory factors. Oligonucleotide-directed mutagenesis was used to identify domains critical to transactivation and putative *tax*:host-protein interactions. Our results demonstrate that *tax* contains several separable functional domains. The role of the individual domains for activation of LTR, and IL-2-R directed gene expression as well as a recently identified phenotype characteristic of *tax* expression in yeast will be presented.

G 330 HIV PROVIRAL DNA: UNUSUAL STRUCTURES IN HOST GENOMIC DNA AND WITHIN THE PROVIRAL GENOME, Ruth M. Ruprecht, Miguel A. Gama Sosa, Jessica C. Hall, Gregory C. Lucaszewics, and Karen Schneider, Division of Cancer Pharmacology, Dana-Farber Cancer Institute, Boston, MA 02115

Unusual DNA structures such as Z-DNA, H-DNA, cruciforms, Pur-Pyr structures, anisomorphic, bent or slipped DNA may represent control regions for replication, recombination and transcription. Such unusual DNA structures are susceptible to cleavage with the single-strand specific nuclease S1.

We have analyzed the plasmid pHXBc2 which contains a complete proviral genome of the human immunodeficiency virus type-1 (HIV-1) as well as neighboring human genomic sequences, for the presence of unusual DNA structures. In native and ethidium bromide gel electrophoresis, RF I pHXBc2 moved more slowly than RF III, whereas chloroquine agarose gel electrophoresis reversed this unusual electrophoretic pattern. S1 nuclease cleavage of RF I pHXBc2 followed by linker insertion mutagenesis revealed two major S1 cleavage sites: Site I was located within a direct repeat sequence of host DNA 5' to the upstream LTR. This DNA region has the capability of forming either slipped DNA, cruciforms or both. A second S1-specific cleavage site was mapped within the HIV genome. This region can assume either slipped or H-DNA forms. Electrophoretic analysis of different linker insertion mutants obtained after S1 cleavage revealed that unusual DNA structures at Site I are responsible for the unusual electrophoretic mobility of pHXBc2 DNA. Unusual DNA structures found in this HIV proviral DNA clone may have important biological functions.

G 331 CO-AMPLIFICATION OF MULTIPLE REGIONS OF THE HIV-1 GENOME BY THE POLYMERASE CHAIN REACTION: POTENTIAL USE IN MULTIPLE DIAGNOSIS, Martin Ruta, Karen Cristiano, C. Ann Hawthorne, J.S. Epstein and Indira K. Hewlett, Division of Blood and Blood Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

We have used the polymerase chain reaction to detect by co-amplification, multiple regions of the HIV-1 genome in infected cells. HIV-1 RNA and DNA were amplified in reactions with and without reverse transcriptase respectively using primer pairs to the gag, envelope tat and nef regions of the viral genome in the same reaction mixture and PCR-products analyzed by liquid hybridization with end labelled oligonucleotide probes followed by gel electrophoresis (oligomer hybridization). The primer pairs were capable of detecting as few as 5-10 copies of HIV-1 RNA and 10-20 gene copies in the samples. The ability to co-amplify several target regions in the same incubation mixture provides a method for confirming the presence of HIV in samples for which limited nucleic acid is available. In addition, it raises the possibility of multiple diagnosis of viral infections such as those that occur in AIDS on the same sample preparation using primer pairs to more than one virus.

G 332 ANALYSIS OF CLUSTERED MUTATIONS, INSERTIONS AND DELETIONS ON TRANSCRIPTIONAL ELONGATION BY *TAT* THROUGH THE HIV-1 LTR.

Mark J. Selby, E. S. Bain, P. A. Luciw and B. M. Peterlin. Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, CA. 94143 and Department of Medical Pathology, University of California, Davis, CA. 95616.

The HIV-encoded *trans*-activator (*tat*) augments HIV gene expression. While the target sequence for *tat* is known, the trans-acting responsive region (*tar*), the mechanism of *trans*-activation remain elusive. Recently, Kao et al (1987) demonstrated that in the absence of *tat*, short RNA species were detected corresponding to premature transcription termination around +57 in the R region of the LTR. In the presence of *tat*, full-length RNA is recovered, suggesting *tat* acts to relieve premature transcription termination. Here, using transient transfections of clustered mutations and 3' deletions in LTR-CAT constructions, we define the region of *tar* critical for *trans*-activation by *tat*; this region corresponds to the top of a proposed stem-loop. Further, cluster and compensatory mutations reveal the contributions of primary and secondary structure to *trans*-activation. We also find a direct relationship between the predicted stability of the stem-loop and *trans*-activation. Analysis of insertions of 11 bp multiples in the 5' end of *tar* demonstrated an inverse relationship between insertions and *trans*-activation, suggesting that *tar* must be in close proximity to the promoter to facilitate efficient *trans*-activation. RNase protection of transfections of 3' deletions and an insertion demonstrate a relationship between the stability of the stem-loop and detection of prematurely terminated species. Moreover, RNA analysis demonstrates that the change in levels of prematurely terminated RNAs reflects the ability to *trans*-activate. We extend these observations to HIV-2, where we also observe full-length and short transcripts with and without *tat*, respectively. We propose a model whereby the stem-loop specifies the association of *tat* or factors induced by *tat* with an active transcription complex which thus becomes resistant to premature termination.

G 333 THE SV40 AND POLYOMA VIRUS T ANTIGENS CAN INHIBIT TRANS-ACTIVATION OF THE HIV-1 LONG TERMINAL REPEAT BY THE HEPATITIS B VIRUS X PROTEIN, Edward Seto & TS Benedict Yen, Department of Pathology, School of Medicine, University of California San Francisco, CA 94143
We have previously demonstrated that the HBV X protein can trans-activate gene expression directed by the HIV-1 LTR using transient transfection assay in Jurkat lymphoblastic T cells (Seto *et al*, Proc Natl Acad Sci, in press). Under identical conditions, we report here that trans-activation was also observed in CV-1 cells but not COS-7 cells which express the SV40 T Ag. Further, cotransfection of plasmids expressing T Ag from either the SV40 or the polyoma early regions suppressed the trans-activation effect by the HBV X protein in CV-1 cells:

plasmid cotransfected with pHIVLTR-CAT	genes present	relative CAT activity
pECE	none	1.00
pECE-X	HBV X	8.00
pECE-X + pSV3neo	HBV X + SV40 T Ag	0.50
pECE-X + pGS3	HBV X + SV40 T Ag	0.88
pECE-X + pSV5neo	HBV X + Py T Ag	1.12

We are currently studying the possible mechanism of this inhibition.

G 334 TRANSACTIVATION OF THE HIV LTR BY EPSTEIN-BARR VIRUS PROTEINS LMP AND EBNA2, Maryanne C. Simurda, Jessica Heimer and Marie-Louise Hammarskjold, Department of Microbiology, State University of New York at Buffalo, Buffalo, NY 14214.
We have shown that the HIV long terminal repeat (LTR) is more active in the EBV-positive cell line Raji than in the EBV-negative cell line Ramos. This suggests that latent EBV gene products may be able to directly or indirectly activate HIV gene expression. We have now shown that both the EBV latent membrane protein (LMP) and the EBV nuclear antigen (EBNA2) are able to transactivate the HIV LTR. Further, we have identified the regions of the HIV LTR involved in the transactivation by each of these proteins. Different mutated forms of the HIV LTR, ligated to CAT as a reporter gene, were used to transfect CV-1, Ramos and Raji cells. These cells were cotransfected with expression vectors containing the genes for LMP or EBNA2. Results showed that most of the effect of the LMP protein could be mapped in the HIV LTR to the binding sites for NF-kB. The EBNA2 transactivation did not work through a single defined cis-acting element. Thus this effect seems similar to that seen with some immediate-early herpesvirus proteins.

G 335 THE EFFECT OF ALPHA INTERFERON ON HIV REPLICATION IN T CELLS: LACK OF INTRACELLULAR ACCUMULATION OF VIRION PARTICLES. Marilyn S. Smith, E. Leigh Brian, Randy Thresher, and Joseph S. Pagano; Lineberger Cancer Research Center, University of N.C., Chapel Hill, NC 27599. Various targets have been proposed for the action of alpha interferons against different retroviruses. In murine retroviral systmes, alpha interferon appears to alter viral assembly or release, resulting in the accumulation of intracellular virions, located in cytoplasmic vacuoles.

We have noted, as have others, the effect of alpha interferon against exogenous infection of T cells (C3): in this system, the EC_{50} was 5.5 U/ml, and the EC_{90} was 274 U/ml. We have attempted to determine if high concentrations of intracellular particles were accumulating by assaying the extracellular RT and p24 levels, the intracellular p24 and viral protein levels; or by processing the cells on the same day for electron microscopy. In cells treated with various concentrations of IFN, the RT levels and extracellular p24 levels decreased proportionally, whereas the intracellular p24 levels remained fairly constant. Western blots of cellular protein showed only at most a 2X decrease in viral proteins. By EM, the cells treated at higher levels (512 U/ml) showed almost no particles budding into extracellular spaces; however, no intracellular particles could be seen, nor any unusual appearance of the cytoplasm. Thus, the mechanism of inhibition of this human retrovirus by alpha interferon does not appear analagous to that seen in the murine retrovirus system.

G 336 SYNTHETIC PEPTIDES OF HIV-1 ENV, GAG AND POL PROTEINS.

E.D. Sprengers, J.J. Schalken and J.A. Hellings, Diagnostics Research Laboratories, Organon International BV, P.O. Box 20, 5340 BH Oss, The Netherlands.

By a variety of methods peptides were predicted to represent immunogenic or antigenic sites of HIV-1 Env, Gag and Pol proteins. These peptides were synthesized by solid phase methods and their reactivity with anti -HIV-1 sera was evaluated in sandwich- and/or inhibition immunoassays. We identified 6 Env, 1 Gag and 3 Pol peptides that were reactive with anti -HIV-1 sera in a sandwich immunoassay with the peptide adsorbed to polystyrene microelisa plates. Epitope specific antisera could be obtained by adsorption of antibodies from polyclonal sera, using immobilised peptides. Peptides were also used to raise monoclonal antibodies against HIV-1 proteins. We evaluated the use of epitope specific antibodies and synthetic peptides in epitope specific immunoassays.

G 337 EFFICIENT EXPRESSION OF HIV 1 ENVELOPE GLYCOPROTEINS IN CHINESE HAMSTER OVARY CELLS P.E. Stephens, S. Thomson, E. Harris, A. Preneta and G.T. Yarranton, Celltech Limited, Slough, Berks. U.K.

The envelope genes from HIV 1 IIIB have been expressed in Chinese Hamster Ovary (CHO) cells. Expression vectors have been constructed which express authentic gp120 exclusively. This has been achieved by the introduction of a translational stop codon at the cleavage site between gp120 and gp41. Following transfection into CHO cells, cell lines were established which secreted gp120 into the culture medium. The gp120 protein has been purified by a one step immunopurification. The identity of the product has been confirmed by Western blot, amino acid analysis and amino-terminal sequencing. In addition this material has been shown to be biologically active in a CD4 binding assay. Low level expression of gp160 in an inducible system has also been achieved by cloning the complete env gene downstream of a regulatable promoter. The effects of the rev gene product of HIV 1 on the productivity of both gp160 and gp120 producing cell lines will be discussed.

G 338 CLONING AND CHARACTERIZATION OF A NON CYTOPATHIC VARIANCE OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I, M. Stevenson, C. LaMonica, C. Meier, V. Vlach, A. Mann, C. Borgeson, A. Wasiak, Molecular Biology Laboratory, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68105

There appears to be two main processes in the HIV mediated cytopathic process. These include syncytition mediated cytopathicity, and single cell killing, where T Cell destruction occurs in the absence of cell fusion. Here a noncytopathic variant of HIV-1 which is unable to induce single cell killing during the course of infection,was isolated from an HIV seropositive asymptomatic hemophiliac. We exploited the phenomenon of interference to select variants which were noncytopathic in highly succeptible cell lines. Screening of the infected cultures through this procedure by southern Blot analysis using a number of restriction enzymes demonstrated the amplification of a variant with a PVUII polymorphism in the gag region of HIV. This was used as a marker for subsequent cloning of infectious DNA clones of cytopathic and noncytopathic HIV variants. Cloned virus stocks obtained by transfection of HELAcells were used to determine cytopathology. The noncytopathic variant maintained itsphenotype such that infection of cells resulted in a persistant noncytopathicinfection rather than the lytic infection as seen with the cytopathic variant. Wehave constructed viral hybrids where different regions of the cytopathic and noncytopathic variants of HIV have been exchanged in an attempt to delineate features of the HIV genome which are important in single cell killing. These features, together with a hypothesis on the mechanism of single cell killing of HIV will be discussed.

G 339 TRANSCRIPTION FACTORS AND MECHANISM(S) INVOLVED IN HTLV-I TAX-MEDIATED TRANS-ACTIVATION. Tse-Hua Tan, Ran Jia and Robert G. Roeder. Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

Human T-cell leukemia virus type I (HTLV-I) is both a T-lymphotropic and a neurotropic human retrovirus. HTLV-I encodes a 40-kd nuclear protein, named tax, that acts in trans to increase the rate of transcription from the HTLV-I and interleukin-2 receptor promoter. The HTLV-I promoter contains three copies of a 21-bp repeat (hereafter called the tax responsive element or TRE) that is involved both in basal-level expression and in tax-mediated induction. We have identified and either partially or completely purified three nuclear factors (TREB-1, TREB-2, and TREB-3) that bind to the TREs within the HTLV-I promoter. The properties of these three factors will be discussed. The "TGACG" motif found in the center of the 21-bp TRE is also present in cAMP responsive elements (CREs) within the cAMP-inducible gene promoters and in ATF-binding sites (adenovirus Ela-responsive) within the adenovirus early gene promoters. Interestingly, the nuclear proteins that bind to the TREs also bind to the CREs and ATF-binding sites. Furthermore, the TREs can confer upon heterologous promoters responsiveness to various inducing agents. The involvement of tax in signal transduction pathway will be presented.

G 340 CELLULAR IMMUNITY IN AIDS WITH PENICILLIOSIS, Deja Tanphaichitra, Mahidol University, P.O. Box 4-217, Bangkok 10400, Thailand

Earlier we reported two cases with penicilliosis in patients with impaired cellular immunity and a case of recrudescent melioidosis with AIDS. We here described a case of heterosexual female with AIDS associated with penicilliosis: A 43-year old female with a history of 30kg. wt loss, cutaneous lesions, dyspnea and impaired memory for two months. She received blood transfusion abroad where she underwent hysterectomy. Physical examination revealed generalized rash at trunk, extremities, hair loss, with cervical/supratrochlear lymphadenopathy. Lungs showed interstitial infiltrates on X-rays. Pertinent laboratory showed WBC, 3,500/mm3, with 9% lymphocyte; T-cell subset ratio, 0.4-0.15. 2,4-Dinitrochlorobenzene reaction was negative. The HIV antibody was positive by Western Blot method. A yeast-like fungus was isolated on specimen of fungal cultures from lymph nodes and from transtracheal aspiration identified to be Penicillium marneffei. She partially responded with amphotericin B before transfer to the special hospital caring AIDS patients.

The occurrence of Penicillium marneffei infection in patient with AIDS was similar to recrudescent melioidosis in patient with AIDS and was not surprising.

Supported in part by USAID

G 341 THE ROLE OF VPU IN THE LIFE-CYCLE OF HIV-1, Ernest Terwilliger, Eric Cohen, William Haseltine, Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston MA.

Recently we identified a ninth gene of the HIV-1 genome, which we called vpu, by demonstrating cross-reactivity of a significant proportion of AIDS patient antisera with an in vitro synthesized translation product of this reading frame. To understand the role of vpu in the HIV-1 life cycle. Isogenic HIV-1 proviruses, identical except for their capacity to utilize the vpu reading frame, have since been constructed and transfected into CD4+ human lymphocytes. Replication of the resulting viruses will be compared in detail. The presence of the native vpu product in infected cultures, and its subcellular localization, will also be identified.

G 342 CHARACTERIZATION OF HIV-1 REVERSE TRANSCRIPTASE EXPRESSED IN E.COLI AND IN BACULOVIRUS INFECTED CELLS, Margaret Tisdale, Brendan A.Larder, Denise M.Lowe, Peter Ertl, Martin Page*, Christopher Bradley, Dorothy J.M.Purifoy, David Stammers, Kenneth L.Powell and Graham Darby, Department of Molecular Sciences and Department of *Molecular Biology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.
We have previously described the cloning of the reverse transcriptase gene of HIV and high level expression of a 66kd polypeptide in E.coli [Larder et al., EMBO J.6 3133-3137, 1987]. Monoclonal antibodies raised to this polypeptide were used in the purification of large quantities of material for functional analysis and crystallization studies. The reverse transcriptase expressed in E.coli was present predominately as a 66kd homodimer, but also as some 66/51kd heterodimer, as seen in virions, with both forms possessing high level RT activity and RNase H activity. This cleavage to a stable heterodimer form could be mimicked by chymotrypsin.
In the baculovirus expression system the RT gene was cloned into a BAMH1 site within the polyhedrin gene in the transfer plasmids pAc373 and p36C-11. Insect cells, Strodoptera frugiperda were cotransfected with wild type baculovirus and the RT plasmid constructs. Plaque purified recombinations were identified with high level RT activity and expressing a 66kd polypeptide. Purification and characterization of this polypeptide from insect cells will be discussed.

G 343 MECHANISM OF LYSIS AND RESISTANCE IN A NEW HUMAN T CELL LINE FOLLOWING INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1, Michel Tremblay, Ronald Rooke, Romas Geluziunas, Art Sullivan and Mark A. Wainberg, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada, H3T 1E2.
A clone from the well-known CEM cell line was fused with normal human peripheral mononuclear cells to increase expression of the CD4 receptor. An adherent CD4-positive T cell line, termed CEM-A, was obtained and was found to be highly susceptible to human immunodeficiency virus type 1 (HIV-1). Following a primary lytic infection, no proviral DNA could be detected by Southern blot analysis in CEM-A cells 60 days after infection, and we determined that the surviving cells were resistant to further HIV-1 infection. A marked decrease in CD4 antigen expression was observed by flow cytometyric analysis, but not of CD3 and transferrrin receptor. This decline in cell surface CD4 antigen was correlated with changes in the structure of the CD4 gene in these cells, and differences were observed in the pattern of CD4 DNA fragments. Quantitative differences in levels of CD4 mRNA were associated with these rearrangements in intrinsic structure of the CD4 gene.

G 344 IMPORTANCE OF CYSTEINE RESIDUES TO HIV-1 ENVELOPE FUNCTION , Erwin Tschachler, Hartmuth Buchow, Robert C. Gallo, Marvin S. Reitz Jr., Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, Md, USA.
Cysteines within the envelope glycoprotein are highly conserved among all strains of HIV and SIV, suggesting that disulfide bonds are important for the tertiary structure,consequencly, the function of the envelope. To help understand the structure- function relationship of the HIV-1 envelope and identify functionally important domains, we mutated various cysteine residues in the amino (positions 131 and 196) and carboxy regions (positions 296, 331, 386, 418 and 445) of gp120 in a biologically active plasmid clone of HIV-1 (pHXB2D). Upon transfection of the altered pHXB2D plasmids into cos-1 cells RT activity was readily detectable in the supernatant in all cases. Although viral particles could be observed with all mutations tested, cell free transmission to CEM cells could only be obtained with the cys386 mutant. Radioimmunoprecipitation assays revealed that 3 of the 5 cysteine substitutions at the carboxy terminus did impair cleavage of the envelope precursor glycoprotein gp160 and prevented secretion of envelope proteins into the supernatant. The data strongly suggests that tertiary structure plays an important role in the processing of the HIV-1 envelope glycoprotein.

G 345 HIGHLY SULFATED DEXTRANS WITH POTENT ACTIVITY AGAINST HIV-1. V. Vlach, C. Meier, R.E. McCarthy, M. Stevenson. Molecular Biology Laboratory, Department of Pathology and Microbiology, UNMC, Omaha NE 68105. Dextran sulfate has received considerable attention recently due to its potent activity against HIV 1 in culture. This antiviral activity appears dependant on the level of sulfation of the dextran sulfate. Here we describe the synthesis of novel highly sulfated dextrans (pustulan and mycodextran). These compounds are highly sulfated with molecular weights of 10,000 for mycodextran and 40,000 for postulan. The antiviral activity of these compounds was evaluated against cytopathic strains of HIV-1 and relative potency was compared with dextran sulfate. A number of parameters were used to compare the relative antiviral activity of these compounds, including minimum dose required to completely block viral infectivity; viral RNA genome copy number at various drug concentrations; viral antigen production and viral cytopathic effect in the presence of the drug. Over a 3 week period, all compounds completely blocked viral infection at concentrations above 30ug/ml. At concentrations of 15 and 7.5ug/ml, infection occurred but the kinetics of replication were significantly reduced when compared to untreated cultures. Pustulan still maintained potent antiviral activity at concentrations as low as 1ug/ml. These effects were most noted at the level of viral RNA genome copy number and relative production of viral antigens in infected cultures. In contrast to dextran sulfate, both mycodextran and postulan appear to have no cytocidal activity, even at concentrations in excess of 200ug/ml. The theraputic value of these novel antiviral compounds remains to be established.

G 346 DETECTION OF HIV INFECTION IN INFANTS BY THE POLYMERASE CHAIN REACTION Karen K.Y. Young, Specialty Laboratories, Inc., Santa Monica, CA 90404 Diagnosis of HIV infection in very young infants born to HIV-infected mothers is a difficult problem. Presence of HIV-specific antibody is not a good criterion for infection, because maternal antibody can traverse the placenta. It is, however, important to identify infected infants, because early treatment with azidothymidine (AZT) can minimize some of the neurological damage associated with HIV infection. It is equally important to rule out infection as AZT has serious side effects. We have tested peripheral blood mononuclear cells from 11 infants for the presence of HIV proviral DNA by the polymerase chain reaction (pcr). The pcr amplifies proviral DNA by repeated cycles of DNA synthesis using HIV-specific oligonucleotide primers and DNA polymerase. Four of the infants were antibody-positive and positive for HIV proviral DNA. Five of the infants were antibody-positive but negative for HIV proviral DNA. Two of the infants (4-1/2 months of age) were antibody-negative but positive for HIV proviral DNA. These infants may have lost the maternally-transferred antibody and have not begun synthesis of their own antibody. The pcr gives promise of differentiating antibody-positive infants who are infected with HIV from those not infected. Furthermore, the pcr is able to detect HIV infection in infants that lack HIV-specific antibody.

G 347 DEVELOPMENT OF A RAPID AND SENSITIVE ENZYME IMMUNOASSAY TO DETECT HIV p24 ANTIGEN. Pierce R. Youngbar, Lynn A. Stinard, Jay M. Hyman, Don E. Lockwood, Rebecca J. Durham*, Patricia M. Watson* and Bryan T. Butman*, Organon Teknika Corporation, Durham, NC 27704, *Bionetics Research, Inc., Rockville, MD 20850. HIV antigen detection is an important tool in AIDS research, therapy and prognosis. An enzyme-linked immunosorbent assay (ELISA) was developed to detect HIV p24 core antigen in serum, plasma, and cell culture fluids. The assay utilizes anti-p24 monoclonal antibodies to capture HIV core antigen, and enzyme-conjugated human anti-HIV immunoglobulin as a detector antibody. The test system is a direct-sandwich ELISA requiring no intermediate amplification step and only 2.5 hours of total incubation time. Interpretation of results may be qualitative or quantitative. Also, by utilizing 100 ul reaction volumes, the consumption of test material (i.e., clinical samples that may be in limited supply) is minimized. Dose response curves, using HIV lysate diluted in normal human serum, indicate a test sensitivity of less than 10 pg/ml of p24 antigen. The assay has been shown to detect very low concentrations of core antigen in clinical plasma samples despite the presence of antibody to HIV. Furthermore, the ELISA has been shown to be 1,000 times more sensitive than the reverse transcriptase assay for monitoring whole virus growth in cell culture systems. Although test sensitivity is greatest for HIV-1, the assay will also detect p24 from HIV-2. This assay offers a rapid technique for HIV p24 antigen detection, that is highly sensitive and simple to perform. A neutralization test, which incorporates the reagents in the screening assay, is used to confirm reactive samples.

Retrovirus-Cell Interactions; Neurological and Neoplastic Complications

G 400 **Ty:HIV VIRUS-LIKE PARTICLES : A NOVEL APPROACH TO VACCINE DESIGN** S.Adams*, N.Burns*, E.Berrie*, M. Richardson*, J.Senior*, S.Kingsman^, M.Edwards* & A.Kingsman^*.
*British Bio-technology Ltd., Brook House, Watlington Road, Oxford, OX4 5LY, U.K. & ^Biochemistry Dept., Oxford University, South Parks REoad, Oxford.

A protein encoded by the yeast retrotransposon Ty can be used as a carrier for recombinant antigens. HIV antigens (gp120, gp41, p17, p24, polymerase, TAT) have been incorporated into specialized expression vectors designed for high level production in yeast. Both complete protein-coding sequences and selected regions have been inserted. Such Ty:HIV fusion proteins form hybrid Ty virus-like particles (VLPs) containing approximately 300 copies of the fusion protein and hence 300 copies of the added antigen. The non-Ty component is presented on the outer surface of the VLP and induces the production of HIV-specific antibodies. The ability of these polyvalent recombinant antigens to induce virus neutralizing antibodies and cellular immune responses is currently being evaluated in rodents and non-human primates.

G 401 **Anti-HIV Compound Assessment by Two Novel High Capacity Assays** Devron R. Averett, Department of Experimental Therapy, Burroughs Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, N.C. 27709

Two novel, semi-automated assays for the assessment of compounds for activity against Human Immunodeficiency Virus (HIV) are described. One assay uses quantitation of DNA fluorescence to monitor reversal by test compounds of HIV-induced growth inhibition. The second assay measures the amount of HIV p24 by an indirect immunofluorescent technique. Both assays are sufficiently sensitive to allow multiple sampling of 96-well plates. Intra- and inter-assay variability were within acceptable limits. The two assays provide comparable results for given compounds. Retrovir® (AZT) protected MT4 cells from HIV-induced growth inhibition, and inhibited the production of HIV p24. Consistent with the results of others, the anti-HIV potency of AZT was dependent on the concentration of the infecting virus. Interestingly, AZT-protected, HIV-infected MT4 cells grew faster than mock-infected MT4 cells, and inclusion of IL2 in the assay eliminated this effect.

G 402 EXPRESSION AND IMMUNOGENICITY OF SOLUBLE gp160..P.W. Berman*, L. Riddle**, G.Nakamura,* O.K.Haffar, R.Byrne+, J.Groopman+, F.G.Klier#, and T. Gregory** Departments of Developmental Biology* and Process Sciences**, Genentech, Inc. S. San Francisco, CA. 94080; The Department of Hematology and Oncology+, New England Deaconess Hospital, Harvard Medical School, Boston, MA. 02215; the Electron Microscopy Laboratory#, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The gene encoding the major envelope glycoprotein, gp160, of HIV-1 was mutagenized so as to direct the synthesis of a truncated protein corresponding to the entire extracellular domain. The variant, termed sgp160, consisted of the entire gp120/gp41 complex with the exception of the transmembrane domain and cytoplasmic tail were deleted. Transfection into CHO cells allowed for the isolation of a stable cell line that constitutively secreted soluble gp160 into the culture media. The sgp160 protein, purified by affinity chromatography, resembled recombinant gp120 in that it was highly glycosylated and reacted with sera from HIV-1 infected individuals. The sgp160 protein differed from recombinant gp120 in that it aggregated to form oligomers. The oligomeric protein possessed a discrete conformation as visualized by electron microscopy after rotary shadowing with tungsten. The structure of gp160 appeared to be functionally relevant as judged by its ability to bind CD4 with high affinity and to react with authentic viral derived gp160, gp120, and gp41. We are currently considering the possibility that the oligomerization of sgp160 is relevant to viral spike formation. Polyclonal and monoclonal antibodies raised against the sgp160 blocked the binding of gp120 to CD4 and neutralized HIV-1 infectivity in vitro. Some of the antibodies raised to sgp160 appear to react with epitopes distinct from those elicited by gp120, and have permitted the identification of novel epitopes involved in virus neutralization and CD4 binding. These studies demonstrate that the sgp160 protein represents unique antigen with immunogenic properties distinct from gp120, and suggest that the sgp160 protein be considered as a component of a potential AIDS vaccine.

G 403 COMPARISON OF TISSUE AND CELL REACTIONS TO HIV INFECTION IN CNS AND LYMPH NODES, **Peter Biberfeld***, Carlo Parravicini**, Anna-Lena Petrén*, Anna Porwit*, R.C. Gallo***, *Immunopathology Lab., Department of Pathology, Karolinska Institute, Stockholm, Sweden, **Cattedra di Anatomia Patologica, Università di Milano, Ospedale "L. Sacco", Milano, Italy, ***Lab. of Tumor Cell Biology, N.C.I., N.I.H., Bethesda.

HIV-induced lesions in brains and lymph nodes of infected individuals were studied by immunohistochemistry and in situ hybridization. Micronodular lesions in the brains were mainly composed of microglial cells, monocyte/macrophages and lymphoid cells, predominantly CD8+.
Gag and env. coded antigens as well as virus RNA were associated with glial cells and monocyte/macrophages. The glial cells and monocyte/macrophages expressed antigens defined by the Mab's KiM6 and 9.4 and to variable degree CD4. In lymphadenopathic nodes only gag antigens were demonstrable and exclusively in germinal centers, mostly associated with dendritic follicular cells (DFC). Rare cells showed evidence of HIV replication also predominantly located to the germinal centers. Follicular involution was associated with destruction of DFC and follicular infiltration of CD8+ cells. Both in CNS and lymph nodes, antigen presenting cells constitute the main source (reservoir) of virus replication. A possible cytotoxic role of CD8+ cells to HIV-infected cells is suggested.

G 404 MURINE MODELS FOR EVALUATING THE THERAPEUTIC EFFICACY OF ANTIVIRAL AGENTS AND BIOLOGICAL RESPONSE MODIFIERS IN TREATING RETROVIRAL DISEASE, Paul L. Black, James T. Rankin, Jr., Michael A. Ussery, and Michael A. Chirigos. Southern Research Inst.-Frederick Research Center, and Virology Div., USAMRIID, Ft. Detrick, Frederick, MD 21701.
We have employed two models of murine retrovirus-induced disease, the Rauscher leukemia virus (RLV) and the LP-BM-5 murine AIDS (MAIDS) virus, to screen antiviral agents and biological response modifiers (BRM) for potential therapeutic activity against AIDS. RLV rapidly produced intense splenomegaly, viremia and immunoglobulinemia, all of which are measures of disease progression. LP-BM-5 produced splenomegaly, lymphadenopathy, and immunosuppression, but not detectable viremia. However, production of infectious LP-BM-5 virus by lymphocytes was demonstrated readily in an infectious centers assay. Furthermore, short-term treatment of LP-BM-5-infected mice with BRM did not reverse the immunosuppression. Due to the rapid disease course and the viremia, which was easily assayed in serum by the UV-XC plaque technique or by reverse transcriptase activity, the RLV model functioned as a rapid primary screen for evaluation of anti-retroviral activity. Fifteen compounds have been evaluated for in vivo therapeutic activity in RLV-infected mice, and five compounds have been further tested in the LP-BM-5 model. Five antiviral agents (AZT, ribavirin, ribamidine, AVS 2575, and AVS 2576) and three BRM (MVE-2, poly[I,C]-LC, and CL-246) have produced antiviral activity. MVE-2 had antiviral activity when given before, but not after, virus inoculation. In addition, the combination of MVE-2 and AZT generated additive antiviral activity.
Research supported in part by NCDDG 1U01AI25619-01 from NIAID.

G 405 A RABBIT MODEL OF PERSISTENT AND ASYMPTOMATIC HTLV-I INFECTION IN HUMANS, G. Cockerell[1], J. Rovnak[1], M. Lairmore[2], B. De[2] and I. Miyoshi[3], [1]Colorado State University, Fort Collins, CO 80523, [2]Centers for Disease Control, Atlanta, GA 30333 and [3]Kochi Medical School, Kochi 781-51, JAPAN
Two groups of rabbits were inoculated intraperitoneally either on the day of birth or at four-weeks of age with an HTLV-I-infected and transformed rabbit cell line (Ra-1), previously shown to induce infection in outbred rabbits. The inoculation procedure was associated with early mortality in rabbits inoculated on the day of birth, however, all rabbits which survived beyond three-weeks post-inoculation (p.i.) seroconverted to HTLV-I when first examined at this time by indirect immunoflourescence, and remained persistently seropositive dur ıg the 22-months p.i. period of study. Seroconversion did not occur in nursing does or saline-inoculated controls maintained in the same cages or room with HTLV-I-inoculated rabbits. Differential patterns of reactivity to specific viral proteins were found when serial serum samples were further tested by western blotting and radioimmunoprecipitation; of all samples from rabbits tested, early and persistent serconversion occurred to p24, p55 and gp68, while reactivity to p19 either never occurred or disappeared by approximately five-months p.i. Direct evidence of HTLV-I in inoculated rabbits could not be demonstrated routinely by standard restriction endonuclease analysis for HTLV-I proviral sequences in freshly isolated peripheral blood mononuclear cells (PBMC), or by immunofluorescent detection of viral antigens in fresh or short-term cultured PBMC. HTLV-I sequences were demonstrable, however, in terminal PBMC samples using the polymerase chain reaction and polymerase primer pairs, while DNA extracts of normal rabbit PBMC remained negative under the same conditions. No qualitative or quantitative changes in leukocytes or erythrocytes were detected in the infected rabbits and no clinical signs could be attributed to the infection. These results indicate the value of the rabbit as an animal model to study the sequence of events leading to persistent, asymptomatic HTLV-I infections in humans.

G 406 INFECTION OF PONIES WITH EQUINE INFECTIOUS ANEMIA VIRUS DERIVED FROM AN INFECTIOUS MOLECULAR CLONE. Leroy Coggins, Michael Jones, Christine Kolmstetter, Linda Whetter, John Dahlberg*, Steve Tronick*, Denis Archambault*, Stuart Aaronson* and Frederick Fuller. Department of Microbiology, Pathology and Parasitology, North Carolina State University, College of Veterinary Medicine, Raleigh, N.Carolina 27606. *Laboratory of Cellular and Molecular Biology, NCI, Building 37, Bethesda, Maryland 20892. Equine infectious anemia virus (EIAV) is an animal lentivirus that persistently infects equine species and causes recurring febrile episodes due to antigenic variation in the viral envelope proteins and virus replication in the presence of a strong host immune response to the virus. We have obtained virus from a full-length, infectious molecular clone of EIAV from an integrated form of the genome and used this virus to infect ponies. The virus used for these studies was derived from a canine thymoma cell adapted isolate of the Wyoming cell culture strain. Ponies infected with virus derived from the infectious molecular clone or a canine thymoma cell adapted isolate developed an immune response to EIAV (as determined by western blot, ELISA and immunodiffusion) but did not become persistently infected with the virus. Twenty ml of whole blood transferred from these infected ponies to susceptible, uninfected ponies showed no evidence of any transfer of infection. We believe that this canine cell adapted virus(from cell culture or the molecular clone) caused an abortive infection in ponies and represents a very interesting attenuated mutant that will provide insights into the molecular basis of viral persistence as well as attenuation of virulence.

G 407 ENDOGENOUS MURINE RETROVIRUS INVOLVEMENT IN MOTOR NEURON DISEASE OF MICE, Christopher H. Contag and Peter G.W. Plagemann, Department of Microbiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Induction of the motor neuron disease, age-dependent poliomyelitis (ADPM) of mice, is dependent upon several well described factors including two murine viruses. Multiple proviral copies of N-tropic, endogenous, ecotropic murine leukemia viruses (MuLV) and the permissive allele for N-tropic viral replication ($Fv\text{-}1^{n/n}$) are genetically linked to the susceptibility of mice to ADPM. We have attributed the loss of motor neurons in ADPM to cytocidal replication of lactate dehydrogenase-elevating virus (LDV, a murine togavirus) in ventral horn motor neurons. The presence of both LDV and MuLV RNA in motor neurons correlate, in time and space, with the other predisposing factors of increasing age and cyclophosphamide treatment as well as with the pathology of ADPM. We show that old age and cyclophosphamide treatment affect the expression of an ecotropic provirus exclusively in motor neurons and propose that this increased retroviral expression predisposes neurons to cytocidal infection by LDV. The pathology of this dual-virus motor neuron disease of mice resembles that of amytrophic lateral sclerosis (ALS) in humans and may therefore serve as a model for this human neurological disease.

G 408 THE ROLE OF CD4 IN THERAPEUTIC AND VACCINE STRATEGIES AGAINST HIV A.G. Dalgleish, D. Wilks, L. Walker and J. Habeshaw, Clinical Research Center, Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ, UK.

Binding studies using peptides and soluble CD4 mutants have shown that the HIV binding site is primarily on the V_1 domain of the CD4 molecule. Moreover, the Leu 3a epitope most clearly defines this binding site at the present time. Using this monoclonal antibody, we have raised anti-idiotypic responses in mice, rats, rabbits and primates. The polyclonal sera from some of these animals has weak anti-HIV activity. We have therefore performed a pilot study in HIV infected patients giving Leu 3a in doses of 1 mg weekly for 6 weeks over a period of 3 months. All three patients have made a specific anti-idiotype response. Further studies using transfected CD4 and a variety of cells show that in different species different epitopes of the CD4 are recognised. This may imply an association of CD4 in human cells with another cellular membrane component. Initial studies have identified a human cellular protein which may be important in the fusion interaction. The gene for this protein has been cloned and transfected into mouse and abnormal human cells which do not express this protein. Soluble CD4 not only binds all HIV-1 isolates, but also neutralizes them to a high titre. Suggesting that this conserved binding site may be used not only for therapy but also for vaccine strategies such as the proposed idiotype approach which may require a more effective antibody than Leu 3a. Two candidates which induce anti-idiotypic responses which cross-react with the variable genes of Leu 3a have now been identified. We have shown that adjuvant is crucial in raising an idiotype response and are now testing muramyl dipeptide, interleukin-2 and a range of other immunogenic approaches which may be extrapolated to human studies.

G 409 MOLECULAR AND ANTIGENIC DIVERSITY IN OVINE LENTIVIRUS STRAINS IS ASSOCIATED WITH VARYING PATHOGENICITY, J.C. DeMartini, M.D. Lairmore, R. Grant, O. Kajikawa, and J. Carlson, Departments of Pathology and Microbiology, Colorado State University, Ft. Collins, CO 80523. Ovine lentiviruses (OvLV) share morphological, structural, and nucleic acid sequence homology with human immunodeficiency virus (HIV). Furthermore, OvLV are naturally and experimentally associated with lymphoid interstitial pneumonia (LIP), a disease syndrome identified particularly in pediatric HIV infections. Groups of newborn lambs were inoculated intratracheally with $1x10^6$ $TCID_{50}$ of two plaque cloned OvLV of differing pathogenicity: isolate 85/34, a highly lytic virus derived from a natural case of LIP and isolate 84/28, a persistent syncytia-inducing virus derived from a case of ovine pulmonary carcinoma. Isolate 85/34 produced more severe and more extensive lesions in a greater proportion of infected animals than did 84/28. Both isolates replicated in alveolar macrophages, but 85/34 induced more rapid syncytia and lysis and reached higher peak titers than did 84/28. Spontaneous interferon production by pulmonary lymphocytes was positively correlated with *in vivo* pathogenicity. To determine viral characteristics that may account for these differences, the isolates were probed using ^{32}P labelled visna virus genome in Southern blot analysis. Differences in restriction endonuclease maps were found. Differences in viral structural antigens also were found when these isolates were compared with each other and with other OvLV strains in SDS polyacrylamide gel electrophoresis and in Western blotting assays. Further analysis of the molecular basis for the differing pathogenicity of OvLV strains may yield new insights into the diverse mechanisms by which lentiviruses induce disease. (Supported by NIH grant AI 25770).

G 410 PRIOR IMMUNIZATION WITH AN HTLV-I SUBUNIT VACCINE PROTECTS *Macaca nemestria* AGAINST A STLV-I CHALLENGE, C. S. Dezzutti, M. G. Lewis, R. M. Bauer, L. Y. Huff, J. R. Blakeslee, D. E. Frazier and R. G. Olsen, The Ohio State University, Department of Veterinary Pathobiology, 1925 Coffey Road, Columbus, Ohio 43210. The purpose of this study was to establish a model for human T cell leukemia virus type I (HTLV-I) infection and to determine the efficacy of a subunit vaccine using two groups of *M. nemestria*. The first set of five monkeys received two vaccinations consisting of peptides from a cell-/serum-free supernatant harvested from an HTLV-I producing cell line prior to the simian T cell leukemia virus type I (STLV-I) challenge. An equal number of monkeys were challenged with STLV-I and were used as controls. Humeral and cellular immune responses were monitored for eight months. After challenge, the control group had a reduced antibody titer to STLV-I as apposed to the vaccinates. Western blot analysis of the sera showed that both groups recognized gag and env proteins, but the vaccinates had a stronger serological response to the env proteins. Additionally, the antibody produced by both groups demonstrated antibody dependent, complement-mediated cytotoxicity toward HTLV-I and STLV-I target cells. The responses of lymphocytes and neutrophils, as measured by lymphocyte blast transformation and chemiluminescence response respectively, showed no apparent difference between the vaccinates and controls. However, when lymphocytes were tested for the presence of viral activity by assaying for reverse transcriptase activity, the control group was positive two weeks after challenge and remained positive while the vaccinated group has remained negative. The data presented here demonstrate that the vaccine was successful in protecting *M. nemestria* from STLV-I infection.

G 411 T-CELL KILLING BY THE FeLV-FAIDS IMMUNODEFICIENCY VIRUS. Peter R. Donahue[1], Edward A. Hoover[2], Sandra L. Quackenbush[2], Julie Overbaugh[1*], and James I. Mullins[1]. [1]Harvard University School of Public Health, Boston, MA and [2]Colorado State University, Ft. Collins, CO. A naturally-occurring strain of feline leukemia virus, designated FeLV-FAIDS, induces fatal immunodeficiency disease in outbred specific-pathogen-free cats. Within this strain are replication competent, non-immunosuppressive viruses (clone 61E is the prototype), and acutely immunosuppressive viruses, most or all of which are replication defective (clone 61C is the prototype). We generated a 61E/61C virus chimera which is replication competent and induces fatal immunodeficiency disease. We now find that 61E + 61C virus mixtures and chimeras are cytopathic for a feline T-cell line *in vitro*. Through generation and testing of additional chimeras we found that the essential determinant for T-cell killing resides within a 21 base pair (7 amino acid) stretch within the extracellular glycoprotein gene (gp70), corresponding to a single amino acid change and a 6 amino acid insertion in the protein. Other changes within gp70 and the viral LTR enhance the efficiency and rate of T-cell killing in vitro and pathogenicity in vivo. T-cell killing occurs along with production of high levels of unintegrated viral DNA and can be blocked by exposing newly infected cells to antisera from cats exposed to the virus. The latter findings suggest that T-cell killing occurs as a result of massive superinfection and that the critical mutations in gp70 result in a failure or delay in the establishment of superinfection interference, possibly through altered interactions with p15E and/or its cellular receptor. * Present address: Regional Primate Research Center, Health Sciences SJ-50, University of Washington, Seattle, WA 98195.

G 412 ANTIBODY-MEDIATED IN VITRO NEUTRALIZATION OF HIV INFECTIVITY FOR CHIMPANZEES, Emilio A. Emini,[1] Peter L. Nara,[2] Jorg Eichberg[3] and Shuzo Matsushita.[4] [1]Merck, Sharp and Dohme Research Laboratories, West Point, PA, [2]NCI-Frederick Cancer Research Facility, Frederick, MD, [3]Southwest Foundation for Biomedical Research, San Antonio, TX, [4]Kumamoto University Medical School, Japan.
Individual titrated chimpanzee challenge inocula of HIV (isolate HTLV-IIIb) were independently incubated in vitro with (1) virus-neutralizing polyclonal IgG from an HIV-seropositive chimpanzee, (2) a specific neutralizing monoclonal antibody, (3) non-neutralizing IgG from an HIV-seropositive human and (4) IgG from an uninfected chimpanzee. Each virus-antibody mixture was inoculated into a susceptible chimpanzee. Both recipients of non-neutralized virus (inocula #3 and 4) became HIV-antibody positive at 6 weeks post-inoculation (p.i.). In contrast, the chimpanzee inoculated with the monoclonal antibody-treated virus (inoculum #2) experienced a delayed seroconversion (at 12 weeks, p.i.) with significantly lower antibody titers. The animal inoculated with the polyclonal IgG-neutralized virus has remained free of all immunological signs of HIV infection. These studies demonstrate a positive effect of in vitro HIV-neutralizing antibodies in inhibiting in vivo HIV infection.

G 413 CYTOMEGALOVIRUS RETINITIS AND ENCEPHALITIS IN PATIENTS WITH NEUROLOGIC COMPLICATIONS OF AIDS, Milan Fiala, Department of Medicine, Eisenhower Medical Center, Rancho Mirage, CA 92270, Vali Kermani, Immunobiogene, Los Angeles, CA 90025, and Harry Vinters, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024. We have studied 60 AIDS patients by ophthalmoscopy, neurological tests, viral cultures, CD4+ cell numbers and HIV p24 antigen assay. The patients with retinitis (n_1=24) had more severe immune deficiency (mean CD4+ co : 46 vs 101 cells/uL, P=0.039), shorter survival (median survival 166 vs 302 days, P=0.054), and excessive rate of encephalopathy (0.7 vs 0.08, P 0.00001) compared to the patients without retinitis (n_2=36). AZT therapy was beneficial in treating neurologic complications only in patients without CMV retinitis. CMV antibody was present at 3 to 6.5 times higher titer in the spinal fluid IgG compared to the plasma IgG. HIV p24 antigenemia was detected at 27 to 114 pg/ml in patients with CMV retinitis but was absent in those without retinitis. CMV viremia was found in 100% of those with CMV retinitis but in only 30% of patients without the retinitis. Neuropathological examination of the brain of a patient with retinitis showed severe CMV ventriculoencephalitis. Certain severely HIV-infected individuals may be more susceptible to disseminated CMV infection involving the nervous system and the eye. CMV causes AIDS encephalopathy by its lytic effect on several cell types in the central nervous system in synergy with the effects of HIV on immune and supporting cells. AZT appears to be effective against HIV infection alone but not in combination with CMV in the nervous system.

G 414 9-(2-PHOSPHONYLMETHOXYETHYL)ADENINE (PMEA) IS A POTENT ANTI-HIV AGENT IN VITRO AND HAS AN ANTIRETROVIRUS ACTIVITY IN VIVO, Ismail Ghazzouli, Joanne J. Bronson, John W. Russell, Lewis J. Klunk, Cathy A. Bartelli, Christine Franco, Michael J. M. Hitchcock and John C. Martin, Bristol-Myers Company, Wallingford, CT 06492.
PMEA is a phosphonate analogue with broad spectrum antiviral activity. In vitro, PMEA was shown to be a potent inhibitor of human immunodeficiency virus (HIV), human cytomegalovirus (HCMV), and murine retroviruses. In vivo, PMEA was found to be significantly effective in a dose-dependent manner in inhibiting splenomegaly in mice infected with high doses of Rauscher murine leukemia virus (R-MuLV). Treatment of R-MuLV-infected mice with PMEA at 80 mg/kg/day for 10 consecutive days reduced splenomegaly and viremia, and increased the survival time. This treatment regimen was far superior with PMEA than with AZT. No toxic side-effects were observed when uninfected mice were treated intraperitoneally with 400 mg/kg/day of PMEA. Biological efficacy and pharmacokinetic results will be presented.

G 415 GENERATION OF ANTI-HIV ANTIBODY RESPONSES USING THE T-INDEPENDENT CARRIER BRUCELLA ABORTUS. Basil Golding, Sue Preston, Elaine Lizzio, Thomas Hoffman. Div. of Blood and Blood Products, FDA, Bethesda MD 20892.

HIV-1 infection is associated with decreased function of CD4+ helper T cells which are required for antibody responses to protein antigens. Thus, in devising a strategy to protect persons infected with HIV-1 it would be neccessary to use antigens that can bypass the requirement for CD4+ T cells. We had previously shown that Brucella abortus (BA) behaves as a T cell independent type 1 carrier in human hapten-carrier B cell responses. Inactivated HIV-1 was therefore conjugated to BA and tested for immunogenicity in normal BALB/c mice. HIV-BA and HIV-1 alone elicited anti-HIV responses detected by ELISA. HIV-BA responses occurred earlier and titers were higher than HIV-1 alone. Immunoblot analysis showed that most of the HIV-1 antigens were recognized by antibodies induced by either antigen. Patterns of IgG subclass responses are determined by the nature of the antigen. Analysis of isotypes revealed that HIV-1 induced similar levels of IgG1 and IgG2a antibodies, whereas the response to HIV-BA was predominantly of the IgG2a subclass. These different IgG subclass patterns suggest that HIV-1 alone behaves as a protein or T cell dependent antigen and that conjugation of HIV-1 to BA converted it into a T cell independent type 1 antigen.

G 416 **Regulated transport and processing of the HIV-1 gp160 in transfected mammalian cells.** O.K.. Haffar, G.R..Nakamura, and P.W. Berman. Dept. of Developmental Biology, Genentech Inc., South San Francisco, CA 94080. We have studied the export and processing of the HIV-1 envelope glycoprotein, gp160, in transfected mammalian cells. When the env gene was transfected into CHO cells in the absence of any other viral genes, gp160 was retained in an intracellular compartment and failed to be proteolytically processed into the mature gp120 and gp41 species (Berman et al., J. Virol. 62:3135, 1988). Based on this initial observation, we considered the possibility that gp160 contained sequences which functioned as a retention signal. Replacement of the transmembrane domain (TM) and cytoplasmic tail of gp160 with corresponding sequences derived from the HSV-1 envelope glycoprotein D generated a chimeric gene, termed d665.gD1. Expression of this chimera in a human embryonic kidney cell line revealed that the d665.gD1 encoded protein was transported to the cell surface and was proteolytically processed in an intracellular compartment to generate mature gp120 and a membrane bound gp41-gD1 protein. The mature gp120 was secreted efficiently into the culture supernatant, and was transported to the cell surface in complex with the gp41 sequences. In contrast, the wild type gp160 although exported to a small extent to the plasma membrane in this transfected human cell line, nevertheless failed to be processed into the mature proteins. The conformation of the chimeric species appeared relevant as measured by its ability to bind to truncated soluble CD4 (Smith et al., Science 238:1704, 1988). Analysis of the deleted HIV-1 sequences revealed the presence of an arginine residue in the TM domain. Replacing this basic residue, by mutagenesis, with a number of different hydrophobic as well as polar uncharged residues did not alter the cellular transport or processing of gp160 in either rodent or human cell lines.. However, when the envelope glycoprotein was truncated at a site correspnding to residue 750 in the cytoplasmic tail, the mutant was transported in the human cells into the appropriate cellular compartment where proteolytic processing generated mature secreted gp120. Our data suggests that sequences in the cytoplasmic region of the HIV-1 gp160, which are absent from the SIV-1 and HIV-2 envelope proteins, play a role in modulating the processing and export of gp160, and thus might play a role in viral maturation and encapsidation.

G 417 RETROVIRUS ASSOCIATED IMMUNODEFICIENCY IN A MURINE MODEL OF PEDIATRIC AIDS, Richard C. Hard, Jr., Medical College of Virginia/VCU, Richmond, VA 23298.

An immunodeficiency syndrome that has many features in common with pediatrics AIDS can be induced in susceptible strains of mice by the perinatal inoculation of F1 hybrid spleen cells. The semiallogenic donor cells are vectors of retrovirus(es) and induce an allogenic Host Versus Graft (HVG) reaction. Like pediatric AIDS, the murine immunodeficiency syndrome is manifested by lymphadenopathy, severe T-cell depletion, polyclonal hyperglobulinemia despite poor primary responses to antigens, immune complexes, thrombocytopenia, lymphomas and death.

Host susceptibility appears to be related to an inability to control infection by exogenous ecotropic murine leukemia virus (MuLV) and the formation of recombinant retroviruses. It is thought that the interaction of the MuLV(s) and the allogenic HVG reaction results in the destruction of host and donor T-cells which release lymphokines that cause proliferation and maturation of B-cells. Recent studies of immunoglobulin isotypes in the early stages of disease have revealed disproportionately high serum levels of IgG1, an indication of a predominant effect on the IL-4 secreting, Th2 subset of T-helper cells prior to the generalized loss of T-lymphocytes.

The model should prove useful for further basic studies of the pathogenesis of retroviral-associated immunodeficiency. It may also be valuable for preliminary in vivo testing of the efficacy and toxicity of drugs with promising in vitro effects.

G 418 TOWARD HIV-REGULATED EXPRESSION OF A DIPHTHERIA TOXIN A GENE IN TRANSFECTED CELLS, Gail S. Harrison, Francoise Maxwell, L. Michael Glode and Ian H. Maxwell, University of Colorado Health Sciences Center, Division of Medical Oncology, Denver, Colorado 80262

Expression of diphtheria toxin A chain (DT-A) from the cloned coding sequence, linked with appropriate transcriptional regulatory elements, can selectively kill specific cell types (Maxwell et al.: Cancer Res. 46:4660,1986; Palmiter et al.: Cell 50:435,1987; Breitman et al.: Science 238:1563, 1987). We reasoned that if DT-A expression can be made stringently dependent on specific trans-activation mechanisms, this may eventually find therapeutic applications in eliminating virus-infected cells before production of viral progeny, with potential application toward the treatment of AIDS. Experiments were therefore initiated using either DT-A or a luciferase coding sequence (de Wet et al.: Mol. Cell. Biol. 7: 725, 1987) as reporter genes adjacent to HIV LTR sequences from -167 to +80 (from the plasmid IIIex7, C. Rosen). A high basal level of expression of both reporter genes was observed, even in the absence of any trans-activation from the HIV-encoded protein tat (from the plasmid pH3tat, C. Rosen). Nonetheless, a significant level of trans-activation of luciferase was demonstrated, shown to be both dose-dependent and time-dependent. We are exploring means to reduce the high basal level of expression by including cis-acting negative regulatory elements from the env portion of the HIV genome in the 3' untranslated region of the constructs (Rosen et al.: PNAS 85, 1988), and by additional strategies which will be discussed.

G 419 EFFECT OF DIETHYLCARBAMAZINE ON PERIPHERAL LYMPHOCYTE COUNTS OF FELV INFECTED CATS, Lynn W. Kitchen, Division of Infectious Diseases Tulane University Medical Center, New Orleans, LA 70112

Diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide [DEC]) is widely used in some tropical regions to prevent and treat filariasis. The antifilarial effect of this drug has been attributed to immunomodulation. Continuous oral DEC treatment given shortly after evidence of FeLV infection slowed the rate of decline of peripheral blood lymphocytes in 2 kittens compared to 2 untreated littermates on a statistically significant basis. In contrast, short-term oral DEC treatment given to 2 adult chronically-infected FeLV cats resulted in transient lymphopenia in both treated cats, whereas no hematologic changes were noted in 2 untreated littermates. Similarly, of 18 outbred naturally-infected adult cats testing positive for FeLV leukocyte antigens that were treated with 1 month oral DEC, 7 (39%) of the 18 cats tested lymphopenic immediately before treatment and 11 (61%) were lymphopenic after treatment. Average survival was prolonged in all treated groups. Further studies are warranted to evaluate whether treatment with DEC could result in elimination of infected lymphocytes in HIV-infected persons. The possibility that DEC may be of benefit in the treatment of HIV infections is supported by a notable inverse correlation between organized filariasis control programs involving administration of DEC and per capita AIDS cases on a country-by-country basis ($p<.0005$).

G 420 ZIDOVUDINE TREATMENT OF ASYMPTOMATIC HIV-INFECTED SUBJECTS, Joep M.A. Lange, Frank de Wolf, Jan W. Mulder, Roel A. Coutinho, Jan van der Noordaa and Jaap Goudsmit, Department of Virology, University of Amsterdam, and Municipal Health Service, Amsterdam, the Netherlands.

The AIDS end-point attack rate in a cohort of 314 homosexual men, who were followed from seroconversion for HIV-Ab or shortly thereafter, after 3½ years was 28.7%. Absence of HIV-core Ab, presence of HIV antigenemia, and CD4+ cell counts $<0.5 \times 10^9/l$ were strongly associated with subsequent development of AIDS (respective attack rates 33%, 54%, and 50%). To eventually come to secondary prevention of HIV-related disease development, the feasibility of long-term antiretroviral treatment was assessed in a subgroup of asymptomatic HIV-infected men at high risk of disease progression. 18 men with persistent HIV-antigenemia were treated with zidovudine 250-500 mg (± acyclovir 800 mg) 6-hourly for 4-12 weeks, and subsequently with zidovudine 500 mg (± acyclovir 1600 mg) 12-hourly for 36 weeks. After 24 weeks 6 additional HIV-antigenemic men were entered and treated directly with zidovudine 500 mg 12-hourly. Over the treatment period serum HIV p24 (HIV-Ag) levels declined in all 24 subjects (mean decline in different treatment groups 63-78%). In a matched control group of 5 men the mean serum HIV-Ag level rose 67%. Initial increases in CD4+ cell counts in the treated men were not sustained. Adverse reactions to the study-drugs were infrequent and mild. Anemia caused symptoms in 2, but serious leuco- or neutropenia were not observed, and there was no progressive hematological toxicity. These data demonstrate that in asymptomatic HIV-infected subjects zidovudine 500 mg 12-hourly is well tolerated and has a prolonged inhibitory impact on viral antigen production. 18-months follow-up results will be presented.

G 421 APPLICATION OF A QUANTITATIVE PLAQUE ASSAY IN A CD4+ HELA CELL LINE TO DETERMINE AZT-SENSITIVITY OF HIV ISOLATES FROM PATIENTS WITH AIDS OR ARC, Brendan A. Larder, Graham Darby and Douglas D. Richman*, Molecular Sciences Dept., Wellcome Research Laboratories, Beckenham, U.K. and *University of California San Diego. Extensive studies have demonstrated Azidothymidine (AZT or Retrovir™) to be clinically effective, significantly reducing mortality in patients with AIDS and ARC. In addition, it has been shown in many patients that AZT therapy has a suppressive effect an p24 antigenaemia. Although these data suggest AZT is having an antiviral effect in patients, to date very few HIV strains have been assessed for AZT-sensitivity. The strains tested were extensively passaged laboratory isolates and not fresh clinical strains. Our aim was to investigate the susceptibility to AZT of HIV strains isolated from patients with AIDS or ARC in order to determine a range of sensitivity values of these strains to the drug. We have prepared isolates in titred pools from a total of 21 patients. To assess AZT-sensitivity we utilized a plaque assay in a CD4-expressing HeLa cell line[1], which provides a 3-day quantitative antiviral assay system for laboratory strains of HIV-1 and HIV-2 as well as isolates from infected subjects. 11 of the isolates tested to date showed very similar sensitivities, giving a mean ID_{50} value of 0.033uM (SD=0.007uM). We shall report data from these isolates and in addition will demonstrate the usefulness of the assay system in evaluating the potency of a range of antiviral components to laboratory strains of HIV-1 and HIV-2.

1. Chesebro, B and Wehrly, K. J. Virol. (1988) 62, 3779.

G 422 EXTENSIVE GENETIC VARIABILITY OF SIMIAN IMMUNODEFICIENCY VIRUS FROM AFRICAN GREEN MONKEYS, Yen Li, Yathirajulu M. Naidu, Muthiah D. Daniel, and Ronald C. Desrosiers, New England Regional Primate Research Center and Department of Microbiology and Molecular Genetics, Harvard Medical School, Southborough, MA 01772, USA
Serological surveys have revealed that 30-50% of wild caught African green monkeys have antibodies reactive to simian immunodeficiency virus (SIV), a retrovirus related to human immunodeficiency virus (HIV). Although the nucleotide sequence of one SIVagm isolate, Tyo1, was recently reported, the extent of genetic variability among SIVagm isolates remains to be determined. Restriction endonuclease mapping of infectious molecular clones of two SIVagm isolates (266 and 385) revealed conservation of only four sites out of thirty-nine across the genome. Partial sequence analysis of the molecular clones revealed only 80% amino acid sequence conservation in the pol gene. Although the three SIVagm isolates, Tyo1, 385, and 266, are more closely related to each other than to other primate lentiviruses, the genetic variation among these three Kenyan SIVagm isolates is much greater than that observed previously among individual HIV-1, HIV-2 or SIVmac isolates. Less variability among HIV-1 and HIV-2 isolates could possibly be explained by recent entry into the human population. The extensive genetic variation in these Kenyan SIVagm isolates should prompt continued examination of SIVagm from other geographic regions; SIVagm strains much more closely related to HIV-1, HIV-2 or SIVmac may be found which would be reasonable candidates for recent cross-species transmission.

G 423 GLQ223: A COMPOUND WITH ANTI-HIV ACTIVITY IN ACUTE AND CHRONIC INFECTION OF T CELLS AND MACROPHAGES, Jeffrey D. Lifson, Kou M. Hwang, Susan E. Caldwell, Paul Wu, Valerie Ng, Suzanne Crowe, Jan Daniels, Isabelle Gaston, Tracy Deinhart, Jane Marsh, Joann Vennari, Hin-Wing Yeung, and Michael S. McGrath, Departments of Cellular Immunology and Medicinal Biochemistry, Genelabs Incorporated, Redwood City, CA 94063, Division of AIDS/Oncology, Department of Medicine, San Francisco General Hospital, San Francisco, CA, 94110 and Department of Biochemistry, Chinese University of Hong Kong, Shaitin, NT, Hong Kong

GLQ223 is a compound with potent anti-HIV activity *in vitro* in assays of acute and chronic HIV infection utilizing both T cells and macrophages as target cells. Selective inhibition of viral replication (documented by HIV p24 antigen capture assay (1), RT assay (2) and quantitative immunocytofluorometric analysis (3)) is observed at concentrations which do not affect viability or proliferation of parallel control cultures of otherwise identical uninfected cells. GLQ223 inhibited HIV replication in acutely infected T lymphoblastoid cells when continuously present during culture (IC_{50} 2.6 ng/ml (1)) or when cells were treated with a single pulsed exposure to the compound (IC_{50} 17 ng/ml (1)). Viral replication was inhibited in acutely infected T cell lines and in activated primary PBMC. GLQ223 also inhibited HIV replication in chronically infected cultures of primary monocyte derived macrophages infected *in vitro* (IC_{50} 10-20 ng/ml (3)) and blocked HIV replication observed when *in vivo* infected cells were cultured. A single pulsed treatment of peripheral blood derived monocyte/macrophages or tissue macrophages from HIV infected donors prevented HIV replication when the cells were subsequently cultured *in vitro* and analyzed. The unique combination of properties of GLQ223--its activity in both acutely and chronically infected cells and its activity in both T cells and macrophages, the two primary *in vivo* targets for HIV--make it an extremely promising agent deserving evaluation *in vivo* for anti-HIV activity.

G 424 **SITE-DIRECTED MUTAGENESIS OF THE IMMUNODOMINANT NEUTRALIZATION EPITOPE OF HIV-1: BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES OF SELECTED MUTANTS** D. J. Looney[1], L. Ivanoff[2], S. Petteway[2], J.R. Rusche[3], S.D. Putney[3], R. Redfield[1], and F. Wong-Staal[4]. [1]Walter Reed Retrovirus Research Group, WRAIR, Washington, D.C. 20307. [2]Smith, Kline, and French Laboratories, King of Prussia, PA 19406. [3]Repligen Corporation, One Kendall Square, Cambridge, MA 02139. [4]Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD 20892.

We previously found that peptides from the RP135/136 region (a.a. 298-330) of gp120 block the neutralizing activity (NA) of type-specific anti-sera raised against gp120 or recombinant peptides. The change of a single residue within this cysteine loop reduced blocking of NA, reflecting the differing neutralization phenotypes of cloned variants of the HIV- 1/NIH/USA/1983/HTLV-III_B isolate.

To further investigate the functional and immunological significance of this area of HIV-1 env, we are constructing mutants differing at specific residues within this loop. Preliminary analysis of characteristics of one such clone suggests that the integrity of this area is important to viral replication and syncytium formation, as well as determination of neutralizing phenotype.

G 425 PHARMACOKINETICS OF RECOMBINANT SOLUBLE HUMAN CD4, Catherine Lucas, Steven W. Frie, Daniel J. Capon, Steven M. Chamow and Joyce J. Mordenti, Genentech Inc., South San Francisco, CA 94080. The gene encoding human CD4, the T helper cell surface marker, was truncated and transfected into a continuous Chinese Hamster Ovary (CHO) cell line to secrete a soluble form of CD4 (rCD4). The protein was purified to greater than 99 % purity as determined by SDS-polyacrylamide gel electrophoresis and injected intravenously into rats, rabbits and rhesus monkeys to determine its clearance from the blood circulation. To measure rCD4 in serum, an enzyme immunoassay (ELISA) was developed with two monoclonal antibodies to CD4. The ELISA was validated for the measurement of rCD4 in rat, rabbit or rhesus serum. The pharmacokinetic profile of rCD4 was biphasic, with a terminal half-life of 16 minutes in rats and rhesus monkeys and 29 minutes in rabbits.

G 426 IN VITRO AND IN VIVO EVIDENCE FOR DIDEOXYCYTIDINE INDUCED DIFFERENTIAL EXPRESSION OF RETROVIRUS BY SELECT TISSUES, Lawrence E. Mathes, Phyllis Polas, Cheryl Swenson, Kate Hayes and Richard G. Olsen, The Ohio State University, Columbus, OH, 43210.

The antiviral activity of 2',3'-dideoxycytidine (DDC) was tested in feline lymphoid and bone marrow cell culture infected with feline leukemia virus (FeLV). Virus expression in lymphoid cells was inhibited at concentrations of 1-2 uM DDC. Bone marrow cultures required concentrations of 35 to 135 uM DDC to induce similar levels of virus inhibition. In in vivo prophylactic experiments, DDC was administered by continuous intravenous infusion into cats at a dose of 10 mg/kg/hr for a period of 4 weeks. FeLV challenge was 2 days after initiation of drug treatment. FeLV viremia induction normally evident by week 2 in challenge controls was delayed 2-3 weeks in DDC treated cats. However, localized expression of FeLV by bone marrow (week 2 or 3) was similar in DDC treated and control animals. The results suggest that DDC differentially inhibits virus expression by as yet unidentified somatic tissue but does not prevent viral infection and expression by bone marrow progenitor cells.

G 427 HIV-1 SPECIFIC CTL IN CHRONICALLY INFECTED CHIMPANZEES, Alison C. Mawle[1], Mitchell R. Ridgeway[1], and Patricia N. Fultz[2], [1]Centers for Disease Control, Immunology Branch, and [2]Yerkes Primate Center, Atlanta, GA 30333.

HIV-1 specific CTL have been demonstrated both in humans and in chimpanzees infected with HIV-1. CTL have been shown directed at the envelope, gag and reverse transcriptase proteins of the virus. However, it is not clear whether those are classical MHC-restricted CTL, or at least in the case of the envelope, a form of ADCC.

We have studied the cytotoxic T cell (CTL) response to HIV-1 in 8 chimpanzees chronically infected with HIV-1. One of these animals has lost antibody to p24, and another is thrombocytopenic. The targets used were autologous EBV-transformed B cell lines infected with vaccinia constructs containing the genes gp160, p24 and nef respectively. Seven of 8 chimpanzees had responses to gp160-infected target cells. However, they were also able to kill allogeneic gp160 infected cells. Since the chimpanzees involved in this study are not related, the response to gp160 appears to be antigen-specific but unrestricted. In contrast, the response to p24 appears to be both antigen-specific and restricted. We have not seen a response to nef in any of these animals. The one chimpanzee with no response to gp160 is the animal lacking antibody to p24.

These data suggest that the response to gp160 is mediated by a non-specific mechanism, possibly ADCC, whereas the response to p24 is mediated by classical CTL.

G 428 COMPARISON OF THE ANTI-HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ACTIVITY OF ANTIRETROVIRAL AGENTS IN LYMPHOCYTES AND MACROPHAGES, Gandis Mazeika*, Patricia Lekas*, Chung K. Chu**, Michael S. McGrath*, and Raymond F. Schinazi***, *AIDS Activities Division, Department of Medicine, UCSF San Francisco General Hospital, San Francisco, CA 94110; **University of Georgia, Athens, GA 300602; ***VA Med Center, Decatur, GA 30322, and Emory School of Medicine, Atlanta, GA 30602.

The aim of these studies was to compare the anti-HIV-1 activity of several antiviral agents in acutely infected human peripheral blood mononuclear cells (PBMC) with their activity in acutely infected normal human macrophages. A series of 2', 3'-dideoxynucleosides with selective anti-HIV-1 activity were selected, all of which are at different stages of clinical and preclinical evaluation. A previous report indicating that macrophages are not susceptible to a chemotherapeutic approach with some 2', 3'-dideoxynucleosides could not be confirmed: all the compounds evaluated were effective inhibitors of HIV-1 in human macrophages at sub-micromolar concentrations. In addition, ammonium-21-tungsto-9-antimoniate (HPA-23) was evaluated and found to be effective in the same dosage range. The decreasing order of inhibitory potencies of the ten compounds evaluated in macrophages were: AZT > 3'-FdT = AzddMeC > AzddU = D2C > AzddC = D4T = D4C = HPA-23 > D2A. The same compounds are currently being evaluated in chronically infected PBMCs and macrophages, and results will be presented.

G 429 STRUCTURE / FUNCTION RELATIONSHIPS OF 2-5A ANALOGUES FOR INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE AND INFECTION *IN VITRO*, William M. Mitchell[1], David C. Montefiori[1], Robert W. Sobol, Jr.[2], Shi Wu Li[2], Nancy L. Reichenbach[2], Ramamurthy Charubala[3], Wolfgang Pfleiderer[3], W. Edward Robinson, Jr.[1] and Robert J. Suhadolnik[2], [1]Department of Pathology, Vanderbilt University Medical School, Nashville, TN 37232; [2]Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA, 19140; and [3]Fakultat fur Chemie, Universitat Konstanz, Konstanz D-7750, West Germany. Replacement of the 3'-hydroxyl of the adenosine moieties of 2', 5'-oliogoadenylate (2-5A) (i.e., cordycepin analogues of 2-5A) or introduction of chirality into the 2', 5'-phosphodiester internucleotide linkages of 5'-phosphate moieties of the 2-5A molecule (i.e., phosphorothioate analogues of 2-5A) convert authentic 2-5A into active inhibitors of HIV-1 reverse transcriptase and, in some cases, potent anti-HIV-1 agents *in vitro*. The minimum structural requirements for maximum inhibition of HIV-1 reverse transcriptase and infection *in vitro* were investigated using a series of 2-5A analogues. Our results suggest that the degree of 5'-phosphorylation, length of 2-5A oligomer and stereoconfiguration are all critical factors for these activities. Delineation of these factors will be presented.

G 430 PROTECTION BY PHOSPHOROTHIOATE OLIGODEOXYCYTIDINE AND POLYANIONIC POLYSACCHARIDE AGAINST ACCESSORY CELL DYSFUNCTION FOLLOWING EXPOSURE TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN VITRO. Hiroaki Mitsuya, Makoto Matsukura, Seiji Hayashi, Shizuko Aoki, Carlo-F. Perno, and Samuel Broder. The Clinical Oncology Program, National Cancer Institute, Bethesda, MD 20892.

Cells within the macrophage/monocyte lineage play a critical role in immune response as accessory (antigen-presenting) cells and such functions may be affected by human immunodeficiency virus type 1 (HIV-1) infection. Indeed, defects in accessory cell function as well as other functional abnormalities in the macrophage/monocyte lineage from patients with acquired immunodeficiency syndrome (AIDS) have been reported.

We have observed that monocytes/macrophages exposed to HIV-1/III_B in vitro failed to generate the antigen-driven proliferation in autologous tetanus-toxoid specific clonal helper T-cells, while monocytes/macrophages not exposed to the virus generated a high magnitude of antigen-driven proliferation. Three DNA-chain terminating anti-HIV agents, 3'-azido-2',3'-dideoxythymidine, 2',3'-dideoxyadenosine, and 2',3'-dideoxycytidine, failed to protect monocytes/macrophages from the accessory cell dysfunction, although these agents completely inhibited the infectivity and replication of HIV-1 in vitro. However, a phosphorothioate analogue of 28-mer oligodeoxycytidine (S-dC_{28}) and dextran sulfate inhibited the replication of HIV-1 and protected monocytes/macrophages against the accessory cell dysfunction, and enabled them to serve as antigen-presenting cells. These data might provide leads in developing new anti-HIV agents to protect accessory cells against HIV-1-caused dysfunction.

G 431 ANALYSIS OF ANTIBODIES TO PEPTIDES REPRESENTING HIV-1 GENE PRODUCTS IN CHILDREN BORN TO HIV-INFECTED MOTHERS

[4,3]Viviana Moschese,[2] P.A. Broliden,[4,1] K. Ljunggren,[4,3] P. Rossi,[4] M. Jondal and[3] B. Wahren
[4] Dept. of Immunology, Karolinska Institute,[2] Dept. of Virology, Natl. Bact. Lab., Stockholm, Sweden and[3] Dept. of Pediatrics, II University of Rome, Italy.

OBJECTIVE: To analyze the pattern of HIV-specific antibody responses in newborns from HIV-infected mothers by site-directed serology to defined peptides representing the HIV gag, pol and env proteins in two different age-based populations.

STUDY POPULATION AND PEPTIDES: All children have been followed for a mean period of 15 months (range 6-24 months) and seroreactivity was sequentially determined by an HIV IgG ELISA and Western Blot. Group 1: 33 children aged 1 day to 6 months. Group 2: 9 children aged 7-15 months. All peptides and recombinant proteins had sequences according to the BH10 clone derived from HTLV-IIIB.

RESULTS: In children who later were classified as HIV negative, 5/19 (26%) of early sera (before 6 months of age) reacted with a 15 aa. env peptide (gp 120/57). None of the early sera from the infected group had this reactivity. In contrast, early sera from infected children had higher antibody titers to the pEnv9 protein, and later sera (from 7 to 15 months of age) had antibodies against a p17 subregion as the first sign of endogeneous antibody production.

CONCLUSIONS: Site-directed serology might be helpful in the prognostic evaluation of children born to HIV-infected mothers.

G 432 NOVEL AIDS MODEL: FUNCTIONAL HUMAN IMMUNE SYSTEMS TRANSFERRED TO SCID MICE, Donald E. Mosier, Richard J. Gulizia, Steven M. Baird and Darcy B. Wilson, Medical Biology Institute, La Jolla, CA 92037.

Human peripheral blood leucocytes (PBL) have been injected into mice with severe combined immunodeficiency (SCID). The human PBL expand in number and spontaneously initiate synthesis of human immunoglobulin. Within a few weeks, reconstituted SCID mice injected with 10 x 10^6 PBL contain up to 100 x 10^6 human cells which are found in all lymphoid organs with the exception of the thymus. In addition, human lymphocytes recirculate in the peripheral blood of the SCID mouse. The ratios of T to B cells and of CD4 to CD8 T cells in these mice is similar to those seen in normal human PBL. These mice have serum human immunoglobulin levels of 100-500 µg/ml. Upon immunization with trinitrophenyl (TNP)-substituted tetanus toxoid, these mice produce both a primary antibody response to TNP and a secondary response to tetanus. The magnitude of the anti-tetanus antibody responses is 1-10% of that seen in intact humans and comparable to the response of an intact mouse. Under these conditions, graft-versus-host disease is not observed. The function of the human immune system has been maintained for at least 8 months following a single injection of PBL. These mice have been infected with HIV to determine the extent of viral replication and the possible expression of pathogenic effects. [supported by NIH grants]

G 433 ANTIBODY TO HTLV AMONG BLOOD DONORS IN THREE CITIES OF THE UNITED STATES, Transfusion Safety Study Group represented by James W. Mosley, University of Southern California School of Medicine, Los Angeles, CA 90032, and other participating institutions. The Transfusion Safety Study (TSS)/National Heart, Lung, and Blood Institute (NHLBI) Donor Repository consists of 200,000 donor sera from New York, Miami, San Francisco, and Los Angeles, collected just prior to licensure of a test for antibody to human immunodeficiency virus type I (anti-HIV-1). Anti-HIV-1 testing identified positive and control donors, and recipients of components from both. All 200,000 specimens are now being screened for antibody to human T-cell lymphotropic virus (anti-HTLV) to characterize donors with HTLV infection, infections among sexual and non-sexual household contacts, and transmission to recipients. Preliminary results of the pilot study in South Florida showing a prevalence of 0.8/1,000 of confirmed positivity have already been presented. Screening under a uniform protocol is now being carried out in the other three cities. The results through October 21, 1988, are as follows:

Area	Total donations	Tested	Repeatably reactive Number	/1,000	Fully confirmed Number	/1,000
New York	74,128	19,500	38	2.0	18	0.9
San Francisco	25,832	20,600	26	1.3	8	0.4
Los Angeles	66,884	18,200	32	1.8	20	1.1

(Supported by Contracts NO1-HB-4-7002 and NO1-HB-4-7003 of NHLBI).

G 434 MOLECULAR BIOLOGY OF LK-3 SIMIAN FOAMY VIRUS INFECTION, Mattias Schweizer, Andreas Jäckle, and Dieter Neumann-Haefelin, Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, Klinikum der Universität Freiburg (FRG)

The DNA of the T-lymphotropic simian foamy virus (SFV) strain LK-3 is present in infected cells as a non integrated, linear, double-stranded molecule of 13 kbp containing a site sensitive to nuclease S1 (J.Gen.Virol.67, 1993-1999, 1986). Repeated sequences of about 1.7 kb, forming the LTRs of this retroviral genome, were detected by restriction analysis and hybridization experiments. In infected cultures, the copy number may be several hundred molecules per cell. So far, no integrated viral sequences could be demonstrated under conditions restrictive for viral cytopathogenicity, as presence of neutralizing antibodies or AZT in the culture medium. Using cloned LK-3 DNA as hybridization probe (Arch.Virol. 99, 125-134, 1988), proviral DNA of SFV type 1, 2, 3, 5, 6, 7, 8, and human spumaretrovirus could be detected and distinguished by their different restriction patterns. Comparison of 12 foamy virus isolates revealed heterogeneity of the DNA restriction patterns probably due to genetic variability of foamy viruses. No cross hybridization occurred to DNA of HIV-1 infected cells. - Supported by the Deutsche Forschungsgemeinschaft (Ne 213/4-3).

G 435 INTERLEUKIN-2 ACTS AS AN ADJUVANT TO INCREASE THE POTENCY OF INACTIVATED RABIES VIRUS VACCINE, Jack H. Nunberg and Michael V. Doyle, Cetus Corporation, Emeryville, CA 94608; Sonia M. York and Charles J. York, BioTrends International, Inc., West Sacramento, CA 95691

Interleukin-2 (IL-2) occupies a central position in the cascade of events involved in the immune response. As such, we were interested to determine if IL-2 could function as an adjuvant to vaccination, to increase the immune response to vaccine immunogens. Using the NIH Test for Potency of inactivated rabies virus vaccine in outbred mice, we have demonstrated that daily systemic administration of IL-2 in conjunction with vaccination can increase the potency of this vaccine at least 25-fold, as measured by survival following virulent rabies virus challenge. Enhanced protection is not correlated with an increase in virus-neutralizing antibody titers, and we suggest that the IL-2 is acting to increase the cellular immune response to challenge.

G 436 MULTIVALENT SYNTHETIC PEPTIDE INOCULUM INDUCES NEUTRALIZING ANTIBODIES AND ANAMNESTIC T CELL RESPONSES TO MULTIPLE HIV ISOLATES, Thomas J. Palker[1], Thomas J. Matthews[2], Alphonse Langlois[2], Jay A. Berzofsky[3], Dani P. Bolognesi[2] and Barton F. Haynes[1], Departments of [1]Medicine and [2]Surgery, Duke University Medical Center, Durham, NC 27712 and [3]Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892

Previous studies have defined neutralizing B cell epitopes and T cell helper epitopes on Human Immunodeficiency Virus (HIV) gp120. To develop a polyvalent, synthetic peptide inoculum that can evoke both neutralizing antibodies and T helper cell responses to more than one HIV isolate, synthetic peptides containing type-specific neutralizing determinants of gp120 (SP10) from HIV isolates HTLV-III_B (III_B), HTLV-III_{MN} (MN) and HTLV-III_{RF} (RF) were coupled to a 16 amino acid T cell epitope (T1) of HIV-III_B gp120 and used to immunize goats. When combined in a trivalent inoculum, T1-SP10 peptides from HIV-1 isolates III_B, MN and RF evoked neutralizing antibody responses to these 3 isolates in goats, and as well, were found to contain 2 distinct T helper sites that induced potent T helper cell activity against all 3 HIV isolates. Multivalent T1-SP10 synthetic peptide constructs were found to be superior to SP10 peptides conjugated to tetanus toxoid in inducing neutralizing antibody responses. Finally, T lymphocytes from goats immunized with the T cell-B cell construct from HIV isolate III B proliferated in vitro to native gp120 IIIB and to the IIIB- recombinant molecule, PBI. Thus, combinations of homologous SP10 region synthetic peptides containing type-specific neutralizing determinants and T-cell epitopes of HIV gp120 may be useful in man to elicit high titered neutralizing B-cell responses and as well T helper cell responses to native HIV gp120.

G 437 ANALOGUES OF VITAMIN D3 INHIBIT HIV REPLICATION IN CULTURED HUMAN MONOCYTES AND MACROPHAGES, C. David Pauza and José Galindo, Developmental Biology Laboratory, The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138.

Persistent HIV infection of monocytes and macrophages is an important element in the pathogenesis of AIDS. These cells comprise a principal reservoir of HIV and, in their role as MHC Class II-bearing antigen presenting cells, constitute a direct route for virus transmission to helper T cells. Therapeutic strategies intended to reduce or eliminate HIV infection must account for virus in the macrophage population. We are concerned with the relationship between monocyte differentiation and HIV replication and antiviral immunomodulatory compounds which act via their effects on monocyte phenotype. Vitamin D3 is an important immunomodulator of this type and we have shown it is also an effective antiviral agent that reduces the rate of HIV production by infected monoblastoid cells and normal human monocytes/macrophages. Several analogues of Vitamin D3 were tested and are ranked according to their relative efficacy in preventing HIV release from these cells. We have also shown that the antiviral activity of Vitamin D3 and its analogues, is related directly to the capacity of these compounds to induce the highly differentiated macrophage phenotype. Vitamin D3-treatment of infected U937 monoblastoid cells causes a reduction in virus production, viral RNA and proviral DNA. Consequently, this interesting antiviral agent promotes the differentiation of monocytes to macrophages and reduces viral gene expression in the infected cells.

G 438 GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) POTENTIATES VIRAL PRODUCTION IN MONOCYTE/MACROPHAGES (M/M) YET ENHANCES ANTIVIRAL ACTIVITY OF AZT AND RELATED CONGENERS, Carlo-F. Perno, Robert Yarchoan, David A. Cooney, Neil R. Hartman, Hiroaki Mitsuya, David G. Johns, and Samuel Broder, Clinical Oncology Program, and Developmental Therapeutic Program, National Cancer Institute, Bethesda, MD 20892.

We have investigated the influence of GM-CSF on the replication of HIV-1 in cells of M/M lineage, and its effect on the antiviral activity of AZT and related congeners. We found that viral replication of both monocytotropic (HTLV-III_{Ba-L}) and lymphocytotropic (HTLV-III_B) strains of HIV is markedly enhanced in fresh elutriated M/M cultivated for 5 days with GM-CSF before infection. No enhancement was seen in lymphocytes and in mature M/M. Moreover, GM-CSF reduced up to 100 times the multiplicity of infection required for productive infection in fresh M/M. On the other hand, GM-CSF potentiates antiviral activity of AZT: 0.1 uM AZT completely inhibited HIV-1 replication in M/M exposed to GM-CSF in spite of the increased viral production, while 1 uM AZT was necessary to induce complete viral inhibition in M/M not exposed to GM-CSF. Similar effect was seen with 3 other thymidine congeners tested: 2'3'-dideoxythymidine, 2'3'-dideoxy-2'3'-didehydrothymidine (D4T), and 3'-azido-2'3'-dideoxyuridine (AZddU). These drugs completely inhibited viral replication in GM-CSF-exposed M/M at concentrations 10-100 fold lower than without GM-CSF. We have also found that GM-CSF markedly increased in M/M (10-50 times) the levels of intracellular parent and mono-, di-, and triphosphate anabolites of AZT and D4T, in the face of limited increase of endogenous deoxythymidine-triphosphate pool (dTTP) (less than 2 fold). Thus, an increase of the AZTTP/dTTP or D4TTP/dTTP ratios in GM-CSF-treated M/M may account for the enhanced activity of such drugs.

G 439 TREATMENT OF HIV TISSUE CULTURE INFECTIONS WITH ANTIBODY-RICIN A CHAIN CONJUGATES, Seth Pincus, Kathy Wehrly, and Bruce Chesebro, NIH, NIAID, LMSF, Rocky Mountain Laboratories, Hamilton, MT 59840

Monoclonal antibody 907, directed against a neutralizing epitope on the HIV envelope glycoprotein, has been conjugated to ricin A chain (RAC). This immunotoxin was used to treat tissue culture cells infected with HIV. The conjugate inhibited protein synthesis in chronically infected cells (8E5 and H9-HTLV III_B) but not in the uninfected parent cells (A3.01 and H9). Using a focal immunoassay, it was demonstrated that the number of cells secreting infectious virus was decreased 100-fold by exposure to the immunotoxin. The 907-RAC conjugate inhibited the spread of HIV infection within an acutely infected population of cells. Exposure of cells to the toxin for as short a period as 20 minutes resulted in toxicity to virally infected cells. Blocking antibodies derived from patient serum only partially inhibited the toxin effect. Variant cells, resistant to the effect of the toxin, arose at a frequency of 0.1-1.0%. None of the variant cells produced any infectious virus. When compared with AZT, the immunotoxin produces significantly greater suppression of viral production with virtually no non-specific toxicity. Additive effects are seen when AZT and 907-RAC are used in combination. These data suggest that there may be a role for immunotoxins in the treatment of HIV infection in vivo.

G 440 INHIBITION OF HIV INFECTION: TARGETING INTERFERON SYNTHESIS TO INFECTED CELLS, Daniel P. Bednarik and Paula M. Pitha, The Johns Hopkins University Oncology Center, Baltimore, MD 21205

The aim of the study is to determine the molecular mechanism by which interferons (IFNs) inhibit HIV replication, and to employ a novel site-directed approach to IFN production. Retroviral expression vectors, in which expression of IFN was limited to HIV-infected cells, were constructed with the HIV-LTR directing α2 IFN. Stable cell lines containing the constructs were established, infected with HIV and IFN HIV inhibition was compared to companion cells receiving exogenous α IFN. The levels of viral mRNA and protein were quantitated and compared to control untreated cultures. Addition of recombinant α2 IFN (500 uml) prior to HIV infection caused a 35% reduction in RT activity after 11 days post-infection. The overall reduction in viral mRNA and viral proteins, in IFN-treated cells, was observed. Several cell lines containing the α-IFN retroviral construct produced low levels of IFN constitutively, however, synthesis was greatly enhanced after transfection with the HIV cloned tat gene or HIV infection. The HIV replication in cells containing IFN constructs was substantially inhibited. HIV replication is sensitive to α2 IFN treatment. Molecular studies indicate that the mechanism of this inhibition may occur at several steps of the virus life-cycle. A new concept of therapy directed against HIV replication is proposed using HIV-activated in situ synthesis of α2 IFN.

G 441 SIMILARITIES OF PARTICLES FROM BREAST CANCER PATIENTS AND BLV Elisabeth Rieping, Pf.130348, FRG-43 ESSEN

	Murine Mammary Tumor Virus	Bovine Leukemia Virus	Particles from breast cancer patients
Characteristics of in vivo infection	particles are produced in vivo	no virus production in vivo	no particle production in vivo
Formation of syncytia	?	Syncytia formation after cultivation	Syncytia formation after cultivation
Ion requirement of reverse transcripta.	Mg	Mg	Mg
Budding	easy to visualize	difficult to visualize	not yet seen
Particle Morphology	eccentric core	central core	central core
Disease in rabitts	?	immune deficency disease	immune deficiency disease watched twice

The DNA of BLV is proven to be integrated in lymphocytes of cows milk. So a check for identity and transfection should be worthwhile.

G 442 ANTI-CD4 ANTIBODIES IN HIV-INFECTED PATIENTS. **Iwao Sekigawa, Randal Byrn, Steven Sherwin, Katherine Lucas, J.Davis Allan and Jerome E. Groopman, New England Deaconess Hospital, Harvard Medical School Boston, MA 02215, Division of Clinical Research, Genentech, Inc, South San Francisco, CA 49080.**

CD4 is the cellular receptor for HIV and soluble recombinant CD4 (rCD4) is being developed as a potential antiviral agent. We have studied patients with AIDS, ARC, and asymptomatic HIV infection and observed approximately 20% of patients in each of the groups had antibodies reactive with CD4 in their circulation. This autoimmune antibody reacted with denatured rCD4 in western blots, partially denatured rCD4 in solid phase ELISA systems and was blocked with purified native rCD4 in a dose dependent manner. No correlation was observed between the total T4 cell number or the T4/T8 ratio in these patients and the presence or absence of anti-CD4 antibodies. This antibody was of the immunoglobulin G class and bound to CD4 on lymphoid cell surfaces but did not substantially block HIV infection in vitro.

G 443 LIMITING DILUTION CULTURE OF HIV-1: ADAPTATION OF MICROCULTURE METHODS TO VIRAL QUANTITATION IN CLINICAL THERAPEUTIC TRIALS, Haynes W. Sheppard[1], Dale V. Dondero[1], Michael S. Ascher[1], Alexandra M. Levine[2], Brian E. Henderson[2], Dennis J. Carlo[3], and Jonas Salk[4]; [1]California Department of Health Services, Viral and Rickettsial Disease Laboratory, Berkeley, CA 94704, [2]USC Cancer Center, Los Angeles, CA 90033, [3]The Immune Response Corporation, San Diego, CA 92037, [4]The Salk Institute, LaJolla CA 92037. Numerous clinical trials are underway to determine the efficacy of therapeutic measures for HIV-1 infected individuals. For most of these studies, changes in the quantity of culturable virus in peripheral blood could be an important index of the effect of a therapy. Current methods for HIV culture are extremely labor intensive. We have modified existing peripheral blood mononuclear cell. (PBMC) co-culture methods so that high efficiency recovery of HIV-1 can be achieved in both 24 well and 96 well microculture trays with as few as $1X10^4$ PBMC. Virus replication is detected by p24 antigen capture ELISA. These methods have facilitated limiting dilution culture of PBMC from patients participating in clinical trials of an immunotherapeutic whole virus vaccine. Preliminary results indicate that HIV can be cultured from about 90% of asymptomatic seropositive individuals by these methods with 1X106 patient PBMC and from approximately 80% with 5X105 PBMC and 35% when 1X105 PBMC are cultured. Studies are underway to evaluate more efficient methods for PBMC separation and additional improvements in the efficiency and sensitivity of these culture methods.

G 444 SIMIAN IMMUNODEFICIENCY VIRUS/DELTA (SIV/DELTA): REPLICATION IN PRIMARY MONOCYTE CULTURES, K. St. Cyr-Coats, V. Traina-Dorge, M. Murphey-Corb, Delta Regional Primate Research Center, Tulane University, Covington, LA 70433. Cells of monocyte/macrophage lineage are major target cells for neurotropic members of the Lentivirus family including human immunodefiency virus (HIV), the causative agent of human AIDS. Latently infected monocytes may be responsible for dissemination of virus to the central nervous system.

We examined the ability of highly pathogenic strains of simian immunodeficiency virus/Delta (SIV/Delta) to infect and replicate in monocytes. SIV/Delta8664 is the non-neurovirulent prototype virus, and SIV/DeltaB670 is a neurovirulent isolate. Purified primary monocytes of human and rhesus origin were infected with the SIV/Delta isolates and cultured in the presence of two cytokines, recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-I (IL-I). Culture supernatants were assayed for reverse transcriptase activity at 3-4 day intervals. The two viruses replicated in both human and rhesus monocytes, but required cytokines for productive infection. No extracellular virus was detected in nonstimulated cultures. Virus production was enhanced to the greatest extent in cultures stimulated with IL-I.

These data demonstrate that like other lentiviruses SIV/Delta is capable of replicating in monocytes. However, the requirement for external stimulation by the cytokines GM-CSF and IL-I possibly indicates a contributor for pathogenesis in the naturally infected animal.

G 445 CLINICAL AND IMMUNOLOGICAL/VIROLOGICAL EFFECTS AND SAFETY OF AMPLIGEN THERAPY IN ARC/PRE-ARC PATIENTS, D.R. Strayer[1], I. Brodsky[1], L. Einck[2], S.M. Miller[3], R.S. Schulof[4], G.L. Simon[4], and W.A. Carter[1]. [1]Hahnemann Univ., Phila., PA; [2]HEM Research, Rockville, MD; Baylor Univ., Houston, TX; [4]George Wash. Univ., Wash., D.C.

38 ARC/pre ARC patients (pts) with a median T4 of 255/μl have been treated with 100-200 mg Ampligen (mismatched dsRNA) twice weekly by IV infusion for 6-24 months (mos) (mean = 14). 76% of pts had improvement of delayed type hypersensitivity (DHR) within 2-12 wks. Mean p24 decreased 33% from pre-treatment level in patients receiving 200 mg Ampligen for 8 months. Decreases ($p<.002$) were seen in the ability to co-culture HIV (OD490) from ARC/pre ARC pts treated for 2-4 mos at 200 mg/dose. Mean T4 cells have stabilized for 4 mos (33 pts) to 20 mos (6 pts). After initiation of Ampligen therapy, pts reported significant improvement in diarrhea (6/8, $p=.05$), night sweats (12/12, $p<.001$), and fatigue (20/22, $p<.002$), and had decreases in lymphadenopathy (11/15, $p<.05$). Ten pts have advanced to AIDS (6 = OI, 3 = KS, 1 = lymphoma) with a mean time to progression of 11 months. No patients have died while on study. Clinical improvement, return of DHR, stabilization of T4 counts, reductions in HIV load, and the absence of clinically significant side effects/drug toxicity, suggest mismatched dsRNA is a candidate for enlarged clinical trials. An update of the AMP101 randomized, placebo-controlled study in ARC patients will be presented.

G 446 A HOST GENE LINKED TO THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CONTROLS THE ENVELOPE GENE STRUCTURE OF TUMOR-ASSOCIATED RECOMBINANT MURINE LEUKEMIA VIRUSES (MuLVs), Christopher Y. Thomas and Michael A. Coppola, Departments of Medicine and Microbiology, University of Virginia, Charlottesville, VA 22908

The biologic significance and the selection for heterogeneity in the envelope genes of the human immunodeficiency virus and other retroviruses is poorly understood. We have studied a mouse model in which host genes influence the envelope gene structure of recombinant MuLVs. The injection of the SL3-3 MuLV into CWD or HRS mice induces lymphoma and the formation of recombinant viruses. The CWD and HRS recombinants contain sequences derived from the 5'portion of the envelope gene (*env*) of highly related endogenous polytropic viruses. However, the HRS viruses retain SL3-3 sequences in the central portion of *env* (class I *env*), while CWD recombinants contain only the polytropic virus sequences in this region (class II *env*). The sequence differences between the class I and class II *env* predict 10 amino acid differences in the aminoterminal portion of the envelope TM protein. Southern blot analysis of recombinant proviruses in tumor DNAs from 24 CWD x HRS and 90 backcross and F2 mice injected with SL3-3 showed that the generation or selection of class I *env* recombinants was dominant and correlated with the inheritance of an allele of the HRS major histocompatibility complex (MHC). HRS mice inoculated with the class II *env* virus CWN-T-25 developed tumors that contained recombinant viruses with class I *env* genes and markers of CWN-T-25. Thus, HRS mice express a gene linked to the MHC on chromosome 17 that selects in vivo for recombinants with class I *env* genes although they are susceptible to infection by class II *env*.

G 447 LYMPHOPROLIFERATIVE DISEASE IN AN AFRICAN GREEN MONKEY ASSOCIATED WITH DUAL INFECTION BY STLV-I AND SIV, V. Traina-Dorge, J. Blanchard, M. Murphey-Corb. Delta Regional Primate Research Center, Tulane University, Covington, LA. 70433. Human T cell leukemia virus (HTLV-I) is an exogenous human retrovirus associated with adult T cell leukemia. Seroepidemiological evidence suggests a variety of old world monkeys are infected with a related virus, STLV-1. Associated disease, however is usually not evident. The simian virus, shares the same genomic arrangement of LTR-gag-pol-pX-LTR, and a DNA sequence similarity of 90-95% with HTLV-I. STLV-I can transform monkey cells *in vitro*, however, no direct viral link with lymphomas or leukemias has ever been established. Several African species are also frequently infected with simian immunodeficiency virus (SIV) without apparent disease.

A spontaneous case of lymphoproliferative disease was observed at DRPRC in a 12 year old female African green monkey (*Cercopithecus aethiops*). Pulmonary thrombi, cryptosporidiosis, and villus atrophy in the small intestines were significant contributors to this animals debilitated condition. Severe enlargement of all lymph nodes and lymphoid tissues due to infiltration by both lymphohistiocytic and syncydial cells was also evident. Electron microscopy revealed lentivirus particles in syncydial cells. Infection by simian immunodeficiency virus (SIV) was confirmed by Western blot analysis of monkey sera.

STLV-I proviral sequences were shown to be monoclonally integrated by Southern blot analysis of hyperplastic lymphoid tissue DNA. Neither SIV or Epstein Barr virus sequences could be detected by this method even under low stringency hybridization conditions. These data indicated the clonal expansion of STLV-I infected cells, and suggest a leukogemic potential of STLV-I *in vivo*. Activation of STLV-I may be necessary for disease, and may have been provided by SIV. This finding may suggest similar pathogenesis in humans dually infected with HTLV-I and HIV.

G 448 POKEWEED ANTIVIRAL PROTEIN-MONOCLONAL ANTIBODY CONJUGATES SELECTIVELY INHIBIT HIV-1 REPLICATION IN HUMAN T-CELLS AND MONOCYTES. Fatih M. Uckun[1], Patricia A. Moran[2], Dorothea E. Myers[1], Joan Sias[2], James D. Irvin[3], Jeffrey A. Ledbetter[2], and Joyce M. Zarling[2]; [1]Tumor Immunology Laboratory, Departments of Therapeutic Radiology-Radiation Oncology, Pediatrics, and BMT Program, University of Minnesota Health Sciences Center, Minneapolis, MN 55455, [2]Oncogen, Seattle, WA 98121, and [3]Southwest Texas State University, San Marcos, TX 78666.

Pokeweed antiviral protein (PAP) is a 30 kDa ribosome inhibitory plant protein which is isolated from leaves and seeds of Phytolacca americana (pokeweed) and shows a broad spectrum of antiviral activity against RNA- as well as DNA-viruses. Pretreatment with PAP at concentrations in excess of 300 nM inhibited HIV-1 production (as measured by antigen capture assays of culture supernatants for expression of gag/p24 protein) in CEM (T-cell precursor line) as well as U937 (promonocyte line) cells that were infected with the LAV-1 isolate of HIV-1, providing unique evidence that the antiviral spectrum of PAP includes HIV-1. The PAP conjugate of G3.7/CD7 MoAb was much more effective in inhibiting HIV-1 replication in CEM cells than unconjugated PAP and elicited significant inhibition of HIV production at non-cytotoxic concentrations of as low as 4 pM. Similarly, the PAP conjugate of F13/CD14 MoAb was highly effective in inhibiting virus replication in U937 cells and completely abrogated detectable HIV production at concentrations as low as 100 pM without detectable cytotoxicty. G3.7-PAP did not affect HIV production by U937 cells and F13-PAP did not affect HIV production by CEM cells. PAP conjugate of B43/CD19 control MoAb did not inhibit viral replication in CEM or U937 cells. To our knowledge, this study represents the first development and application of antiviral agent-MoAb conjugates for selective inhibition of HIV production by infected cells bearing target surface antigens and may provide the foundation for a novel treatment strategy for AIDS.

G 449 CESSATION OF ZIDOVUDINE LEADS TO INCREASED VIRAL BURDEN, Mark A. Wainberg, Mary Fanning, John Gill, Karen Gelmon, Julio S.G. Montaner, Michael O'Shaughnessy, Chris Tsoukas and John Ruedy, Jewish General Hospital, Montreal, Quebec, Canada, H3T 1E2, and the Canadian Multicentre Study.

We have used zidovudine to treat 72 HIV-1-infected adults (CDC stages II and III). Isolation of HIV-1 from circulating mononuclear cells of each subject was carried out both prior to the start of therapy and at regular intervals thereafter. Subjects received 600 mg of drug per day for 18 weeks, 900 mg per day for a subsequent 9 weeks, and 1200 mg per day for a further 9 week period following. After 36 weeks of therapy, subjects received no drug for a 6 week wash-out period. Drug was then restarted at 42 weeks, at a dose of 1200 mg per day. HIV was successfully isolated from about 40% of patients, both prior to and during initial therapy, with average time to culture positivity under 20 days. In contrast, at the end of the 6 week wash-out period, and during the 6 months of therapy which followed, HIV-1 was isolated at least 95% of the time, with time to positivity about 12 days. These findings are reflected by data obtained on p24 antigen levels in these individuals over the course of this study. These studies suggest that discontinuation of zidovudine for 6 weeks may result in increased viral burden, and that shorter wash-out periods should be employed, whenever possible, to overcome the toxic effects of this drug.

G 450 TEMPORAL EXPRESSION OF HIV-ASSOCIATED ANTIGENS DETECTED BY QUANTITATIVE IMMUNOFLUORESCENCE USING INTERACTIVE LASER CYTOMETRY, Jonathan T. Warren, James B. McMahon, Owen S. Welslow, Rebecca Kiser, and Michael R. Boyd, Program Development Research Group, National Cancer Institute and Program Resources, Inc., Frederick Cancer Research Facility (NCI-FCRF), Frederick, MD 21701

We are investigating the feasibility of quantitative immunofluorescence techniques for rapid, large-scale screening assays of possible anti-HIV compounds. An Anchored Cell Analysis and Sorting (ACAS) interactive laser cytometer (Meridian Instruments, Okemos, MI) gave quantitative immunofluorescence data on the expression of the HIV-1 envelope glycoprotein, gp120, and the HIV-1 core protein, p24. The ACAS permitted quantitative fluorescence detection and evaluation not possible with conventional fluorescence microscopy. The expression of viral-derived gp120 and p24 appear to be early events subsequent to HIV-1 infection. In the case of gp120, these are important molecules associated with the tissue selectivity and cytopathic effects (i.e., syncytium formation) of HIV-1 infection. Therefore, these markers were particularly appropriate for our initial immunofluorescence analyses. In indirect immunofluorescence assays using a panel of anti- gp120 and p24 monoclonal antibodies, we used the ACAS system to analyze the fluorescence signals generated by cell lines (i.e., H9 and CEM) infected with the rf strain of the Haitian variant of HIV-1 (HIV-1rf). Since this method proved to be particularly sensitive in detecting gp120 and p24 expression, the antibodies were used to examine expression of gp120 and p24 at several different time points after infection with HIV-1rf. This methodological approach permited (i) early and very sensitive non-subjective and statistically based distinctions in the expression of gp120 and p24 by HIV-infected target cells, (ii) evaluation of additional quantitative morphological information (i.e., mean fluorescence intensity and cell size) not generally possible with other fluorescence detection systems, and (iii) such analyses on target cells stained after fixation (an important safety consideration).

G 451 INITIAL CLINICAL STUDIES OF 2',3'-DIDEOXYADENOSINE (DDA) AND 2',3'-DIDEOXYINOSINE (DDI) IN PATIENTS WITH AIDS OR AIDS-RELATED COMPLEX (ARC)

Robert Yarchoan, Rose V. Thomas, Hiroaki Mitsuya, Carlo-Federico Perno, James M. Pluda, Neil R. Hartman, David G. Johns, and Samuel Broder, National Cancer Institute, Bethesda MD 20892.

DDA and DDI are dideoxypurine analogues which inhibit HIV replication in T cells and macrophages *in vitro* at concentrations of 1 to 10 μM. DDA is rapidly converted to DDI on an equimolar basis by the ubiquitous enzyme adenosine deaminase, so for many purposes they can be considered alternate forms of the same drug. We administered DDA at doses of 0.4 or 0.8 mg/kg intravenously every 12 hours for 2 weeks to 7 patients with AIDS or ARC. In a separate ongoing study, we administered DDI intravenously at 0.2, 0.4, or 0.8 mg/kg every 12 hours to 9 patients with AIDS or ARC, followed by 4 weeks of oral therapy with twice the intravenous dose. Peak serum levels of 0.6 to 2.5 μM DDI were obtained with these regimens. Also, when given with antacids, both drugs were found to be well absorbed from the gastrointestinal tract (bioavailability approximately 40%). Some patients on either trial received extended therapy with the agents (maximum 14 weeks). The drugs were well tolerated with little or no toxicity observed. 6 of the 7 patients on DDA had increases in their T4 cells over 2 weeks (mean Δ of 66 ± 40 T4 cells/mm3, mean ± SEM), and each of 3 patients who were serum HIV p24 antigen positive at entry had decreases on therapy. While the patients on the lowest dose of DDI had unchanged T4 cells, 5 of 5 on the 2nd and 3rd doses had increases during the 2 weeks of intravenous therapy (mean Δ 91 ± 48 T4 cells/mm3) which were generally sustained during oral dosing. These preliminary results suggest that these agents may have clinical activity against HIV with little or no toxicity and that they warrant further clinical testing for use in HIV infection.

Vaccines and Therapeutic Intervention; Animal Models

G 500 **NOVEL MECHANISM BASED INHIBITORS OF HIV**, Jeffrey J. Blumenstein, Terry D. Copeland, Stephen Oroszlan, Christopher J. Michejda,

Laboratory of Chemical and Physical Carcinogenesis, and Laboratory of Molecular Virology and Carcinogenesis, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

In an effort to develop non-nucleoside antivirals we have synthesized a new class of compounds designed to act upon critical viral enzymes other than reverse transcriptase. Initial efforts have focused upon retroviral proteases. Utilizing synthetic HIV-2 protease and well defined cleavage site peptides we have been able to conveniently screen a number of these compounds. At practical concentrations (ca. 2 mmol) a number of these compounds exhibit significant inhibition of the protease specific cleavage of the Tyr-Pro peptide bond. Further studies on HIV cytopathic effects in vitro suggest that these compounds may be active through more than one mechanism.

G 501 BIOLOGICAL CHARACTERIZATION OF PAIRED HIV-1 ISOLATES FROM BLOOD AND CEREBROSPINAL FLUID OF PATIENTS WITH VARYING SEVERITY OF HIV-1 INFECTION, Francesca Chiodi*, Antonio Valentin*, Barbara Keys*, Stefan Schwartz*, Birgitta Åsjö*, Susan Gartner#, Mikulas Popovic#, Jan Albert* **, Vivi-Anne Sundqvist**, and Eva M. Fenyö*, Departments of Virology, Karolinska Institute* and National Bacteriological Laboratory**, Stockholm, Sweden and National Cancer Institute#, National Institutes of Health, Bethesda, Maryland. HIV-1 isolates were obtained from the blood and cerebrospinal fluid (CSF) of three asymptomatic carriers, one patient with lymphadenopathy syndrome, two with AIDS-related complex (ARC) and three with AIDS. The 5 patients with ARC and AIDS also showed neurologic/psychiatric complications. Whereas all isolates but one (from the CSF of an asymptomatic carrier) replicated in peripheral blood mononuclear cell cultures after cell-free transmission, few isolates replicated in H9 or U937 clone 2 cells. Viruses isolated from blood and CSF of the same patient could be distinguished by their replicative capacity in different cell lines, by the type of cytopathic effect and by protein profile as tested by radioimmunoprecipitation. When tested on monocytes, all isolates replicated and gave a cytopathic effect involving extensive syncytia formation. Isolates from the CSF of two patients with dementia showed increased monocyte tropism as compared to the corresponding blood isolates. The results indicate that variant viruses with distinct biological characteristics may be isolated from the blood and CSF of the same patient.

G 502 CD4-DERIVED SYNTHETIC PEPTIDES INHIBITING HIV ENVELOPE/CD4 INTERACTIONS INVOLVED IN HIV INFECTIVITY AND VIRALLY MEDIATED CYTOPATHOLOGY, Lee E. Eiden, Kou M. Hwang, Peter L. Nara, Dianne Rausch, Anne-Helene Voltz, Mary Padgett, Nancy Dunlop, Blair Fraser, Vaniambadi S. Kalyanaram and Jeffrey D. Lifson, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892, Departments of Cellular Immunology and Medicinal Biochemistry, Genelabs Incorporated, Redwood City, CA 94063, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701, and Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, Bionetics Research Incorporated, Rockville, MD 20850

Synthetic peptides derived from the CD4 molecule have been tested for their ability to inhibit HIV infectivity and virally mediated cytopathology, in vitro. Modified synthetic peptides corresponding to residues 81-92 of CD4 have been synthesized and shown in pure form to inhibit HIV envelope/CD4 interactions involved in HIV infectivity and viral cytopathology. These activities were dependent on both the correct primary sequence of the amino acid backbone of the peptide and on specific modification (derivatization) of particular amino acid side chains. Peptides which block HIV infectivity and viral cytopathology do not appear to block in vitro assays of CD4-dependent immune functions at concentrations in excess of those required for complete blockade of HIV/CD4 interactions. Peptides have also been evaluated in a modified form of the CEM-SS quantitative syncytia forming assay of HIV infectivity (Nara, et al, AIDS Res Hum Retrovir, 3:283, 1987) for activity in distinct kinetic phases of HIV infection, as well as for their ability to interfere with HIV envelope/CD4 interactions in direct co-immunoprecipitation-based binding assays. Results of these studies will be discussed. The requirement for modification of the peptide for the observed biological activity may reflect a need for stabilization of the peptide backbone of CD4(81-92) in a conformation which optimizes its ability to competitively inhibit HIV envelope interactions with the native CD4 protein.

G 503 DIFFERENCES IN EPSTEIN-BARR VIRUS INVOLVEMENT AT DISTINCT TUMOR SITES IN AN AIDS-RELATED LYMPHOMA, Ellen G. Feigal,Nathaniel A. Brown,Chen-Ren Liu, and Michael S. McGrath, Department of Medicine, University of California, San Francisco, San Francisco, CA. 94143, Recently there has been a marked increase in the incidence of high grade B cell non-Hodgkin's lymphoma (NHL)in individuals at risk for AIDS. Because Epstein-Barr virus (EBV) has been implicated in B cell transformation *in vitro*, in African Burkitt's lymphoma, and in the B lymphoproliferative disorders and B cell lymphomas afflicting immunodeficient persons, we investigated the contributory role for EBV in an AIDS NHL patient's tumor. Normally, the EBV genome persists as a circularized plasmid within infected B cells, the ends of the linear virion DNA having become covalently fused. This fusion event causes the length of the EBV terminal region to vary among individual EBV infected cell lineages. The presence of a clonal EBV terminal region within a monoclonal tumor cell population suggests that EBV entered the tumor cell at or near the time of malignant transformation. Using Southern blot hybridizations we analyzed DNA samples from two metastatic tumor foci taken at autopsy from a patient with AIDS NHL. One focus revealed a single-sized EBV terminus, indicating that it was a clonal expansion of an EBV infected progenitor cell. The other focus did not contain any detectable EBV DNA using five different EBV gene probes. Both foci had identical and monoclonal patterns of immunoglobulin gene rearrangement. These results suggest that EBV may not have been present in the patient's progenitor tumor cell, when it underwent malignant transformation. Multiple distinct tumor foci should be analyzed when assessing a possible contributory role for EBV in tumor formation.

G 504 REPLICATIVE CAPACITY OF SEQUENTIAL HIV-1 ISOLATES FROM PATIENTS WITH PROGRESSIVE HIV-1 RELATED DISEASE, Eva M. Fenyö*, Jan Albert**, Linda Morfeldt-Månson* and Birgitta Asjö*, Departments of Virology, Karolinska Institute* and National Bacteriological Laboratory**, Stockholm, Sweden. We have shown previously that according to their capacity to replicate in vitro, human immunodeficiency virus type 1 (HIV-1) isolates can be divided into two major groups, rapid/high and slow/low. The viruses able to replicate efficiently are regularly isolated from immunodeficient patients, whereas poorly replicating isolates are often obtained from individuals with no or mild disease. To study whether the replicative capacity of viruses isolated from one individual changes in the course of the infection, we followed six patients with sequential virus isolations during a 30 months observation period. One patient with stable lymphadenopathy syndrome (LAS) yielded slow/low viruses on repeated isolations for one year. Viruses isolated from two patients during the period of clinical progression from LAS to AIDS-related complex (ARC) showed increasing replication potential. In two other patients progressing from LAS to ARC, the replication potential of sequential virus isolates fluctuated during the observation period. Similarly, changes from slow/low to rapid/high and back to slow/low replication pattern, could be observed in sequential virus isolates from two patients with ARC. One patient showed a rapid development of severe immunodeficiency, progressing from LAS to AIDS within one year. Viruses isolated during this period replicated with increasing efficiency in vitro. The results show that HIV-1 isolates with distinct replicative capacity may be obtained from the same individual over time. Rapid clinical progression is often accompanied by the emergence of variant viruses with increased replicative capacity.

G 505 INDUCTION OF HIV-1 VIRUS PRODUCTION FROM LATENTLY INFECTED CNS-DERIVED CELL LINES, Mark A. Laughlin, Janet M. Harouse, James A. Hoxie, F. Gonzalez-Scarano

A number of CNS-derived cells including MED 217, a medulloblastoma line and U373, a glioblastoma line, can be infected with HIV-1. Unlike infection of most lymphoid and monoblastoid cells, infection of these two cell lines results in latent infections from which virus can be recovered by co-cultivation but which do not yield infectious virions in the supernatant under usual circumstances. Antigen capture assay for p24 in either the supernatant of infected cells or intracellularly as well as fluorescence immunocytology indicate that *gag* products are not produced either. On the other hand, virus can be consistently rescued by co-cultivation with some, but not all CD4 positive lymphoid lines. Furthermore, cell-to-cell contact is required, indicating that rescue is not due simply to efficient capture of an occasionally released virion.

We have looked at the initiation of virus production in these cells by several methods: (1) the induction of transcription (2) the production of p24 antigen in the supernatant and (3) the production of infectious virions. Standard methods for the induction of latent retroviral genomes, like treatment with phorbol esters (TPA), result in increases in transcription, $p24^{gag}$ antigen production and in the production of infectious virus. Transcription of the viral genome can be induced by treatment with epidermal growth factor (since the MED 217 contain EGF receptors), by contact with SUP-T1 cells (a CD4 positive lymphoid line) and by exposure to supernatant from uninfected primary macrophages.

These results indicate that CNS derived cells can serve as a reservoir for virus, and can be induced to produce infectious virions by diverse biologically relevant stimuli, including treatment with growth factors and contact with cells of lymphoid and monocytic/macrophage lineage.

G 506 ASSOCIATION OF HIV RETROVIRUSES WITH A SPECIFIC SUBSET OF HUMAN B CELL LYMPHOMAS FROM AIDS PATIENTS. Angela Goodacre, Joel Bresser, F. Cabanillas, R.J. Ford. M.D. Anderson Cancer Center, Houston, TX 77030. AIDS patients have an increased risk for the development of B cell Non-Hodgkins (NHL-B) lymphomas, predominantly of high grade type with extranodal presentation. The spectrum of disease, karyotypes and immunophenotypes are similar to those of NHL-B associated with immunodeficiency. However, we have observed a syncytial variant of small non-cleaved cell lymphoma, characterized by cytoplasmic vacuolation and giant cells, in a minority of AIDS patients. Cell lines established from large cell syncytial variant AIDS associated lymphomas show distinct phenotypes, exhibiting intracytoplasmic vacuolation and giant cell formation, reminiscent of the cytopathic infection of CD4+ T lymphocytes. These cell lines show positive hybridization with subgenomic HIV probes using RNA in situ, dot blot and Northern blot analysis. Although negative by Southern blot, polymerase chain reaction revealed HIV sequences in extracted DNA. Ultrastructural study revealed a population of cells containing retroviral particles within cytoplasmic vacuoles, resembling HIV infected cells of the monocyte/macrophage lineage. Immunophenotypic studies identified the presence of HIV peptides. It appears that this subset of AIDS associated NHL is directly infected by HIV. This retroviral infection may either contribute to the pathogenesis of the lymphomatous disease process or modify the neoplastic cells morphologically in this large cell syncytial variant of AIDS related NHL-B.

G 507 VARIABILITY OF PROVIRUS FROM HTLV-I-ASSOCIATED TROPICAL SPASTIC PARAPARESIS AND ADULT T-CELL LEUKEMIA DETECTED BY GENE AMPLIFICATION Steven J Greenberg, Steven Jacobson, Mark A Abbott, Satyakam Bhagavati, Dale E McFarlin, Bernard J Poiesz, Thomas A Waldmann, Garth D Ehrlich, NCI and NINCDS, Natl Institutes of Health, Bethesda, MD 20892, Department of Medicine SUNY Syracuse, NY 13210, Department of Neurology, SUNY Brooklyn, NY 11203 The human T-cell leukemia virus type I (HTLV-I) exhibits pleiotropia; it is linked to a hematologic malignancy, adult T-cell leukemia (ATL), a neurologic disorder, tropical spastic paraparesis (TSP), and an immunosuppressed state. Such variation in disease expression may derive from differences in proviral genomic sequences. To detect genetic diversity, ATL and TSP blood mononuclear cell DNA was subjected to enzymatic amplification and screened for homology to LTR, gag, pol and env regions of prototype HTLV-I provirus. Within the env domain the region encoding gp21E protein displayed considerable variability in both ATL and TSP populations. Diversity was also noted in the pol region. By contrast, uniformly positive amplification in other regions of the env and in the LTR and gag domains suggested relative genetic conservatism. The gp21E sequence encodes a transmembrane protein homologous to the Moloney murine leukemia virus p15E protein which has immunosuppressive properties. Alterations of the genetic message within the gp21E region may account, in part, for the disparity in immunosuppression seen in different patients.

G 508 PRODUCTIVE BUT NOT 'LATENT' INFECTION AFFECTS ACCESSORY CELL FUNCTION OF MONOCYTIC CELLS, R.A. Gruters, F.G. Terpstra, C. van Noesel, M. Tersmette, F. Miedema, Central Lab. Netherl.Red Cross Blood Transf.Service and the Lab. of Exp. and Clin. Immunol. of the Univ. of Amsterdam, Amsterdam, The Netherlands
In the early HIV infection HIV is believed to be present in a latent proviral form mainly in cells of the macrophage/monocytic lineage. Immunological abnormalities occur early in infection (Miedema et al. J.C.I., in press), which may implicate that this latent infection affects the immune system. We studied whether latent infection of monocytic cells exists and whether this influences accessory-cell functions of these cells in a previously described model system using U937 cells (Petit et al. J.I., p.485, 1988) and RC2a cells. With respect to kinetics of viral replication, two types of isolates were distinguished. Fast replicating isolates showing virtually no lag time between infection and virus production and slow replicating isolates with a lag time of 3-6 weeks. In this lag time only a small percentage of the cells was infected as monitored by titration, and proviral DNA was not detectable by Southern blotting. At that time the cells has normal accessory function in an alpha-CD3 driven T-cell proliferation or in allogenic T-cell stimulation. In virus-producing cultures 100% of the cells were infected, accessory function was disturbed and viral DNA/RNA was easily detected.
In contrast to U937, RC2a cells in spite of the low CD4 expression were killed upon HIV infection. Our results show that accessory functions of monocytic cells are affected only when virus replication occurs.

G 509 EVIDENCE FOR A ROLE OF VIRULENT HIV STRAINS OBTAINED FROM STUDIES ON A PANEL OF SEQUENTIAL HIV ISOLATES, M. Tersmette, J.G. Huisman, F. de Wolf*, R.E.Y. de Goede, R.A. Gruters, J. Goudsmit**, F. Miedema, Central Lab. Netherl.Red Cross Blood Transf.Service and the Lab. of Exp. and Clin. Immunol. of the Univ. of Amsterdam, *Municipal Health Service, **Dept. of Virol., Acad. Med. Center, Amsterdam, The Netherlands
We studied the properties of sequential HIV isolates, recovered from 20 asymptomatic individuals, 9 of whom eventually progressed to ARC or AIDS. In 16 individuals only non-syncytium-inducing (NSI) isolates with variable replication rates were observed. NSI isolates could only be propagated in peripheral blood mononuclear cells. From two individuals throughout the period of observation high-replicating syncytium-inducing (SI) isolates were observed. In two others, during the study period a transition from NSI to SI isolates were observed. Three of these four individuals developed ARC or AIDS 9-15 months after the first isolation of an SI isolate. In contrast, to NSI isolates, SI isolates could also infect two T-cell lines, H9 and Sup T1, as well as two promonocytic cell lines, U937 and RC2A. A significant correlation was found between the replication rate of isolates and the two in-vivo rate of CD4+ cell decrease. In individuals with low-replicating HIV isolates no significant CD4+ cell loss was observed. Recovery of high-replicating isolates, however, in particular when these isolates were syncytium-inducing and had a broad host range, was associated with rapid decline of CD4+ cell numbers and development of disease.

G 510 INDUCTION OF CD4 AND SUSCEPTIBILITY TO HIV-1 INFECTION BY THE HTLV-1 LIKE VIRUS DERIVED FROM A PATIENT WITH TROPICAL SPASTIC PARAPERESIS BUT NOT BY THE HTLV-1 FROM HUT 102, Steven Jacobson, Steven J. Greenberg, Scott Koenig and Dale E. McFarlin, Neuroimmunology Branch, National Institutes of Health, NINCDS, Building 10, Room 5B-16, Bethesda, MD 20892 We have extended our observations on the HTLV-1 like virus that is associated with TSP and have identified additional biological differences between the viruses derived from individuals with TSP and prototype HTLV-1 (Hut 102). We have found that the PEER T cell line which has a cell surface phenotype that is CD4-, CD8-, T cell receptor alpha/beta -, and TcR gamma/delta +, could be induced to express CD4 and CD8 by co-infection with a T cell line derived from a Colombian patient with TSP but not by conventional HTLV-1 (the Hut-102 cell line). In addition, there was an induction of the alpha/beta TcR on the TSP co-cultured PEER cell line. Expression of the CD4 molecule on the surface of these co-cultured cells was associated with development of a permissive and lytic infection with HIV-1. Since both HTLV-1_{TSP} and HIV-1 are endemic in similar geographical regions, it raises the possibility that individuals infected with HTLV-1_{TSP} may be at greater risk of infection with HIV-1 by this previously unrecognized mechanism.

G 511 INDICATIONS FOR RETROVIRUS IN MULTIPLE SCLEROSIS, Slavenka Kam-Hansen*, Lu Chuan Zen, Sten Fredrikson & Yi Qing, Department of Neurology, Karolinska Institutet, Huddinge University Hospital, S-141 86 Huddinge, Stockholm, Sweden, *Present address: Department of Cancer Biology, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115

Bone marrow might be a reservoir of retroviruses in humans infected with such viruses, similarly to what has been observed in e.g. visna infection of sheep or feline leukemia virus infections in cats. With the assumption that multiple sclerosis, chronic demyelinating disease of central nervous system, is a retrovirus associated disease, we performed over 100 long term cultures of mononuclear cells from bone marrow and peripheral blood from 16 patients with multiple sclerosis. Different mitogens and known as well as presumed inducers of retrovirus expression were used to facilitate virus replication. Cocultures with T, B and histiocytic cell lines have been done. Presence of reverse transcriptase, the enzyme indicative of retrovirus expression, was demonstrated in primary bone marrow and peripheral blood mononuclear cell cultures as well as in cocultures. Reverse transcriptase activity was transferable with riverse transcriptase positive supernatantes to uninfected cells. Strategies for identification and characterisation of putative retrovirus in multiple sclerosis will be discussed.

G 512 HIV REPLICATION IN MACROPHAGES IS PREVENTED BY INTERFERONS (IFN) AND BACTERIAL LIPOPOLYSACCHARIDE (LPS). R.S. Kornbluth, P.S. Oh, J.R. Munis, P.H. Cleveland, and D.D. Richman. Univ. Calif. San Diego and the V. A. Medical Center, San Diego, CA 92161.

Monocyte-derived macrophages were treated with various substances and then infected with a macrophage-tropic strain of HIV-1 (HTLV-III Ba-L/85). Pretreatment with IFN-α, IFN-β, IFN-γ, or LPS prevented viral replication in macrophages. In treated cultures, little or no infectious HIV or p24 core antigen was released into the supernatant, no virions were seen by electron microscopy, no viral RNA or DNA was detectable in the cell lysates, and no cytopathology (as determined by multinucleated giant cell formation) occurred. In contrast, pretreatment with a wide dose range of recombinant IL-1β, IL-2, IL-4, IL-6, M-CSF, TNF-α, or lymphotoxin failed to protect macrophages from productive infection by HIV. A consistent effect of GM-CSF on HIV replication in macrophages was not observed.

Pretreatment with ~100 U/ml of IFN-α, ~ 10 U/ml of IFN-β, or ~100 U/ml of IFN-γ was sufficient to prevent virion release maximally and to prevent cytopathology completely. Even when added three days after infection with a multiplicity of 1 $TCID_{50}$/cell, these treatments markedly reduced virion release, suggesting that these agents do not affect the early events of virus binding, penetration, and uncoating. Polymerase chain reaction assays are underway to assess the effects of IFN on provirus formation.

These data indicate that HIV replication in previously uninfected macrophages may be regulated by an inducible host cell mechanism. These findings may explain the restricted replication of HIV in macrophages *in vivo* and suggest an antiviral role for interferons in the therapy of HIV infection.

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G 513 INFECTION OF HUMAN FETAL DORSAL ROOT GANGLIA GLIAL CELLS WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INVOLVES AN ENTRY MECHANISM INDEPENDENT OF THE CD4 T4A EPITOPE, Charles Kunsch and Brian Wigdahl, Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

HIV-1 has been implicated in the generation of acquired immunodeficiency syndrome (AIDS)-dementia complex and the presence of CD4 in the adult human nervous system may be involved in the susceptibility of selected neural cell populations to HIV-1 infection. We have previously demonstrated that human fetal dorsal root ganglia (DRG) glial cells are susceptible to HIV-1 infection with the subsequent expression of at least a fraction of the virus genome. Treatment of these cells with antibody directed against the T4A epitope of the CD4 molecule, but not the T4 epitope, resulted in a partial reduction in HIV-1 specific gag antigen expression. In addition, pre-incubation of the HIV-1 inoculum with HIV-1 neutralizing antiserum prior to infection resulted in no reduction of HIV-1 gag antigen expression. As determined by ribonuclease protection assay, a protected CD4-specific RNA fragment was detected in RNA isolated from human fetal DRG as well as spinal cord tissue. In addition, RNA blot hybridization analysis of total cellular RNA isolated from human fetal DRG and spinal cord utilizing a CD4-specific RNA probe demonstrated specific hybridization to two unique RNA species with a relative molecular size considerably greater than 5.1 kilobase. However, we have been unable to detect the synthesis or physical presence of the CD4 molecule in neural cell populations derived from DRG. These data suggest that HIV-1 infection of human fetal DRG glial cells may use a mechanism of viral entry independent of the T4A epitope. Current studies are directed at examining the structure, protein coding capacity, and potential role in HIV-1 DRG glial cell infection of these unique CD4-related RNA species.

G 514 EX VIVO QUANTITATION OF ACTIVELY EXPRESSED AND/OR GENOMIC HIV ASSOCIATED WITH MONONUCLEAR CELLS FROM PATIENTS, D. Mathez, J. Leibowitch, Unité d'Immuno-Virologie, Hôpital Raymond Poincaré, Université René Descartes Paris-Ouest, France

The cumulated mass of HIV expressed over time in one individual carrier could be the main factor for HIV-related clinical morbidity. Co-cultivation techniques where patient's cell-associated HIV load is amplified from patient's living cells by indicator PHA-blasts does not discriminate between either of two species of HIV: 1) one expressed from patient's cells before cultivation artifacts, indicative of the clinically relevant pool, 2) the other, which after in vitro mitogenic stimulations is recruited to express virus from latent pro-viral HIV genomes.

We have devised a co-cultivation system where the yields of cell-associated virus from patient's cells in vitro can be ascribed to either species of HIV. Serial specimen from individual patients with a progressive immuno-deficiency course tested with this system do show a progressive increase in expressed, cell-associated, HIV load, especially within a population of non-dividing, vessel-adherent cells. AZT treatment only partially reverses such a documented progression.

G 515 HIV-1 INFECTION OF A NEURONAL CELL LINE BY A CD4-INDEPENDENT PATHWAY,Xi Ling Li,Tarsem Moudgil,David D. Ho,Cedars-Sinai Medical Center and UCLA School of Medicine

Six neuronal cell lines were examined for susceptibility to HIV-1 infection in vitro. One particular cell line (SK-N-MC) was found to be susceptible to productive infection by HTLV-IIIRF with multiplicity of infection (MOI) ranging from 0.002 to 1.0. Other cell lines were not infectable even at MOI of 1.0. HTLV-IIIRF infection of SK-N-MC has become persistent for over 170 days. During this period, HIV-1-specific antigens were demonstrated in the cells by immunofluorescence on multiple occasions, as was the finding of HIV-1-like particles by electron microscopy. Furthermore, virus was rescued from cluture supernate on all three attempts. Interestingly, SK-N-MC was not susceptible to infection by other HIV-1 isolates, including HTLV-IIIB, AL, AC, ARV-2 and a brain isolate. Characterization of SK-N-MC show that these cells express dopamine hydroxylase and neuron-specific enolase,but not S-100 protein, glial fibrillary acidic protein, and CD4. These results suggest that SK-N-MC cells are indeed neuronal in origin. Infection of SK-N-MC by HTLV-IIIRF was blocked by anti-gp120 antibodies and soluble CD4; however, it was not blocked by OKT4A and Leu3A monoclonal antibodies.These experiments show that certain cells of neuronal origin are infectable in vitro by select strains of HIV-1 via a CD4-independent mechanism.

G 516 ACCELERATED VIRAL EXPRESSION AND CELLULAR DEATH RATE IN HUMAN CD4+ T LYMPHOCYTES COINFECTED BY HIV-1 AND HHV-6, Paolo Lusso, Erwin Tschachler, Phillip D. Markham and Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda MD, 20892.

The human herpesvirus 6 (HHV-6) has been detected in the vast majority of patients with AIDS and is markedly infectious and cytopathic for the same major target cell population as HIV-1, i.e., CD4+ T lymphocytes. To study the possible role of HHV-6 as a cofactor in AIDS, we investigated the interaction between HIV-1 and HHV-6 *in vitro*, by coinfecting human normal peripheral blood mononuclear cells or established CD4+ T-cell lines with the two viruses. In both types of culture, productive dual infection of individual lymphocytes by HIV-1 and HHV-6 was observed by two-color indirect immunofluorescence and electron microscopy. The time-course of viral expression and cellular death was consistently accelerated in dually infected cultures, compared to those infected with either virus alone. In addition, chronic infection by HIV-1 induced more rapid HHV-6 expression and cytopathic effect in a CD4+ T-cell line, compared to homologous, previously uninfected cells. These results, combined with evidence for HIV-1 LTR transactivation by HHV-6 (B. Ensoli *et al.*, this symposium), are consistent with the hypothesis that HHV-6 can play a cofactorial role in the depletion of CD4+ T cells observed in the course of HIV-1 infection.

G 517 ANALYSIS OF HIV-INDUCED NEUROPATHOLOGY: THE USE OF HUMAN FETAL CENTRAL NERVOUS SYSTEM ORGANOTYPIC CULTURES, William D. Lyman, Maria Tricoche, Yvonne Kress, Fung-Chow Chiu and Ruy Soeiro, Albert Einstein College of Medicine, Bronx, NY 10461.

Neurologic disease is a common manifestation of the acquired immunodeficiency syndrome (AIDS). Human immunodeficiency virus-1 (HIV-1) infection of central nervous system (CNS) tissue is associated with these manifestations but, as yet, it has not been determined if the neuropathology is a consequence of "bystander" mechanisms related to inflammation or direct infection of neurons or glia. To investigate the latter possibility, human fetal CNS tissue was established in organotypic culture to determine if HIV-1 can infect neuroectodermal cells. Fetal CNS tissue cultures were exposed to titered inocula of HIV-1 for differing periods of time. The cultures were then either examined for morphologic changes by light and electron microscopy or analyzed using molecular biologic probes centered on the polymerase chain reaction and neurochemical techniques exploiting Western blot technology. Results show that HIV-1 can induce significant neuropathology consistent with that observed in the brains of pediatric AIDS patients. The pathology is characterized by a significant depletion of cells in the presence of edema. The polymerase chain reaction verified infection of the fetal tissue by HIV-1 and the neurochemical analyses demonstrated an alteration in cell-type specific proteins in exposed cultures.

G 518 ROLE OF HIV INFECTION IN PRODUCTION OF TNFα AND IL-1β BY A MONOCYTIC CELL LINE, **Jean-Michel Molina, Randal Byrn, Charles A. Dinarello and Jerome E. Groopman, New England Deaconess Hospital, Harvard Medical School and New England Medical Center Hospital, Tufts University School of Medicine, Boston, MA 02215.**

Recent studies demonstrated high levels of TNFα in serum or in culture supernatants of monocytes from AIDS patients and suggested a role for TNF in the pathogenesis of HIV infection. To address the issue of whether HIV itself provokes TNF secretion or if TNF is produced in response to secondary infectious stimuli, we measured the production of TNFα and IL-1β by monocytic cells (THP-1) using specific radio-immunoassays.The cells, chronically infected with HIV-1 or non-infected were stimulated for 24 hours with endotoxin(0.5 to 500μg/ml) and/or γIFN(100 U/ml). We found a dose-response curve of TNF(0.1 to 3 ng/ml) and IL-1 (60 to 500 pg/ml) production with endotoxin, and a marked increase of both cytokines production when the cells were also treated with γIFN. There was however, in this system, no significant difference in cytokine production by HIV-infected or non-infected cells. This suggests that HIV itself does not increase TNF and IL-1 secretion by monocytic cells.

G 519 INFECTION OF B CELL LINES BY HIV-1, HIV-2, SIV_{Mac}, SIV_{SM}, and SIV_{AGM}, Carel Mulder*,**, James E. Monroe*, Alain Calender***, and Gilbert M. Lenoir***, *University of Massachusetts Medical School, Worcester, MA, USA, **Institute for Cancer Research, London, UK, ***International Agency for Research on Cancer, Lyon, FRANCE

HIV appears to be able to infect an ever expanding range of cells. As has been shown before, Epstein-Barr Virus immortalized (EBV^+) human B cell lines can be infected by HIV-1. We have now tested a panel of 25 human B cell lines, including 6 EBV^- Burkitt's Lymphoma cell lines and their EBV-converted counterparts, 5 EBV producer and 14 EBV non-producer cell lines. This panel of cell lines was infected with HIV-1, HIV-2, SIV_{Mac}, SIV_{SM}, and SIV_{AGM}. Infection was monitored by the presence of cytoplasmic HIV or SIV RNA and by formation of syncytia upon cocultivation with a CD_4^+ T cell line. SIV's do not form syncytia readily with most human T_4 cell lines.[4] We isolated a cell line, M8166, (a subclone of C8166) which forms syncytia with SIV-infected cells as readily as with HIV-infected cells.

All 25 cell lines could be infected productively with HIV-1; most cell lines also produced HIV-2 and all three SIV's. No correlation was found between infectibility and the presence or absence of EBV DNA in the B cells. Many cell lines appeared CD_4^- by immunofluorescence; however, infection could be blocked by anti-CD_4 antibodies in the cell lines tested.

G 520 RECEPTOR-VIRAL RELATIONSHIP BETWEEN HIV AND BRAINCELLS, Thea Pugatsch, Department of Virology, Hebrew University-Hadassah Medical School, POB 1172, 91010 Jerusalem, Israel

Although astrocytes and oligodendrocytes seem to be CD4(-) they are infectible by HIV, both in vivo and in vitro. The binding of HIV to these cells might nevertheless be mediated by gp 120, presumably at a lower affinity. Gp 120 peptides can be used to study the specific binding to the surface of established astrocyte cell lines (U343, U118MG, U138MG) thereby forming a gp 120-receptor complex. This complex is being studied and the putative non-CD4 receptor will be analysed. The results of these studies will be discussed.

G 521 TWO DIFFERENT MODES OF CELLULAR EXPRESSION OF BOVINE LEUKEMIA VIRUS IN BLOOD CELLS FROM INFECTED ANIMALS. K. Radke, D. Grossman, L.C. Kidd and D. Lagarias. Department of Avian Sciences, University of California, Davis, California, 95616.

Bovine leukemia virus (BLV) is an oncogenic, B-lymphotropic retrovirus that is closely related to human T-cell leukemia viruses I and II. Sheep are susceptible to experimental infection and tumorigenesis by BLV. A small fraction of blood mononuclear cells from sheep with latent BLV infections can be induced to express viral genes when freshly prepared blood cells are cultured in the presence of fetal calf serum. The number of virus-inducible cells varies episodically, ranging from 0.0002% to 0.05% of blood mononuclear cells. Cells from one set of animals act as infectious centers, producing BLV that induces synctium formation among indicator cells. Infected cells from a second set of animals support the immediate synthesis of BLV-specific RNA that is detected by in situ hybridization, but most of the cells fail to score as infectious centers in culture. When contact between mononuclear cells is prevented by culturing them in methylcellulose medium, an increased number of cells can act as infectious centers, indicating that cell killing may eliminate some virus-producing cells in liquid cultures, or that a diffusible factor induced by cell contact blocks progression through the viral replication cycle in some host cells. However, syncytium-inducing cells still represent only a fraction of the RNA-inducible cells. Intermittent viral replication must occur in vivo, since these animals maintain titers of neutralizing antibodies as high as those from the first group of animals. We are currently investigating why these cells fail to produce infectious virus in culture.

G 522 MOLECULAR ANALYSIS IN NODE BIOPSIES FROM HIV INFECTED PATIENTS WITH LAS AND LYMPHOMA, Giuseppe Saglio, Robin Foa', Angelo Guerrasio, Paola Francia di Celle, Giorgio Palestro, *Alessandro Sinicco, Umberto Mazza. Dipartimento di Scienze Biomediche e Oncologia Umana and *Clinica delle Malattie Infettive, University of Turin, Italy.

DNAs obtained from 20 node biopsies of patients suffering from LAS and from three patients who developped a Burkitt's lymphoma or leukemia were analyzed by Southern blot in order to establish: a) the pattern of the immunoglobulin (Ig) gene rearrangement, b) the genomic structure of the c-myc locus, c) the presence of clonally integrated HIV and EBV sequences. In all LAS polyclonality was the prevalent pattern of Ig gene rearrangement observed, but in 30% of the cases we were able to identify discrete bands of rearrangement correponding to the presence of monoclonal or oligoclonal cell populations. In none of the specimen tested c-myc structural alterations or HIV genome presence were detected. By contrast, the presence of EBV-related sequences was observed in all LAS biopsies, although we could not establish the presence of a clonal integration of the virus in the cells. As expected, all three Burkitt's tumors (two L-3 type ALL and one lymphoma) displayed a clonal pattern of Ig gene rearrangement. The c-myc was found altered in two cases which presented a truncation of the gene beginning within a very short region of the first intron. On the contrary the breakpoint positions on chromosome 14 were mapping in different regions of the Ig loci. All tumors were lacking the presence of HIV sequences, but were positive when tested for EBV-genome sequences.

G 523 DISAPPEARANCE OF SYNCYTIUM-INDUCING ISOLATES IN EARLY HIV INFECTION, M. Tersmette, R.E.Y. de Goede, J. Goudsmit*, R. Coutinho**, J.G. Huisman, F. Miedema, Central Lab. Netherl.Red Cross Blood Transf.Service and Lab. Exp. and Clin. Immunol. of the Univ. of Amsterdam, *Dept. of Virol., Acad. Med. Center, **Municipal Health Service, Amsterdam, The Netherlands

Two types of HIV isolates can be distinguished: syncytium-inducing (SI), high-replicating isolates with a broad host range and non-syncytium-inducing (NSI) isolates only replicating in peripheral blood mononuclear cells (PBL) (Tersmette et al., J.Virol., 1988, 62: 2026-2032). From stable asymptomatic seropositive persons only NSI isolates were recovered. SI isolates were only recovered from persons progressing to ARC or AIDS. The absence of SI strains in asymptomatics seems paradoxical since it would be expected that such isolates can be recovered from individuals infected by a SI strain carrier. Therefore, we studied isolates obtained from 10 persons from seroconversion on. Eight of them yielded NSI isolates, in only 2 persons SI isolates were observed. Three months later, however, these two persons yielded NSI isolates. From one of them, who remained asymptomatic, thereafter only NSI isolates were recovered. In the other person SI isolates again were detected 4 months before development of AIDS. These findings suggest that early in HIV infection high-replicating SI isolates are suppressed, presumably by some immunological mechanisms. Re-appearance of SI-isolates associated with rapid progression to disease may occur after insidious attenuation of the immune system by low-replicating NSI strains.

G 524 HIV-1 EXPRESSION IN MACROPHAGES IN THE SPINAL CORDS OF AIDS PATIENTS WITH MYELOPATHY. B. Weiser, D. Eilbott, N. Peress, D. LaNeve, R. Seidman, H. Burger. SUNY Stony Brook, Stony Brook, N.Y. 11794

Spinal cord disease is common after HIV-1 infection, and is found in 10-30% of AIDS autopsies. Postmortem exam shows a vacuolar myelopathy characterized by vacuolation of spinal cord white matter and macrophage infiltration, pathologically distinct from HIV-1 encephalopathy. To determine the presence and localization of HIV-1 RNA expression in the spinal cords of AIDS patients with vacuolar myelopathy, we used the technique of combined in situ hybridization and immunohistochemical staining of the same slide. Spinal cord sections were hybridized in situ for RNA using an HIV-1 specific riboprobe. A study of 17 cords obtained from AIDS patients at autopsy showed that HIV-1 RNA was detected in 6 of 10 with vacuolar myelopathy, 0 of 5 without spinal cord pathology, and 0 of 2 with other spinal cord disease (toxoplasmosis and lymphoma). 3 control cords obtained from HIV negative patients with other spinal cord disease showed no HIV RNA. In the 10 AIDS cords with vacuolar myelopathy, the amount of HIV-1 RNA expression correlated with the extent of pathology. Combined in situ hybridization and immunohistochemical staining on 3 of the cords with detectable HIV-1 RNA by in situ hybridization showed HIV-1 expression in mononuclear and multinucleated macrophages localized mainly in areas of myelopathy in spinal cord white matter. Immunohistochemical staining showed myelin within macrophages. These data support a role for HIV-1-infected macrophages locally in the pathogenesis of HIV myelopathy and systemically in the development of disease in HIV-1-infected patients.