# HIV AND AIDS: PATHOGENESIS, THERAPY AND VACCINE

Organizers: Samuel Broder and Flossie Wong-Staal

March 31-April 6, 1990

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## HIV and AIDS: Pathogenesis, Therapy and Vaccine

### *HIV Regulation and Pathogenesis*

**L 001** MECHANISM OF ACTION OF THE HIV-1 REV TRANS-ACTIVATOR, Michael H. Malim, Laurence S. Tiley, David F. McCarn and Bryan R. Cullen, Howard Hughes Medical Institute and Department of Microbiology and Immunology, Duke University Medical Center, Durham, N.C. 27710.

HIV-1 encodes a nuclear trans-activator, termed Rev, that is required for the expression of viral structural proteins and, hence, for viral replication. The Rev protein acts posttranscriptionally by inducing the nuclear export of unspliced HIV-1 mRNA species that are otherwise excluded from the cell cytoplasm. Sequence specificity is conferred by a viral RNA target site, the Rev Response Element (RRE), that coincides with a highly structured RNA sequence element located within the viral env gene. Data will be presented demonstrating direct, sequence specific binding by Rev to the viral RRE element. In support of the functional significance of this interaction, mutations that affect RRE function in vivo are also observed to impair Rev:RRE binding in vitro. Mutational dissection of the rev gene product demonstrates the existence of two distinct functional domains that appear comparable to the "binding domain" and "activation domain" described for several eukaryotic transcription factors. The activation domain of Rev has a minimal size of ~7 aa and is located towards the carboxy-terminus. Mutations that affect this element result in Rev proteins that display a dominant negative phenotype. The binding domain of Rev is a highly basic region with a minimal size of ~33 aa. This protein domain shares significant sequence identity with both the "Arg-rich" RNA binding motif defined by Lazinski et al. (Cell 59, p207, 1989) and with known protein nuclear localization signals. Mutations that affect this domain result in a recessive negative phenotype and, in some cases, aberrant subcellular localization. The effect of mutations in this region on Rev/RRE binding is under investigation.

**L 002** REPLICATION AND PATHOGENESIS OF HIV-1.

William A. Haseltine, Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115

A brief overview of recent studies of the molecular biology of HIV-1 will be presented. The current status of the laboratory's studies on the non-essential regulatory genes, vpr, vpu, and nef will be presented. The effect of these genes on virus replication and cytopathic effect will be summarized as will be the current understanding of mechanisms of action of these gene products.

An update of the laboratory's studies of the virus structural genes, gag and env will also be presented. Extensive analysis of consequences of selected mutants on each of the gag proteins, p17, p24, p9 and p6 will be discussed. The role of conserved amino acid sequences and glycosylation sites in HIV-1 env functions will also be discussed.

## L 003 STRUCTURAL ORGANIZATION AND REGULATION OF EXPRESSION OF HIV-1, George N. Pavlakis

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HIV-1 has the general structural and functional characteristics of the lentiviruses. It encodes additional regulatory factors necessary for its expression. The study of these factors clearly indicates the importance and complexity of post-transcriptional processes in regulating gene expression. The production of many proteins from the HIV promoter is achieved by three different mechanisms: ribosomal frameshifting (for the production of Gag-Pol polyprotein), alternative splicing, and production of bicistronic mRNAs. Although all three mechanisms are used in other retroviruses, only the lentiviruses make such extensive use of alternate splicing and bicistronic mRNAs. Infection of human lymphoid cells in culture results in the production of more than 30 different species of viral mRNAs. Functional studies have shown that several HIV mRNAs are bicistronic, including all the mRNAs producing Env.

Two regulatory proteins, Tat and Rev, are necessary for viral replication. These small nuclear proteins accumulate primarily in the nucleoli and act on HIV via sequence specific elements found on the viral RNA. Both the Tat responsive element (TAR) and the Rev responsive element (RRE) map within regions of strong RNA secondary structure. Several experiments indicate that specific structures within TAR or RRE are necessary for function. While all viral mRNAs contain TAR, only the mRNAs producing HIV structural proteins contain RRE. Tat increases the levels of all viral mRNAs. Its function is complex and involves transcriptional and possibly post-transcriptional steps. Rev promotes the transport of viral mRNAs containing RRE from the nucleus to the cytoplasm and increases the half-life of these viral mRNAs. The Rex protein of HTLV-1 can substitute for Rev. Rex acts on the RRE element in a similar fashion, but the targets for Rev and Rex within RRE are distinct. Tat and Rev together with a third factor, Nef, are integrated in a feedback regulatory network that results in the balanced expression of viral components.

To study the Rev function and find ways to inhibit Rev-RRE interaction we have generated mutant Rev proteins. Some of the mutants inhibit the function of Rev in transfected and infected human cells, and could be useful for the inhibition of HIV replication.

## L 004 TRANSCRIPTIONAL REGULATION OF HIV GENE EXPRESSION

B. Matija Peterlin, Shaw-Yi Kao, Paul A. Luciw, Mark J. Selby, and Sandra Tong, Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA 94143, and Department of Medical Pathology, University of California, Davis, CA 95616

The long terminal repeats (LTRs) of HIV1 and 2 are transcriptionally activated following T cell or macrophage activation, growth, and proliferation. Cellular *trans*-acting factors that participate in this response include nuclear factors κB (NFκB) and of activated T cells (NFAT-1), as well as AP3. Following activation, HIV LTRs are *trans*-activated by their respective *trans*-activators (*tats*). These act upon sequences 3' to the sites of initiation of viral transcription called TAR. The primary mechanism of *trans*-activation by *tat* appears to involve the conversion of an unstable to a stable (i.e. elongation competent) transcription complex. Evidence for efficient loading of RNA polymerase II at the HIV promoter and utilization of *tat* at TAR will be presented. By mixing and matching HIV LTR sequences, differences in activation and *trans*-activation are revealed that might explain distinct clinical courses of HIV1 and HIV2 infections. In sum, HIV developed complementary transcriptional strategies that respond rapidly to extracellular stimuli and result in efficient viral replication and gene expression.

Genes Dev **3**: 547-558 (1989)
J Immunol **142**: 702-707 (1989)
Nature **330**: 489-493 (1987)
PNAS **83**: 9734-9738 (1986), **84**: 6845-6849 (1987), **85**: 8286-8290 (1988)

**L 005** HIV Regulatory Genes: Trans-activation and Beyond

Flossie Wong-Staal*, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892

The HIV genome encodes a number of accessory genes, two of which have been shown to be absolutely essential for function. These genes, tat and rev, are novel transactivators, with RNA, rather than DNA as their primary targets. The functional domains of these proteins as well as the structures of the cis-acting sequences in the respective pathways have been well-dissected, and their mechanisms of action are being unraveled. Recent studies also indicate that these small regulatory molecules are found extracellularly and can be taken up into non-expressing cells. Furthermore, exogenous tat has been shown to stimulate the growth of in vitro cultured KS-derived cells (See abstraction by Ensoli, et al., submitted for this meeting). This expanded repertoire of activities for these proteins have major implications for the pathogenesis of HIV infection.

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## *Cellular Targets for HIV Infection*

**L 006** THE IMPORTANCE OF THE gp120/CD4 INTERACTIONS IN THE PATHOGENESIS OF AIDS, Angus Dalgleish, Clinical Research Centre, Harrow, UK, Fabrizio Manca, Dept. of Immunology, Genoa, Italy, John Habeshaw, Clinical Research Centre, Harrow, UK.

The nature of the immunodeficiencies seen in AIDS suggest that memory T cell control of indigenous infection is lost prior to new antigen recognition. In vitro antigen presented by antigen presenting cells (APC) to T cell lines in the presence of HIV or gp120 leads to a failure to see the specific antigen. The fact that these cells are not deleted by virus or gp120 can be demonstrated by recovering these anergic cells and demonstrating that the effect is reversible. Because only the CD3 recognition pathway appears to be involved this raises the question as to whether recognition of self is perturbated in the presence of gp120. This would lead to a chronic allogeneic disease, the predicted features of which include many of those seen in HIV infection. If this is an important mechanism of pathogenesis then interference with the gp120 CD4 interaction will be required in both therapy and vaccine strategies.

**L 007** ENVELOPE GLYCOPROTEINS OF HIV-1, GP120, INHIBIT NORMAL T CELL FUNCTION.

Savita Pahwa, Narendra Chirmule, Naoki Oyaizu, and Vaniambadi S. Kalyanaraman. Department of Pediatrics, North Shore University Hospital- Cornell University Medical College, Manhasset, NY; Department of Cell Biology, Bionetics Research Inc., Kensington MD.

Depression of antigen-specific T cell responses is a characteristic feature of AIDS. We have investigated the effect of native and recombinant envelope glycoprotein gp120 on functions of human peripheral blood lymphocytes (PBL) and $CD4^+CD8^-$, tetanus antigen-specific, MHC class II restricted, exogenous IL-2 independent T cell clones. Lymphoproliferative function of T cells was tested by stimulation of the CD3-Ti receptor complex with antigen (tetanus) or anti-CD3 (SP34) mAb. Alternatively, T cell proliferation was elicited by anti-CD2 (9.6 + 9-1) or anti-CD28 (9.3) mAbs or by a combination of phorbol ester PMA plus calcium ionophore, ionomycin. Preincubation of PBL or the T cell clones with both preparations of gp120 inhibited tetanus antigen- or anti-CD3 mAb-induced proliferation in a dose-dependent manner. Half maximal inhibitory dose was 0.1 ug/ml for native gp120 and 1 ug/ml for recombinant gp120. At these concentrations the gp120 also suppressed secretion of IL-2 and cell surface expression of IL-2 receptor alpha chain. The inhibitory effects of gp120 could be prevented by soluble CD4 and reversed by exogenous IL-2. While gp120 treated PBL recovered their proliferative function after 72 hours in culture, the proliferative function of cloned T cells did not recover as long as 7 days after exposure to gp120. Gp120 failed to inhibit T cell proliferation when the stimulation was elicited via CD2 or CD28 cell surface molecules or by the combination of PMA plus ionomycin. Signal transduction via CD3-Ti receptor complex was impaired by gp120 pretreatment as evidenced by failure to generate intracellular inositol phosphates and $[Ca^{++}]i$. In gp120 treated T cell clones, mRNA for IL-2 was greatly reduced, while that for IL-2 receptor and actin were unaffected. We conclude that gp120 interferes with an essential function of the CD4 molecule in T cell activation and that the inhibition is specific for signals delivered via the CD3-Ti receptor complex. The mechanism of suppression has not been completely elucidated and is associated with impaired signal transduction and suppression of IL-2 mRNA.

Supported by Grant #AI28281.

**L 008** BIOLOGICAL VARIABILITY OF HIV-1, Mikulas Popovic, Kunihiro Ohashi, and Suzanne Gartner, Division of Virology/Immunology, PRI-New Mexico State University, P. O. Box 1027, Holloman AFB, NM 88330.

Genomic heterogeneity is a hallmark characteristic of HIV-1. The biological significance of this variability, however, especially with respect to pathogenicity, requires further investigation. To address this question, we quantitatively assessed the susceptibility and permissivity of normal human peripheral blood-derived monocyte/macrophages (M/M) and T lymphocytes and neoplastic monocytoid and lymphoid cell lines to various HIV-1 isolates. Also, we examined the replicative potential and syncytia induction of progeny virus populations from fresh HIV-1 isolates for evidence of biological heterogeneity using both normal T cells and M/M) as target cells. Furthermore, we investigated the role of host cell modifications as important determinants of viral infectivity. The results suggest: (1) "Fresh" isolates recovered from patients and propagated only in normal host cells exhibit a dual tropism for both M/M and T cells, regardless of their tissue of origin or the cell-type from which they were isolated. (2) The repeated passage of an isolate through normal M/M does not generally result in the loss of the ability to infect normal T cells nor vice versa. (3) The majority of fresh HIV-1 isolates do not infect neoplastic cells of either origin, and those that do show no preference for monocytoid or lymphoid targets, regardless of their cell origin. (4) Progeny virus populations exhibited significant variation in replicative potential. (5) HIV-1 can be adapted to heterologous primate cells; this adaptation results in an increased host range of the virus and changes in its antigenic determinants.

*Genetic and Biologic Variants of HIV*

**L 009** MOLECULAR DETERMINANTS OF HIV-2 PATHOGENICITY, P. Kumar[1], J.C. Kappes[1], H. Hui[1], J.M. Jin[1], M.L. Mixon[1], J.A. Conway[1], J.A. Hoxie[2], G.M. Shaw[1], and B.H. Hahn[1]. [1]University of Alabama at Birmingham, Birmingham, Alabama 35294, [2]University of Pennsylvania, Philadelphia, PA 19104.

Naturally-occurring strains of human immunodeficiency virus (HIV) can vary considerably in their *in vitro* biological properties, and such differences may also be reflected in their *in vivo* pathogenesis. In an attempt to define genetic determinants of viral pathogenicity, we have molecularly cloned, sequenced and characterized an attenuated isolate of HIV-2 (HIV-2/ST) that differs from prototype HIV-2 strains in its inability to fuse with and kill susceptible CD4-bearing target cells. A proviral clone, termed JSP4-27, was identified to be transfection-competent and to fully exhibit the noncytopathic and nonfusogenic properties of its parental isolate. Nucleotide sequence analysis of JSP4-27 revealed a genomic organization very similar to that of cytopathic HIV-2 strains and amino acid sequence comparison confirmed the integrity of all major viral gene products. Since inspection and comparative analysis of the JSP4-27 sequence failed to identify genetic changes likely to be responsible for its attenuated phenotype, we molecularly analyzed two additional strains of HIV-2/ST which represented *in vitro* generated fusogenic and cytopathic variants of this virus. Both fusogenic strains, termed ST/24.1C and ST/24.2C, were originally derived from a biologically-cloned subculture of HIV-2/ST (ST/24), that produced noncytopathic and nonfusogenic virions biologically indistinguishable from the parental isolate. After serial cell-free transmissions of ST/24 supernatant to uninfected SupT1 cells, large and numerous syncytia were observed on two independent occasions, which indicated the emergence of fusogenic progeny virus in the culture. To determine whether the phenotypical differences of those fusogenic variants were the result of alterations in their envelope fusion region, we PCR amplified, cloned and sequenced this envelope domain from genomic DNA of ST/24.1C, ST/24.2C as well as ST/24, and compared the results with JSP4-27. The analysis failed to reveal significant changes in the envelope fusion domain of all viral strains analyzed, but identified the presence of genotypic variants in the ST/24.1C and ST/24.2C cultures. We therefore molecularly cloned ST/24.1C and ST/24.2C as well as their parental strain ST/24, and isolated 19, 15 and 6 full-length recombinant viral clones, respectively. Preliminary transfection experiments have identified one full-length clone derived from ST/24.1C to be replication competent as well as highly cytopathic and fusogenic. Comparative sequence analysis as well as the construction of chimeric viruses is underway to further map the genetic regions and changes responsible for the phenotypical differences observed among cytopathic and noncytopathic HIV-2 viruses. Finally, the fact that cytopathic mutants of HIV-2/ST evolved by cell-free passage on two independent occasions indicates the presence of strong selective pressures for cytopathic and fusogenic viruses *in vitro*. It is possible that similar pressures are also present *in vivo* which may favor the emergence of more virulent strains in infected individuals over time.

**L 010 EPIDEMIOLOGY AND NATURAL HISTORY OF HIV-2**

Kanki, Phyllis*; Marlink, R.*, M'Boup, S**, and Essex, M*, et. al.
*Harvard School of Public Health, Boston, MA, **University of Dakar, Dakar, Senegal.

Since 1985, we have followed a large group of registered female prostitutes (n>1500) in Dakar, Senegal to better understand the epidemiology and pathobiology of HIV-2. all Physical examinations, questionnaires regarding risk and sexual behavior were administered and serum samples obtained for STD and retrovirus examination semi-annually.

Currently ,the overall seroprevalence in 1500 female prostitutes was 9.8% HIV-2, 1.8% HIV-1 and 0.4% HIV-1/2. A serostatus of HIV-1/2+ was confirmed with both immunoblot and radioimmunoprecipitation. In some cases, antibodies to both the vpu (HIV-1) and the vpx (HIV-2) confirmed dual infection by both retroviruses. The majority of women were native Senegalese (73%), with 21% from Ghana and 6% other nationalities. The mean age was 34 yrs, range 21-68, and the mean years of prostitution 6.7 yrs, range 1-19. HIV-2 positive prostitutes were more likely to be older (>33 yrs; OR=2.58 ) and to be non-Senegalese (OR=1.79), indicative of an endemic virus infection perhaps with lower pathogenicity in contrast to HIV-1. The number of years of prostitution was not significantly related to serostatus, inclusion of all variables in a multivariate logistic model did not modify these results The spread of HIV-2 in this population was slow as judged by less than 1.5% seroconversion with 1400 person-years of observation in sequential bleeds. Previous history of cervicitis or genital ulcer disease was not found to be a significant risk factor for HIV-2 infection in contrast to HIV-1. 160 person-years of follow-up on HIV-2 positive women has failed find evidence of the development of AIDS . None of the HIV-2 positive women had absolute T4 lymphocytes below 400 cells/$mm^3$. In comparison of HIV+ versus HIV- values a trend towards higher levels of absolute T8 counts were noted in HIV-2 prostitutes, but the difference from the controls were not as dramatic as those seen in HIV-1 prostitutes in the same cohort. There was no significant difference in cutaneous anergy between HIV-2 + and HIV-2- prostitutes, but HIV-1+ prostitutes were much more likely to be anergic as compared to seronegative prostitutes.

The epidemiology of HIV-2 in a high risk population followed over time appears to differ significantly from that of HIV-1. These differences include age-specific seroprevalence, risk factors for infection, rate of sexual transmission and clinical outcome.

L 011 IS THE VARIATION EPIDEMIC-DRIVEN?, Gerald Myers, James M. Hyman and E. Ann Stanley, Theoretical Division, Los Alamos National Laboratory Los Alamos, NM 87545.

The title of this talk poses a question for which we do not yet have an answer. Nevertheless, it is not premature to summarize what is known about the rate of variation and what we still need to discover.

Time-calibration of phylogenetic trees constructed from HIV-1 sequences, correlated with the likely time of entry of viruses into the U.S., raises the possibility that the rate and extent of HIV variation may depend upon the number of infected individuals or, more precisely, upon the frequency of transmission. A phenomenon of this kind appears to have been associated with a polio outbreak in 1978. Moreover, the microscopic "swarming" tendencies of HIV are consistent with such a macroscopic picture.

The general mathematics for modeling epidemic-driven variation, and the time constraints within which we must work to eliminate the empirical uncertainties, can be stated. However, the inquiry is complicated by AZT therapy and the possible additional contribution of AZT-resistant strains to the overall variation.

*Pathogenic Mechanisms and Immune Responses in HIV Infection (joint)*

L 012 IMMUNOPATHOGENESIS OF HIV INFECTION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Infection with HIV in vivo is characterized by a long and variable asymptomatic stage during which time the virus either remains latent or replicates at a very low level. In vitro, a variety of agents, including mitogens, antigens, and heterologous viral genes, have been shown to upregulate HIV expression. In addition, we and others have recently shown that cytokines can induce HIV expression in chronically infected T cell and monocytic cell lines. In this regard, it is becoming increasingly evident that tumor necrosis factor-alpha may play an important role in the induction of HIV expression. Tumor necrosis factor-alpha functions as part of a complex network of cytokines involved in the regulation of immune responses. Elevated levels of TNF-alpha have been found in the sera of AIDS patients, and elevated levels of TNF-alpha are produced by monocytes from HIV-1 infected individuals. We have shown that TNF-alpha can upregulate HIV expression in chronically HIV-infected cell lines and that HIV-infected cells have higher levels of TNF receptors that their uninfected counterparts. We have also shown that PMA can induce both TNF-alpha and HIV expression and that PMA-induced upregulation of HIV expression can be blocked by anti-TNF antibodies. In addition, exogenous TNF-alpha synergizes with PMA in its own induction. We have shown that the molecular mechanisms of HIV activation by TNF-alpha involve the induction of cellular DNA-binding proteins that interact with the kB enhancer sites that are located just upstream of the HIV promotor. Further studies have shown that both IL-6 and GMCSF can upregulate HIV expression and that TNF-alpha can synergize with both IL-6 and GMCSF in the induction of HIV. The molecular mechanisms of HIV induction by IL-6 will be discussed. Studies on the role of TGF-beta in suppressing both mitogen and cytokine-induced expression of HIV will also be presented. Studies of cytokine-induced expression of HIV suggest that normal homeostatic mechanisms of the immune response may function in an autocrine/paracrine manner to activate HIV in vivo; such studies may be useful in the delineation of strategies to suppress viral activation.

**L 013** HIV IN HEMATOPOIESIS, Jerome E. Groopman, Division of Hematology/Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215

We have addressed two major questions with respect to regulation of hematopoiesis following HIV infection. The first question involves the effects of HIV or its envelope glycoprotein on production of hematopoietic growth factors and other regulatory cytokines. Studies using different isolates of HIV, as well as purified recombinant gp120, failed to demonstrate any induction of interleukin-1, interleukin-6, tumor necrosis factor, GM-CSF or G-CSF. These studies were done both in monocyte cell lines as well as in fresh peripheral blood monocytes and peripheral blood lymphocytes. We are also interested in studying whether important components of the bone marrow environment, particularly nonhematopoietic stromal cells, are susceptible to HIV infection. We have succeeded in infecting bone marrow mesenchymal cells of fibroblast origin with HIV-1 and HIV-2. These cells are negative for surface CD4 protein as well as CD4 messenger RNA. The mechanism of HIV entry into target mesenchymal fibroblasts is not known. Further studies are in progress regarding regulation of cytokine production by mesenchymal cells.

**L 014 HIV INFECTION OF THE SCID-hu MOUSE.** J. M. Mc Cune, Reiko Namikawa, Chu-Chih Shih, Linda Rabin, and Hideto Kaneshima. HIV Group, SyStemix, Inc, Palo Alto, CA 94303. During HIV infection of man, cells of the hematolymphoid system are rendered dysfunctional or destroyed. Most of these cells are normally closely apposed within organ structures of that system. In an attempt to study this process from the time of acute infection, interactive human hematolymphoid organs have been surgically implanted into the immunodeficient C.B17 scid/scid mouse, creating the SCID-hu mouse (1). After vascularization and growth, human thymus, lymph node, and skin of the SCID-hu are found to assume histologic characteristics indistinguishable from those found in man. Growing within the human thymus, structures similar to normal human bone marrow maintain human hematopoiesis for periods of time as long as 20 months. T-lineage precursors may be observed to differentiate through the thymus. CD4+ and CD8+ T cells, in a normal ratio, are found in the peripheral circulation for periods of time as long as 20 months. B cells are found to differentiate within primary follicles of lymph nodes to plasma cells secreting IgM and IgG, at high levels and for long duration. Well-characterized isolates of HIV (cloned, tissue-culture adapted, and/or carrying specific mutations) have been introduced into SCID-hu mice by a variety of routes, in both cell-free and cell-associated form. As a function of time, the infectious process may be carefully observed by histologic techniques and PCR. Viral replicative pathways may be described in cells of different lineages and the impact of viral infection on discrete cell subpopulations within the human organs may be analyzed. This animal model offers previously unavailable insights into the pathogenic mechanisms of HIV infection.

1. Mc Cune JM, Namikawa R, Kaneshima H, et al. The SCID-hu mouse: murine model fo the analysis of human hematolymphoid differentiation and function. **Science** 241:1632-1639, 1988.

**L 015** VACCINATION WITH A LIVE RETROVIRUS: THE NATURE OF THE PROTECTIVE IMMUNE RESPONSE, Ruth M. Ruprecht[1,3], Richard C. Hom[2,3], Steve Mullaney[1], Lisa D. Bernard[1], Miguel A. Gama Sosa[1,4] and Robert W. Finberg[2,3], [1]Division of Cancer Pharmacology and [2]Laboratory of Infectious Diseases, Dana-Farber Cancer Institute, and [3]Department of Medicine or [4]Department of Pathology, Harvard Medical School, Boston, MA 02115.

Post-exposure chemoprophylaxis with 3'-azido-3'-deoxythymidine (zidovudine or AZT) combined with recombinant human interferon-αA/D (rHuIFN-αA/D) protected all mice (in a group of 234 animals) exposed to a lethal dose of live Rauscher murine leukemia virus (RLV) from viremia and disease. After completing a 3-week course of therapy, 96% of these mice resisted re-challenge with live virus, thus demonstrating acquired immunity to RLV. Only animals with prior exposure to RLV, but not mock-infected mice given the treatment with AZT and rHuIFN-αA/D, were immune.

The nature of this protective immune response was analyzed by transferring cells or serum from immune mice to naive animals which were challenged with live RLV. Only a very high titer of immune serum was protective against RLV challenge.

To test for cellular immunity, adoptive cell transfer of splenocytes from immune mice was carried out. Recipient animals resisted live RLV challenge after a 1:1 transfer of splenocytes (splenocytes from one immune donor mouse per one naive recipient). Next, B cells and macrophages were removed from the immune splenocytes by passage through nylon wool columns. A dose of $4 \times 10^7$ T-cells obtained from immunized mice fully protected naive recipients from viremia and disease after RLV challenge. The same number of T cells derived from naive animals was not protective. However, when the immune T-cells were depleted *in vitro* of either the $CD4^+$ or the $CD8^+$ cell populations prior to adoptive transfer, naive recipients were only partially protected from RLV challenge, even when given $4 \times 10^7$ $CD4^+$ or $CD8^+$ T-cells. Full protection against RLV challenge was seen again when the depleted T-cell subsets from immunized mice were recombined and transferred at a normal CD4:CD8 ratio.

We conclude that vaccination with a live retrovirus whose replication was blocked by pharmacological intervention induces a strong cellular immune response capable of providing full protection against a lethal retrovirus-induced disease in the primary animals, and against viral challenge in secondary recipients. Both $CD4^+$ as well as $CD8^+$ immune T-cells are needed to provide full protection against RLV challenge.

**L 016** IMMUNE REACTIVITY OF HIV MAY BE OF DAMAGING OR BENEFICIAL VALUE TO THE HOST, Hans Wigzell, Department of Immunology, Karolinska Institute, 10401 Stockholm, Sweden.

Immune reactivity against HIV may proceed to reduce viral replication but can also cause sizeable harm to the individual. Certain specific parts of the immune response against HIV can be linked to positive prognosis of the individual, either in the form of a reduced likelihood for infection or as an indicator of better prognosis with regard to survival time after infection. Prior exposure to HIV antigens or antibodies may also change the progress of disease subsequent to infection, sometimes acting in certain experimental systems to delay or maybe even eliminate the onset of disease. The understanding of this reactivity pattern allows certain strategies in relation to clinical situations such as HIV-seropositive pregnant women or the finding of a HIV PCR-positive, seronegative individual.

## HIV and AIDS: Pathogenesis, Therapy and Vaccine

### *Molecular Targets for Therapy*

**L 017** DEVELOPMENT OF GENE THERAPY FOR THE TREATMENT OF AIDS. Richard A. Morgan*, Flossie Wong-Staal#, Robert C. Gallo#, and W. French Anderson*. *National Heart, Lung and Blood Institute, and #National Cancer Institute, National Institutes of Health, Bethesda MD 20892.

We are applying the technology of retroviral vector mediated gene transfer as an anti-HIV agent. The most promising feature of retroviral vectors as anti-HIV agents, is their ability to deliver a gene or gene product precisely where it is needed, at or in the cell. This ability can have advantages for biological compounds which may only function in the intracellular environment, for proteins with a limited biological half-life, or for compounds which need to be present in a high local concentration. We have demonstrated the feasibility of using retroviral vectors as potential anti-HIV agents by designing vectors which express the soluble CD4 (sCD4) protein. A variety of vectors have been produced and used to transduce both established and primary human cell lines. Transduced cells were shown to express sCD4 and in a coculture protection experiment, protection of human T-cells from infection by HIV-1 was demonstrated. Further developments in this system include producing hybrid CD4 molecules by fusion of the HIV binding domains of CD4 to long-lived serum proteins. The choice of the cellular delivery system for vector based anti-HIV therapies will be of importance for the success or failure of this approach. Two potential cell delivery systems are currently under investigation: direct transduction of lymphocytes and cell implantation technology. Experiments designed to evaluate different vector designs and cell delivery systems can be done in appropriate AIDS animal models (SIV infection of primates and HIV infection of Hu/SCID mice). In summary, we have shown that retroviral mediated gene transfer can be used as an anti-HIV system, thus affording the realistic potential for a gene therapy approach for the treatment of AIDS.

**L 018** TARGETED THERAPY AGAINST HUMAN IMMUNODEFICIENCY VIRUS. Hiroaki Mitsuya, Robert Yarchoan, Shizuko Aoki, Takuma Shirasaka, Mary O'Brien, Michael Currens, and Samuel Broder. The Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

The acquired immunodeficiency syndrome (AIDS) remains a significant and worsening medical problem since it was first described as a clinical entity about nine years ago. The causative agent of this disease, human immunodeficiency virus (HIV), has nine known genes, and each gene/product could provide an opportunity for therapeutic intervention. Indeed, in these six years progress has been made in the chemotherapy of HIV infection.

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 or zidovudine), a member of 2',3'-dideoxynucleosid family. Recently, another member of the dideoxynucleoside 2',3'-dideoxyinosine (ddI), has also been shown to suppress the replication of HIV-1 in patients. However, it is 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.

The development of AZT has increased the momentum of basic and clinical research that should be essential before we approach the curative therapy of this disease. From a practical point of view, the identification of the dideoxynucleosides has already stimulated efforts of searching more effective antiviral drugs for therapy of HIV infection. Indeed, for certain potential antiviral compounds including 2',3'-dideoxynucleosides, a great deal of knowledge in terms of structure/activity relationships has emerged. Also, pathophysiological aspects of HIV-infection have been analysed during antiretroviral therapies. For example, we have utilized the polymerase chain reaction (PCR) technique to quantitate the viral load in patients with AIDS or AIDS-related complex. We have recently found a significant decrease in the proviral DNA content in peripheral blood cells following therapy with ddI.

The thus far successes of antiretroviral therapy against HIV infection may also provide a new rational and perspective for the chemotherapy against hepatitis B virus infection. Indeed, we and others have seen significant antiviral effects of some dideoxynucleosides against hepadnaviruses in vitro and in vivo. As we learn more about the life-cycle and biology of HIV and we define the structure/activity relationships of potential drugs, we should be able to improve our options for therapy against AIDS and its related diseases.

**L 019** INTERACTION OF THE HIV ENVELOPE WITH THE HUMAN CD4 RECEPTOR, Raymond W. Sweet, James Arthos, Mona Ivey-Hoyle, Keith C. Deen, Margery A. Chaikin, Hanne Johansen, and Martin Rosenberg, Biopharmaceutical R&D, Smith Kline Beecham, 709 Swedeland Road, King of Prussia, Pennsylvania 19406-2799

Soluble derivatives of the human CD4 receptor bind the HIV envelope protein with high affinity and thereby inhibit virus binding to CD4 cells, viral infection and syncytia formation in vitro. Through truncation and substitution mutagenesis of soluble CD4, we have defined the high affinity gp120 binding site. This site overlaps a sequence analogous to the surface exposed CDR2 region in an Ig light chain which participates in antigen recognition. However, in contrast to antigen recognition by Ig, the analogous CDR1 and CDR3 regions of CD4 do not appear to directly participate in gp120 interaction. The data is consistent with the presence of a loop on CD4 which could be accommodated within a pocket on gp120. Further deletion and point mutagenesis identifies specific residues at the N and C termini of the CD4 V1 domain, which are important to the folding and presentation of the CDR2 loop region such that it retains high affinity for gp120.

In addition, we have developed a Drosophila cell based expression vector system which efficiently expresses and secretes HIV 1 gp120 and gp 160 envelope proteins. The system allows either constitutive or regulated expression in continuous culture of genes stably inserted at high copy number in a single transfection-selection event and, thus, has certain advantages over the more commonly used baculovirus-based insect cell vectors. We demonstrate that the gp120 produced is an appropriate mimic of the viral envelope in that it is highly glycosylated, binds to CD4 with high affinity, efficiently inhibits syncytia formation and is recognized by both mono- and polyclonal antibodies against viral gp120. Most interestingly, we find that the Drosophila cell expression of gp160 protein but not the mRNA is dependent on concomitant expression of the HIV rev protein.

## *Primate/Animal Models of AIDS (joint)*

**L 020** SIV/SMM-PBJ14: PROPERTIES OF INFECTIOUS MOLECULAR CLONES AND PROTECTION AGAINST ACUTE LETHAL DISEASE. Patricia N. Fultz*, James I. Mullins**, Stephen Dewhurst**, Daniel C. Anderson*, A. A. Ansari*, and Harold M. McClure*, *Yerkes Primate Research Center and Department of Pathology, Emory University, Atlanta, GA 30322, **Department of Cancer Biology, Harvard University School of Public Health, Boston, MA 02115.

The SIV/SMM-PBj14 isolate is unique among the lentiviruses because rather than inducing a slowly progressive disease like its parent virus, SMM-9, and other SIV isolates, infection of both pig-tailed macaques and mangabey monkeys results in an acute lethal disease and death within 2 weeks. In addition to its pathogenicity, SMM-PBj14 also differs from SMM-9 in multiple biologic properties that include the ability to replicate in resting macaque and mangabey PBMC, cytopathic effects on mangabey $CD4^+$ lymphocytes, and induction of PBMC proliferation in vitro. As a first step to identify those regions of the viral genome that are determinants for the various biologic properties of SMM-PBj14, molecular clones were generated and were shown to possess at least some of the properties of SMM-PBj14, including induction of acute disease, characterized by severe bloody diarrhea, lymphoid hyperplasia, and death within 2 weeks. Correlations between in vivo and in vitro properties of SMM-9 and SMM-PBj14 are evident and will be used to screen molecularly cloned chimeric viruses prior to inoculation of animals. In addition, the differential pathogenesis of SMM-9 and SMM-PBj14 was exploited to determine whether prior infection of pig-tailed macaques with SMM-9 would prevent infection or acute disease and death following challenge with SMM-PBj14. Following challenge with 100 $TCID_{50}$ of SMM-PBj14, acute lethal disease was prevented in nine macaques infected for either 3 weeks, 3 months or 7 months with SMM-9 whereas all three uninfected control animals died within 12 days. In addition to protection against acute disease in SMM-9-infected macaques, no increases in antibody titers to SIV/SMM or in apparent viral loads were detected, and there was no evidence for replication of SMM-PBj14 as assessed by syncytia formation between PBMC from the challenged animals and SUP-T1 cells (a property of SMM-PBj14, but not of SMM-9). Whether neutralizing antibodies were present at the time of challenge currently is under investigation. These data indicate that infection with a less pathogenic strain of SIV can protect against lethal disease induced by a highly pathogenic variant of SIV and suggest that vaccination against lentivirus-induced disease is possible. (Supported in part by NIH grants AI-27136, AI-85007, CA-01058 and RR-00165 and by AmFAR.)

**L 021** REGULATION OF GENE EXPRESSION IN PRIMATE AND ANIMAL LENTIVIRUSES, Paul A. Luciw*, John Elder**, E. Ellen Sparger*, and Niels Pedersen*, *University of California, Davis, CA 95616 and **Scripps Clinic and Research Foundation, LaJolla, CA 92037.

Simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV) are T-cell tropic cytopathic lentiviruses associated with fatal AIDS-like disease in rhesus macaques and domestic cats, respectively. The genomes of both viruses encode transactivators that affect viral gene expression. Several host cell regulatory factors that play a role in controlling transcription directed by the long terminal repeat (LTR) have been identified. SIV and FIV replicate in macrophages as well as in CD4+ T-lymphocytes of their host species. Cell activation signals in both macrophages and T-cells act through promoter elements in the viral LTRs to augment viral transcription. In addition, regulatory genes of heterologous viruses (e.g., immediate early gene of cytomegalovirus) transactivate the SIV and FIV LTRs. Elucidation of the mechanisms regulating lentiviral gene expression may provide a basis for understanding viral latency and disease progression.

**L 022** INFECTION OF hu-PBL-SCID MICE WITH HIV-1: Donald E. Mosier*, Richard J. Gulizia*, Stephen A. Spector†, Deborah H. Spector†, Stephen M. Baird†Δ, & Darcy B. Wilson*, *Medical Biology Institute, La Jolla, California 92037, †University of California at San Diego, San Diego, California 92103, †ΔVeterans Administration Medical Center, La Jolla, California 92161

SCID mice reconstituted with human peripheral blood leucocytes (PBL) contain all of the cellular elements of the human immune system for several months and maintain some human immune functions for over one year. CD4+ T cells persist in the peritoneal cavity, spleen, peripheral blood, and lymph nodes. These animals, which we term hu-PBL-SCID mice, can be infected with the LAV-1/Bru strain of HIV-1 either by injection of cell-free virus or virus-infected T lymphoblasts derived from the original PBL donor. Infection has been established by injection of HIV-1 2-8 weeks following PBL engraftment, and virus has been isolated from animals for up to 16 weeks post-infection. HIV-1 can be recovered from the spleen, peripheral blood, and lymph nodes of infected mice by co-cultivation with fresh human T cell blasts. Infection of human cells within the hu-PBL-SCID spleen has been confirmed by *in situ* hybridization and amplification of HIV DNA sequences using the polymerase chain reaction (PCR). HIV infection of hu-PBL-SCID mice caused a 90% reduction in human immunoglobulin levels when compared to uninfected controls. Total number of human cells also was reduced in HIV-infected animals. These results document the potential utility of the hu-PBL-SCID model for studies of the pathogenesis and treatment of AIDS in that both viral infection and resultant immunodeficiency are observed. [supported by NIH grants AI 27703 and AI 29182].

*Candidate Vaccines*

**L 023** AN IMMUNODOMINANT CYTOTOXIC T CELL EPITOPE OF THE HIV-1 ENVELOPE THAT PROVIDES HELP FOR ITSELF: ROLE OF VIRAL VARIABILITY. Jay A. Berzofsky, Hidemi Takahashi, Richard Houghten*, Scott D. Putney†, Bernard Moss‡, and Ronald N. Germain**, Metabolism Branch, National Cancer Institute, and ‡Laboratory of Viral Diseases and **Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; *Torrey Pines Institute for Molecular Studies, San Diego, CA 92121; †Repligen Corp., Cambridge, MA 02139

We have identified an immunodominant cytotoxic T-cell determinant of the HIV-1 envelope in the hypervariable V3 loop, residues 315-329, that is recognized by both murine and human CTL. In the BALB/c mouse, this is the only site in the whole envelope protein that is recognized by class I MHC-restricted CTL. The homologous region is a CTL epitope in several viral isolates, but the high sequence variability leads to noncrossreactivity between most of these. Single amino acid substitutions in the IIIB isolate sequence demonstrated that a mutation at positions 322 or 324 can interfere with binding to the class I MHC molecule, whereas a mutation at residue 325 affects recognition by the T-cell receptor. Furthermore, a single amino acid interchange at residue 325 between the Val of the IIIB isolate and the Tyr of the MN isolate is sufficient to completely and reciprocally interconvert the specificities of the IIIB and MN peptides for IIIB- and MN-specific CTL, despite five other sequence differences between these peptides. The MN and IIIB isolates share residues 322 and 324 involved in MHC molecule interaction. Therefore, residue 325 plays a major role in determining T-cell specificity, when the MHC binding residues are held constant. Thus, virus variation in human hosts involves both residues interacting with the class I MHC molecule and residues interacting with the T-cell receptor of CTL, both types of mutations potentially capable of aiding the virus escape from the immune system. This same peptide also provides help for itself for induction of CTL in vitro in immune BALB/c spleen cell populations. Spleen cells from mice immunized with a recombinant vaccinia virus expressing the HIV-1 envelope protein require restimulation in vitro for expression of CTL activity. In BALB/c mice, peptide alone is sufficient for this restimulation, without addition of lymphokines. Peptide restimulation is abrogated by depletion of CD4 T cells or by blocking with anti-I-A monoclonal antibodies, but in both cases is restored by exogenous IL-2 in the culture. Spleen cells from immune B10.A mice, which share the same class I H-2D molecule but differ in class II molecules from BALB/c mice, behave like CD4-depleted BALB/c spleen cells. Therefore, the same short peptide containing the immunodominant CTL epitope seen with class I H-2D also contains a helper T-cell epitope seen with class II I-A molecules that elicits help for CTL induction. The juxtaposition of both functional activities in the same segment of protein sequence may contribute to its immunodominance.

**L 024** THE IMMUNOBIOLOGY OF THE HIV ENVELOPE, Dani P. Bolognesi, Duke University Medical Center, P.O. Box 2926, Durham, North Carolina 27710

The envelope of HIV is probably the major focus of attention with regard to current vaccine strategies. Scattered throughout the envelope are a number of discrete sites that represent targets for immune attack by neutralizing antibodies, antibodies that mediate ADCC and cytotoxic lymphocytes. Of considerable interest is that variable regions of the envelope constitute some of the dominant immunogenic epitopes and one in particular, the V3 loop includes all three categories of epitopes cited above. A number of studies suggest that this region is necessary for virus infectivity, perhaps representing a shield which protects the hydrophobic fusion domain of the virus. It is somewhat enigmatic that the virus could maintain a hypervariable structure as an essential component. Analysis of the fine structure of this element indicates that not all regions are variable and that there appears to be selective pressure to conserve segments within the crown of the loop among the isolates thus far studied. Further clarification of the structure/function relationships within this segment of the envelope and how they relate to processes involved with virus binding and entry into target cells will further clarify the value of this domain as a component vaccine design.

**L 025** ANTIGENIC VARIATION OF HIV-1 AND ITS ROLE IN THE PATHOGENESIS OF AIDS, Jaap Goudsmit*, Jacques de Jong*, Antony de Ronde*, Matthijs Tersmette° and Peter Nara#; *Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands; °Central Laboratory of the Netherlands Red Cross Transfusion Service, Amsterdam, the Netherlands; #National Cancer Institute, FCRF, Frederick, Maryland, USA.

Transmission of HIV-1 infected cells and cell free virus in its natural host results in an almost "clonal expansion" of HIV-1 pre-seroconversion, as has been shown by sequence analysis of the viral genome in peripheral blood mononuclear cells prior to culture. This homogeneous virus population has a genome identical in serum RNA (genomic RNA) and cellular DNA, indicating selection for replication competence. This selected virus population appears to attract the attention of the immune system. The first 6-9 months after antibody seroconversion in both humans and chimpanzees, who remained asymptomatic for extended periods of time, antibodies are elicited that neutralize exclusively the infecting virus strain and its relatives. Human seroconverters who developed AIDS within 4 years rarely showed measurable neutralizing antibody titres to the input virus, indicating a role for neutralizing antibodies in hampering spread of viruses with high replication potential and by doing so slowing down disease progression. Such early antibodies in healthy HIV infected subjects have been shown to neutralize the input virus through binding to the third variable domain (V3) of the external envelope. Within the first year, the neutralizing antibody response broadens to include viruses with heterologous V3 domains; during this period the neutralizing antibodies to the input virus binding its V3 domain persist at rising titres. V3-specific antibodies select *in vivo*, as tested in chimpanzees, virus variants without significant changes in the V3 domain, indicating conformation-dependence of this epitope. Such mutants escape immune surveillance both by changes in antigenic properties as well as changes (low/slow) in virus expression and replication. These mutants appear only to trigger memory cells, enhancing the selective pressure on the early fast-replicating virus population, paving the way for the escape mutants to spread and demolish the immune system. As soon as the initially effective immune response to the fast-replicating viruses diminishes, these viruses are allowed to reappear. Such reappearance heralds disease progression. The role of natural antibodies to the V3 domain and V3 antigenic variants in this cascade of events is elucidated by the production of recombinant viruses with exchanged V3 domains and the study of longitudinal sets of homologous and heterologous sera for their ability to block infectivity of such recombinant viruses.

**L 026** BROADLY NEUTRALIZING ANTIBODIES INDUCED BY THE HYPERVARIABLE PRINCIPAL NEUTRALIZING DOMAIN OF THE HIV-1 ENVELOPE, Thomas Matthews, Kashi Javaherian, Alphonse Langlois, Sandra Silver, Albert Profy, Dani Bolognesi, Scott Putney. Department of Surgery, Duke University Medical Center, Box 2926, Durham, NC 27710, [2] Repligen, Boston, MA.

A major target for isolate restricted neutralization of HIV falls in the third variable domain (V3) of the HIV gp120. The apparent blocking activity of antibodies directed at this variable domain is particularly strong in in vitro neutralization assays and a preliminary experiment in chimpanzees (Emini et al) is consistent with the possibility that these antibodies may also be protective in vivo. If this site on the envelope of gp120 however, can be considered as a component of an HIV vaccine approaches to overcome the sequence diversity must be developed. Studies in that direction include the cataloguing of V3 sequences of field isolates (see Putney et al, this meeting) and searches for conserved sequences or structures within the otherwise hypervariable region. In the latter case we have observed broadly neutralizing antibody in several animals immunized with a single V3 based synthetic peptide. Clearly this type of response is distinct from the isolate restricted antisera that is more commonly observed. We will describe experiments aimed at mapping the epitopes involved in the cross isolate recognition and our efforts to use these sites to more reproducibly induce broadly neutralizing antibody.

**L 027** BIOSYNTHESIS and ASSEMBLY OF RECOMBINANT HIV PROTEINS and PARTICLES, Bernard Moss*, Patricia Earl*, Robert Doms*, Sekhar Chakrabarti*, Velissarios Karacostas*, Kunio Nagashima#, and Matthew Gonda#, *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda MD 20892 and #Laboratory of Cell and Molecular Structure, Program Resources, Inc., Frederick Cancer Research Facility, Frederick, MD 21701.

We have prepared live recombinant vaccinia viruses that express the structural proteins of HIV-1 and HIV-2 in vitro and in vivo. The env proteins are transported to the surface of infected cells where they can mediate fusion with CD4-bearing human cells. The proteins are synthesized as glycosylated gp160 precursors that assemble posttranslationally into dimers and higher order tetrameric forms. Oligomerization persists even after cleavage to gp120 and gp41 and has important implications for intracellular transport, CD4 binding, and immunogenicity. Studies with mutated env proteins indicated that regions within the first 129 amino acids of gp41 are primarily responsible for oligomer stability. Thus, deletion of the cytoplasmic and transmembrane domains of gp41 to produce a secreted form of the env protein does not interfere with oligomerization whereas free gp120 is monomeric. When the entire HIV gag-pol gene was expressed by vaccinia virus, the gag and pol precursor proteins were transported to the cell surface where budding occurred. Particle formation did not depend on either expression of the env proteins or on the presence of HIV genomic RNA. Processing of the gag and pol precursors with formation of active reverse transcriptase occurred during or after particle release. Morphologically, the budding and released particles resembled immature and mature HIV, respectively. The recombinant vaccinia viruses, used as live vaccines or to prepare oligomeric env proteins and HIV-like particles in tissue culture, might have important safety advantages over either attenuated or inactivated human retroviruses.

**L 028** IMMUNIZATION AGAINST PRIMATE LENTIVIRUSES IN MACAQUE MONKEYS, Erling Norrby[1], Per Putkonen[2], Jan Albert[3], Gunnel Biberfeld[2] and Eva-Maria Fenyo[1]: [1]Department of Virology, Karolinska Institutet, School of Medicine, [2]Department of Immunology and [3]Virology, National Bacteriological Laboratory, Stockholm, Sweden.

The level of immunity obtained by vaccination against primate lentiviruses may vary. Under optimal conditions the presence of high level circulating and local immunity completely blocks virus replication. Such a sterilizing immunity is difficult to obtain and it is in fact not seen even with the effective conventional viral vaccines available to date. Alternatively, the level of immunity is infection-permissive. Virus replication occurs but it is markedly restricted. As a consequence, development of disease may be prevented. In this presentation it is proposed that the latter form of immunity may represent the most realistic approach to prevention of lentivirus-associated diseases. Neutralization tests with consecutively collected virus strains and serum from HIV-infected individuals demonstrate effective blocking of replication of early virus isolates but a rapid emergence of neutralization escape variants. An early impairment of the capacity to mount primary immune responses preceeding the occurrence of significant reduction of CD4-positive cells is postulated. The selection process eventually yields virulent virus causing immunosuppressive disease. The partly related SIV and HIV-2 can infect cynomolgus macaques, but only SIV causes a rapid reduction in CD4 cells and leads to development of lethal disease. Immunity deriving from the mild replication of HIV-2 was found to markedly restrict the replication of subsequently infecting SIV. Replication of SIV occurred but there was no reduction of CD4 positive cells or any other symptoms indicating extensive virus replication. This finding is one of four observations in different laboratories that immunity against SIV can be induced in macaque monkeys. These findings give hope for the ultimate development of a vaccine preventing the occurrence of AIDS in man.

*Clinical Trials*

**L 029** RECENT TRENDS IN ANTIVIRAL CHEMOTHERAPY OF AIDS, L.Corey, A. Collier, R.W. Coombs, University of Washington.

Recent developments in antiviral chemotherapy for HIV infection have extended the use of the nucleoside analogs to zidovudine and shown that reduced dosages have less toxicity. Multicenter studies performed by the AIDS Clinical Trials Group among patients with CD4 counts less than 500 regardless of whether they have asymptomatic and/or symptomatic infection, have shown that zidovudine reduces by about fifty per cent the frequency of progression to AIDS and/or symptomatic illness. In addition, dosages of zidovudine (averaging 500-600 mg/day) are associated with less toxicity and better outcome than "standard" doses of 1200 mg/day. A recent dose comparison study between 600 vs 1200 mg of zidovudine daily in AIDS patients have shown better survival and less toxicity with the 600 mg daily doses. A recent phase II study has shown that 300 mg total daily dose of zidovudine has as good a p24 antigen lowering effect and a better CD4 elevating affect among ARC patients than either 600 or 1500 mg/day. These data are illustrative of the fact that the dosages originally projected to reduce p24 Ag may not be the optimal CD4 elevating dosages of nucleoside analogs. The in vivo effects of nucleoside analogs may occur at lower dosages than predicted by current in vitro assays. Nucleoside analogs produce only palliative treatment reducing the incidence of progression by about fifty percent. As such, combination chemotherapy with either additional nucleoside analogs that have either additive effects, reduce the subsequent frequency of in vitro resistance or that have different mechanisms of action, offer the potential of enhancing the therapeutic efficacy of patients with HIV. Currently combination therapy of AZT with ddI, AZT with interferon and AZT with CD4 are undergoing Phase I testing. The challenge of combination chemotherapy is to develop in vitro and in vivo laboratory perimeters that are predictive of enhanced therapeutic efficacy. Discussion of the current state of clinical trials with combination therapy will be emphasized.

**L 030** HIV DRUG RESISTANCE, Douglas D. Richman, University of California San Diego and San Diego VA Medical Center, USA.

AZT resistant isolates of HIV can be isolated from the blood of patients with AIDS or AIDS-related complex during prolonged therapy with the drug (Larder, Darby and Richman, Science 243:1731, [1989]). These observations were made possible by a plaque reduction assay with a CD4+ HeLa cell line. Fifty percent inhibitory ($ID_{50}$) values of isolates from untreated individuals ranged from 0.01 to 0.05$\mu$M. In contrast most isolates from patients who had received AZT for 6 months or longer exhibited decreased sensitivity. Isolates from several patients exhibited progressive, step-wise increases in resistance which has been accounted for by the cumulative appearance of amino acid substitutions in at least 4 positions in the reverse transcriptase gene (Larder, et al., in press). Cross resistance to AZdU and AZG was observed in all 5 AZT resistant isolates, but no cross resistance has been observed to ddC, ddI, d4T, 3'F-ddT, carbovir, foscarnet, or interferons $\alpha$, $\beta$, or $\gamma$. In contrast to the almost uniform change in resistance phenotype after 6 months in patients with AIDS or late ARC, resistance appears at a significantly slower, but finite, rate in patients at earlier stages of HIV infection. Studies with isolates from children on AZT therapy are in progress, as are studies utilizing PCR to detect the resistance mutations directly in cells from patients. Several chemotherapeutic trials are in progress to address this problem, including the use of regimens combining AZT with ddC or the CD4-IgG hybrid molecule.

**L 031** THE EFFECTS OF DIDEOXYNUCLEOSIDES ON THE VIROLOGIC, IMMUNOLOGIC, AND NEUROLOGICAL MANIFESTATIONS OF HIV INFECTION, Robert Yarchoan, Hiroaki Mitsuya, James M. Pluda, Pim Brouwers, Rose V. Thomas, Kathy S. Marczyk, Carlo Federico Perno, and Samuel Broder, Clinical Oncology Program, National Cancer Institute, Bethesda, MD 20892

A number of dideoxynucleosides have been shown to have potent *in vitro* activity against HIV in T cells and in monocytes (Mitsuya, et al., PNAS, 1985, 83, 7096; Mitsuya and Broder, PNAS, 1986, 83, 1911; Perno, et al., J. Exp. Med., 1988, 168, 1111). One of these compounds, 3'-azido-2',3'- dideoxythymidine (AZT, zidovudine), is now an approved therapy for severe HIV infection, while a second, 2',3'-dideoxycytidine (ddC), was found to have activity against HIV in Phase I testing and is now being evaluated ina large Phase II trial. Both AZT and ddC are pyrimidine analogues. In July of 1989, we initiated the first Phase I trial of a dideoxypurine, 2',3'-dideoxyinosine (ddI), in 37 patients with AIDS or AIDS-related complex (ARC) in an ascending dose study. Patients received from 0.4 to 25.6 mg/kg/day of ddI intravenously (given in 2 or 3 does) for 2 weeks, followed by twice the dose given orally (with antacids). 11 dose regimens in total were studied. ddI was well absorbed orally and entered the cerebrospinal fluid. The patients overall had an increase in their mean CD4 cells from 114/mm3 at entry to 161/mm3 at week 6 (p=0.00004 compared with entry). These rises have been sustained for over 1 year in certain patients. The patients also had increases in their total lymphocytes, CD4/CD8 ratios, and CD8 cells. 16 of 18 patients who had detectable serum HIV p24 antigen at entry had a decrease in this parameter by week 6 (p=0.0034). Patients reported increased appetite and energy and gained weight. The dose limiting toxicities at very high doses were peripheral neuropathy and sporadic pancreatitis; however, such toxicity was observed in only 1/17 patients at doses of 6.4 to 9.6 mg/kg/day (doses which were active against HIV). We also recruited four patients who had HIV-induced cognitive impairment to study the effect of ddI on this disease manifestation. One patient who was very impaired had a substantial improvement in memory (-3.95 SD to 0.33 SD), conceptualization (-2.00 SD to -0.08 SD), and other functions during the first 6 weeks of therapy. The other three also had evidence of improvement in memory (mean memory IQ increased from 95 to 110) and reasoning (mean IQ increased from 83 to 94) when retested after approximately 10 weeks on ddI. These data indicate that ddI can induce immunologic, virologic, and neurologic improvement in patients with HIV disease and they provide a rationale for initiation of large scale Phase II/III testing at doses of 200 to 750 mg/day.

## *Kaposi's Sarcoma/AIDS Associated Malignancies*

**L 032** KAPOSI'S SARCOMA: THE LESSONS LEARNED FROM THE EPIDEMIC OF HUMAN IMMUNE DEFICIENCY VIRUS (HIV), Bijan Safai, M.D., D.Sc., Memorial Sloan-Kettering Cancer Center, New York, NY 10021. One of the striking features of the epidemic of the HIV infection is the association with and the increased incidence of Kaposi's Sarcoma (KS) among the infected individuals with this virus. To date over 16,000 cases of KS have been reported among HIV infected individuals which accounts for almost 14% of all the reported cases of Acquired Immune Deficiency Syndrome (AIDS). Contrary to what was believed previously, data obtained from AIDS-associated-KS indicate that immune deficiency is not a prerequisite for the development of Kaposi's Sarcoma. Furthermore, it is most likely the disturbance of the regulatory mechanism of the immune system that is the basic underlying cause for the development of KS. The data on HLA indicates that in the classical KS a high frequency of HLA DR5 is observed, while such frequency is not seen in AIDS-associated-KS. This obviously does not negate the role of genetic factors in KS. Further investigation into the role of genetic susceptibility and KS is needed. The cell of origin of KS is believed to be the endothelial cell of the lymphatic, however, the histologic structure of the KS lesion suggests proliferation of a number of elements, mostly of blood vessel and lymphatic origin. Finally, recent observations suggest the possible role of growth factors in the development and maintenance of KS-like cell line and a possible autocrine and paracrine phenomenon to be operative in this interesting tumor. Thus KS appears to be a proliferative process rather than a malignant tumor. As a result of these new understandings one may be able to develop more effective modes of therapy for KS.

*HTLV-I and Other Virus Cofactors*

**L 033** HUMAN HERPESVIRUS 6 AS A POTENTIAL COFACTOR IN AIDS

Paolo Lusso, Barbara Ensoli, Andrea De Maria, Flossie Wong-Staal and Robert C. Gallo, Laboratory of Tumor Cell Biology, NCI, and Laboratory of Immune Regulation, NIAID, Bethesda, MD 20892.

Although HIV-1 is the causative agent of AIDS and related disorders, other mechanisms are likely to play a role as cofactors in the development and/or progression of the disease. In this respect, a suitable candidate is human herpesvirus 6 (HHV-6), a recently discovered DNA virus classified within the herpesviridae family, which can be directly identified by different techniques in PBMC from the majority of patients with AIDS. Both in vitro and in vivo studies demonstrate that HHV-6 is predominantly tropic and cytopathic for CD4+ T lymphocytes, a major target cell population also for HIV-1 infection. However, several observations indicate that HHV-6 penetrates into susceptible cells via CD4 independent mechanism(s). The interaction between HHV-6 and HIV-1 can be investigated by simultaneously infecting normal or neoplastic human T cells with the two viruses. HHV-6 and HIV-1 are able to productively coinfect individual CD4+ T lymphocytes, resulting in an accelerated time-course of HIV-1 expression and cytopathicity. Consistent with these observations, HHV-6 trans-activates the LTR of HIV-1, as well as that of other related retroviruses. The sequences required for this activation are distinct from those responsive to the tat protein of HIV-1 and map to a region extending from -103 to -48 of the HIV-1 LTR. In addition, infection by HHV-6 selectively upregulates the expression of the CD4 antigen (i.e., the HIV-1 receptor) on the surface membrane of infected T cells. We hypothesize that HHV-6, by direct (e.g., CD4+ T cell killing) and/or indirect (e.g., HIV-1 LTR trans-activation, CD4 upregulation) mechanisms, may accelerate the course of HIV-1 infection toward AIDS. To definitively establish the role of HHV-6 in AIDS, suitable animal model systems for coinfection by HIV-1 and HHV-6 are necessary. In this respect, only chimpanzees (Pan troglodytes), among several non-human primate species tested, are susceptible to in vitro infection by HHV-6. As seen in humans, CD4+ T lymphocytes from chimpanzees can be productively coinfected by HHV-6 and HIV-1, undergoing an accelerated cytopathic effect. The chimpanzee may therefore represent a suitable in vivo model to elucidate the role of HHV-6 in the course of HIV-1 infection.

**L 034** MOLECULAR INTERACTION OF HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN CYTOMEGALOVIRUS, Deborah H. Spector[1], Valerie Koval[1], Mahima Vaishnav[1], Charles Clark[1], and Stephen A. Spector[2], Departments of Biology[1] and Pediatrics[2], University of California, San Diego, La Jolla, CA 92093.

Although Human Immunodeficiency Virus (HIV) is the etiologic agent of AIDS, other factors likely play an important role in the disease. Human cytomegalovirus (HCMV), in particular, has been implicated as a potential cofactor, because it is one of the principal pathogens in AIDS and is known to infect the same cell populations as HIV. One difficulty in studying the full range of biological interactions between HIV and HCMV in tissue culture is that only human fibroblasts and the human glioblastoma-derived cell line U373MG are fully permissive for HCMV replication. Since these cells are $CD4^-$, HIV replication is inhibited at the adsorption step. To bypass this difficulty, we devised a way to create HIV pseudotypes with expanded host range. This was accomplished by coinfecting $CD4^+$ cells with HIV and a murine amphotropic retrovirus which resulted in the formation of phenotypically-mixed particles with the HIV genome and amphotropic retrovirus glycoproteins. These HIV pseudotypes were able to infect and replicate to high titers in both $CD4^-$ human fibroblasts and the U373MG cell line.

With these HIV pseudotypes we have begun to examine the interactions between HIV and HCMV in human foreskin fibroblasts. Our results indicate that in the coinfected cell there is a marked effect on the replication of both viruses. Although HCMV early and late gene expression and viral titers seemed to be normal, HCMV immediate early gene expression was inhibited by the HIV infection. Additionally, the titers of HIV were substantially reduced, and it appears that HCMV inhibits HIV gene expression both at the transcriptional and posttranscriptional levels. This transcriptional inhibition is particularly surprising in view of the finding that HCMV infection stimulates transcription from an HIV LTR-CAT construct. Studies are currently in progress to determine the molecular basis of these effects.

**L 035** ACTIVATION AND REPRESSION OF HIV EXPRESSION BY HETEROLOGOUS VIRAL GENES, A. Srinivasan*, A. Velpandi*, C.E. Monken*, S.A. Plotkin*, W. Ho*, E. Gonczol*, A.M. Ventura[1], M.R. Arens[1] and G. Chinnadurai[1], *The Wistar Institute, Philadelphia, PA 19104; [1]Institute for Molecular Birology, St. Louis, MO 63110

Epidemiological studies clearly indicate that a wide variation exists with regard to the onset of AIDS from the time of exposure to the human immunodeficiency virus (HIV). The predominant state of viral infection in HIV-1 infected individuals is latency; productive virus infection occurs initially after primary infection and continues only until sufficient immune responses are mounted. HIV replication resumes later in these individuals but the mechanisms underlying active replication are not clear. A role for cofactors in the progression of the disease, by accelerating virus replication, has been proposed. Evidence from experiments using HIV-1 LTR-directed transient expression assays from ours and other laboratories support such a hypothesis. Bidirectional activation of HIV and HCMV was studied in cell lines stably expressing HIV tat and HCMV IE genes. The results reveal the effect of tat on HCMV early and late gene expression. In the course of the studies with immediately early genes of a number of viruses, we have found that trans-dominant E1a mutant suppressed the basal activity of the LTR as well as transactivation of the LTR by HCMV IE gene or DNA damaging agents such as mitomycin C and UV. Trans-dominant E1a mutant also abrogated HIV tat function and is also effective in inhibiting virus production in cotransfection experiments with HIV proviral DNA. The activation and repression functions of heterologous viral genes offer potential approaches that can be utilized to develop strategies for suppressing HIV replication.

## HIV and AIDS: Pathogenesis, Therapy and Vaccine

### *HIV Genes and Gene Products*

**L 100** HIV1 RNA AND PROTEIN SEQUENCES INVOLVED IN VIRAL RNA PACKAGING, Anna Aldovini and Richard A. Young, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142

To identify RNA and protein sequences involved in packaging of Human Immunodeficiency Virus type 1 (HIV1), various mutations were introduced into the viral genome. Portions of the HIV1 genome between the first splice donor site and the gag initiation codon were deleted to investigate the RNA packaging site (Ψ). Point mutations that alter cysteine residues in one or both zinc finger motifs of p7, a cleavage product of the gag precursor, were created to study the role of the gag zinc fingers in packaging. The Ψ site mutants and the gag mutants exhibited similar phenotypes. Cells transfected with the mutant genomes, while expressing normal levels of HIV1 RNA and proteins, produce viral particles that are normal in protein content but lack detectable viral RNA. These mutant virions are unable to productively infect cells. The combination of HIV1 packaging mutations should minimize fortuitous assembly of infectious virus and may provide a means to produce noninfectious particles for candidate vaccines. We are currently investigating the molecular details of the interaction of p7, tRNA and genomic RNA in the packaging process.

**L 101** TOXICITY OF HIV PROTEASE IN *ESCHERICHIA COLI*: A MEANS TO GENERATE MUTANT PROTEASE. Ellen Z. Baum*, Geraldine A. Bebernitz, and Yakov Gluzman, Molecular Biology Section, Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y. 10965.

The protease encoded by the *pol* gene of human immunodeficiency virus was found to be toxic to *E. coli* strain BL21(DE3). This toxicity provided a convenient selection for isolating mutants of the protease which are non-toxic and appear to be enzymatically inactive. A total of 24 missense mutations and 7 nonsense mutations were identified. A subset of these mutations are in regions of the protease which are highly conserved among retroviral proteases. Additional mutations identify other regions which are apparently critical for protease function.

**L 102** SEQUENCE CONSERVATION AROUND THE ENVELOPE CLEAVAGE SITE OF HIV-1 BIOLOGICAL CLONES WITH DIFFERENT IN VITRO CHARACTERISTICS, Marnix L. Bosch, Matthijs Tersmette*, Martijn Groenink* and Albert D.M.E. Osterhaus, dept. of Immunobiology, Natl. Inst. for Public Health and Environmental Protection, Bilthoven, The Netherlands and *Centr.Lab.Netherl. Blood Transf. Service and Lab of Exp. and Clin. Immunology of the Univ. of Amsterdam, Amsterdam, The Netherlands.

Within the HIV envelope a number of functional regions have been defined. One of these functional sites, the fusion peptide, is responsible for HIV induced membrane fusion in viral infection and virus-induced syncytium formation. We have shown previously that mutations in the SIVmac envelope fusion peptide can dramatically alter the fusogenic potential of the envelope gene products when expressed in vitro. Since HIV isolates can be characterized on the basis of their ability to form syncytia we decided to study the fusion peptide sequences of well defined biological clones of HIV-1. To this end we cloned PBL's of HIV infected individuals by limiting dilution and cultured their viral progeny. Using PCR we amplified the HIV fusion peptide sequences of these biologically cloned viruses and determined their nucleotide and predicted amino acid sequences. We will present these sequences and show that the fusion peptide regions of these viruses are highly conserved. We conclude that fusion peptide variation is not responsible for the different in vitro characteristics of the studied viruses. Approaches to define other regions of biological interest on the evelope glycoproteins of HIV and related viruses will be discussed.

**L 103 HIV-1 TAT ACTIVATES EXPRESSION VIA AN RNA TARGET**

Martin Braddock, A. Chambers, A.Thorburn, R.Docherty, L.Capsey, G.Elliott, A.J.Kingsman and S. M. Kingsman. Department of Biochemistry, University of Oxford and British Biotechnology, Cowley, England.

TAR-containing RNA (TAR+ RNA) and control TAR-less RNA are not expressed when injected into the nucleus of Xenopus oocytes. TAR+ RNA is not translated after cytoplasmic injection whereas control RNA is translated. TAT overcomes the block to translation of TAR containing RNA but only when both the RNA and TAT are present in the nucleus (Braddock et al, 1989). TAR+ and TAR-less RNA are equally stable and are both exported to the cytoplasm after nuclear injection. TAT has no influence on these parameters. TAR+ RNA is simply converted to the TAR-less phenotype by mutations that disrupt the upper stem loop structure. These mutations allow the formation of an alternative stable stem loop. Mutant RNAs are translationally inhibited after nuclear injection but they are non-responsive to TAT and they are translated after cytoplasmic injection. We suggest that any pre-synthesised RNA is inherently untranslatable after nuclear injection unless the TAR sequence and TAT are provided. TAT therefore functions in the nucleus to activate TAR RNA for translation and thereby bypasses a block that is imposed on TAR-less RNA. The importance of the nuclear translation block in normal and HIV directed gene expression and the role of the TAT-TAR interaction in HIV replication will be discussed.

**L 104** STRUCTURE/FUNCTION OF HUMAN IMMUNODEFICIENCY TYPE I TAT: IDENTIFICATION AND CHARACTERIZATION OF TAT/CELLULAR PROTEIN INTERACTIONS. David A. Brake and Christine Debouck. Department of Molecular Genetics, SmithKline and French Labs, King Of Prussia, PA. 19406

The tat gene product from HIV-1 is absolutely required for viral gene expression and replication and thus constitutes an attractive therapeutic target. A therapeutic approach is not trivial since the precise mechanism(s) by which tat regulates HIV-1 gene expression and viral growth is still unknown. Several lines of experimental evidence suggest that tat interacts with cellular proteins to carry out its function. In an effort to identify such factors, we have undertaken the following experiments. A panel of murine monoclonal antibodies (MAbs) to HIV-1 tat protein were produced and fully characterized by epitope mapping and functional activity. The MAbs were then coupled to KLH and used to immunize mice in an attempt to generate anti-idiotypic antibodies that could mimic tat itself. The anti-idiotypic sera were used to screen a lambda gt11 expression cDNA library from human monocytes, positive clones were identified and are presently being characterized. In other experiments, $^{125}$I-labelled tat was shown to bind specifically to cultured cells. Experiments are in progress to further define this tat/host cell membrane interaction and possibly elucidate the mechanism(s) of tat cellular uptake/exogenous transactivation. This work was supported in part by NIH grant Ai24845.

**L 105** HIV-1 ISOLATES FROM CLOSE CONTACTS: NUCLEOTIDE SEQUENCES EXHIBIT THE LEAST DIVERSITY SEEN TO DATE IN VIRUSES FROM DIFFERENT INDIVIDUALS. H. Burger[1], R. Gibbs[2], P.N. Nguyen[2], K. Flaherty [1], J. Gulla[1], A. Belmani, and B. Weiser[1] [1]SUNY, Stony Brook, Stony Brook, N.Y., [2]Baylor College of Medicine, Houston TX.

Studies of the molecular diversity of strains of the human immunodeficiency virus type I (HIV-1) are important for vaccine design as well as for understanding HIV-1 pathogenesis and evolution of the AIDS epidemic. To examine the nucleotide sequence diversity in HIV-1 isolates from close contacts, including those with a known time of infection, we studied a family of five in which both heterosexual and perinatal transmission of HIV-1 had occurred. The index case, a woman with a history of intravenous drug abuse in New York City until 1978, transmitted HIV-1 to her daughter perinatally in 1977, and to her lover via heterosexual contact during the period from 1978-1987. The daughter has been infected 12 years, and her mother for longer, both exceptionally long documented periods of HIV-1 infection without clinical AIDS. HIV-1 isolates were cultured from the infected family members, then sequenced directly from PCR-amplified viral DNA from hypervariable regions of the envelope gene. An evolutionary tree was constructed by progressive sequence alignment and demonstrates that the daughter's isolate diverges more (5.5%) from her mother's than the lover's isolate does (3.3%). The sequence data from these close contacts show that the isolates from the index case and her lover are more closely related than isolates from any 2 different individuals characterized to date. The greater but still low level diversity seen between the strains from the index case and her daughter, who was infected 12 years ago, supports the concept of an increase in HIV-1 heterogeneity over time.

**L 106** DETECTION OF HIV-1 PROVIRAL SEQUENCES IN LIVER CELLS BY POLYMERASE CHAIN REACTION (PCR). Yunzhen Cao, Yaoxing Huang, Alvin E. Friedmen-Kien, Michael Mirabile, JianJun Li, Tarsem Moudgil*, Douglas Dieterich, Patricia A. Thomas and David D. Ho*, Department of Microbiology, Medicine and Pathology, NYU Medical Center, New York and Infectious Diseases Division, Cedars-Sinai Medical Center, Los Angeles, California*.

Liver biopsy tissues from 9 patients with AIDS or ARC were examined by PCR for HIV-1 DNA, along with their corresponding peripheral blood lymphocytes and monocytes. Liver and blood samples from 9 non-HIV patients served as controls. DNA extracted from liver cells, lymphocytes, and monocytes were subjected to PCR with gag primers (SK 38,39) and specific viral sequences were then detected by hybridization with a $^{32}$P-labeled probe (SK 19). The results are summarized in the table. HIV-1 DNA was detected in the liver of 6 of 9 patients, but in none of the 9 controls. Qualitatively, the amount of HIV-1 DNA in liver cells correlated with those detected in lymphocytes and monocytes. In addition, the PCR results are being compared with the patients' clinical features and liver histopathology. Further studies are now in progress to determine the specific cell type in the liver carrying HIV-1.

TABLE

| PATIENT NUMBER | PCR LIVER | PCR LYMPHOCYTE | PCR MONOCYTE |
|---|---|---|---|
| 1 | ++ | +++ | ++ |
| 2 | − | ++ | − |
| 3 | + | +++ | + |
| 4 | + | +++ | + |
| 5 | − | ± | ± |
| 6 | ± | ++ | ++ |
| 7 | ± | ± | − |
| 8 | − | − | ND |
| 9 | ++ | ++ | ± |

**L 107** THE REQUIREMENT FOR PROTEOLYTIC CLEAVAGE IN THE V3 LOOP OF gp 120 FOR VIRAL INFECTIVITY? G.J. Clements, P.E. Stephens, S. Thomson, P. Murray C. Sutton, G.T. Yarranton and E. Harris.

As part of the MRC Aids Directed Programme recombinant HIV-1 BH10 111B gp120 has been successfully expressed in Chinese Hamster Ovary (CHO) cells and purified from conditioned culture supernatants using a single step antibody affinity chromatography step. Analysis of the final purified product by Coomassie blue stained SDS-PAGE under both reducing and non-reducing conditions indicated that the predominant band corresponded to intact gp120. However, under certain cell culture conditions additional bands were seen of 70 and 50 kDa. These bands were only seen under reducing conditions indicating that they were held together by disulphide bridges. Amino terminal sequence analysis of the 50 kDa band demonstrated that cleavage occurred between the arginine and alanine residues at the tip of the V3 loop (residues 315 and 316). This loop has been previously identified as a hypervariable region in the gp120 protein, the tip of which constitutes an epitope capable of eliciting type specific neutralising antibodies. The significance of a proteolytic cleavage within this region will be discussed.

**L 108** **STRUCTURE/FUNCTION STUDIES OF PURIFIED RECOMBINANT HIV-1 GP120 AND PROTEASE,** Jeff Culp, Brian Hellmig, Mike Minnich, Sam Franklin, Preston Hensley, Mitch Lewis, Annie Hassell, John Keller, Christine Debouck, Jim Strickler, Hanne Johansen, Bruce Vickroy, Shing Mai, Marge Chaikin, Kalyan Anumula, Claudine Bruck*, Tom Matthews#, and Martin Rosenberg, Departments of Protein Biochemistry, Molecular Genetics, Biological Process, Gene Expression, Macromolecular Sciences, SmithKline & French, King of Prussia, PA 19406, *Molecular Biology, SmithKline-RIT, Belgium, #Duke Univ. Med. School, Durham, NC

The envelope protein of HIV-1 3B deleted in the amino terminal 31 amino acids was expressed in and secreted from Drosophila. gp120 was isolated to 95% purity and 74% recovery using S-Sepharose and an immunoaffinity resin. Purified gp120 bound soluble CD4 with the same affinity and inhibited syncytia formation of HIV-infected H9 cells with the same efficacy as viral gp120. Analysis of carbohydrate structures suggests that soluble CD4 binding and syncytia inhibition are unaffected by differences in carbohydrate structure.

In an effort to design a safe and effective protease inhibitor, large quantities of protease protein have been purified for structural characterization. HIV-1 protease was expressed in E. coli in soluble form and purified to 95% homogeneity and high specific activity by chromatography on Superose 12, Q-Sepharose, and S-Sepharose. At high concentration, protease was found to undergo autolysis in the absence of inhibitor whereas in the presence of inhibitor, protease is stable for 100 hours at 25°C. Ultracentrifugation studies suggest that the inhibitor stabilizes the dimer form of the protease. Diffraction quality crystals of the protease-inhibitor complex have been obtained. These will be used for determination of a 3-D structure of the complex.

**L 109** THE REV PROTEIN OF HIV I BINDS TO THE mRNA OF THE REV RESPONSIVE ELEMENT, Simon Daefler, Mary E. Klotman Jim Rusche and Flossie Wong-Staal. Laboratory of Tumor Cell Biology, NIH, Bethesda, Maryland and Duke University, Durham, N.C.. Rev, a regulatory protein of HIV I functions post-transcriptionally to increase the expression of incompletely spliced mRNAs coding for structural proteins by promoting the nuclear export of these mRNAs (Malim et al., 1989). We proposed that Rev might facilitate this transport by directly binding to the RNA of the essential Rev responsive element (RRE). The 234 base pair RRE as well as 5' and 3' deletions were placed downstream from the T7 polymerase promoter using polymerase chain reaction methodology and $^{32}$P-labeled as well as unlabeled transcripts were synthesized in vitro. Purified Rev protein from an E. coli expression vector bound to the RRE transcripts as demonstrated by significant retardation in a gel mobility assay. The protein failed to cause similar retardation of other RNAs having secondary structure. Deletion analysis mapped this specific binding to a minimum of the first ninety bases of the RRE. The binding occurred over broad salt concentrations(60-200mM) and protein concentrations (125ng-1ug). These data suggest that Rev facilitates transport of incompletely spliced structural mRNA from the nucleus by direct binding to the RRE in the RNA found in these transcripts.

**L 110** MONOMERIC FORM OF HIV-1 PROTEASE IS ACTIVE, Lenora Davis, Carolyn DiIanni, Paul Darke, Nancy Kohl and Richard Dixon, Department of Molecular Biology, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486. Processing of human immunodeficiency virus 1 (HIV-1) gag and gag-pol precursor polyproteins by a virally-encoded protease is essential for viral replication. In HIV-1, the protease coding region is positioned at the 5' end of the pol gene and encodes a 99 amino acid polypeptide. The mature enzyme is a dimer composed of two 99 amino acid subunits related about a 2-fold axis of symmetry. Structural and biochemical studies indicate that HIV-1 protease is an aspartic acid protease. We have constructed a dimeric protease gene in which the coding regions for the two subunits of the mature enzyme are linked. The polypeptide obtained by expression of this construct in bacteria exhibits wild-type activity in-vitro and has the same approximate molecular weight as the native dimer. Mutation of the active site aspartyl residue in one of the linked subunits renders the enzyme completely inactive. Further characterization of the wild-type and mutant single polypeptide enzymes is in progress.

**L 111** FUNCTIONAL ANALYSIS OF CAR, THE TARGET SEQUENCE FOR THE REV PROTEIN OF HIV-1; Andrew I. Dayton[1], Douglas N. Powell[1,2] & Elahe T. Dayton[1]; [1] The National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 & [2]Genetics Department, George Washington University, Washington, D.C.

Expression of high levels of the structural proteins of the human immunodeficiency virus type 1 (HIV-1) requires the presence of the rev protein and its associated target sequence CAR (*cis* anti-repression sequence) which is present in the *env* region of viral RNA. This study demonstrates that CAR has a complex secondary structure consisting of a central stem and five stem/loops. Disruption of any of these structures severely impairs the rev response, but at least three of the stem/loops may be individually deleted without effect. The data suggest that the material in the unnecessary stem loops is present for functions unrelated to the CAR/rev axis of regulation and that it must be retained in these structures to avoid destabilizing adjacent structures critical for CAR function. Probably no more than two of the described structural components are involved in sequence-specific recognition by regulatory proteins.

**L 112** INHIBITION OF REVERSE TRANSCRIPTASE ASSAY BY DNase I TYPE ACTIVITY IN EXTRACELLULAR SUPERNATANTS OF HIV-I INFECTED T-CELLS

M. Diane Dorsett[1], Thomas Rowe[2], & Thomas M. Folks[2] Department of Experimental Pathology, Emory University, Atlanta, GA; Retrovirus Diseases Br., Centers for Disease Control[2], Atlanta, GA.

DNase I is a well characterized deoxyribonuclease that has been isolated from many cell types. Although the enzyme has been well characterized, no cellular function has yet been attributed to the enzyme. We have identified a DNase I type activity, present in high concentrations in the extracellular supernatant of several T-lymphocyte lines. The enzyme activity has been identified as DNase I based on substrate specificity, pH optima, heat liability at 56°C, requirement for Mg+2 and Ca+2 and the corresponding total inhibition of activity in the presence of 50mM EDTA. The DNase I type activity level in the supernatants of certain T-cell lines infected with HIV-I is sufficient to prevent quantitation of virus titer by the reverse transcriptase assay described by Wiley et al. The enzyme degrades the oligo-dT primer component of the assay to mono, di and tri-nucleotides; the poly-rA template is cleaved to a lesser degree. Addition of equal amounts of HIV-I to supernatants from different uninfected T-cell lines results in widely varying levels of reverse transcriptase activity reflecting the DNase I activity present in these supernatants. Preliminary data suggest that HIV-I infection induces DNase I levels several fold in A3.01, HUT78 and human peripheral blood lymphocytes. We are currently investigating the regulatory mechanisms of this induction.

**L 113** Biosynthesis and oligomerization of HIV-1 envelope protein. Patricia L. Earl*, Robert W. Doms*, and Bernard Moss*, *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md. 20892.

A set of recombinant vaccinia viruses expressing wild type or mutant forms of the HIV-1 envelope protein was constructed. The mutant env proteins include one that is not processed to gp120/41 and two carboxyl terminal truncations. These proteins are glycosylated, folded, transported to the cell surface, and bind CD4. Using these recombinant viruses we have shown that env protein is synthesized as a monomer and forms dimers and higher order oligomers post-translationally. Env molecules remain oligomeric but become less stable following cleavage to gp120/41. Analysis of truncated forms of the env protein has shown that the 129 amino terminal amino acids of gp41 is sufficient for oligomer formation and stability. Furthermore, the truncated molecule lacking the transmembrane anchoring sequence is secreted from the cell as an oligomer. The ability of oligomeric env protein to bind several molecules of CD4 has been studied. In addition, we have examined the kinetics of oligomerization, cleavage, secretion, and acquisition of CD4 binding potential.

**L 114** SYNTHESIS AND EVALUATION OF "ABASIC" OLIGODEOXYRIBONUCLEOTIDE PHOSPHOROTHIOATES AS ANTI-HIV-1 AGENTS, William Egan, R.P. Iyer, B. Uznanski, J. Boal, C. Ronald, G. Zon[1], A. Wilk[2], W. Stec[2], M. Matsukura[3], S. Broder[3]

Center for Biologics Evaluation and Review, FDA, Bethesda, MD; [1]Applied Biosystems, Inc., Foster City, CA; [2]Polish Academy of Sciences, Lodz, Poland; [3]National Cancer Institute, NIH, Bethesda, MD.

Oligodeoxyribonucleotide phosphorothioates (**S-ODNs**) have been shown to block the *de novo* infection of susceptible cells by HIV-I and to inhibit viral replication in already infected cells. The blocking of *de novo* infection appears to be a general property of **S-ODNs**, but not phosphoric acid diester linked oligonucleotides, whereas the inhibition of expression in infected cells is specific to sequences, both phosphoric acid diester and phosphorothioate linked, that are anti-sense to viral RNA. These observations suggest that it is the phosphorothioate linkage that is responsible for the potent anti-HIV activity of **S-ODNs**. Because of their potential as new therapeutics, and to shed light on the anti-viral mechanism of **S-ODNs**, we have synthesized and evaluated the anti-HIV activity of two, stable, abasic compounds, $d[C_{PS}(E_{PS})_{26}]C$ and $d[C_{PS}(V_{PS})_{26}C$ where E and V derive from 1,2,-dideoxy-D-ribofuranose and butane-1,2-diol, respectively. These data are reported.

**L 115** EXPRESSION IN E. COLI AND PURIFICATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 MAJOR CORE PROTEIN (p24), Lorna S. Ehrlich, Hans-Georg Kraüsslich, Eckard Wimmer and Carol A. Carter, Department of Microbiology, State University of New York at Stony Brook, N.Y. 11794. Capsid protein (p24;CA) of Human Immunodeficiency Virus type 1 (HIV-1) was expressed in E. coli and purified in a form that is able to self-associate, a property expected of a protein that forms the shell of the virus core. Expression was done in BL21(DE3) cells carrying the plasmid FS II which allows translation of the *pol* gene in the *gag* reading frame resulting in efficient processing of matrix (MA), capsid (CA), nucleocapsid (NC) and proteinase (PR) from one polyprotein. Capsid protein expressed in this system is soluble, recognized by monoclonal antibodies directed against HIV capsid, and has an N-terminal sequence that is identical to that of capsid purified from HIV. Purification of recombinant CA was done under mild conditions where co-expressed HIV PR retains enzymatic activity. Milligram quantities of 90% pure CA were obtained after chromatography on DEAE cellulose followed by facilitated aggregation of CA in the unbound fraction. Precipitated CA readily dissolves in low ionic strength aqueous buffers. In solution, purified CA existed in oligomeric form. We believe that HIV capsid protein purified in this manner retains its native conformation because of its ability to self-associate. This material should be useful in obtaining structure-function information that can guide the design of antiviral drugs that target events occuring during viral assembly.

**L 116** CHARACTERIZATION OF INFECTIOUS MOLECULAR CLONES DERIVED FROM AN HIV-1 ISOLATE WITH RAPID/HIGH REPLICATIVE CAPACITY, Eva Maria Fenyö*, Robert Fredriksson*, Per Stålhandske*, Agneta von Gegerfelt*, Birgitta Lind*, Pierre Åman**, and Eric Rassart***, Departments of Virology* and Bacteriology**, Karolinska Institute, Stockholm, Sweden and Department of biological Sciences, University of Quebec at Montreal, Canada***. HIV-1 has been isolated from a patient with AIDS. The isolate, designated 4803, has shown a high replicative capacity in peripheral blood mononuclear cells (PBMC) as well as in T-lymphoid and monocytoid cell lines. Infected cultures showed extensive syncytia formation. Molecular cloning was performed from high molecular weight DNA of 4803 infected PBMC. The cellular DNA was cut with Xba-1, appropriate size fragments selected on a sucrose gradient and cloned in the bacteriophage λ-dash. Eight recombinant phages were identified using the BH10.R3 probe. Sac-1 fragments of one of the clones were subcloned into the vector pT3/T7α19 and used as probes in restriction enzyme analysis. The clones were found to be highly related to each other and differed only at 1 or 2 restriction sites (of 28). Compared to HXB-2D, the difference with any one clone was at 7 or 9 restriction sites (of 31). Differences were more often found over the env fragment than over the gag-pol fragment.
The molecular clones were transfected into various cells by electroporation. Four clones yielded infectious virus in PBMC, three in U937-2 and two in Hut-78. Virus recovered from PBMC cultures replicated in PBMC and Jurkat-tat cells only, a characteristic of slow/low viruses. The replicative pattern, however, appeared to be unstable and following the third passage in PBMC, all clones were able to replicate in U937-2. Infectious virus recovered from U937-2 cells replicated in U937-2 cells but not in T leukemia cell lines CEM and Jurkat. Further characterization of the clones is under progress.

**L 117** RETROVIRAL MEDIATED TRANSFER OF THE *TAT* AND *REV* GENES OF HIV, J. Victor Garcia and A. Dusty Miller, Program in Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104. The *tat* and *rev* genes of HIV regulate LTR transcription and the expression of the viral structural genes. An unspliced fragment of ARV-2 containing the *tat*, *rev*, and envelope genes was cloned into pLXSN. After transfection into PE501 cells, an ecotropic producer line, virus supernatant was used to establish amphotropic producer lines. Both lines, PA317/LTATSN and PA317/LREVSN, have a single unrearranged spliced provirus and a titer >$10^6$ PFU/ml on NIH3T3 cells. Virus supernatant has been used to establish human and murine lines that constitutively express either gene. Expression of the *tat* gene was monitored by an HIV LTR-CAT construct, whereas *rev* expression was monitored using a *rev*$^-$ HIV provirus. Constitutive expression of either gene does not seem to grossly affect cell growth or CD4 expression. The human T-cell lines obtained should be helpful in studying the role of constitutive expression of *tat* and *rev* before and during HIV infection.

L 118 NATURAL VARIANTS IN THE LONG TERMINAL REPEATS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1: FUNCTIONAL ROLE?, Jan L.M.C. Geelen, Sabine E.C. Koken, Wilco Keulen, Leon G. Epstein, and Jaap Goudsmit, Human Retrovirus Laboratory, AMC, Amsterdam, the Netherlands.
The long terminal repeat (LTR) is an important element in regulation and cell specificity of human immunodeficiency virus. Therefore, we decided to analyse the LTRs of HIV-1 derived from different tissues. DNA was extracted from brain and spleen tissue of HIV-1 infected children, and analyzed for the presence of complete LTRs by the polymerase chain reaction, using primers which are located in the nef region and the gag region. As these primers bracket the LTR region only LTRs in the single LTR conformation (eg one LTR circular DNA) will be amplified. Amplification of DNA from brain and spleen tissue resulted in two main groups of LTRs: one group with three sp-1 sites and a minority of LTRs with four sp-1 sites. The promotor/enhancer efficiency of these two types of LTRs was analyzed in a number of cell lines (monocytes, T- and B- lymphocytes) by chloramphenicol acetyl transferase assay. In all cell systems tested the natural occurring four sp-1 LTR variant proved to be the most efficient.

L 119 ORDERED SEQUENCE OF SPLICE ACCEPTOR UTILIZATION DURING HIV-1 INFECTION OF CULTURED CELLS. John C. Guatelli, Thomas R. Gingeras, Douglas D. Richman. University of California San Diego, San Diego VA Medical Center, Salk Institute of Biotechnology/Industrial Associates, Inc.
The sequence of appearance of alternatively spliced RNA sequences was examined after high-multiplicity infection of CEM cells with the LAV-$1_{Bru}$ strain of HIV-1. A cDNA/polymerase chain reaction technique was used to generate DNA fragments reflecting specific splice events. This analysis revealed that the splice acceptor at position 5555, common to nef and env transcripts, is dominant. The splice acceptors for tat (position 5356) and rev (position 5533) were utilized after the nef/env acceptor and appeared to be utilized less efficiently at all time points. Abundant accumulation of singly-spliced env -specific sequence was coincident with appearance of the rev-splice and with an increase in cell association p24 antigen. The accumulation of all spliced sequences was accelerated and compressed under conditions of increased multiplicity of infection. We suggest that the relatively low strength of tat and rev splice acceptors may determine a level of transcription required to activate viral encoded positive regulatory mechanisms.

**L 120 ASSEMBLY OF HIV-LIKE PARTICLES BY A RECOMBINANT VACCINIA VIRUS EXPRESSION SYSTEM, OMAR HAFFAR, JACQUES GARRIGUES, BRUCE TRAVIS, PATRICIA MORAN, MOLLY SMITHGALL, JOYCE ZARLING, and SHIU-LOK HU, ONCOGEN, SEATTLE, WA 98121.** We report on the assembly of HIV-like particles in African Green Monkey kidney cells infected with two recombinant vaccinia viruses carrying either the HIV-1 gag or env genes. The gag gene used in these experiments included the virus protease coding sequence. The particles were isolated from culture supernatants of doubly infected cells by sedimentation at 120,000xg. Biochemical analysis of the isolated particles revealed that they were composed of gag proteins, primarily p24, as well as the env proteins gp120 and gp41, in ratios similar to HIV virion. Thin section immunoelectron microscopy showed that the sedimented particles were 100-120nm in length, were characterized by the presence of cylindrical core structures, and displayed the mature gp120/gp41 complexes on their surfaces. EM analysis of infected cell monolayers showed that particle assembly and budding occured at the plasma membrane. Dot blot hybridization suggested that the particles packaged only the gag mRNA and not the env mRNA. Furthermore, the particles did not replicate in CEM lymphoblastoid cells. Finally, initial results indicated that immunization with the recombinant-made particles elicited a pronounced immune response in rabbits, comparable to immunization with inactivated virus. Therefore, the nonreplicating HIV-like particles provide a novel and attractive approach for vaccine development.

**L 121** EXPRESSION OF FULL-LENGTH RECOMBINANT GP41 FROM HIV-1 IN MAMMALIAN CELLS: EVIDENCE FOR CELL SURFACE EXPRESSION AND OLIGOMERIZATION, Nancy L. Haigwood, Christine B. Barker, Kimberly A. Mann, and Gregory K. Moore, Carl J. Scandella and Kathelyn S. Steimer, Chiron Corporation, Emeryville, CA 94608.
The HIV-1 gp41 gene was engineered for cell surface expression independent of the gp120 sequences by the addition of an exogenous signal sequence. Expression constructs were made to test four different amino-terminal sequences, two retaining the amino-terminal hydrophobic fusion domain and two omitting this domain. Transient experiments in COS-7 cells showed that constructs encoding full length and truncated proteins could direct expression, as measured by a gp41-specific ELISA on the cell lysates. Expression correlated with the presence of the exogenous signal sequence, suggesting that the hydrophobic fusion domain cannot by itself serve as a signal. Permanent CHO cell lines were engineered for three gp41 constructs, and they were positive for cell-surface expression of the gp41 protein by surface immunofluorescence using human HIV-positive sera. Western blot analysis of these lysates with human HIV-positive sera or with a gp41 monoclonal antibody revealed the presence of proteins migrating in the 41 kDa range. To test for the formation of oligomers of gp41, we added a membrane-impermeable reversible cross-linking agent to CHO cell cultures. Lysates were analyzed with and without reducing agent on western blots processed with human HIV-positive sera. These experiments showed the presence of high molecular weight species in the nonreduced lanes that are reduced to a gp41 monomer upon the addition of reducing agent. The sizes of the higher molecular weight species are consistent with dimers, trimers, and tetramers of gp41.

**L 122** IDENTIFICATION OF A *cis*-ACTING ELEMENT IN HIV-2 THAT IS RESPONSIVE TO THE HIV-1 REV AND THE HTLV-I AND II REX PROTEINS. Marie-Louise Hammarskjöld, Nancy Lewis and David Rekosh, Depts of Microbiology and Biochemistry, University at Buffalo, Buffalo, NY 14214.
A SV40 late replacement vector encoding HIV-1 gp120 (pGP120) was used to define a region within the HIV-2 genome that could work as a *rev* responsive element (RRE). Our previous work showed that gp120 expression in this system required a functional RRE *in cis* , and the rev protein *in trans* ( Hammarskjöld et al.(1989) J. Virol 63,1959). Using pGP120 we first mapped a RRE to a 1042bp Sau3a fragment in the *env* region of HIV-2. Both HIV-1 *rev (rev1)* and HIV-2 *rev (rev2)* could work in conjunction with this fragment. Further mapping showed that a 272bp subfragment within the1042bp region was sufficient as an RRE. Surprisingly, the smaller fragment worked only with the rev1 protein and not with its homologous rev2 protein. In addition, the rev2 protein failed to function together with the RRE from HIV-1. We also utilized this system to examine the ability of the *rex* genes of HTLV-I and II to functionally substitute for *rev*. These experiments showed that complementation by both rexI and rexII required the presence of a RRE. The rex proteins worked well in conjunction with either the HIV-1 or the HIV-2 RRE (the1042bp as well as the 272bp fragment).

**L 123** STUDY OF THE NON-ESSENTIAL GENES OF HIV-1: vpu and vpr

**W.A. Haseltine, E.A. Cohen, and E. Terwilliger. Division of Human Retrovirology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115**

Human immunodeficiency virus type 1 (HIV-1) encodes at least six regulatory genes which are not found in classical animal retroviruses. Two of these genes tat and rev are essential for virus replication whereas the remaining genes nef, vif, and vpu are not required for viral replication.

To investigate the role of vpu and vpr in the replication and cytopathicity of HIV-1, infectious proviruses isogenic except for the expression vpr or vpu were constructed.

It will be shown that the vpu protein increases greatly the export of virus particles from infected cells and reduces the accumulation of cell-associated viral proteins. Information relevant to the sub-cellular localization of the vpu protein will also be presented as will be the implications for the mechanism of action of the vpu protein.

Vpr is shown to encode a 96-amino acids 15Kd protein that acts in trans to accelerate virus replication and cytopathic effects in T cells. Experiments showing that the HIV-1 viral particle contains multiple copies of the vpr proteins will also be presented. This observation raises the possibility that vpr acts to facilitate the early steps of infection before de novo viral protein synthesis occurs.

**L 124** INVESTIGATION OF SELECTED HUMAN RETROVIRUS AND DNA VIRUS SEQUENCES N KAPOSI'S SARCOMA BIOPSIES. Yao-oi Huang, Yunzhen Cao, JianJun Li, Bernard Poiesz*, Alvin Friedman-Kien, Department of Microbiology, NYU Medical Center, New York and *Division of Hematology and Oncology, State University of New York, and the Veterans Administration Medical Center, Syracuse, New York.

The most common neoplasm associated with AIDS is Kaposi's Sarcoma (KS). The etiology of this neoplasm is unknown. Nucleic acid sequences of several viruses, such as human immunodeficiency virus type I (HIV-I), hepatitis-B virus (HBV), human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV) have not been detected in KS tissues by Southern hybridization. Polymerase chain reaction (PCR) has been used to detect genomic sequences of other viral agents that might be present at very low copy number on a per cell basis which would otherwise be undetectable by other methods. To test the possibility that known infectious agents might be involved in KS pathogenesis, we examined fresh tissue specimens from 18 AIDS-associated KS (AIDS-KS) biopsies using PCR for several viruses which have been implicated in the etiology of KS. PCR analysis revealed the presence of HIV-I sequences in 17/18 AIDS-KS tissue samples. The sequence of a virus-like infectious agent (VLIA) was also detected in 9/18 AIDS-KS biopsies. Other virus sequences studied, including human herpes virus 6 (HHV-6) and human T cell leukemia virus type II (HTLV-II) were not detected in any of the 18 AIDS-KS specimens. These results support the hypothesis that HIV-I is involved in the pathogenesis of KS in patients with AIDS. Our data suggest that a VLIA may also play a role in the etiology of AIDS-KS.

**L 125** MUTATIONS WITHIN THE HIV env GENE THAT BLOCK GLYCOPROTEIN FUNCTION, Eric Hunter, John Dubay and Hae-ja Shin, Univ.of Alabama at Birmingham, Birmingham, AL 35294.

Point mutations have been inserted into the 3'-end (C-terminal domain) of the HIV env gene sequence which introduce premature stop codons into the env open reading frame. They result in the synthesis of progressively truncated proteins lacking from 18 to 191 amino acids. None of the mutations prevented normal levels of env gene product biosynthesis, or the assembly and release of virus at wild-type levels, even though proteins truncated by 172 and 191 amino acids were secreted from the cell. When the mutant env genes were expressed in a pol-deficient HIV genome in HeLa-T4 cells, we showed that all of the glycoproteins which retained the membrane spanning domain were able to induce large syncytia. Thus gp41 molecules truncated by 18 to 138 amino acids can still be inserted in a biologically active form in the membrane. Nevertheless, all of the truncations in the C-terminal domain of gp41 abrogated infectivity of the mutant viruses. Experiments underway will determine if this is the result of inefficient incorporation of the surface-expressed glycoprotein complex into virions.

During transport of gp160 to the plasma membrane it is cleaved to gp120 and gp41 after a sequence of basic amino acids (K-A-K-R-R-V-V-Q-R-E-K-R). Since this sequence contains two consensus cleavage sequences, we have explored the possibility that both might serve as cleavage sites. Mutation of the REKR sequence to REER or of the KAKRR sequence to KAERS had no effect on precursor cleavage or on cell fusion in HeLa-T4 cells. In contrast, an env gene containing both mutations yielded a product that was transported normally but which remained uncleaved and was unable to induce cell fusion. These results indicate that both cleavage sites can be utilized, protein sequence analyses of the mutated gp41 molecules will confirm this possibility.

**L 126** REV-DEPENDENT EXPRESSION OF GP160 IN DROSOPHILA SCHNEIDER 2 CELLS, Mona Ivey-Hoyle, Hanne Johansen, and Martin Rosenberg, Department of Gene Expression Sciences, Smith Kline & French Laboratories, King of Prussia, PA 19406

Stable cell lines allowing regulated expression of HIV-1 IIIB rev protein, gp120 and gp160 have been obtained using Drosophila Schneider 2 cells. Constructs were made which encoded either the authentic rev protein, or a gp120 or gp160 molecule whose signal sequence was replaced by the signal sequence of the human tPA gene. In these constructs, expression of the HIV protein is under the control of the inducible metallothionein promoter. These constructs were transformed singly and/or in combination into Drosophila cells, and stable lines were selected. Upon metal induction of these cell lines, gp120 production was achieved at up to 5 mg/liter and did not require co-expression of rev. In contrast, gp160 expression could only be detected in cell lines where rev was co-expressed.

Although gp160 protein production was clearly rev-dependent, an analysis of RNA production indicates that gp160 RNA is produced at similar levels in both the presence and absence of rev protein. However, in cells expressing rev protein, gp160 RNA is clearly present in the cytoplasm, whereas in cells lacking rev, cytoplasmic gp160 RNA is dramatically reduced. Apparently, the regulation of gp160 expression by rev, known to occur in mammalian cells, is also operative in Drosophila Schneider 2 cells. This regulation is eliminated by simply removing from the transcription unit gp41-encoding sequences containing the rev response element.

**L 127** BIOCHEMICAL AND CRYSTALLOGRAPHIC STUDIES OF HIV REVERSE TRANSCRIPTASE,

Alfredo Jacobo-Molina[1], Arthur D. Clark, Jr.[1], Raymond G. Nanni[1], Gail Ferstandig Arnold[1], Andrea L. Ferris[2], Stephen H. Hughes[2], and Edward Arnold[1], 1-Center for Advanced Biotechnology and Medicine (CABM) and Rutgers University Chemistry Department, 679 Hoes Lane, Piscataway, NJ. 08854-5638, 2-NCI-Frederick, Cancer Research Facility, P.O. Box B, Frederick, MD 21701. HIV reverse transcriptase (HIV RT), essential for replication of the AIDS virus, is a promising target for antiviral agents; unlike all of the known cellular polymerases, retroviral RTs (including HIV RT) operate in the cytoplasm as opposed to the nucleus. The determination of the structure of HIV RT will substantially improve the chances of designing specific drugs that will not interfere with normal cellular functions. Recombinant HIV RT has been isolated from an E. coli expression system (Hizi et al., 1988) in its two dimeric forms, p66/p66 homodimer and p66/p51 heterodimer. A number of crystal forms of both forms of HIV RT have been obtained, and X-ray diffraction has been observed from small crystals at the Cornell High Energy Synchrotron Source (CHESS). However, crystals have not yet been obtained that diffract to high resolution. Additional crystallization strategies being pursued include cocrystallizations of p66/p66 homodimer or p66/p51 heterodimer with double-stranded DNA oligomers that mimic the primer-template complex (using sequences corresponding to human $tRNA^{Lys III}$), and complexes of HIV RT with Fab fragments purified from anti-HIV RT monoclonal antibodies.

Nine monoclonal antibodies (Mab) raised against p66/p66 homodimer were used to prepare immunoaffinity columns to identify structural differences between p66/p66 homodimer versus p66/p51 heterodimer. Two Mabs, one directed against the C-terminal and the other to the middle portion of the polypeptide, bind tightly to homodimer but very weakly to heterodimer, while the other Mabs recognize both HIV RT forms. These results suggest that certain portions of the molecule experience significant conformational changes, and that others remain essentially unaltered. However, these affinity differences might be partially explained by differences in HIV RT dimerization constants. One of these columns has been used to resolve mixtures of homodimer and heterodimer, and its use will potentially simplify purification of HIV RT from E. coli extracts.

Finally, we propose a model for the overall spatial arrangement of HIV RT polymerizing and RNase H activities in the functional reverse transcription complex.

**L 128** EXPRESSION AND CHARACTERIZATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 NEF GENE.

Kaminchik, J., N. Bashan, D. Pinchasi, B. Amit, N. Sarver*, M.I. Johnston*, M. Fischer, Z. Yavin, M. Goreki and A. Panet, Biotechnology General LTD Kiryat Weizmann Rehovot, 76326 Israel. *National Institute of Allergy and infectious Diseases,National Institutes of Health, Bethesda, Maryland, 20892.

Nef genes from HIV-1 isolates BH10 and LAV1 were expressed in E.coli under the *deo* operon promoter. The two proteins found in the soluble compartment of the bacterial lysate were purified by ion exchange column chromatography to apparent homogeneity. Under native conditions the recombinant Nef protein is a monomer of 23 kD. In denaturing polyacrylamide gels however, BH10 and LAV1 derived Nef migrates as 28kD and 26kD proteins respectively. Purified recombinant BH10 Nef was used as an immunogen to elicit murine monoclonal antibodies. A series of monoclonal antibodies were obtained which reacted with sequences at either the amino or carboxy terminus of Nef or with a conformational epitope on the native protein. Using the later antibody we were able to detect structural differences between LAV1 and BH10 Nef proteins. GTP binding and GTPase activity were monitored during Nef protein purification. These activities did not copurify with the recombinant Nef protein from either the BH10 or LAV1 isolate.

**L 129** ANALYSIS OF THE PROCESSING OF THE GAG PRECURSOR OF HIV: EVIDENCE FOR A SECOND PATHWAY INVOLVING CYTOPLASMIC PROCESSING, ANDREW H. KAPLAN AND RONALD SWANSTROM, DEPARTMENT OF BIOCHEMISTRY, AND LINEBERGER CANCER RESEARCH CENTER, UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, 27599. Processing of the retroviral *gag* gene product is required for the production of infectious virions. The processing of the Gag precursor of the human immunodefficiency virus (p55) gives rise to the structural proteins of the viral capsid, p17 (MA), p24 (CA), p7 (NC), and p6. The location in the cell where these cleavages occur (ie. intracellularly in the cytoplasm, at the membrane, or within the virion) and the order of cleavage have not been well-characterized. We combined pulse-chase labelling of acutely infected CEM cells together with cell fractionation to address these questions. The initial capsid species to appear is p25, indicating that the initial cleavages occur between amino acids 132 (Y) and 133 (P) (MA/CA) and the two methionines at 377 and 378 (p25/NC). Further, we have determined that there are two distinct processing pathways for the p55 Gag precursor. A substantial amount of processing occurs in the cytoplasm. This pathway results in a cytoplasmic accumulation of p25 and p24 representing about one-third of the total radioactivity present in viral Gag related proteins at the end of the pulse. These species remain in the cytoplasm even after prolonged chase periods. In addition, the cytoplasmic fraction contains equivalent amounts of p24 and p25. The second pathway, which results in virion formation, reveals primarily p24 moving through the membrane at one hour of chase. There is no turnover of Gag precursors in the virion, suggesting either that little or no processing takes place after virus is released from the cell or that processing is extremely rapid after virus release.

**L 130** THE EFFECT OF EXTENDED PASSAGE ON THE EXPRESSION LEVELS OF THE HIV-1 nef PROTEIN, James A. Lautenberger[1], Sharon Bladen[2], Garrett C. DuBois[2], David Hodge[1], Kenneth P. Samuel[2], Stephen D. Showalter[2], Martin Zweig[2], and Takis S. Papas[1], [1]Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD 21701, [2]Program Resources, Inc., NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701. The HIV-1 nef protein was analyzed by use of monoclonal antibodies raised against a recombinant nef protein expressed in bacteria. Two forms of nef were seen in HTLV-III-infected H9 cells, $p25^{nef}$ and $p27^{nef}$. Cells that had been passaged continuously for over one year contained relatively high levels of $p25^{nef}$ and lower levels of $p27^{nef}$. In contrast, low passage cells contained lower levels of nef with the p27 form predominating. When nef proteins were analyzed on H9 cells recently infected with virus isolated from high passage infected cells, high levels of nef were observed with the p25 form predominating. These results indicate that continuous passage selects for viral genomes expressing high levels of the $p25^{nef}$. Concomitant with the high levels of nef, high passage cells contained reduced amounts of viral proteins and messenger RNA. These results are consistent with the proposed negative regulatory function for nef. A full length nef protein has been expressed in a baculovirus system and will be used to elucidate the biochemical properties of the HIV-1 nef gene.

**L 131** STRUCTURE- FUNCTION STUDIES OF AUTHENTIC RECOMBINANT HIV-1 REVERSE TRANSCRIPTASE: CHARACTERIZATION OF ITS INTERNAL PROCESSING BY HIV-1 PROTEASE Gary Lazarus, Steven Carr, Gerald Roberts, Michael Moore, and Christine Debouck, Smith Kline Beecham Pharmaceuticals, King of Prussia, PA

Reverse transcriptase (RT) is essential for the replication of HIV-1 and its integration into the host cell genome. It is the target for the only approved drug for the treatment of AIDS. Both viral and recombinant HIV-1 RT have been shown by us and other investigators to exist as a heterodimer of polypeptides of molecular weight 66 and 51 kDa, respectively. Previously, we showed that these subunits shared a common amino terminus. In this study, we identify a single carboxyl terminus for the p51 subunit of RT as Phe 440. The peptide AETFYVD, corresponding to amino acids 437-443 of RT, is recognized by HIV-1 protease and cleaved between Phe 440 and Tyr 441. This suggests that HIV-1 protease may be responsible for the generation of p51 from p66. Currently, we are generating mutations at the Phe 440 and Tyr 441 and will examine these mutants for their ability to be processed by HIV-1 protease. We will purify unprocessed mutants to examine the structure-function characteristics of the p66 subunit in the absence of p51. We have also produced an authentic form of the p51 subunit by deletion mutagenesis. We have purified this p51 protein and are now examining its subunit structure and activities.

This work was in part supported by NIH grant GM39526.

**L 132** U1 RNA PLAYS A ROLE IN THE PROCESSING OF REV DEPENDENT UNSPLICED HIV1 ENV mRNA. Xiaobin Lu, David Rekosh and Marie-Louise Hammarskjöld. Depts of Biological Sciences, Biochemistry and Microbiology, University at Buffalo, Buffalo,NY14214.

We have previously shown that rev is required for HIV env protein expression from a SV40 late replacement vector and that rev functions to promote nuclear export of *env* mRNA ( Hammarskjöld et al., J.Virol. 63,1959,1989). The *env* mRNA made in this system is not spliced. Further analysis of the requirements for env expression showed that in addition to rev, an upstream 5' splice site had to be present in the *env* mRNA. Deletion or mutation of this splice site resulted in loss of env expression. No *env* mRNA was detected in the cytoplasm of cells transfected with 5' splice site mutants and the levels of nuclear env mRNA were dramatically reduced. Env expression could be restored by the insertion of a heterologous 5' splice site derived from the EBV EBNA1 gene. Furthermore near wild type levels of env protein were obtained when a construct containing a point mutation at the +5 position in the splice site was cotransfected into cells together with a vector expressing a U1 suppressor snRNA that restored U1 base pairing at the splice site. These experiments demonstrate a direct role for U1 RNA in the processing of a non spliced mRNA and suggest that rev may directly interact with components of the splicing machinery.

**L 133** TOXIN GENE REGULATION BY HIV-1 Tat AND Rev, I.H. Maxwell, F. Maxwell, C. Long, C. Rosen* and G.S Harrison, University of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262, and *Roche Institute of Molecular Biology, Nutley, NJ 07110

We are exploring HIV-regulated expression of a diphtheria toxin A gene (DT-A) for killing HIV-infected cells before production of viral progeny. The applicability of this toxin gene therapy for AIDS will ultimately depend on: (i) minimizing basal expression of HIV-regulated DT-A, and (ii) efficient delivery of the toxin gene into cells. Towards the first aim, we have constructed luciferase reporter and DT-A constructs which include the HIV-1 LTR (from -167 or -453 to +80), and, in some cases, the negative regulatory *crs* sequences from the *env* region, inserted in the 3' untranslated region. In transient transfection of HeLa or Jurkat cells, we have demonstrated low basal expression of DT-A and significant *trans*-activation by cotransfected Tat and Rev expressing plasmids. In further experiments, HeLa LUC19, a cell line stably transformed with an HIV-regulated luciferase construct, exhibited very low basal expression and >100-fold *trans*-activation by Tat and Rev. We are attempting to generate analogous cell lines stably transformed with HIV LTR--DT-A--*crs* plasmids using either wild-type DT-A or a mutant with reduced toxicity. We hypothesize that such cell lines will be unable to support HIV production due to cell death upon activation of the early viral gene products.

**L 134** MECHANISM OF ACTION OF THE RIBONUCLEASE H ACTIVITY OF HIV-1 REVERSE TRANSCRIPTASE, Valerie Mizrahi, Lindsay R. Dudding and Alexis Harington, Molecular Biology Laboratory, The South African Institute for Medical Research, Hospital Street, P.O. Box 1038, Johannesburg 2000, South Africa.

The ribonuclease H (RNase H) activity associated with recombinant p66/p51 HIV-1 reverse transcriptase (RT) has been analyzed by using homogeneous RNA.DNA hybrid substrates which consisted of SP6 transcripts from the gag region of HIV-1 hybridized to complementary ss-DNA from an M13 or a phagemid subclone. Denaturing gel electrophoretic analysis of the products generated by HIV-1 RT, M-MuLV RT and E. coli RNase H indicated significant differences in the cleavage site selectivities, and hence in the product distributions of the three enzymes. Endoribonucleolytic cleavage by HIV-1 RT in the absence of DNA synthesis was observed using substrates that consisted of a short (20 nt) oligodeoxynucleotide internally hybridized to a T7 gag-derived RNA. This observation suggests that the hybrids formed between viral RNA and antisense oligonucleotides may be selectively hydrolyzed by the viral RNase H, providing a potentially novel mode of action of these reagents in the inhibition of viral expression. Substitution of the highly conserved Asp-443 residue by Asn in the putative COOH-terminal RNase H domain of the p66 subunit affected neither the RT nor the RNase H activity of the p66/p51 enzyme.

**L 135** **KINETICS OF EXPRESSION OF STRUCTURAL AND REGULATORY GENES OF HIV-1 DURING SINGLE-CYCLE REPLICATION IN MACROPHAGES.**

James R. Munis, Richard S. Kornbluth, John C. Guatelli, and Douglas D. Richman. Departments of Medicine and Pathology, University of California, San Diego, and Veterans Administration Medical Center, San Diego, CA 92161.

The sequence of appearance of several components of HIV-1 replication was examined during a synchronous, high multiplicity (m.o.i.=6) infection of human monocyte-derived macrophages with a macrophage-tropic strain of HIV-1 (HTLV-$III_{Ba-L/85}$). In addition to assays for formation of provirus, core antigen production, viral infectivity, and cytopathic effects (CPE), a modification of the polymerase chain reaction technique was used to assess the time-course of HIV-1 regulatory gene expression during synchronized infection. These studies demonstrated: A) detection of provirus at 2 hours after infection; B) transcripts representing rev and nef/env gene expression between 12 and 24 hours; and C) transcripts representing tat and singly-spliced env gene expression between 24 and 48 hours after infection. Coincident with tat and env expression between 24 and 48 hours, the development of CPE (multinucleated giant cells), a 3-log increase in supernatant viral infectivity, a 1.5-log increase in supernatant p24 antigen, and a 1-log increase in cell-associated p24 antigen occurred. Additional experiments using both high (10) and low (1) multiplicities of infection demonstrated that an increased m.o.i. accelerates the appearance of all regulatory transcripts, but does not alter their sequence of expression.

**L 136** POLYMERASE CHAIN REACTION (PCR) STANDARDIZATION PROGRAM, Linda M. Muul and Gregory Milman, Pathogenesis Branch. Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
The polymerase chain reaction (PCR) is a sensitive method for amplifying small amounts of specific DNA or RNA to produce detectable quantities. Early results from studies by various research groups using PCR for HIV detection have raised important questions about the application of PCR in HIV infection. PCR may be useful to detect the presence of HIV prior to seroconversion, to determine the HIV infection status of infants born to seropositive mothers, and to follow the levels of HIV after treatment with anti-viral agents. In collaboration with Cetus and several AIDS Clinical Trial Groups (ACTG), the Pathogenesis Branch is coordinating efforts to examine the reproducibility and sensitivity of PCR in clinical research laboratories. The ACTG laboratories tested a uniform PCR protocol with common primers and probes using samples containing known quantities of HIV. Both technical and procedural problems were encountered. Thirty three percent of the laboratories had severe problems with DNA carry-over of HIV positive DNA to negative samples and controls. Although PCR has the capability of amplifying single molecules of HIV, only 50% of the laboratories were able to detect 60 copies of HIV DNA. Problems identified during the first phase of the PCR Standardization Program indicate that revised procedures and the development of new methods are required for PCR to be a reliable clinical tool.

**L 137** PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT HIV-1 rev PROTEIN Carlo M. Nalin*[1], Douglas Antelman[2], Ming-Chu Hsu[2], Departments of Protein Biochemistry[1], and Oncology and Virology[2], Hoffmann-La Roche Inc.Roche Research Center, Nutley NJ 07110
Recombinant Human Immunodeficiency Virus-1 rev protein expressed in E. coli has been purified by ion exchange and gel filtration chromatography. Specific binding of the purified protein to the rev-responsive element of the viral RNA can be demonstrated. Gel filtration chromatography of the protein in aqueous buffer or in 8 M urea and 2 M NaCl indicates that the protein has an apparent molecular weight of 53,500. These results suggest that the protein in solution forms a stable tetramer consisting of monomers having molecular mass of 13,000. Optical spectroscopy has also been used to characterize the protein in solution. Fluorescence measurements of the single tryptophan residue in rev indicate that $Trp_{45}$ is located on the protein surface and in an aqueous environment . Together with chemical quenching measurements, the results indicate that protein prepared by this method is homogeneous and suitable for biological and structural studies.

**L 138** THE ABILITY TO PRODUCTIVELY INFECT BLOOD MONONUCLEAR PHAGOCYTES BY HIV-1 IS GENETICALLY DETERMINED AND INVOLVES ENTRY, William A. O'Brien, Yoshio Koyanagi, Jerome A. Zack and Irvin S.Y. Chen, Division of Infectious Diseases, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024
The mononuclear phagocyte is a principal target cell for infection with HIV-1. However, high titer virus production does not occur following infection of these cells with all viral isolates. Utilizing molecular clones of HIV-1 strains that differ in ability to replicate in mononuclear phagocytes, we have constructed recombinant HIV-1 strains in order to determine if there are genetic domains responsible for mononuclear phagocyte tropism. Substitution of sequences from the env region of the primary isolate $HIV\text{-}1_{JR\text{-}FL}$ into pNL4-3 gives rise to hybrid HIV-1 strains that replicate as efficiently in mononuclear phagocytes as the parental isolate, $HIV\text{-}1_{JR\text{-}FL}$. Analysis of DNA from infected cells by polymerase chain reaction (PCR) suggests that the block to infection for poorly tropic strains is at entry.

**L 139** HIV-1 INFECTS FRESHLY-ISOLATED MONOCYTE/MACROPHAGES VIA THE CD4 RECEPTOR, *Kunihiro Ohashi, *Suzanne Gartner, **Vaniambadi S. Kalyanaraman and *Mikulas Popovic, *Division of Virology/Immunology, PRI-New Mexico State University, P. O. Box 1027, Holloman AFB, NM 88330, **Department of Cell Biology, Bionetics Research, Inc., Rockville, MD 20850.

Human CD4+ T lymphocytes and monocyte/macrophages (M/M) are the major targets and viral reservoirs for HIV-1 infection. Virus entry into these cells takes place through the binding of the viral envelope (gp120) to the CD4 receptor molecule. This virus-host cell interaction has been mainly investigated using CD4+ normal and neoplastic T cells. Little is known about the interaction between HIV-1 and the surface of M/M. To investigate the early steps in the infection of M/M, inhibition experiments were carried out using freshly isolated M/M and M/M-tropic HIV-1 (HTLV-III Ba-L) with (1) monoclonal antibodies against CD4 and (2) purified gp120 and gp160 from HIV-1. Both monoclonal antibodies and the purified viral glycoproteins significantly blocked M/M infection. In addition, we also demonstrated that the binding of HIV-1 to M/M takes place through interactions between the gp120 and the CD4 molecule on these cells as evidenced by precipitation of complexes of gp120-CD4. These results indicate that the CD4 molecule represents a primary receptor for HIV-1 entry into M/M.

**L 140** PHYSICAL BIOCHEMICAL STUDIES OF THE STRUCTURE-FUNCTION RELATIONSHIPS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REVERSE TRANSCRIPTASE (HIV-RT), George R. Painter, Lois L. Wright, Sam Hopkins and Phillip A. Furman, Burroughs Wellcome Co., Research Triangle Park, NC 27709. Tryptophan has been used extensively as a fluorescent probe to monitor protein conformation in solution with the assumption that the fluorescence quantum yield of a trp residue in a protein depends on its local environment. The heterodimeric (51 KD plus 66 KD) transcribing form of HIV-RT contains thirty trp residues within the polymerase and ribonuclease domains. The addition of a single stranded DNA or a double stranded DNA-RNA hybrid to a solution of HIV-RT decreases the fluorescence intensity. Apparent dissociation constants (Kapp) for the binding reactions were calculated from the observed quench. Hill plots of the binding data indicated there is a single class of energetically equivalent DNA binding sites within the heterodimer. Kapp is affected minimally by changes in $[Na^{+}]$ and $[Mg^{+2}]$ indicating binding to be nonelectrostatically driven. Rather than extensive, direct interaction between the negatively charged lys and arg residues on the protein, formation of the HIV-RT DNA complex may involve either dipole-dipole interaction or interaction with hydrogen bond donating and accepting groups in the major and minor groves of DNA. A similar analysis has been carried out for dNTP substrate binding in the presence and absence of saturating amounts of complementary template-primer. The order of the reaction with respect to dNTP is one, indicating there is a discrete binding site(s) for the substrate on the heterodimer. The four natural substrates appear to compete for the same site on the protein.

**L 141** BASE-SUBSTITUTION MUTAGENESIS BY HIV-1 REVERSE TRANSCRIPTASE, Bradley D. Preston, Department of Chemical Biology, Rutgers University College of Pharmacy, Piscataway, NJ 08855.
HIV-1 shows extensive genetic variation within populations, within individuals and over periods of time. This hypervariability may be central to the pathogenesis of AIDS and its resistance to therapy. The molecular basis of HIV mutagenesis is poorly understood, but presumably originates in mechanisms unique to the HIV life cycle. In an effort to identify pathways contributing to HIV mutagenesis, we have studied the fidelity of purified HIV-1 reverse transcriptase (RT) during DNA polymerization in vitro (Preston et al., Science 242:1168, 1988). Measurements of errors at a single template A residue in a $\phi$X174-based fidelity assay showed that HIV-1 RT introduces base-substitutions with unusually high frequency (error rate $\approx$1/4,000) and unique specificity (70% A $\rightarrow$ T transversions, 25% A $\rightarrow$ C transversions and 5% A $\rightarrow$ G transitions). By using four $\phi$X174 templates containing either A, C, G or T at position 587, we have measured the rates of formation of all twelve possible mispairs at a single template site. The average misincorporation frequency for all mispairs at this site was 1/19,000. However, HIV-1 RT showed a strong preference for the formation of certain mispairs (frequency for G•T and T•T mispairs = 1/2,000) and not others (frequency for G•G mispair = 1/110,000). A comparison of HIV-1 RT with the mammalian replicative polymerase $\alpha$ (pol-$\alpha$) showed that these enzymes form nucleotide mispairs with distinct frequencies and specificities. Moreover, HIV-1 RT, but not pol-$\alpha$, efficiently extends 3' terminal mispairs following initial insertion of noncomplementary nucleotides (Perrino, Preston et al., PNAS 21:8343, 1989). The high error rate of HIV-1 RT points to the likely importance of this enzyme in viral evolution and suggests that HIV-1 RT is unusually promiscuous in its recognition of nucleotide and template substrates.

**L 142** CONSTRUCTION OF A RETROVIRAL PARTICLE CONTAINING AN INTERFERING HIV-1 GENOME
G.B. Rossi, M. Federico, F. Titti, S. Butto, A. Orecchia, F. Carlini, B. Taddeo, I. Saggio, and P. Verani.
Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy.

We have isolated an HIV infected non-producer Hut-78 clone (F12) exhibiting an altered viral protein pattern, i.e. uncleaved env gp160 and gag p55, and the remarkable presence of a p19, possibly related to an overproduction of the rev protein. As for other HIV infected cells, the CD4 HIV receptors are down-regulated in F12 cells, as determined by the FACS analysis using five different anti-CD4 monoclonal antibodies. In addition, we are studying in F12 cells a possible modulation of CD4 specific mRNA. The F12 clone appears resistant to HIV-1 and HIV-2 superinfection, even at high-multiplicity. Experiments are now in progress to detect the HIV replication after transfection with an HIV infectious molecular clone. To verify if this phenomenon of homologous interference could be reproducible in other cellular systems, we have cloned the HIV provirus from an SstI F12 genomic library and then subcloned it in retroviral vectors as pLJ, Ntk or NSV, all harboring the neo resistance gene. In order to recover a recombinant retrovirus containing the provirus originally present in F12 genome, the retroviral vector construct has been transfected in the amphotropic retrovirus packaging line PA 317. With the supernatants from G418 resistant PA 317 clones, we have infected HIV sensitive (H9, CEM, H937) and resistant (NIH 3T3, He-La) cell lines and then selected the neomycin resistant clones. HIV infection and/or transfection of cell clones expressing the F12 genome is in progress. This will allow us to verify if the viral interference occurs in cell systems other than Hut-78 cells.

**L 143** HIV-1 RECOMBINANT REV PROTEIN BINDS SPECIFICALLY TO THE REV RESPONSIVE ELEMENT *IN VITRO,* James R. Rusche, Thomas J. Daly, Kathleen Sue Cook, Gary Gray, and Theodore E. Maione, Repligen Corporation, One Kendall Square, Building 700, Cambridge, MA 02139.

The human immunodeficiency virus type 1 (HIV-I) genome encodes two regulatory proteins which are required for viral replication. One of these trans-acting proteins, designated REV, is a 13,000 dalton protein synthesized from fully processed viral transcripts prior to synthesis of HIV-1 structural proteins. REV has been postulated to exert control within the nucleus at the level of mRNA processing. A cis-acting sequence has been identified within a region of the envelope gene of the viral transcript that appears to control mRNA processing in a REV dependent manner. This REV responsive element (RRE) appears necessary, in concert with REV, to control HIV mRNA processing and production of viral structural proteins. DNA transfection studies by Malim et al. demonstrated that REV-dependent inhibition of processing was unaffected by moving the RRE to varying locations within the mRNA transcript when the RRE was kept in the sense orientation. The RRE RNA (210 nucleotides) is the most highly conserved region of the envelope gene and has been predicted to possess an extensive secondary structure containing stem-loops. Genetic studies have identified REV gene mutants with dominant phenotypes, supporting the hypothesis that REV interacts directly with the RRE. In the present study, we demonstrate that REV protein, purified from *E. coli,* binds in a sequence specific manner to the REV responsive element *in vitro.* Studies demonstrating activity of mutant REV proteins will also be presented.

**L 144** EXPRESSION OF HIV-1 PROTEIN AND mRNA IN LYMPHOID TISSUE AFTER HIV-1 INFECTION, by Henk-Jan Schuurman and Jaap Goudsmit, Division Immunopathology, University Hospital, P.O. Box 85.500, 3508 GA Utrecht, and Academic Medical Centre, Amsterdam, The Netherlands.

The presence of p17 and p24 core, and gp41 env, proteins, and gag/pol and env mRNA segments of HIV-1 was studied on frozen sections of lymph node and thymus. In lymph node, follicles revealed protein (especially core) in staining patterns like those of immunoglobulin and complement: presumably the proteins are present in immune complexes. Interfollicular areas showed solitary protein$^+$ cells, especially for gp41. Such cells were HLA-class 2$^+$: protein expression may be related to antigen presentation by these cells. mRNA$^+$ cells occurred in low numbers without distinct compartmentalization. Thymus simularly showed scattered mRNA$^+$ and protein$^+$ cells, including epithelium. Some a-p17 and a-p24 reagents labeled epithelium in thymus from HIV-1$^-$ persons: this phenomenon could not be exclusively explained by cross-reactivity to thymic peptides like thymosin. Both thymus and lymph node showed protein and mRNA in endothelium. In conclusion, immuno- and hybrido-histochemistry provide valuable tools to study HIV-1 infection in the histologic approach.

**L 145** HIV-CYTOPATHICITY: LEVELS OF VIRAL RNA AND PROTEIN IN HOST CELLS.
Mohan Somasundaran* and H. L. Robinson+, Departments of Pediatrics* and Pathology+, University of Massachusetts Medical Center, Worcester, MA 01655. Since HIV can undergo both latent and active infections in CD4-positive T-cells, we established a C8166 culture system where one could follow synchronous HIV infections to study the mechanism of HIV-cytopathicity. Cytoblot analyses of C8166 cells, synchronously infected with HTLV-IIIb showed that they express very high levels of viral RNA (close to or higher than that of cellular RNA) two to four days prior to their death. Pulse labeling studies on infected C8166 cells during this post-infection period revealed the expression of high levels of viral gag (pr55) protein which accounted for approximately 40% of the total proteins synthesized. Similarly, high levels of viral RNA and gag protein were expressed in non-synchronously infected cultures of CEM cells, H9 cells and peripheral blood lymphocytes. However, levels of viral RNA and gag protein in 8E5 cells (a continuous cell line expressing HIV) were 10-100 fold lower than those cultures undergoing cytopathic infections. Studies on protein synthesis in HIV-infected C8166 cells suggest that HIV has the potential to inhibit cell functions. Similar analyses on persistently infected cells are underway to confirm that high levels of viral RNA and protein is a general characteristic of HIV-cytopathicity and also to identify the precise mechanism by which HIV inhibits cell functions.

**L 146** KINETICS OF RNA SYNTHESIS IN HIV-1 INFECTED MONOCYTE-MACROPHAGES,
Secondo Sonza, Anne L. Colvin, Sarah H. Burgess, Katherine A. Silburn and Suzanne M. Crowe, Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Victoria, 3078, Australia

Monocyte-macrophages, purified from buffy coat preparations by Ficoll-Hypaque centrifugation and glass adherence, were cultured in suspension in teflon containers for 1-2 weeks and then infected with HIV-1 (DV) at a multiplicity of ~0.1. At various time intervals from 4h to 1 month post-infection (p.i.), total cellular RNA was prepared and analysed by Northern hybridisation using a radiolabelled HXB-2 DNA probe. Three HIV-1 specific RNA bands were detected. The largest band, ~9.5kb, the size expected for gag and pol mRNA and genomic RNA, was first detectable at low levels at 10 days, reached maximal levels at 15 days and was undetectable after 21 days p.i. This is consistent with the production of p24 antigen which is very low prior to 7 days and which peaks at 15 days p.i. A broad band at ~4.3kb, a size consistent with env mRNA and messages such as those for vif, vpr and partially spliced regulatory transcripts, was the most abundant throughout and was still detectable at low levels 1 month p.i. The smallest band, ~2kb, the size expected for tat, rev and nef regulatory gene mRNAs, showed kinetics similar to the larger bands but at lower levels throughout. Interestingly, the 2kb transcripts were not detectable early on in infection but were still just detectable 1 month p.i. We are currently analysing mRNA production and also chromosomal and extra-chromosomal DNA for evidence of integration and at what stage this may occur in monocyte-macrophages infected with HIV-1.

**L 147** PRODUCTION OF RECOMBINANT HIV gp120 IN ANIMAL CELLS
P.E. Stephens, G. Clements, S. Thomson, P. Murray, M. Doyle, G.T. Yarranton and E. Harris.

As part of the MRC Aids Directed programme a process has been developed for the production and purification of recombinant HIV-1 BH10 111B gp120 from Chinese Hamster Ovary (CHO) cells. Details of the cloning, expression and cell line development will be discussed along with the single step purification process. Additionally work carried out to characterise this product will be described. Expression levels of the gp120 protein were relatively low (1mg/L) and attempts to increase the productivity by coexpressing the rev gene or using alternative host cell lines will be discussed.

A similar process is being developed for the production and purification of gp120 derived from the HIV-2 strain ST as part of the NIAID Aids programme. HIV-2 ST was originally isolated from a healthy Senegalese prostitute by Hahn et al. HIV-2 ST did not cause cell death or induce cell fusion in peripheral blood lymphocytes or in any of the CD4 cell lines tested. The molecular basis of the attenuation is unknown but may well involve alterations in the envelope protein. Steps involved in the cloning and expression of this protein in CHO cells will be described together with approaches to its purification.

**L 148** ELECTRON MICROSCOPIC VISUALIZATION OF HIV REVERSE TRANSCRIPTASE FREE AND BOUND TO DNA. Deborah Thomas, Jack Griffith, Phillip Furman* and George Painter*, Lineberger Cancer Research Center and Department of Microbiology, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514, and * Division of Virology Burroughs Wellcome Co. Research Triangle Park, N.C. 27709. A native bacterially expressed HIV reverse transcriptase consisting of an equimolar mixture of the p66 and p51 peptides and exhibiting the enzymatic properties of the enzyme isolated from virus particles has been examined by electron microscopy both free and bound to various nucleic acid templates. To examine the shape and appearance of the enzyme, samples were briefly adsorbed to a thin carbon support, flash frozen at -170°, freeze-dried and rotary shadowcast with tungsten or tantalum in a fully cryopumped vacuum system. The enzyme alone appears as an oblong particle approximately 55A by 85A. Often two domains (presumably the p51 and p66 subunits of the heterodimer) can be distinguished and many particles show a longitudinal groove or cleft running lengthwise. When the enzyme was incubated with duplex DNA, single stranded DNA, or an RNA/DNA hybrid duplex, protein particles having the same size and shape as the unbound enzyme were seen bound along the nucleic acid. The number of enzyme molecules bound along the DNA or DNA/RNA hybrids was sparse and appeared to be relatively independent of salt or nucleotide cofactors. A strong preference for binding to the ends of the DNA was not observed. The possibility of two different arrangements of bound protein will be discussed.

**L 149** EXPRESSION OF HIV IN PRIMARY CNS CELL CULTURES. B. Watkins, W. Kelly, H. Dorn, R. Armstrong, C. Kufta* and M. Dubois-Dalcq. Laboratory of Viral and Molecular Pathogenesis and *Surgical Neurology Branch, NINDS, NIH, Bethesda, MD. 20892.

In an attempt to understand how HIV causes the pathological changes seen in the brains of AIDS patients, we have inoculated primary cultures of normal and neoplastic adult human brain with several strains of HIV1 and HIV2. The release of infectious virus was measured by reverse transcriptase (RT) activity and HIV core antigen (p24) in the cell-free supernatant. The only viral strains that actively replicate in these neural cultures grown in medium supplemented with giant cell tumour supernatant (provided by Dr. N Sarver, NIH) are macrophage tropic strains - one of them, the AD87 strain (provided by Dr. B. Potts, NIH) produces peak levels of RT activity ($2x10^5$ cpm $^{32}$P/ml) and p24 antigen (47 ng/ml) at 2-3 weeks post infection (PI), while T-cell tropic and HIV2 strains did not produce measurable levels of RT or p24 antigen. The release of infectious virus from infected cultures was confirmed by the ability of cell-free supernatant to infect other primary brain cultures and also by electron microscopy which revealed typical HIV buds on the plasma membrane and free virus in the extracellular space. To determine which cell - types present in these primary cultures were infected, we used a triple label technique in which microglial cells are identified by binding of DiI-LDL (DiI labeled low density lipoprotein), astrocytes by labeling with rabbit antiserum to GFAP (glial fibrillary acidic protein), and infected cells by immunostaining with mouse monoclonal antibodies to HIV p24 or p17 *gag* proteins. Starting at 4-5 days PI, AD87 caused progressive fusion of cells which stained with DiI-LDL and expressed *gag* proteins, but not GFAP. In contrast, GFAP$^+$ astrocytes did not express viral antigens and did not fuse. Macrophage tropic strains also caused a progressive and dramatic loss of microglial cells: at 3 weeks the number of LDL$^+$ cells was reduced by 80-90% compared with controls. These results indicate that microglia, but not astrocytes, present in primary adult human brain cultures are productively infected by macrophage tropic strains of HIV1, and suggest that microglia may be infected *in vivo* by virus released from macrophage. This resident population of cells, scattered throughout the CNS, may thus favor the spread of virus. Moreover, the progressive death of these cells may cause neurological dysfunction in a direct or indirect manner.

## *Cellular and Viral (non-HIV) Factors that Influence HIV Production*

**L 200** CORRELATION OF MONOCYTE-MACROPHAGE DIFFERENTIATION AND KINETICS OF HIV REPLICATION Birgitta Åsjö(1), Antonio Valentin (1) and Shunji Matsuda (1,2), (1) Department of Virology and (2) Department of Immunology, Karolinska Institute, Stockholm, Sweden

Monocyte cultures prepared from peripheral blood mononuclear cells (PBMC) recovered by Ficoll Hypaque gradient centrifugation were infected with viruses isolated from HIV-seropositive individuals 0,5,7,10,15,30 and 60 days after initiation of culture. Infections were monitored by reverse transcriptase (RT)-activity and presence of viral p24 antigen in culture medium. Cocultivation with PHA-stimulated normal PBMC was done to rescue virus from non-producing cultures.

HIV-infection could be established at all time points. However, the mode of replication varied according to the cellular differentiation at the time of infection. Monocytes infected at day 0 always yielded RT-positive cultures. Fifteen days old or older cells (up to 60 days) were susceptible to infection but in contrast to younger cells, the virus replication was at a low level only detectable by antigen assay. HIV-infection between day 5 and day 10 yielded a heterogenous pattern; some cultures showed high virus replication while others showed a low virus production.

Conclusions: Normal blood monocyte-macrophages are susceptible to HIV-infection at all stages of differentiation. However, the kinetics of virus replication is affected by the stage of maturation of the cells at the time of infection.

**L 201** **INACTIVATION OF THE HIV LTR BY DNA CpG METHYLATION: EVIDENCE FOR A ROLE IN LATENCY,** *D.P. Bednarik, *P.C. Guenthner, †J.A. Cook, †P.M. Pitha, *Centers for Disease Control, Retrovirus Diseases Branch, Atlanta, GA 30333 †The Johns Hopkins Univ. Sch. of Med. Oncology Center, Baltimore, MD 21205 Infection of cells by HIV can result in a period of quiescence or latency which may be obviated by treatment with inducing agents such as 5-azacytidine. Evidence from these experiments demonstrate the existence of two CpG sites in the HIV LTR enhancer region which can silence transcription of both reporter genes and infectious proviral DNA when enzymatically methylated. This transcriptional block was consistently overcome by the presence of the transactivator tat without significant demethylation of the HIV LTR. In vitro DNase protection experiments showed increased binding of nuclear factors to the core enhancer region between -36 to -55 nucleotides upstream from the cap site, when the HpaII site located at -146 was methylated. In contrast, binding of other nuclear factors to this region and upstream DNA sequences was inhibited by methylation at this site. These results suggest that DNA hypermethylation of the HIV LTR facilitates differential binding of cellular factors which suppress expression of the HIV LTR and modulates viral expression.

**L 202** ALTERATIONS IN AND HIV INDUCTION OF IL-6 IN VIVO AND IN VITRO, Deborah Birx, Robert Redfield, Kathleen Tencer, Maryanne Vahey, Donald Burke, and Giovanna Tosato, Walter Reed Institute of Research and the FDA, Washington DC, Bethesda,MD.

Interleukin 6, a multifunctional cytokine produced in monocytes and other cell types is induced by a variety of stimuli, including a laboratory strain of HIV. In vitro, IL-6 promotes the proliferation of EBV-infected B cells and Ig secretion in activated B cells. We examined whether HIV isolated from infected individuals induces IL-6 secretion in vitro, and whether HIV-infected individuals have abnormally elevated serum IL-6 levels.

Using an in vitro culture system for the isolation of HIV, we found that HIV-positive cultures contained significantly greater amounts of IL-6 bioactivity.IL-6 secretion in normal monocytes cocultures with PBMC

| Test sup | N | p24(pg/ml) | IL-6 U/ml (geo.mean) |
|---|---|---|---|
| Normal | 5 | <10 | 7 x/÷ 5 |
| HIV sero(+) | 12 | <10 | 5 x/÷ 2 |
| HIV sero(+) | 12 | >250 | 48 x/÷6 ($p<0.04$) |

Production of IL-6 in mononuclear cell cultures replicating natural HIV was confirmed by immunoprecipitation.

In further studies, we determined that mean serum levels of IL-6 bioactivity were abnormally elevated in HIV seropositive individuals with early stage 1/2 disease (15.8 x/- 2 U/ml) and stage 3/4 disease (25.1 x/- 1.6 U/ml) when compared to normals (1.6 x/- 1.2 U/ml). In addition, serum IL-6 levels in this patient population correlated directly with serum IgG levels ($r=0.6$, $p<0.003$).

Thus, HIV-infected individuals who often display abnormally elevated serum IgG levels and abnormally elevated numbers of EBV-infected B cells in the circulation, may exhibit these abnormalities as a consequence of abnormally elevated IL-6 levels induced by HIV.

**L 203** INTEGRATED HIV-1 LTR SEQUENCES ARE NOT TRANSACTIVATED BY HERPES SIMPLEX VIRUSES WITH DEFINED MUTATIONS IN THE GENE ENCODING THE IMMEDIATE-EARLY PROTEIN, IE110 (ICP0). E. D. Blair[1], C. M. Roberts[1], and R. D. Everett[2]. Wellcome Research Labs, Beckenham[1] and Institute for Virology, Glasgow[2], UK. Herpes simplex virus infection of cells harboring HIV LTR sequences, linked to the E. coli lacZ gene, resulted in transactivation of the retroviral promoter. LTR-driven B-gal expression was also induced by plasmids expressing HSV-1 IE protein, IE110, but not plasmids expressing IE175 or IE63. A series of plasmids with mutations in the IE110 gene showed a spectrum of trans-activation activities; mutations in region 1, including a potential $Zn^{2+}$-coordination domain, failed completely to transactivate, mutations preventing IE110 nuclear localization also failed to transactivate, whereas C-terminal deletions of the IE110 protein hypertransactivated B-gal expression. Recombinant HSV carring the same IE110 mutations in the viral genome, which results in high particle/pfu values, showed similar relative levels of transactivation, compared to plasmid transactivation, at equivalent particle /cell ratios. These defects were manifest within individual plaques where wild type virus gave rise to blue staining infected cells under X-gal overlay whereas mutant IE110-containing viruses had white plaques or deep blue plaques. We are examining the ability of these mutant viruses to induce $NF_\kappa B$ activity to determine whether $NF_\kappa B$ induction correlates with IE110-mediated transactivation of the HIV LTR.

**L 204** DIFFERENTIAL SUSCEPTIBILITY OF U-937 CLONAL DERIVATIVES TO HIV INFECTION, François Boulerice, Romas Geleziunas, Alla Lvovich and Mark A. Wainberg, Jewish General Hospital, Montreal, Quebec, Canada, H3T 1E2
In this study, single cell clones were obtained from the cell line U-937 by limiting dilution technique. These cells were studied for their susceptibility to HIV infection. We found that two clones (UC12, UC18) could easily support HIV replication compared to parental U-937, showing a rapid increase in immunofluorescence for the p24 viral core antigen, while another clone (UC11) remained negative throughout a ten week culture period. This differential susceptiblity, as defined by longer or shorter periods of latency between initial infection and appearance of p24-positive cells, did not correlate with the expression of surface CD4. First, all cell types showed similar FACS profiles when screened for the presence of membrane CD4, as detected by Leu3a mAb reactivity. In addition, relative CD4 mRNA expression in each clone was comparable to parental U-937. Finally, the level of binding of radiolabeled HIV did not correlate with higher or lower susceptibility to infection. Blocking experiments using Leu3a mAb totally abbrogated infection on all cell types, suggesting that no other receptors were recognized by HIV. Furthermore, polymerase chain reaction analysis of cellular DNA revealed the presence of proviral DNA in all clones, including the one that was poorly susceptible (UC11), suggesting the existence of a mechanism regulating HIV replication after viral entry. This system represents a good model to study HIV latency in cells of monocytic origin and may provide a useful tool for studying the role of cellular factors restricting or enhancing viral replication.

**L 205** ANTIBODIES TO THE CD2 RECEPTOR ACTIVATE THE HIV LTR IN NORMAL PERIPHERAL BLOOD LYMPHOCYTES, Peter B. Bressler, Giuseppe Pantaleo, and Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892. Numerous physiologic agents previously have been shown to activate HIV in vitro including tumor necrosis factor-alpha and lymphotoxin in T cell lines and tumor necrosis factor-alpha, lymphotoxin, interleukin-1 and interleukin-6 in monocytic cell lines. Additionally, stimulation of the CD3-TCR complex by monoclonal antibodies resulted in activation of the HIV LTR in T cell lines. The CD2 T lymphocyte surface glycoprotein (the natural ligand for LFA-3) is involved in cell to cell adhesion and CD2 stimulation by itself or in conjunction with TCR stimulation tranduces signals resulting in T lymphocyte activation. In this study we have investigated the effect of CD2 stimulation on transiently transfected PHA-activated T lymphocytes. Using cells transfected with HIV LTR-CAT plus HIV LTR-TAT, we demonstrate that treatment with anti-CD2 antibodies results in the activation of the HIV LTR. Furthermore, deletion analysis of the LTR-CAT construct demonstrates that this activation occurs through activation of the NFkB enhancer region. These studies suggest that interaction of CD2 with its natural ligand, LFA-1, in vivo may play an important role in regulation of latent HIV expression.

**L 206** EFFECT OF RECOMBINANT LYMPHOKINES ON NATURALLY HIV-1 INFECTED $CD4^+$ T CELLS IN VITRO. Jan E. Brinchmann, Gustav Gaudernack and Frode Vartdal, Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway.
Lymphokines affect cell metabolism through alteration in cellular proteins. Malignantly transformed cell lines may be less suitable than freshly isolated human cells for studies of the effects of the different lymphokines. We have used positively isolated $CD4^+$ T cell from asymptomatic HIV infected individuals to study the effect of the recombinant interferons $\alpha$, $\beta$ and $\gamma$ and of TNF-$\alpha$ on cell proliferation and HIV replication.
Results: rIFN-$\alpha$ inhibited the replication of HIV in $CD4^+$ T cells stimulated with an anti-TCR mAb in the presence of rIL2 in a dose-dependent manner. None of the other lymphokines, alone or in combination, inhibited the replication of HIV. rIFN-$\beta$ and rTNF-$\alpha$ had a co-stimulatory effect, while rIFN-$\alpha$ had an inhibitory effect on cell proliferation. rTNF-$\alpha$ could, in some instances, induce cell proliferation and HIV replication in the $CD4^+$ T cells in the absence of activation signals other than rIL2.
Conclusion: rIFN-$\alpha$ can inhibit the replication of HIV in naturally infected $CD4^+$ T cells in vitro, while rTNF-$\alpha$ can induce cell proliferation and HIV replication in the absence of other activation signals.

**L 207** MODIFIED OLIGONUCLEOTIDES AS INHIBITORS OF HIV-1 IN VITRO

S. Burgess, O. Kemal, P. Simmonds, A.J. Leigh Brown, J.O. Bishop & T. Brown, Institute of Genetics, University of Edinburgh, Scotland. The poly-cytidine phosphorothioate oligonucleotide $SdC_{28}$ has been synthesised, and its effect on the infection of T-cells, by HIV-1 in vitro, determined using a range of virus titres and $SdC_{28}$ concentrations. Although the observed inhibition of HIV-1 infection by $SdC_{28}$ does not appear to occur through an antisense mechanism, phosphorothioate oligonucleotides complementary to the rev gene of HIV-1 have been shown to act in a sequence specific manner and thus offer potential as anti-HIV agents. However, although S.ODNs were shown, by $^{35}S$ labelling studies, to be relatively stable in culture compared to unmodified oligonucleotides, the thio backbone substitution is thought to reduce the thermal stability of duplexes formed with unmodified oligonucleotides. In order to increase the thermal stability of S.ODN-ODN duplexes, and hence the efficiency of their antisense effect, certain chemically modified bases have been synthesised for incorporation into S.ODNs and testing against the expression of HIV-1 in chronically infected T-cells in vitro.

**L 208** **ENHANCEMENT OF TNF-ALPHA PRODUCTION FROM CHRONICALLY HIV-1 INFECTED HL-60 CELLS.**

**Salvatore T. Butera, Victor L. Perez, and Thomas M. Folks.** Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA 30333.

As a model of HIV-1 infection in hematopoietic progenitor cells, we have developed and characterized an HIV-1 infected HL-60 cell line (OM). After recovery from cryopreservation, OM cultures remain virus negative for several weeks and then undergo a spontaneous activation of HIV-1 expression. The HIV-1 titers of these cultures continue to rise until a maximum level is achieved (> 25 ng/ml p24 antigen by ELISA).

Directly associated with this rise in virus titer is also the finding of enhanced secretion of TNF-alpha in response to PMA stimulation. OM cultures during the initial HIV-1 dormant stage and parental uninfected HL-60 cultures secrete only low detectable amounts (< 5 U/ml) of TNF-alpha when induced with PMA. However, as the OM cell cultures progress through the viral production stages, they become increasingly more responsive to PMA induced TNF-alpha production (> 30 U/ml).

The addition of HIV-1 to PMA-stimulated HL-60 and early OM cell cultures did not enhance TNF-alpha production. Therefore, extracellular HIV-1 particles are not required for the inductive signal. Furthermore, classical intracellular protein kinase C dependent pathways are not involved in this induction. H-7, a specific protein kinase C inhibitor, had no inhibitory effect upon TNF-alpha production by HIV-1 expressing OM cultures. In these same cultures, H-7 was able to significantly reduce the constitutive and PMA-stimulated expression of HIV-1.

HIV-1 expressing OM cultures are activated by TNF-alpha to increase their level of HIV-1 production. The possibility of TNF-alpha, liberated in response to PMA stimulation, being involved in autocrine enhancement of HIV-1 production was investigated using an anti-TNF-alpha polyclonal antibody. The addition of this antibody to HIV-1 expressing OM cultures neither reduced the constitutive expression of HIV-1 nor altered the induction of HIV-1 in response to PMA stimulation.

These data suggest that the mechanisms governing HIV-1 activation from latency may also influence cellular gene expression and the liberation of host factors which are intricately involved in the virus life cycle.

**L 209** CHARACTERISATION OF CELLULAR PROTEINS WHICH INTERACT WITH THE NEGATIVE REGULATORY ELEMENT OF THE HIV-1 LTR.

Mary Collins[1], Kim Orchard[1,2], Neil Perkins[1], Caroline Chapman[1,3], Julian Harris[1,3], Vincent Emery[2], Graham Goodwin[1] and David Latchman[3], [1]Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, [2]Department of Virology, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG, [3]Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, U.K.

Expression from the HIV-1 LTR is known to be regulated by host cell responses such as T lymphocyte activation, lymphokine action and macrophage differentiation. These events have the potential to critically influence the progress of disease in infected individuals. A number of cellular proteins capable of binding to the HIV-1 LTR have been identified, and one of these, NF .B, binds to the enhancer region of the LTR and increases viral transcription in response to several stimuli. In contrast, however, the region of the LTR upstream of -278 has been identified as a negative regulatory element, the removal of which increases LTR expression in the Jurkat T cell line, and which may therefore play a role in the maintenance of HIV latency. We have defined the two major binding sites within this region, both of which bind previously undescribed human T cell proteins. Further structural and functional characterisation of these proteins will be presented.

**L 210** INFECTION OF MONOCYTE-DERIVED MACROPHAGES IS CD4-DEPENDENT
R. Collman, B. Godfrey, H. Friedman, S. Douglas, F. Gonzalez-Scarano, N.Nathanson

Work in several laboratories has established that human macrophages can be infected with HIV-1, although virus isolates differ in their ability to establish a productive infection. The role of the macrophage entry pathway as a determinant of this selective tropism has received wide attention. To determine whether HIV-1 strains that infect macrophages utilize the CD4 molecule as their entry pathway, primary human monocyte-derived macrophages and peripheral blood lymphocytes (PBL) were prepared and infected with HIV-1 isolate SF162, a strain that replicates in both macrophages and lymphocytes.

Immunoreactive CD4 was detectable by Western blot in fresh monocytes and in differentiated macrophages just prior to infection (7-10 days). HIV-1/SF162 infection of both macrophages and lymphocytes was inhibited by the anti-CD4 monoclonal antibody Leu3a, but not by antibodies directed against HLA-DR or other control antibodies. Similarly, preincubation of virus with soluble recombinant CD4 (courtesy of R. Sweet, Smith, Kline and French Labs.) inhibited infection of macrophages as well as infection of PBL's.

These results suggest that CD4 functions as the receptor for macrophage infection. Thus the determinants of macrophage tropism are not likely to reside in envelope-CD4 binding.

**L 211** **Cellular Requirements for HIV Infection, Kathleen A. Daher and Dan R. Littman, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143**

HIV selectively infects human cells that bear the T4 surface antigen. Mouse cells that have been manipulated to express T4 can bind HIV but are unable to internalize and/or uncoat the virus. This suggests that there are cellular factors other than T4 which are required for the early events of HIV infection. We have designed a system which allows dominant selection of cells permissive for the early events of HIV infection. An infectious molecular clone of HIV was made replication-defective by deletion of the envelope gene and insertion of a drug resistance gene in its place. The defective genome is packaged by co-transfection of this construct along with an envelope expression construct. Virus produced in cells transfected with both constructs is phenotypically normal but contain a defective genome that encodes drug resistance. Subsequent infection of cells with the defective virus allows selection of susceptible cells due to their acquisition of the drug resistance gene. We intend to identify cellular genes required for infection by introducing human genes into T4 positive, non-infectable cells. The rare cells which acquire an infectable phenotype in this manner will be selected using the defective virus system. When such cells are isolated the introduced genes will be rescued, cloned and analysed. This system has also been used to test the ability of various viral envelopes to complement the env(-) defective virus. The ability of non-HIV viral envelope glycoproteins to pseudotype with HIV is being tested.

**L 212** MODULATION OF HIV-1 MULTIPLICATION BY rIFN-α2 AND AZT TREATMENT OF MONOCYTIC CELLS,
Martine Dubreuil, Lucy Sportza, Judith Lacoste, Ronald Rooke, Mark A. Wainberg and John Hiscott, Lady Davis Institute, Jewish General Hospital and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada H3T 1E2

To examine the effect of rIFN-α2, either alone or in combination with the known reverse transcriptase inhibitor AZT, on transmission of HIV-1, U937 cells were infected with HTLV-IIIB or chronically infected U937 cells (U9-IIIB) were mixed with U937 at a ratio of 1:100. The progressive spread of HIV-1 through these cell populations was monitored by p24 antigen indirect immunofluorescence, reverse transcriptase and Northern blot hybridization of HIV-1 RNA. Usually 21-28 days were required to transmit virus to 90% of the cells in the culture. p24 antigen immunofluorescence and Northern blot analysis of intracellular RNA suggested modest (2-3 fold) reductions in the number of infected cells and the total amount of viral RNA per cell as a result of IFN treatment alone; the effect was most dramatic at a time period coincident with the rapid increase in virus spread through the cell culture (days 10-11). IFN decreased the extracellular levels of HIV-1 as measured by p24 ELISA antigen capture, suggesting that an additional point of IFN action may be at the level of virus budding from the cell surface. AZT was not an effective agent in limiting virus spread in the cell-cell transmission experiments, nor did it act synergistically with rIFN-α2 in reducing virus transmission.

L 213 **PRODUCTION OF INTERLEUKINS IN HIV-1 REPLICATING LYMPH NODES.** D. EMILIE, °PEUCHMAUR, M., MAILLOT, M.C., CREVON, M.C., DELFRAISSY, J.F. and GALANAUD, P. INSERM U131 & Service de Médecine Interne, °Service d'Anatomopathologie, Hôpital Béclère, Clamart, France.

In order to document the *in vivo* interactions occuring between the immune system and HIV replicating cells, we analyzed using *in situ* hybridization the production of IL-1beta, IL-6, IL-2 and INF-gamma in hyperplastic lymph nodes from 9 patients infected with HIV-1. Numerous IL-1beta and IL-6 producing cells associated in clusters were detected in sinuses, with few individual IL-1beta and IL-6 producing cells being present in interfollicular and follicular areas. IL-2 and INF-gamma producing cells were detected in all lymph node compartments, with a selective enrichment in germinal centers. These cytokine producing cells were not specific for HIV replicating lymph nodes, as they were observed in a similar distribution in HIV unrelated hyperplastic lymph nodes. The amount of IL-1beta , IL-6 and IL-2 producing cells was not different between HIV and non HIV lymph nodes. In contrast, HIV lymph nodes exhibiting a florid hyperplasia displayed a high level of INF-gamma production as compared to HIV lymph nodes presenting follicular lysis and to non HIV lymph nodes. The $CD8^+$ cells which accumulate in germinal centers of HIV lymph nodes (and not in non HIV germinal centers) were actively involved in this INF-gamma production. They were in direct contact with cells containing HIV core antigens and HIV RNA. *Thus a high INF-gamma production may characterize some forms of anti-HIV* T cell immune response, potentially contributing to control of viral spreading as well as to the development of follicle lysis.

L 214 DIFFERENTIAL EFFECTS OF THE IMMEDIATE-EARLY GENE REGION 2 OF HUMAN CYTOMEGALOVIRUS ON THE PROMOTERS OF HUMAN IMMUNODEFICIENCY VIRUS I AND HUMAN T-CELL LEUKEMIA VIRUS TYPE I IN PRIMARY T LYMPHOCYTES. Ronald B. Gartenhaus, Mary E. Klotman and Flossie Wong-Staal. Laboratory of Tumor Cell Biology, National Institutes of Health, Bethesda, Md 20892 and Duke University. Human cytomegalovirus (HCMV), Human Immunodeficiency Virus I(HIV I) and Human T-cell leukemia virus type I (HTLV I) all have been shown to infect OKT4+ T-lymphocytes in vitro as well as in vivo. We investigated the effects of HCMV IE2 gene on HIV and HTLV I promoters in primary lymphocytes, the presumed in vivo target cell. Lymphocytes were co-transfected by electroporation with plasmids containing the HCMV IE2 gene under its own promoter and the HIV I and HTLV I promoters upstream from the chloramphenicol acetyl-transferase gene. The HCMV IE2 gene product stimulated the HIV promoter in contrast to the HTLV I promoter which was significantly down regulated. These types of viral interactions in primary lymphocytes may have important implications for disease expression in the host.

L 215 MOLECULAR INTERACTIONS BETWEEN TAT, TAR AND CELLULAR FACTORS, Anne Gatignol and Kuan-Teh Jeang, Laboratory of Molecular Microbiology, N.I.A.I.D., National Institutes of Health, Bethesda, MD 20892.

The expression of the Human Immunodeficiency Virus (HIV-1) is regulated through multiple pathways including activation by the virally-encoded Tat protein. Tat trans-activates HIV-1 through a target called TAR which is located in the R region of the LTR between nucleotides +1 and +80. Different observations suggest that TAR could be functional as an RNA element and that cellular factors could be involved in Tat-mediated trans-activation.

We have identified cellular proteins that bind to the TAR RNA in nuclear extracts from Tat producing and non-producing cell lines. In RNA gel shift and UV crosslinking experiments, we have characterized 3 proteins that bind to the TAR RNA. One (100 kDa) was found to bind specifically.

A bacterially produced functional Tat protein was tested for its nucleic acid binding properties. We observed binding of this protein to TAR RNA but also to mutated TAR RNA, unrelated RNA and TAR DNA. Using different synthetic peptides, we found that the binding activity was located in the basic amino acid region of Tat between amino acids 37 and 62.

Since trans-activation by Tat is less efficient in mouse cells compared to human cells, we have assayed the binding properties of proteins from different cells to the TAR RNA. Because the binding of Tat alone to TAR RNA does not seem to be functionally relevant, we believe that Tat and cellular proteins act together through TAR RNA in the trans-activation process.

**L 216** THE ROLE OF AP-1 SITES IN VISNA VIRUS TRANSACTIVATION, Susan L. Gdovin [1,3] and Janice E. Clements[2,3], Department of Immunology and Infectious Diseases[1], Department of Molecular Biology and Genetics[2], Division of Comparative Medicine[3], The Johns Hopkins University, Baltimore, MD 21205 Visna virus is an ovine lentivirus that infects cells of the monocyte/macrophage lineage. The virus life cycle is tightly regulated by both cellular and viral factors. Cellular factors present in activated macrophages appear to be important for transcriptional activation of the visna viral LTR. In addition the visna virus promoter is activated, in trans, by a viral protein tat. Our original studies implicated AP-1 sites as possible cis elements in virus-mediated transactivation.

Studies were done to examine the molecular mechanism of action of the visna tat protein and the role of AP-1 sites and other cis-acting sequences. A vero cell line that constitutively expresses visna tat was established. Expression from the visna promoter, as well as heterologous viral promoters, is activated in the visna tat cell line. Promoter deletions identified AP-1 sites as important for this transactivation. The visna tat expresser line also transactivates other AP-1 containing promoters, including cellular promoters that may be involved in pathogenesis.

**L 217** DIMINUTION OF CD4 PROTEIN SYNTHESIS BUT NOT mRNA LEVELS IN MONOCYTES INFECTED BY HIV-1, Romas Geleziunas, Stephan Bour, François Boulerice and Mark A. Wainberg, Department of Microbiology and Immunology, McGill University, and Jewish General Hospital, Montreal, Quebec, Canada, H3T 1E2

The function of the CD4 molecule in human monocytes/macrophages, in contrast to lymphocytes, is not understood. However, this protein serves as a specific receptor for HIV-1 in both cell types. We have analyzed both CD4 mRNA and protein levels in a HIV-1 chronically infected monocytic cell line, U-937, that no longer expressed cell surface CD4. Steady state CD4 mRNA levels were unaltered in these infected cells, in contrast to the decline of CD4 mRNA seen in infected lymphocytes. Neither enhanced viral expression following PMA-induced differentiation of infected U-937 cells, nor replication of highly cytopathic or defective HIV-1 viruses in this cell line affected CD4 mRNA accumulation, despite the consistent absence of cell surface CD4 in each case. In contrast, OKT4-immunoprecipitable CD4 levels were diminished in the infected U-937 population. In addition, OKT4 co-precipitated viral gp120, suggesting that CD4-gp120 complexes are present in infected monocytes as well as lymphocytes. Reduced CD4 protein and CD4-gp120 complex formation are thus responsible for the absence of membrane-associated CD4 in HIV-1-infected U-937 cells, and diminution of CD4 gene expression is not a factor in cell surface depletion of CD4 in these cells. Depletion of cell surface CD4-receptors, which is the basis for retroviral interference, is apparently mediated by mechanisms that differ according to cell lineage.

**L 218** INTEGRATION IS NOT NECESSARY FOR EXPRESSION OF HIV-1 DNA. Sheryl A. Haggerty, Carolyn A. Lamonica, Craig M Meier, Siao-Kun Welch*, Andrzej J. Wasiak, and Mario Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha NE 68105; *Shering Health Ctr, Omaha NE 68114. A common feature in the lifecycle of cytocidal retroviruses, including HIV-1, is the accumulation of large amounts of unintegrated viral DNA. As yet, the role of unintegrated viral DNA in the cytopathogenesis of cytocidal retrovirus infections remains unresolved. Mutants of HIV-1 which were deleted in the integrase/ endonuclease gene and which were unable to establish an integrated form of the virus were constructed. Despite an inability to integrate, these mutants were fully competent templates for HIV-1 antigen production. HIV-1 antigen could be detected in the supernatants of lymphocyte cultures infected with HIV-1 integrase mutants. However, an inability to rescue infectious virus from these cultures indicated that HIV-1 integration was required for production of infectious HIV-1. Based on the ability of unintegrated HIV-1 DNA to serve as a template for HIV-1 antigen production, it is plausible that unintegrated viral DNA can contribute to the HIV-1 antigen pool during HIV-1 replications. This suggests a role for unintegrated DNA in the cytopathogenesis of retroviral infections.

L 219 INTERACTION OF THE HEPATITIS B VIRUS (HBV) WITH THE HUMAN IMMUNODEFICIENCY VIRUS (HIV). Georg Hess, Siegbert Rossol, Rita Voth, Ralph Clemens, Norbert Drees, Klaus Gutfreund, Karl-Hermann Meyer zum Büschenfelde I.Med. Dept. Universitiy of Mainz, 6500 Mainz, Fed. Rep. Germany

The HIV and the HBV are transmitted similarly and are known to interact e.g. via they regulatory proteins. We have followed 452 HIV infected persons. The HIV disease of HBsAg carriers did not progress more rapidy when compared to HBsAg negative persons as analysed by clinical symptoms, number of CD 4 positive cells and p24. Spontaneous loss of viral replication was noted less frequent in HIV infected persons when compared to HIV negative individuals. Similarly, HIV infected persons rarely responded to alpha interferon (1/8) or a combination of alpha interferon and azidothymidine (0/6) given for replicative (HBeAg and HBV-DNA positive) chronic type B hepatitis. The HIV disease remained stable during these four months treatment courses. Moreover, anti-HIV positive individuals (n=13) responded poorly to a hepatitis B vaccine (obtained from Smith Kline Dauelsberg, Munich) while good responses with development of anti-HBs to this vaccine were observed in HIV negative persons at risk for HIV (n=7). Peripheral blood cells were obtained from healthy subjects (n=26) and HIV infected persons (n=141) and stimulated with 6.2µg/ml PHA or 5 µg/ml HBsAg. The in vitro studies indicated that HBsAg activated T cells as assessed by gamma interferon production, analysed by enzyme linked immunoassay. Gamma interferon production was stage dependent less pronounced in HIV infected individuals when compared to healthy controls. In vivo fall of CD 4 positive cells was similar in vaccinated and in unvaccinated HIV infected individuals, indicating that vaccination against hepatitis B was not harmful to HIV infected persons. In summary, there are a multitude of interactions between HIV and HBV that may open new insights into the natural history of both infections. Further studies are required to further define these interactions.

L 220 CHARACTERIZATION OF CYTOKINE GENE EXPRESSION DURING HIV-1 INFECTION OF MONOCYTIC CELLS. John Hiscott, Mario D'Addario, Lucy Sportza, and Mark A. Wainberg, Lady Davis Institute, Jewish General Hospital and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada H3T 1E2

HIV-1 infection of CD4 positive cells of the monocyte/macrophage lineage occurs at high frequency and may contribute to spread of virus to peripheral tissues. To determine whether chronic infection of monocytic cells by HIV-1 leads to dysregulation of cytokine gene expression, the production of interferon and cytokine mRNA production was characterized in HIV chronically infected U937 cells (U9-IIIB) by PCR mRNA phenotyping. Opposing primers corresponding to different IFN-$\alpha$ subtypes, IFN-$\beta$, TNF-$\alpha$ and various interleukins were used to measure cytokine transcription in uninfected U937, chronically infected U9-IIIB, and U9-IIIB superinfected with Sendai virus. PCR amplification detected IFN-$\alpha$1, -$\alpha$2, -$\beta$, IL-1$\beta$, IL-6 and TNF-$\alpha$ mRNA induction by Sendai virus infection of U937. Infection of U9-IIIB with Sendai virus had the effect of superinducing RNA production from several cytokine genes including TNF-$\alpha$, IL-1$\beta$, IL-6 and IFN-$\beta$ suggesting that HIV-1 infection had the effect of priming cells for cytokine expression. To examine the kinetics of cytokine gene activation, the progressive spread of HIV through the cell population was monitored by p24 antigen analysis and Northern blot analysis of HIV-1 RNA. After a refractive period of 10-13 days, virus spread through the U937 population was detectable, coincident with activation of multiple cytokines. These experiments indicate that unscheduled cytokine gene expression in monocytic cells may contribute to virus pathogenesis by restricting viral multiplication, leading to persistent infection.

L 221 **MOLECULAR CLONING AND CHARACTERIZATION OF HIV ISOLATES WITH DISTINCT IN-VITRO BIOLOGICAL PROPERTIES. J.G.Huisman, M.Groenink, R. Fouchier, R.E.Y de Goede and M.Tersmette. Centr.Lab of the Netherlands Red Cross Blood Transfusion Service,Lab for Exp and Clin Immunology,Univ. of Amsterdam,The Netherlands.**

**HIV isolates posses different biological properties in terms of their replication rate, syncytium-inducing (SI) capacity and host range. However, little is known of the variation at the molecular level responsible for the observed phenotypical differences. Therefore, HIV isolates obtained from an individual who developed AIDS within 6 months after seroconversion were used for molecular cloning. Xba-DNA fragments of 10 - 20 kB obtained from HMW DNA from infected cells were inserted into lambda GEM-11 arms. After several rounds of cloning, subcloning was carried out in XbaI sites of pUC19. Sofar 46 molecular clones have been obtained. Five out of seven molecular clones, obtained from a single time point, tested thusfar appeared to be infectious and included both the syncytium-inducing and the non-syncytium inducing phenotypes. Data on the characterization of these molecular clones in relation to their biological properties will be presented.**

L 222 DOWN-REGULATION OF HLA CLASS I ANTIGENS IN HIV INFECTED CELLS, Thomas Kerkau, Christian Kneitz, Renate Schmitt-Landgraf, Anneliese Schimpl, Eberhard Wecker, Institute of Virology and Immunbiology, University of Würzburg

By means of indirect immunofluorescence analysis we investigated the effect of HIV 1 infection of HLA class I surface antigens. We report here that in CD $4^+$ HeLa cells, in H 9 cells and in peripheral T lymphocytes HLA class I antigens are down-regulated following infection with HIV 1. The down-regulation is effected at a post-transcriptional level since the amounts of HLA class I specific mRNA are similar in infected and uninfected cells. This phenomenon is not only correlated with the state of infection, i.e. the presence of p 24 of HIV 1 in the cells, but also depends on the time of infection. Upon HLA class I down-regulation by HIV infection, the specific lysis of peripheral blood cells by allogenic CTL is reduced.

L 223 FACTORS AFFECTING CELLULAR TROPISM OF HIV.

Sunyoung Kim, Kenji Ikeuchi*, Jerome Groopman*, and David Baltimore.
Whitehead Institute, and Department of Biology, MIT; *New England Deaconess Hospital and Harvard Medical School.

Certain strains of HIV grow well in T cells but poorly in monocytes/macrophages, while others exhibit an opposite growth pattern. As a model system, we compared the growth of a well-characterized HIV strain in the T-lymphocyte and monocyte cell lines, H9 and U937 respectively. Although we found that U937 cells had a higher percentage of CD4-positive cells than H9 cells, our HIV strain grew much less efficiently in the monocytic cell line. Surprisingly, we found that viral tropism was primarily determined during the early stages of the virus cycle; that is, sometime between binding of the virus to the cell surface and reverse transcription of viral genomic RNA. Once the virus entered the host cell, reverse transcription, use of the LTR, RNA expression and production of virus particles seemed to be as efficient in U937 cells as in H9 cells.

L 224 ALTERATION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION IN THE DEVELOPING HUMAN FETAL PERIPHERAL NERVOUS SYSTEM: A POTENTIAL MECHANISM OF NEUROTOXICITY IN HIV-ASSOCIATED NEUROLOGIC DYSFUNCTION, Beth Boyer Kollas and Brian Wigdahl, Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.
Recent studies have demonstrated the presence of products potentially secreted from activated or HIV-infected cells in the cerebrospinal fluid of patients infected with HIV. It has been proposed that a variety of cytokines may be involved in the neuropathological dysfunction observed in HIV-infected individuals. Although previous studies by other investigators have demonstrated a low level of MHC class I and II expression in the human nervous system, treatment of neural cell populations with interferon (IFN) or infection with neurotropic viruses induces the expression of MHC class I and II antigens potentially allowing these cells to participate in an immune response. In the present studies, we have examined human fetal dorsal root ganglia (DRG) cells for MHC class I and II antigen expression by fluorescence-activated flow cytometric analysis and have demonstrated that between 59 and 87% of human fetal DRG neural cells expressed detectable levels of MHC class I antigen while between 13 and 26% expressed class II antigen. In addition, RNA hybridization analyses have demonstrated detectable levels of MHC class I- and II-specific RNA in total RNA isolated from human fetal DRG neural cell populations. Additional studies examining the effects of cytokines on MHC antigen expression have shown that treatment of human fetal DRG neural cell populations with $\gamma$-IFN or $\alpha$-IFN altered the levels of MHC class I- and II-specific RNA and cell surface MHC antigen expression. These studies suggest that cytokines such as $\gamma$-IFN and $\alpha$-IFN may alter the ability of specific neural cell populations present in the developing human nervous system to participate in immune reactions by modulation of MHC class I and II antigen expression.

**L 225** HIV-INFECTED MACROPHAGES RESIST SUPERINFECTION BY VESICULAR STOMATITIS VIRUS (VSV) BUT DO NOT PRODUCE INTERFERON: EFFECTS OF 2-AMINOPURINE. Richard S. Kornbluth and Douglas D. Richman, Infectious Diseases, Univ. Calif. San Diego and the V. A. Medical Center, San Diego, CA 92161

Monocyte-derived macrophages (MDM) can be protected from lytic infection with VSV in 48 h by 18 h pretreatment with IFN-$\alpha$, -$\beta$, or -$\gamma$. Surprisingly, MDM infected with HTLV-III$_{B9\text{-}L/85}$ resisted lysis by VSV even without IFN treatment. By virus yield assay, VSV failed to replicate in HIV-infected MDM whereas supernatants from uninfected cells contained $10^{2.7}$ $TCID_{50}$ of VSV. No IFN, however, was detectable in the supernatants of these HIV-infected MDM using a VSV/MDBK cell assay. Furthermore, in the presence of AZT, 18 hour pre-incubation with supernatants from HIV-infected MDM failed to protect uninfected MDM from VSV, suggesting that cellular infection is required for this effect.

Since the interferon-inducible PI/eIF-2$\alpha$ protein kinase is believed to be essential for the anti-VSV effects of interferons, we tested the effects of 2-aminopurine (2-AP), an inhibitor of this kinase, in this system. At a dose 1 mM, 2-AP abrogated the protective effect of HIV infection on VSV-induced cell lysis. Furthermore, although doses of 1-10 mM were minimally toxic to uninfected MDM, 2-AP accelerated the ballooning degeneration and cellular death of HIV-induced multinucleated giant cells. Cultures of HIV-infected MDM that survived a 24 hour pretreatment with 10 mM 2-AP released only 32% of the p24 as did untreated cultures.

Taken together, these results suggest that the PI/eIF-2$\alpha$ protein kinase may be functionally altered by HIV infection, in the absence of interferon production. Since *TAR* RNA has been shown to activate this kinase *in vitro*, this experimental system may provide a means to examine the functional consequences HIV infection on the regulation of this enzyme.

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**L 226** ANALYSIS OF A NONPRODUCTIVE HIV-1 INFECTION OF HUMAN FETAL DORSAL ROOT GANGLIA GLIAL CELLS IN VITRO, Charles Kunsch and Brian Wigdahl, Dept. of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Direct infection of glial cells by HIV-1 has been implicated as one of several potential mechanisms responsible for the severe neurologic complications observed in both neonates and adults with the acquired immunodeficiency syndrome. Previously, we have shown that HIV-1 infection of primary human fetal dorsal root ganglia (DRG) neural cells results in HIV-1 gag antigen expression in approximately 70% of the glial cell subpopulation. The expression of HIV-1 gag antigens and viral mRNA reaches a maximum by 2-3 days postinfection and declines thereafter to minimally detectable levels. Polymerase chain reaction amplification in conjunction with DNA blot hybridization analyses of DNA isolated from HIV-1-infected DRG neural cell populations demonstrates the retention of HIV-1 proviral DNA in the infected cell population. However, infection of the fetal DRG neural cells appears to be abortive or nonproductive with little, if any, infectious progeny virus production. Furthermore, protein immunoblot analysis of HIV-1-infected DRG neural cell extracts demonstrated that although considerable quantities of gag antigens (both precursor and processed forms) are expressed in the neural cell population, no env gene products (gp41 and gp120) were detected, implying selective genomic expression in the DRG neural cell population. In addition, although transmission electron microscopic analysis has suggested the absence of intracellular viral particles, highly electron-dense cytoplasmic inclusions were observed in the HIV-1-infected DRG glial cells which appear similar in nature to gag-reactive cytoplasmic inclusions seen by immunofluorescence analysis. These results suggest that incomplete or selective genomic expression may account for the cytoplasmic accumulation of HIV-1 structural proteins without assembly and release of mature virus. The mechanism accounting for the nonproductive HIV-1 infection of human fetal DRG glial cells is currently under investigation.

**L 227** DIFFERENTIAL ACTIVATION OF NF-$\kappa$B BINDING AND HIV GENE TRANSCRIPTION BY TNF-$\alpha$ AND HTLV-1 TAX PROTEIN, Judith Lacoste, Jean François Leblanc, Lucie Cohen, Steven Xanthoudakis and John Hiscott, Lady Davis Institute, Jewish General Hospital and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada H3T 1E2

Inducible binding of the NF-$\kappa$B transcription factor and transcriptional activation of the HIV-1 enhancer and the PRDII domain of the IFN-$\beta$ promoter has been examined in different hematopoietic cell lines and in T lymphoid cells expressing the HTLV-1 Tax protein. To explore the role of TNF-$\alpha$ in the activation of the HIV-LTR cells were transfected with CAT constructs composed of different domains of the HIV-LTR or IFN-$\beta$ promoter. TNF-$\alpha$ treatment enhanced transcription of the transfected plasmids containing the HIV-LTR, the HIV enhancer element or the PRDII domain but not the IFN-$\beta$ promoter in myeloid and lymphoid cells; this enhancement correlated with TNF-$\alpha$ induced binding of NF-$\kappa$B like factors to the HIV enhancer. Co-transfection of these plasmids with a HTLV-1 Tax expressing plasmid constitutively stimulated expression of the reportor plasmids but to differing degrees. The HIV enhancer and PRDII containing plasmids were trans-activated 50-100 fold, while the HIV-LTR was trans-activated 3-4 fold; the IFN-$\beta$ promoter was not trans-activated by the Tax protein. In Jurkat cells stably expressing the Tax protein, binding of NF-$\kappa$B to the HIV enhancer was increased about 5 fold when compared to non-expressing Jurkat cells; in both cell types, NF-$\kappa$B factor binding and reporter gene activity were still inducible by PMA treatment. By UV-crosslinking and DNA affinity chromatography three proteins have been identified which interact with the PRDII domain and cross-react with the HIV enhancer. The role of these proteins in differential activation of the HIV enhancer is currently under investigation.

**L 228** SYNTHETIC INHIBITORS OF HIV-1 PROTEASE INHIBIT MATURATION AND INFECTIVITY OF VIRIONS FROM CHRONICALLY INFECTED T-CELLS. D. M. Lambert, J. Leary, T. J. Matthews, T. D. Meek, G. B. Dreyer, T. Hart, B. W. Metcalf and S. R. Petteway, Jr., Departments of Antiinfectives, Medicinal Chemistry and Ultrastructural Pathology, Smith Kline & French Laboratories, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406; Department of Surgery, Duke University Medical School, Durham, NC 27710.

Proteolytic processing of the gag and gag-pol polyprotein precursors of HIV-1 into functional virion proteins and enzymes is accomplished by the HIV-1 protease. Inhibition of this protease therefore comprises a potential target for anti-retroviral therapy. We have analyzed the effects of synthetic peptide analogues, which are inhibitors of HIV-1 protease in vitro, on processing of virion polyproteins within a T-lymphocyte culture chronically infected with HIV-1. Using Western blot and immune precipitation techniques, we have shown that purified virions from treated cell cultures, in contrast to virions from untreated cultures, contained $Pr55^{gag}$ in addition to processing intermediates of $Pr55^{gag}$. A corresponding diminution of the mature protein products p24 and p17 was seen. Moreover, electron microscopy revealed that virtually all of the virion particles produced from the inhibitor-treated cells were of an aberrant morphology correlating with the molecular effects due to inhibition of polyprotein processing. Virions from treated chronically-infected cells had a reduced capacity to infect susceptible T-cells. In addition, acute infection of susceptible T-cells was greatly reduced or completely blocked during treatment with these protease inhibitors.

**L 229** REGULATION OF HIV-1 TRANSCRIPTION AT MULTIPLE LEVELS: EFFECTS OF HIV-1 TAT AND ADENOVIRUS E1A ON TRANSCRIPTIONAL INITIATION AND ELONGATION, Michael F. Laspia, Andrew P. Rice and Michael B. Mathews, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

We have characterized regulation of HIV-1 transcription by Tat using a recombinant adenovirus vector containing an HIV-1 LTR-promoted CAT gene. For comparative purposes we also studied regulation of HIV-1 transcription by a general transcriptional activator, the adenovirus E1A protein. In the absence of transactivators, two classes of correctly initiated cytoplasmic RNAs were detected, one class extending into the CAT gene and another class ending 55 and 59 nucleotides from the site of initiation. Both Tat and E1A increased the level of full length RNA more than 100-fold. The level of the short RNAs was not increased by Tat but was increased by E1A. These results suggest that both Tat and E1A act primarily to increase the level of HIV-1 transcription. Since the short transcripts were not increased by Tat, but were by E1A, it appears that Tat is not acting solely to increase initiation. However, because the total amount of RNA was increased by Tat, it is unlikely that Tat acts only to suppress termination.

Measurement of RNA synthesis rates by nuclear run-on assay indicated that Tat produced a large increase in transcription (15-fold) in the immediate vicinity of the promoter, as did E1A. In the absence of transactivators, transcription exhibited a marked polarity, with RNA polymerase density declining sharply as a function of distance from the promoter. Tat partially suppressed transcriptional polarity while E1A did not. Mutations in the TAR element did not influence basal transcription rates or the response to E1A but eliminated the Tat response. Thus, Tat appears to increase RNA polymerase density in the promoter proximal region by increasing transcription initiation, and, in addition, reduces transcription polarity by stabilizing elongation. On the other hand, the general transactivator E1A acts to increase transcriptional initiation but does not stabilize elongation. We propose that Tat acts through TAR to regulate transcription at two levels, by increasing initiation complex formation and by stabilizing elongating complexes.

**L 230** MOLECULAR CLONING OF FULL-LENGTH HIV-1 PROVIRUSES FROM UNCULTURED BRAIN TISSUE OF A PATIENT WITH AIDS DEMENTIA COMPLEX. Yuexia Li[1], Richard W. Price[2], George M. Shaw[1], and Beatrice H. Hahn[1]. [1]University of Alabama at Birmingham, Birmingham, AL 35294, [2]University of Minnesota, Minneapolis, MN.

The pathogenic mechanisms responsible for AIDS Dementia Complex (ADC) are poorly understood. In order to study HIV infection of the brain on a molecular level, we cloned HIV-1 proviral sequences directly from uncultured brain tissue obtained from a patient with ADC. Screening approximately $8 \times 10^6$ lambda phage recombinants, we isolated a total of 10 HIV-1 clones, nine of which represented unintegrated, circular HIV-1 proviruses cloned in their permuted form. The other clone represented the 5' half of an integrated provirus flanked by cellular sequences. Restriction enzyme analysis of the permuted circular forms identified four distinct species of cloned HIV-1 inserts: Three recombinant clones contained a full-length HIV-1 provirus with a single copy of the long terminal repeat (LTR); one clone contained a full-length HIV-1 provirus with two tandem LTRs; three clones represented defective proviral genomes with various deletions in *gag, pol* or *env* genes; and two clones contained inverted LTR sequences within their *gag* and *pol* genes. The boundaries of these self-integrated LTRs were confirmed by sequence analysis, which revealed the presence of an inverted, but intact LTR in one clone and a deleted LTR (9 bps) in the other clone. From these data we conclude (i) that a substantial portion of proviral sequences in the brain is defective, (ii) that unintegrated as well as integrated proviruses exist in the brain, and (iii) that unintegrated HIV-1 circles exist *in vivo* both with one and two LTRs. Our studies also show, for the first time, that replicative intermediates of HIV-1 can self-integrate and that this happens relatively frequently in HIV-1 infected brains.

**L 231** REPRESSION OF THE TAT FUNCTION THROUGH MULTIPLE TAR RNA SEQUENCES, Julianna Lisziewicz[1], Jay F. Rappaport[2] and Ravi Dhar[1], [1]Laboratory of Molecular Virology, NIH, NCI, Bethesda, MD; [2]Laboratory of Tumor Cell Biology, NIH, NCI, Bethesda, MD 20892. Tat is a powerful trans-activator of HIV gene expression and is essential for viral propagation. Tat stimulates gene expression via a target responsible (TAR) sequence located within the HIV LTR. Trans-activation by tat can be inhibited at the molecular level using transdominant inhibitors of tat function. Another method of inhibition is to use multiple target sequences that can soak up trans-activators. To demonstrate this we constructed plasmids containing multiple copies of TAR sequences in tandem under the control of HIV LTR. Our results show that multiple TAR RNA sequences are capable of downregulating trans-activation and this inhibition is proportioned to the number of TAR sequences expressed. The construct containing seven copies of TAR RNA sequences in tandem cotransfected with LTR-CAT downregulates CAT expression by 90% in COS cells. In a cotransfection assay, multiple TAR DNA sequences or LTR alone showed no significant effect on CAT expression, thus, showing the requirement for transcription of these sequences into RNA.

HIV LTR expresses low levels of viral message in the abscence of tat. The trans-activation of HIV LTR can be diminished with these multiple TAR sequences, therefore, this can be utilized for gene therapy.

**L 232 THE ROLE OF DNA BINDING MOTIFS IN TRANSACTIVATION OF THE HIV LTR BY HSV-1.** David M. Margolis, Carmen Parrott, John Leonard, Arnold B. Rabson, Jeffrey M. Ostrove, Laboratories of Clinical Investigation and Molecular Microbiology, NIAID, NIH, Bethesda, MD 20892.

As DNA binding motifs are thought to play an important role in the molecular mechanism of transactivation of the HIV LTR by HSV-1, we constructed plasmids carrying the HIV LTR with deletions of NF-kB and/or Sp1 binding sites, linked to a CAT indicator gene. These constructs were transfected into Vero or A3.01 cells in combination with plasmids carrying the HIV tat gene and/or the HSV-1 immediate early genes ICP4 or ICP0. In addition some cells were infected with HSV-1 KOS strain. We found that deletion of the NF-kB regions did not significantly alter the activation of the HIV LTR by tat, ICP4, ICP0, or HSV-1, while deletion of the Sp1 regions caused a slight but consistent decrement in LTR activation. Deletion of both sites, however, abrogated the ability to activate the LTR. This may indicate a gross transcriptional defect, as this mutant has recently been shown to be replication incompetent (Leonard et al. J. Virol. 63:4919, 1989). The NF-kB and Sp1 sites alone do not appear to play an essential role in the mechanism of transactivation of the HIV LTR by HSV-1.

**L 233** DIFFERENTIAL REGULATION OF HUMAN IMMUNODEFICIENCY VIRUSES: A NOVEL HIV-2 REGULATORY ELEMENT RESPONDS TO T CELL RECEPTOR STIMULATION. D.M. Markovitz, A. Freedman, and G.J. Nabel. Departments of Internal Medicine and Biological Chemistry and the Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor,MI, and St George's Hospital, London, England

Although it is a distinct retrovirus, the human immunodeficiency virus type 2 (HIV-2) shares nucleic acid and protein similarity to HIV-1. First described in West Africa, HIV-2 is also a causative agent of the acquired immunodeficiency syndrome (AIDS), and has begun to appear throughout the world. Although the enhancer regions of HIV-1 and HIV-2 have some elements in common, it was unknown whether these immundeficiency viruses display different transcriptional regulation. In this study, the cis-acting sequences in HIV-2 which respond to T cell activation have been analyzed. We report that the regulation of the HIV-2 enhancer differs from HIV-1: activation of the HIV-2 enhancer is partly mediated by an upstream site not found in HIV-1. In contrast to HIV-1, HIV-2 contains a novel regulatory element, termed CD3R, that is responsive to stimulation of the T cell receptor complex. This site also contributes to HIV-2 activation by phorbol myristate acetate (PMA) and phytohemagglutinin (PHA). In contrast, HIV-1 is minimally responsive to stimulation of the T cell receptor complex, and stimulation by PMA and PHA is mediated solely by κB, the major regulatory element of its enhancer. These findings suggest that a nuclear factor responsive to triggering of CD3, NF-CD3R, contributes to HIV-2 induction, and raise the possibility that different co-factors may determine the latency of HIV-1 and HIV-2 associated AIDS.

**L 234** NON-CYTOPATHIC INFECTION OF NORMAL CD8+ LYMPHOCYTES CONCOMITTANT WITH THE ACCUMULATION OF UNINTEGRATED HIV-1 DNA, Louis Mercure, Alla Lvovich, Denis Phaneuf and Mark A. Wainberg, Lady Davis Institute - Jewish General Hospital and Department of Infectious Diseases, Université de Montréal

Although the cellular tropism of HIV-1 is dependent on the expression of CD4 molecules at the surface of target cells, it is controversial whether CD4 may always be necessary for infection. There is conflicting evidence as to whether CD8+ lymphocytes can be infected by HIV-1. To distinguish between direct infection of CD8+ lymphocytes and cell-to-cell transmission, we have first purified CD8+ cells from normal PBMCs which were stimulated for 3 days with PHA, and then co-incubated them with HIV-1. In a second protocol, we have infected PBMCs with the $III_B$ strain of HIV-1, after 3 days of exposure to PHA. Twenty days later, CD8+ cells were sorted and purified by a biomagnetic system using Leu 2a MAbs. Purity of CD8+ lymphocytes by flow cytometry was 98-99%. In both cases, CD8+ cultures were maintained for up to two months without any apparent cytopathic effect. The production of viral particles was demonstrated by reverse transcriptase activity. The presence of proviral and unintegrated viral DNA (UVD) was evaluated in CD8+ cultures by PCR using specific *env* primers. Infection of CD8+ cells was also determined at the single cell level with both double immunofluorescence and double flow cytometric analysis, using Leu 2A MAbs, anti p24 MAbs and a partially purified pool of sera from seropositive patients. By each method, the data show that normal CD8+ lymphocytes can be productively infected by HIV-1. Despite the presence of UVD, CD8+ cells remained non-susceptible to cytopathic effects.

**L 235** **Differentiation of THP-1 cells induced by PMA renders them permissive for a macrophage-tropic strain of HIV-1.** Pascal R. Meylan, Richard S. Kornbluth, Celsa A. Spina and Douglas D. Richman. Departments of Medicine and Pathology, University of California, San Diego, and Veterans Administration Medical Center, San Diego, CA 92161.

HIV isolates vary in their ability to replicate in different cell types. However, the relationship between any particular cell tropism and the pathogenic role of HIV is not clear. Investigations on macrophage-tropic strains of HIV-1 are hampered by the unavailability of a cell line susceptible to infection with such strains. For instance, the prototypic macrophage-tropic isolate $HTLV\text{-}III_{Ba\text{-}L/85}$ is unable to infect productively the monocytoid cell lines such as U 937, THP-1 or MonoMac 6. We have observed that as the THP-1 cells differentiated toward a macrophage-like phenotype after phorbol myristate acetate (PMA) stimulation, they became permissive for $HTLV\text{-}III_{Ba\text{-}L/85}$ replication. $10^{-8}$ M PMA proved to be the optimal concentration to induce permissiveness for replication of this macrophage-tropic strain of HIV-1. Two weeks after infection, the supernatants of PMA-stimulated cells contained more than 400 fold more p24 antigen (137,200 pg/ml) than those of unstimulated cells (290 pg/ml). This permissiveness to the $HTLV\text{-}III_{Ba\text{-}L/85}$ strain did not develop in the THP-1 cells until 4 days after PMA stimulation. FACS analysis demonstrated that permissiveness appeared despite a downregulation of surface CD4 antigen expression. PMA did not render U 937 cells for the $HTLV\text{-}III_{Ba\text{-}L/85}$ strain in parallel experiments. Experiments are now underway to determine with polymerase chain reaction whether provirus is formed inside undifferentiated THP-1 cells or whether the virus life cycle is aborted at an earlier step. This experimental system seems appropriate to further delineate the viral and cellular determinants underlying macrophage tropism.

**L 236** ESTABLISHMENT OF TRUE HIV-1 LATENCY BY INFECTION OF THP-1 MONOCYTES Judy A. Mikovits, Martin Ruta and Francis W. Ruscetti, Biological Carcinogenesis Development Program, PRI and Laboratory of Molec. Immunoreg., BRMP, NCI-FCRF, Frederick, MD., and Division of Virol., CBE, Bethesda, MD.

The cellular and molecular mechanism(s) underlying the long clinical latency following infection by the human immunodeficiency virus (HIV) is not well understood. The existence of true HIV latency (i.e. complete absence of viral expression) has been suggested but not proven. In studying the role of the monocyte in HIV persistence, a human monocytoid cell line, THP-1, was infected with several isolates of HIV-1 at a low MOI. Initially, all infections were productive. On continuous passage, several cultures showed no extracellular viral production. Southern analysis of the DNA from these cultures revealed the preseence of HIV DNA. However, no viral RNA, proteins or virions could be detected. PCR analysis using primer pairs to the gag, env, tat and nef region of HIV-1 failed to detect viral RNA. Agents such as LPS, PMA, TNF and GM-CSF which can augment HIV-1 expression in chronic low level producers of THP-1, U-937 and primary monocytes failed to stimulate viral expression in cells with latent virus. Since previous work showed that the transfected HIV-LTR was methylated, 5-Azacytidine (10 uM) was used to reactivate viral expression in these cells even after 12 months of culture. Co-culture of these activated cells with Hut-102 produced a massive infection in ten days. This virus retained the morphological, biological, and biochemical characteristics of the original strain. Latency was seen with T-cell and monocytotropic strains. After induction by 5-Azacytidine, viral expression could be enhanced by LPS, PMA and TNF showing that true latency is distinct from chronic low-level expression.

**L 237** INFECTION OF NEW WORLD MONKEY T CELL LINES BY HIV AND SIV. Carel Mulder, James E. Monroe, Eva Szomolanyi, David DeGrand, and Richard Nelson, Departments of Pharmacology and Molecular Genetics & Microbiology, University of Massachusetts Medical School, Worcester, MA 01655, USA.
No $CD4^+$ established T cell lines from Old World Primates are available for SIV research. Therefore, we tested several T cell lines from New World Monkeys for the presence of CD4 and for their ability to be infected by HIV's and SIV's. These cell lines were established by immortalizing in vitro peripheral blood lymphocytes from cottontopped Tamarins and common Marmoset with Herpesvirus ateles or H.saimiri. All cell lines were found to be CD8 positive by antigen and mRNA tests; 7/9 lines were also CD4 mRNA positive although only about half expressed detectable levels of CD4 antigen. These seven T cell lines could be infected productively with most or all of HIV-1, HIV-2 and $SIV_{Mac}$. In contrast, $SIV_{Mnd}$ and $SIV_{AGM}$ could not infect any of these cells. Most of these cell lines were persistently infected with the lentiviruses. After prolonged passage in at least one of these Tamarin cell lines, $SIV_{Mac}$ (originally carried in human cell lines) acquired a considerably altered hostrange. For use in animal experiments, virus recovered from these persistently infected monkey T cell lines might possibly be more appropriate than virus propagated in human cells. Molecular and biological differences of $SIV_{Mac}$ grown on human or Tamarin cell lines will be presented.

**L 238** **POSSIBLE ASSOCIATION BETWEEN HIV INFECTION IN MONOCYTES AND THE MONOCYTE CELL SUFRACE ANTIGENS, CD13 AND CD14.** **Nicholson, J.K.A., Sasso, D.R., and Melendez, L.M. Centers for Disease Control, Atlanta, GA 30333.**
**Monocytes/macrophages (MO) can be infected with human immunodeficiency virus (HIV) in vitro, however, the requirements for this infection are not well-understood. CD4, which is found in low density on the MO surface is involved in infection of monocytes, however, the role of other molecules involved in virus entry and release is not known. We examined the role of CD13 and CD14 molecules (found on mature MO) in HIV binding to MO and in vitro infection of MO with HIV. In addition, we monitored expression of these surface antigens on HIV-infected monocytes in culture. We found that the expression of CD14 is decreased in HIV-infected MO cultures. Monoclonal antibodies to CD13 (MY7) and CD14 (MY4, LeuM3) were able to partially inhibit HIV binding to MO. To determine whether these MoAb have an effect on infection of MO by HIV, we inoculated MO with HIV in the presence or absence of anti-CD13 or -14, then monitored the cultures for viral antigens in an antigen-capture ELISA. We found that anti-CD13 suppressed infection of monocytes in a dose-dependent manner. Experiments are in progress to further evaluate the role of CD13 and CD14 in in vitro HIV infection of MO.**

**L 239** RELATIONSHIP BETWEEN THE kB SITES IN THE HIV AND SV40 TRANSCRIPTIONAL ENHANCERS, William Phares, Robert Franza and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. The sequence homology between the kB sites present in the enhancer core of the HIV LTR and in the C element of the SV40 enhancer has been examined. Unlike other sequences in the HIV LTR, the kB sites will promote SV40 replication in a permissive monkey cell line, and in an orientation-independent manner. The two HIV kB sites, as well as the wild type SV40 C16 element and several well characterized mutations, were tested in a number of cells lines in transient assays for transcriptional enhancing activity. Results indicated a broad distribution of activity, and in addition an inducible activity in certain lymphoblast cells. Extracts of stimulated Jurkat cells were analyzed for those proteins which specifically associate with each of the sites tested for enhancer function.

**L 240** THE ROLE OF INTERFERONS AND CYTOKINES IN HIV INFECTION AND AIDS SYNDROME, Paula M. Pitha, Waldemar Popik, and Morella Rodriguez-Ortega, The Johns Hopkins University School of Medicine Oncology Center, Baltimore, MD 21205
We have recently shown that human immunodeficiency virus (HIV) replication in vitro could be inhibited but not aborted by treatment with both α- and β-interferons. However, in cells in which high levels of α- and β-interferons could be induced by HIV infection, HIV replication was completely aborted. To examine the basis of the differential effect of exogenous and endogenous interferon on HIV replication, we constructed cell lines expressing the mature α-interferon that is not released from these cells, and studied the HIV replication in these cell lines. In addition, the effect of exogenous and endogenous interferon on the tat, HSV-1, or NFκB-induced trans-activation of HIV transcription was examined. The results of these studies will be discussed.

**L 241** HIV-1 CORE PROTEIN PRECURSORS MADE FROM A SV40 LATE REPLACEMENT VECTOR ARE PROPERLY PROCESSED AND ASSEMBLED INTO VIRUS-LIKE PARTICLES. David Rekosh, Alan J. Smith and Marie-Louise Hammarskjöld, Departments of Microbiology and Biochemistry, University at Buffalo, Buffalo, NY 14214
The expression of the HIV-1 *gag* and *pol* genes has been studied using fragments of the BH10 clone of HIV inserted into a SV40 late replacement vector. An initial construct containing the entire coding regions of *gag, pol* and *vif* produced only minute amounts of the gag precursor (p55). However, high level expression was obtained when an additional sequence from the *env* gene (the rev-responsive element) was inserted 3' of *vif* in the correct orientation, and rev was provided in trans from a second vector. Western blot analysis of transfected cells showed the presence of large amounts of both gag and gag-pol precursors as well as all of the expected cleavage products. Electron microscopy of thin sections of transfected cells showed a multitude of virus-like particles. Both immature particles in the process of budding and particles containing the condensed core characteristic of HIV were observed. Analysis of the released virus-like particles showed the presence of reverse transcriptase. Sucrose gradient analysis of particles produced from $^{3}$H-uridine labeled cells indicated a peak of radioactivity which co-sedimented with a peak of p24, suggesting that the particles contained RNA.

**L 242** HTLV-IIIb DNA SYNTHESIS IN T-CELLS, H.L. Robinson and D.M. Zinkus, Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655
HTLV-IIIb DNA synthesis has been followed in a synchronous active infection in 8166 cells in the presence and absence of superinfection. By 48 hours after infection, cultures undergoing active superinfection contained 75 to 100 copies whereas those grown in the absence of superinfection contained only 3 to 4 copies of HTLV-IIIb DNA. In a single cycle infection, a MOI of 0.5 produced 1.5 copies of linear DNA by 12 hours after infection and 0.7 copies of proviral DNA by 24 hours after infection. We conclude that the accumulation of viral DNA in T-cells results from superinfection and that HTLV-IIIb DNA synthesis is similar to that observed for C-type retroviral infections.

**L 243** THIOLS INHIBIT HIV REPLICATION IN AN IN VITRO MODEL SYSTEM: POTENTIAL THERAPEUTIC USE OF N-ACETYL CYSTEINE. Mario Roederer, Frank J. T. Staal, Paul A. Raju, Monty A. Montano, Leonore A. Herzenberg, and Leonard A. Herzenberg, Department of Genetics, Stanford University, Stanford, CA.

Glutathione (GSH) plays important roles in many cellular processes, including maintenance of a reduced state of small molecules and proteins as well as protecting cells against peroxides, free radicals, and other reactive oxygen intermediates (ROIs). GSH levels are important in many lymphocyte functions, and are crucial in T cell activation. Reduced GSH levels in unfractionated leukocytes and blood plasma have recently been correlated with the progression of AIDS (Eck et al, *Biol. Chem. Hoppe-Seyler*, **370**, 101-108, 1989). Decreased GSH levels are seen first in HIV-positive asymptomatic patients, and further decrease in ARC and later stages of AIDS. Levels of Tumor Necrosis Factor α (TNF-α) are also increased in AIDS patients.

TNF-α has many postulated activities, including upregulation of HIV production via activation of an enhancer site in its promoter, and induction of the "oxidative burst," which results in the production of peroxides, free radicals, and other ROIs. Since GSH is the primary protective agent against ROIs, and based on our postulation that the stimulation of HIV is dependent upon production of ROIs, we used thiols such as N-acetyl cysteine (NAC) to try to counteract the effect of TNF-α on HIV. Using cell lines with stably integrated HIV LTR-*lacZ* fusions, we found that the stimulation by TNF-α or PMA can indeed be blocked by addition NAC. Additionally, NAC inhibited the production of HIV in acutely infected Molt4 cells. These data provide a rationale for the treatment of AIDS with GSH-replenishing drugs such as NAC. NAC has a long history of therapeutic uses in people with no known toxic side effects.

**L 244** CHRONIC LOW LEVEL HIV-1 EXPRESSION IN MONOCYTES INVOLVES NEGATIVE REGULATION OF VIRAL TRANSCRIPTION, Francis W. Ruscetti, Judy A. Mikovits, Nancy C. Lohrey and Raziuddin, Laboratory of Molecular Immunoregulation, BRMP, NCI-FCRF and Biological Carcinogenesis Development Program, PRI, NCI-FCRF, Frederick, MD.

The mechanism(s) of the human immunodeficiency viruses' (HIV) persistence in the host are not well understood. Previous work involving animal and human lentiviruses suggests that the monocyte/macrophage plays a major role in HIV persistence. To study this, a human monocytoid leukemic cell line, THP-1, has been infected with several isolates of HIV-1 and HIV-2. Initially, all infections were productive with HIV-1 titers much higher than HIV-2. On continuous passage, several cultures showed markedly reduced levels of extracellular viral production by several parameters. This chronic low level (restricted) expression has been observed by others in U-937 cells and primary monocytes. This restricted phenotype was seen with several viral isolates, stable over several months and related to the low MOI of the initial infection. Failure to detect to much extracellular virus was associated with intracytoplasmic sequestration of virions and lower levels of viral RNA. Levels of 9.1 Kb genomic RNA were much reduced and a novel 7.5 Kb RNA detected. In LTR-directed nuclear run-off assays, components of nuclei from cells with restricted HIV expression negatively regulated viral transcription. Using gel shift assays, nuclear extracts from these cells completely blocked tat-associated DNA binding complex formation to the enhancer-tar region suggesting that initiation of transcription was negatively regulated. Thus, in monocytes with restricted HIV expression, viral production is negatively regulated at multiple levels either by virally induced cellular or viral factor(s).

**L 245 INDUCTION OF HIV IN MACROPHAGES IN THE CONTEXT OF T-CELL ACTIVATION,** Rachel D. Schrier, Allen McCutchan, Jay A. Nelson, and Clayton A. Wiley. University of California, San Diego and Scripps Clinic, La Jolla California

Macrophages are major reservoirs of HIV in the tissues of infected humans. As monocytes in the peripheral blood do not show high levels of infection, we have investigated the expression of HIV in T-cell activated, differentiated macrophages. Peripheral blood mononuclear cells were isolated from HIV seropositive individuals, the T-cells were stimulated with antigens or mitogens, then removed from the cultures. Macrophages were cultured alone and HIV expression was assessed. Results from p24 antigen capture assays, electron microscopy, and immunocytochemisty concurred and indicated that the presence of T-cells and an activating agent such as Con A or allogeneic cells during only the initial 24 hours of culture induced monocyte differentiation and HIV expression in 21/30 HIV seropositives within 3 weeks. Antigens such as HSV and CMV usually failed to induce HIV expression in macrophages. These herpes virus antigen responses are impaired at a relatively early stage of disease while mitogen and allogenic responses are maintained. Thus, the inability of antigens to induce HIV may reflect either the level of T-cell activity in these immunodeficient subjects, or quantitative differences between antigen and mitogen/allogenic responses. For most donors, direct contact between the monocytes and autologous T-cells was required for HIV expression. Supernatants from allogenic response failed to trigger HIV and antibody to HLA DR abrogated the response, suggesting that the induction of HIV in macrophages is a genetically restricted event. Since very little free (or T-cell associated) virus is present in the initial 24 hours of culture, it is unlikely that the T-cells are the source of virus in this system. However, the possibility remains that HIV could be specifically transferred to autologous macrophages via class II interactions. Collectively, the data suggest that, in the context of a classical immune response, T-cells activate silently infected monocytes to differentiate and become active producers of HIV.

**L 246** HIV REPLICATION IN PRIMARY CD4 LYMPHOCYTE CULTURES: INDUCTION OF REGULATORY AND STRUCTURAL GENE EXPRESSION BY CELL ACTIVATION AND DNA SYNTHESIS. Celsa A. Spina, John C. Guatelli, and Douglas D. Richman. UCSD School of Medicine and the VA Medical Center, San Diego, CA 92161.

Although T cell activation is thought to be a requirement for a productive infection cycle by HIV, it is unclear which steps in the T cell activation cascade are essential and how each of these steps influences the regulatory control of HIV replication. To study this question, we have chosen an *in vitro* model system using purified, primary CD4 lymphocyte cultures from HIV seronegative donors, and acute infection with the LAV-$1_{Bru}$ strain of HIV-1. Our previous studies with this experimental system showed that: (1) HIV can infect resting, unstimulated CD4 cells and establish a proviral state; (2) incomplete cell activation with peak expression of IL-2R but no DNA synthesis does not result in a complete HIV replication cycle; and (3) stimulation of cellular DNA synthesis in the presence of IL-2 is required for induction of a productive cycle of HIV replication and release of cell-free virions. Our current studies used a modification of the polymerase chain reaction (PCR) technique to study the influence of cell activation on the expression of HIV regulatory and structural RNA transcripts in this model cell system. We found that transcripts corresponding to *nef* appeared very early and remained predominant up to 6 days after the infection of unstimulated CD4 cells. Transcripts corresponding to *tat* and *rev* were also detectable but, constituted minor components. With incomplete cell activation, increases in *tat* expression, with corresponding decreases in *rev* expression, were seen 24-48 hours after cell induction. In contrast, complete cell activation resulted in increased expression of all regulatory transcripts within 24 hours of exogenous IL-2 addition. Detection of singly-spliced *env* transcripts corresponded to increases in soluble p24 levels at 72 hours after cell stimulation. Delineation of how different events in cell activation influence regulation of the different stages of HIV replication should aid in the understanding of HIV-induced pathology and in the design of rational, therapeutic interventions.

**L 247** HIV-1 REPLICATION IS CONTROLLED AT THE LEVEL OF T-CELL ACTIVATION AND PROVIRAL INTEGRATION, Mario Stevenson, Trevor L. Stanwick, Michael P. Dempsey, and Carolyn A. Lamonica, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68105. During progression of the Acquired Immune Deficiency Syndrome (AIDS), the human immunodeficiency virus type 1 (HIV-1) is harbored in CD4 positive T-cells, which act as the primary reservoir for the virus. *In vitro*, HIV-1 requires activated T-cells for a productive infection; however, *in vivo*, the number of circulating T-cells in the activated state which are potential targets for HIV-1 infection is low. We have investigated the ability of HIV-1 to infect resting T-cells, and the consequences of such an infection. T-cell activation was not required for HIV-1 infection, however, viral DNA was unable to integrate in resting T-cells and was maintained extrachromosomally. Subsequent T-cell activation allowed integration of extrachromosomal forms and led to a productive viral life cycle. Extrachromosomal forms of viral DNA were found to persist for several weeks after infection of resting T-cells and, following T-cell activation, these forms maintained their ability to integrate and act as a template for infectious virus. Several lines of evidence, including temporal analysis of HIV-1 replication and analysis of an HIV-1 integrase deletion mutant, indicated that extrachromosomal HIV-1 DNA genomes were transcriptionally active. These results are compatible with a model whereby HIV-1 can persist in a non-productive extrachromosomal state in resting T-cells until subsequent antigen-induced or mitogen-induced T-cell activation, virus integration and release. Thus agents which induce T-cell activation may control the rate of HIV-1 replication and spread during AIDS progression.

**L 248** IDENTIFICATION OF HIV 1 TAR- AND TAT TARGET mRNA SEQUENCES FOR POTENT TRANSLATION ARREST BY ANTISENSE OLIGONUCLEOTIDES, Udo Stropp, Joerg Baumgarten, Axel Kretschmer, Antonius Löbberding and Wolfgang Springer, Zentrale Forschung, Bayer AG, D-5090 Leverkusen, Fed. Rep. Germany.

Sequence specific hybridization of antisense oligonucleotides complementary to mRNA interferes with post transcriptional mRNA processing events, translation and /or other mechanisms of mRNA metabolism thus inhibiting gene expression. The inhibition of HIV replication rate by antisense oligonucleotides was demonstrated in tissue culture (1,2). We could show by testing 40 overlapping antisense oligonucleotides (20 mers) complementary to the 5' - end (nt= 1-80) and the first coding tat exon (nt= 5358-5625) of HIV 1 mRNA that three short sequence regions are extremely susceptible for translation arrest.

The translation arrest was quantitatively measured in an in vitro reticulocyte lysate translation assay and in cloned transfected HeLa cell lines expressing HIV 1 LTR-human growth hormone as a reporter gene construct and LTR-tat. Consistent results were obtained with phosphorothioate oligonucleotides instead of DNA oligomers. Inhibition of protein formation was sequence specific, dose dependent and greater than 90% at 1 - 10 µM concentrations. (1) Matsukura, M. et al. PNAS 86, 4244 (1989); (2) Agrawal,S. et al. PNAS 85, 7071 (1988).

**L 249** COMPLEMENT-MEDIATED ANTIBODY-DEPENDENT ENHANCEMENT OF HIV-1 INFECTION IN EBV-CARRYING B CELLS, Michel Tremblay[1,2], Sylvain Meloche[2], Rafick P. Sekaly[2], and Mark A. Wainberg[1], [1]Lady Davis Inst., Montreal, H3T 1E2, and [2]Laboratory of Molecular Immunology, IRCM, Montreal, Canada, H2W 1R7. We tested whether enhancement of HIV-1 infection via an alternative receptor, other than the CD4 molecule, could be present in EBV-positive B cells. Enhancing activity was demonstrated when these cells were coincubated with HIV-1 and sera from HIV-1 antibody-positive individuals in presence of human complement. The phenomenon of complement-mediated ADE of HIV-1 detected in EBV-carrying B cells required the implication of the CD4 receptor, the complement receptor type 2 (CR2), and the alternate pathway of complement. The exact significance of such mechanism in the natural pathogenesis of HIV-1 infection is not known but our results suggest that HIV-1 may spread *in vivo* to EBV-containing B cells with more efficiency through a CD4-CR2 receptor complex and may then circumvent the effect of neutralizing antibodies.

**L 250** VIROLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CELL CLONES CHRONICALLY INFECTED BY, BUT NOT PRODUCING, HIV-1 FROM PBLs OF ASYMPTOMATIC SEROPOSITIVE SUBJECT, P. Verani, T. Fausto, A. Borsetti, M. Federico, L. Sernicola, T. Maggiorella, N. Sulli, S.G. Parisi, R. Bona, G.B. Rossi, Lab. of Virology, Istituto Superiore di Sanità, Rome, Italy.

PBLs from an asymptomatic HIV-1 seropositive and HTLV-1 seronegative subject were cloned by limiting dilution in presence of IL-2. The past medical history of the subject was characterized by recessive LAS (3 years ago) with virus isolation from lymphnodes. Further attempts, also at time of cloning, to reisolate virus were negative. Cell subset characterization was made using monoclonal antibodies anti-T and -B lymphocytes, monocytes and macrophages membrane antigens. Two cell clones (URS1, URS4) carrying but not expressing the HIV-1 genome were isolated and established in vitro. Clone URS4 shows giant cells and an absolute requirement for exogenous IL-2. Cytofluorymetric analysis indicates that these cells are not terminally differentiated. Both clones are negative for spontaneous or BudR-induced extracellular virus production, and even if negative for CD4 receptor and mRNA, they are susceptible to homologous superinfection. URS1 and URS4 show a spontaneous production of IFN-. (200 U/ml and 50 U/ml respectively). EBV genome is present in URS1 but not in URS4 and both are negative for HTLV-I genome. Enzyme restriction pattern (SstI, EcoRI) shows the presence of a detectable HIV genome only in URS1 clone. PCR-DNA analysis (URS4) using the gag primers SK01-SK02, SK38-SK39, $CC_1$-$CC_2$ (782-1092) and the env primers SK68-SK69, $CO_1$-$CO_2$ followed by hybridization with oligonucleotide probes was positive. PCR-DNA analysis using the LTR primers SK29-SK30, 52N-600N (59-600) was negative. Preliminary PCR-RNA analysis indicated the absence of active transcription of HIV-1 genome.

## *Related Viruses and Animal Models*

**L 300** **HIV p24-SPECIFIC HELPER T-CELL CLONES FROM IMMUNIZED PRIMATES RECOGNIZE HIGHLY CONSERVED REGIONS OF HIV-1** Sally E. Adams[1], Kingston H.G. Mills[2], Peter A. Kitchin[2], Bernard P. Mahon[2], Amanda L. Bernard[2], Susan M. Kingsman[3] and Alan J. Kingsman[1,3]. [1] British Bio-technology Ltd., Watlington Road, Oxford. [2]National Institute for Biological Standards and Control, South Mimms, Herts. [3]Virus Molecular Biology Group, Department of Biochemistry, Oxford University, Oxford.

The ability to induce HIV-specific T-helper (TH) cells is likely to be an essential requirement of any candidate vaccine designed to prevent or control HIV infection. TH cells are a vital component of the immune system and are required for both antibody and cytotoxic responses. We have investigated TH cell recognition of the HIV core protein p24 using CD4+ T-cell clones derived from cynomolgus macaques immunized with hybrid HIV p24:Ty virus-like particles (VLPs). Immunization of macaques with hybrid HIV p24:Ty-VLPs induced serum antibodies and proliferative T-cells specific for HIV p24 as well as the Ty carrier protein. MHC Class II-restricted T-cell lines and clones have been derived from the immunized animals and used to delineate two TH epitopes on the HIV p24 protein. The two epitopes are both highly conserved between twelve HIV-1 isolates and may therefore be useful components of a combination vaccine against HIV.

**L 301** ENHANCEMENT OF SIVagm INFECTION BY SOLUBLE RECEPTORS. Jonathan S. Allan, Evelyn Whitehead, and David W. Buck, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX 78284 and Becton-Dickinson Monoclonal Antibody Center, Mountain View, CA 94043.

Studies have shown that the family of immunodeficiency viruses including HIV-1, HIV-2, SIVmac, and SIVagm utilize CD4 as the primary receptor for T cell infection. Penetration of HIV-1 occurs by pH-independent fusion of the viral membrane with the cell surface. The fusigenic region of the viral envelope is theorized to involve a peptide sequence in the amino-terminal domain of the transmembrane protein. SIVagm strains were analyzed to determine the relation of SIVagm to HIV-1 in the mechanism of cell entry and replication. In contrast to the blocking effects of recombinant soluble CD4 with HIV-1 infection, rCD4 enhanced SIVagm infection of susceptible CD4-bearing human T-cells by as much as 17 fold over infection in the absence of rCD4. This effect was both concentration-dependent and could overcome primary CD4 receptor requirements on the T cell-surface. Enhancement by rCD4 could also be demonstrated with other SIVagm strains and in other T cell lines indicating that this effect is not limmited to one virus or one cell line. Inhibition of rCD4-enhancement of SIVagm infection could be demonstrated with antibodies from naturally and experimentally infected monkeys. Inhibitory titers ranged from 1:2000 to 1:10,000 although neutralizing antibodies could not be detected. In addition, a monoclonal antibody to a region of CD4 not inovlved in gp120 binding was able to prevent infection only in the presence of rCD4 suggesting that this antibody sterically hinders subsequent fusigenic events. Theoretically, rCD4 may enhance SIVagm infection by activating secondary viral domains important for viral penetration.

**L 302** SEROLOGICAL IDENTIFICATION AND BACULOVIRUS EXPRESSION OF STRUCTURAL GENE PRODUCTS OF THE BOVINE IMMUNODEFICIENCY-LIKE VIRUS (BIV), Jane K. Battles, Lynn Rasmussen, Marie Y. Hu, and Matthew A. Gonda, Laboratory of Cell and Molecular Structure, PRI, NCI-FCRF, Frederick, MD 21701.

BIV is an infectious pathogenic virus of the lentivirus subfamily of retroviruses. It is structurally, antigenically, and genetically related to HIV. Biologically active proviral molecular clones of BIV have been isolated and sequenced. The open reading frames (ORFs) of the major structural genes, as well as those with enzymatic or putative regulatory function, have been identified. Sera from both experimentally and naturally infected animals, as well as sera from animals immunized with virions and synthetic peptides, were used to identify putative BIV gag and env structural proteins in radioimmunoprecipitations and Western blots. ORFs for the structural genes were subcloned into baculovirus expression vectors. Immature virus-like particles, devoid of envelope, were made and excreted into the supernate by budding, when only the gag ORF was expressed. Expressed gag gene products were recognized by antisera that were reactive to authentic viral proteins; hyperimmune sera produced to these expressed proteins also recognized authentic BIV viral proteins. These and other genetically engineered proteins will be valuable as diagnostic reagents and for preparing vaccines and immunomodulating reagents in future studies using BIV as an animal model of lentivirus pathogenesis.

**L 303** EFFECTS OF SPERM ON MURINE AIDS (MAIDS)

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We have studied the effects of sperm and on the generation and course of MAIDS. Disease was induced by viruses obtained from cell line LP-BM5-Scl in male C57BL/6J, B10A, ATL and ATH strains. Allogeneic sperm cells ($1-3 \times 10^7$) were injected intravenously prior to and following the viral inoculations. Marked prolonged suppression of splenocytes proliferation to mitogens and allogeneic stimulation as well as splenomegaly were observed following a single intravenous sperm injection. When suboptimal amounts of virus preparations not sufficient to induce MAIDS were used, disease developed only in those animals injected with sperm in addition to the virus. This was also observed when virus and sperm were given to the animals rectally. Sperm injections did not help in inducing disease in animals of MAIDS resistant strains. . These results suggest that sperm may function as a cofactor of MAIDS induction probably by it general immunomodulating effects and possibly also by its functions as vector. This has a clear relevance to the pathogenesis of human AIDS and probably also to the increased risk of HIV infection associated with receptive anal sex.

**L 304** INFECTION OF CYNOMOLGUS MONKEYS WITH HIV-2 PROTECTS AGAINST SIV INDUCED IMMUNODEFICIENCY AND PATHOLOGY. G, Biberfeld, P Putkonen, R Thorstensson, J Albert, E Norrby, P Biberfeld, National Bacteriological Laboratory and Karolinska Institute, Stockholm, Sweden.
Three cynomolgus monkeys were experimentally infected with HIV-2 strain SBL-K135 and 168 days later challenged with 10-100 animal infectious doses of the closely related $SIV_{sm}$ to study protective immunity. At the time of challenge the HIV-2 infected monkeys were seropositive, but virus could no longer be recovered and they had no clinical symptoms or decrease of the CD4/CD8 cell ratio. After 5 months of follow up all three HIV-2 infected monkeys were found to be protected against SIV induced immunodeficiency (no CD4/CD8 cell decrease) and lymphadenopathy but not against SIV infection (presence of virus and anamnestic antibody response). Four naive control monkeys which were inoculated with the same dose of SIV developed a decrease of the CD4/CD8 cell ratio and marked lymphadenopathy. Immunohistochemical examination showed that viral antigen was abundant in lymph node biopsies from the SIV infected monkeys but was lacking in the biopsies from the three HIV-2 preinfected and SIV superinfected monkeys. Vaccination against HIV may be possible since pre-infection with a non pathogenic HIV-2 seems to protect against SIV induced immunodeficiency and pathology in cynomolgus monkeys.

**L 305** IDENTIFICATION AND CHARACTERIZATION OF HIV-RELATED ENDOGENOUS SEQUENCES IN HUMANS AND NON-HUMAN PRIMATES BY PCR. Michael Boyce-Jacino, Marc S. Horwitz and Anthony J. Faras. Institute of Human Genetics and Department of Microbiology, University of Minnesota, Minneapolis, Minnesota.
We have begun to analyze disperse eucaryotic species for the presence of human-retrovirus-related sequences such as HIV. Families of sequences related to HIV have been detected in a variety of species using the polymerase chain reaction to target the characterization of normal genomic sequences related to specific regions of HIV-I. When oligomers derived from specific regions of the viral env gene were used in PCRs, amplified sequences which cross-hybridized with viral derived probes were produced. For each PCR done distinct band patterns were produced for each species template on ethidium bromide stained gels and on Southern blots of PCR products using retrovirus-derived probes. These variations indicate that the PCR products produced were specific to the species DNA used as template. Data on the size and distribution of PCR products generated with several oligomer combinations will be presented. These results show that there are families of putative HIV-I related sequences in normal cell DNA which are readily detectable by oligomer-specific PCR targeting.

**L 306** The Role of Viral and Cellular Proteins in Transcriptional Activation of the HTLV-1 LTR, John N. Brady, Paul F. Lindholm, Mike F. Radonovich, Scott D. Gitlin and Susan J. Marriott, Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Transcription of the Human T-Cell Leukemia/Lymphoma Virus (HTLV-I) is controlled through the interaction of viral and cellular proteins with regulatory elements located in the viral long terminal repeat (LTR). The viral regulatory protein $Tax_1$ has been shown to activate transcription of the LTR through at least two independent elements, the 21 base pair repeats (TRE-1) and an element located from -117 to -163 (TRE-2). Although $Tax_1$ does not bind DNA directly, we have previously demonstrated that a cellular protein can mediate the indirect interaction of $Tax_1$ with TRE-2. We have recently purified a 36 kD nuclear protein from HeLa cells which binds to TRE-2 and allows the indirect interaction of $Tax_1$ with this element. This protein is thus referred to as "$Tax_1$ interaction factor 1" (TIF-1). We have also shown that TIF-1 is able to activate transcription from the viral LTR in vitro. Both $Tax_1$ and TIF-1 interact with a zinc affinity chromatography column, suggesting the possibility that interaction between the two proteins is mediated by zinc or that the interaction of TIF-1 with TRE-2 requires zinc. No detectable difference was observed. The requirement for zinc in $Tax_1$ - TIF-1 heterodimer formation is currently under investigation as are the specific domains of Taxl responsible for zinc binding and transactivation. These results as well as the contribution of protein-protein interactions to $Tax_1$-mediated transactivation of the HTLV-I LTR will be discussed.

**L 307** ACTIVATION BY UV B RAYS OF THE HIV-1 LTR DIRECTED EXPRESSION IN TRANSGENIC MICE, Catherine Cavard, Alain Zider, Gisèle Grimber and Pascale Briand, Hôpital Necker-Enfants malades, 149 rue de Sèvres 75015 Paris. France.
One of the characteristics of HIV-1 is the extended time frame from initial HIV infection to clinically detectable immunologic abnormalities and disease manifestations. It has been previously shown in vitro that the HIV LTR is activated by UV irradiation. In order to demonstrate if a similar effect could occur in vivo, adult and 10 day transgenic mice carrying the B-galactosidase reporter gene under the control of the HIV-LTR were irradiated at 300 nm. These mice spontaneously expressed the transgene in the skin and eyes of both adults and embryos. In the eye, the B-galactosidase activity is restricted to fiber lens cells at the anterior part of the lens. In the skin, two types of cells have shown expression, one located in the hair follicle and the other in the stratum corneum. Irradiation at 300 nm caused a significant increase in skin B-galactosidase activity as determined by skin biopsy. Identification of cells expressing the transgene with and without irradiation is under progress.

**L 308** CROSS COMPLEMENTATION OF HIV-1 REV-DELETION MUTANTS WITH $SIV_{MAC}$ REV, Sandra Colombini, Robert C. Gallo and Flossie Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
Human immunodeficiency virus type 1 (HIV-1) encodes a transactivator termed REV that is required for the expression of viral structural proteins. The $SIV_{MAC}$ REV gene cDNA was isolated and characterized. We examined its ability to rescue different HIV-1 REV deletion mutants and to transactivate HIV-1 REV responsive elements placed upstream of the CAT gene. Moreover, we constructed HIV-1/$SIV_{MAC}$ chimeric REV in order to define the region of the protein important for function. We observed different levels of complementation of HIV-1 REV defective mutants by $SIV_{MAC}$ REV depending on the cell lines used and the presence of homologous or heterologous TAT in the assay. These results will be presented.

**L 309** IDENTIFICATION OF THE FUSION DOMAIN OF THE VISNA VIRUS ENVELOPE: ROLE OF GLYCOPROTEIN CLEAVAGE AND HYDROPHOBIC RESIDUES, Sharon E. Crane,[1,2] Patrick Kanda[3], Charles Flexner[4] and Janice E. Clements,[1,2], Department of Molecular Biology and Genetics[1], Division of Comparative Medicine[2], Department of Medicine[4], The Johns Hopkins University School of Medicine, Baltimore, MD 21205, Department of Virology and Immunology[3], Southwest Foundation for Biomedical Research, San Antonio, TX 78284 Visna virus is an ovine lentivirus which causes cytopathic effects in vitro. Virus-induced cell fusion occurs several hours after infection at high multiplicities of infection, and is mediated by the envelope glycoprotein. The epitope responsible for inducing fusion has been identified using a twenty-four amino acid peptide which is present at the amino terminal end of the transmembrane portion of the glycoprotein. This region is analogous in position to the HIV fusion domain, but lacks a phe-leu-gly sequence. The peptide induces fusion of goat synovial membrane cells; antiserum to the peptide blocks virus induced cell fusion. A vaccinia virus recombinant expressing the visna virus envelope glycoprotein induces fusion of many cell types. Site directed mutagenesis of the fusion region of the transmembrane protein has been done to examine the significance of cleavage of the env polyprotein and of specific amino acids in the hydrophobic region in the fusion process.

**L 310** MACAQUE NEUTROPHIL CHEMILUMINESCENCE RESPONSES SUPPRESSED BY HIV-gp41 SYNTHETIC PEPTIDES BUT ENHANCED BY HTLV VACCINE, Charlene S. Dezzutti, J. Michael Marr, Mark K. Pollman, Patrick Kanda, Louis J. Lafrado and Richard G. Olsen, The Center for Retrovirus Research, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210 and The Southwest Foundation for Biomedical Research, San Antonio, TX Five pig-tailed macaques were utilized in the present study. Previously, three macaques were immunized with a human T-cell lymphotropic virus (HTLV) vaccine; the other two macaques received vehicle control. Both groups were subsequently challenged with a simian T-cell lymphotropic virus-infected cell line. To date no disease has been documented in either group. Neutrophils from these macaques were tested for their ability to generate a chemiluminescene (CL) response against whole vaccine and the synthetic peptides 735-752 and 846-860 from HIV-gp41. The two highest dilutions of HTLV vaccine (100 $\mu$g and 10 $\mu$g) increased the CL responses in the vaccinated macaque neutrophils while had no effect on control macaque neutrophils. Lower vaccine concentrations had no effect on the vaccinated macaque neutrophil CL response. Both synthetic peptides suppressed the CL response from both macaque groups. Immune cell dysfunction has been consistently documented in retrovirus infections. The ability of the neutrophil to generate a CL response parallels the cell's bacteriocidal activities. A dysfunction in this cell type may predispose the animal to infectious agents.

This work was supported in part by the NIH grant CA 40714 and The Center for Retrovirus Research, Columbus, OH 43210.

**L 311** HTLV I/II IN INTRAVENOUS DRUG USERS IN SAN FRANCISCO: RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY, Ellen G. Feigal,Edward Murphy,Karen Vranizan,Peter Bacchetti,Richard Chaisson,James Drummond,William Blattner,Michael McGrath,John Greenspan,Andrew Moss,
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Risk factors for seropositivity for the serologically indistinguishable human T cell lymphotropic viruses types 1 and 2 (HTLV I/II) infection were assessed in 702 intravenous drug users (IVDUs) in San Francisco between 1985 and 1987. All sera were tested by western blot, enzyme linked immunosorbent assay (ELISA), and p24 radioimmunoassay (RIA). One hundred and sixty-five participants were positive by at least one assay for HTLV I/II. Sixty-nine were positive by all 3 assays. Fifty-one were positive in only 1 assay: 24 by western blot, 13 by ELISA, and 14 by RIA. The lack of a gold standard serologic test makes any definition of seropositivity arbitrary. Using a positive rsult by any of the 3 assays will yield the most false positives, while requiring all 3 to be positive will produce the most false negatives. For this report, we defined a seropositive as being positive in a minimum of 2 out of 3 assays. When we looked at risk factors for HTLV I/II seropositivity, duration of IV heroin use was the strongest association: 11-20 years OR = 3.6 (95% CI 1.6, 8.5), 21 plus years OR = 6.1 (95% CI 2.2, 17.5), coompared to 10 or fewer years of heroin. Additional independent risk factors included black or Hispanic race, female sex, and the use of a shooting gallery in the past. Coinfection of HTLV I/II and HIV was less frequent than expected. In 154 IVDUs tested serially, the age- and race-adjusted seroconversion rate was 3.4% (95% CI 1.3, 8.9) per person year. This seroconvesion rate is consistent with an observed increase in the cross-sectional seroprevalence over the 3 years of the study (1985, 8.7% 95% CI 4.7, 12.7; in 1986 13.2% 95% CI 9.5, 16.9, and in 1987 15.0% 95% CI 11.8, 18.2).

Our study found some similarities and several marked distinctions between risk factors important in HTLV I/II infection and those observed to be important for HIV infection. Analogous to HIV infection, HTLV I/II infection was significantly associated with black or Hispanic race. In contrast to HIV infection, recent drug usage, or recent drug behavior such as cocaine injection, were not significantly associated with HTLV I/II infection.

**L 312** ANTIGENICITY AND IMMUNOGENICITY OF A RECOMBINANT HIV-1 GP160 EXPRESSED IN INSECT CELLS, M. Francotte[1], C. Thiriart[1], D. Gheysen[1], F. Van Wijnendaele[3], P. Desmons[2], M. De Wilde[1] and C. Bruck[1], Molecular and cellular Biology (1), Human Vaccine Development (2)and Protein Purification (3) Departments, Smith Kline Biologicals, 89 rue de l'Institut, B-1330, Rixensart, BELGIUM

The human immune deficiency virus (HIV-1) envelope gene was efficiently expressed in Spodoptera frugiperda insect cells using a Baculovirus expression system. Upon infection with the env recombinant virus, a major band with an apparent molecular weight of 160 kD was detected in the infected cell lysates. Western Blot and ELISA analyses revealed that this protein was immunoreactive with a panel of gp41 and gp120 specific monoclonal antibodies as well as with HIV-positive human serum. The recombinant gp160 protein was purified from the infected insect cells and injected into guinea pigs in conjunction with a number of adjuvant formulations. Serological responses were analyzed by ELISA, Western Blot, RIPA and neutralization assays. Repeated immunizations with adjuvanted gp160 resulted in a high anti-HIV antibody response in some guinea pigs as determined by several sandwich ELISA using either native viral glycoproteins or env recombinant proteins expressed in different systems as antigens. These guinea pig antisera were shown to contain type specific neutralizing antibodies, with titers depending on the number of immunizations and the adjuvant formulation used.

**L 313** HUMAN CD4 GENOMIC DNA AS A POTENTIAL TRANSGENE IN GENERATING HIV-I SUSCEPTIBLE ANIMALS, Frances Gillespie, James Robl, and Cha-Mer Wei, Transgenic Sciences, Inc., Worcester, MA 01605. We have isolated the CD4 gene in a single 45 kb genomic fragment from a human placenta cosmid library. This genomic clone includes approximately 5 kb of 5' flanking sequences and 13 kb of 3' flanking sequences, and has been designated pCD4-17A.2. This DNA clone was transfected into NIH 3T3 cells and colonies were selected in G418. Isolated colonies contain an estimated 2-20 copies of intact CD4 DNA , and express abundant quantities of a c. 3 kb mRNA which hybridizes to a CD4 cDNA probe. We are currently in the process of analyzing these cell lines by immunofluorescent staining techniques to detect any CD4 receptor on the cell surface. To study tissue specific expression of the human CD4 gene in other animals we are microinjecting pCD4-17A.2 into mouse embryos to generate transgenic mice. Various tissues will be isolated and analyzed from the transgenic mice which express human CD4 protein.

Recently, rabbits have been shown to be capable of being infected by HIV-I (Filice, G. et al, 1988, Nature 335: 366; Kulaga, H. et al, 1989, J. Exp. Med. 169: 321) However, high titers of virus and/or human HIV-I producer cells are required for successful infection. We are attempting to produce transgenic rabbits carrying human CD4 DNA, which may be more susceptible to HIV-I infection than non-transgenic counterparts.

**L 314** MOLECULAR ANALYSIS OF SEVERAL OVINE LENTIVIRUSES WITH DISTINCTLY DIFFERENT PATHOGENIC POTENTIALS. Richard F. Grant*, Jonathan O. Carlson*, Osam Kajikawa^, James C. DeMartini^. Department of Microbiology* and Department of Pathology^, Colorado State University, Ft.Collins,CO 80523 Ovine lentiviruses (OvLV) are being used as a model to study the molecular mechanisms involved in lentivirus pathogenicity. Like the human immunodeficiency viruses, OvLV strains vary in pathogenic potential and differ in nucleotide sequence. Earlier work performed in our laboratories has shown that plaque purified isolates from field cases of ovine progressive pneumonia can be used to induce OvLV associated disease in experimentally infected lambs and that in vitro growth characteristics of certain OvLV strains correlate with in vivo pathogenicity. We have found antigenic differences between rapidly lytic isolates and slow, persistent isolates in both the gag and env gene products. The polymerase chain reaction was used to amplify viral LTR's and a portion of the polymerase gene from four of our isolates and the amplified products were directly sequenced and cloned. Comparison of nucleotide sequences from the LTR and polymerase genes shows that our isolates are distinct from one another and from other known lentiviruses. The LTR sequences that are unique to each isolate may be important in determining viral replication and pathogenicity.

**L 315** NEUTRALIZING ANTI-HIV ANTIBODIES ARE INDUCED IN RHESUS MONKEYS IMMUNIZED WITH HYBRID SYNTHETIC PEPTIDES (T1-SP10) CONTAINING T AND B CELL EPITOPES FROM HIV gp120. M.K. Hart, T.J. Palker, T.J. Matthews, A.J. Langlois, R.M. Scearce, M.E. Martin, C. McDanal, N. Lerche[1], D.P. Bolognesi, and B. F. Haynes. Duke University Medical Center, Durham, NC 27710; [1]University of CA Davis Primate Center, Davis, CA 95616. T1-SP10 synthetic peptide constructs containing a T helper cell epitope (T1) and type-specific neutralizing determinants (SP10) derived from gp120 sequences of HIV-IIIB, MN, and RF isolates have previously been used to induce neutralizing antibody responses in goats. To determine whether primates also generate HIV-specific neutralizing responses following immunization with synthetic peptides, rhesus monkeys were immunized with T1-SP10IIIB peptides (not conjugated to any additional carrier molecules) using PolyA:PolyU, alum, threonyl-muramyldipeptide (T-MDP), or incomplete Freund's (IFA) as adjuvants. Monkeys immunized with peptides in PolyA:PolyU generated weak proliferative and antibody responses. Monkeys receiving the other three adjuvants gave strong proliferative responses and generated antibody responses against all three T1-SP10 peptides. Neutralizing antibody responses were detected after three boosts in monkeys that received either T-MDP or IFA as adjuvant. Using truncated peptides to map the neutralizing and non-neutralizing antibody responses, it was observed that most of the antibody responses were directed against the SP10 portion of the peptide. These studies indicate that carrier-free synthetic peptides containing neutralizing epitopes (SP10) and T helper cell epitopes (T1) from HIV gp120 can induce both proliferative and type-specific neutralizing antibody responses in primates.

**L 316** IMMUNOLOGICAL AND VIROLOGICAL STATUS OF AN HTLV-1 INFECTED ARC PATIENT TREATED WITH ZIDOVUDINE. Toshio Hattori, Toshio Murakami, Atsushi Koito, Yosuke Maeda, Shuzo Matsushita and Kiyoshi Takatsuki. 2nd Dept. Intern. Med. Kumamoto Univ. Med. School.

Frequent infection with HTLV-1 in patients with AIDS(58%) but not in carriers of HIV-1(23%) among Japanese hemophiliacs was reported by us(JAIDS 2, 272, 1989). In this report, an efficacy of anti-retroviral agent against an individual infected with both viruses was evaluated. Zidovudine, known to suppress replication of both viruses in vitro was given to an ARC patient co-infected with HTLV-1(200mg/6h). Weight gain (3Kg) was observed by the therapy, however the therapy was discontinued at day 50 due to neutropenia and general malaise. HIV-Ag was found to be positive(55.2pg/ml) prior to the therapy, and was negative(8.4pg/ml) at day 4, but was again positive(33.1pg/ml) at day 35. Provirus DNA of both viruses were examined by PCR technique before and after the therapy. Provirus of HIV-1, positive before therapy was negative after the therapy, but that of HTLV-1 was still detectable even after the therapy. A significant change of T4 counts was not observed during the therapy, but HLA-DR-Ag-positive T8 cells decreased by the therapy. Above findings suggest that HTLV-1 might be more resistant to zidovudine in vivo, and did not indicate that the therapy using anti-viral agent in co-infected individuals, is promising.

**L 317** ANTIBODIES TO $SIV_{agm155}$, $SIV_{agm\ ver\text{-}1}$, $SIV_{mac251}$, HIV-1, HIV-2, AND HTLV-1 IN WILD AFRICAN GREEN MONKEYS FROM AFRICA; 1957-62 AND 1980-85, AND THE CARIBBEAN; 1980-85, R. Michael Hendry, GV Quinnan, DE Parks, J Allen, VN Hirsch, and PR Johnson, Center for Biologics Evaluation & Research, FDA, Bethesda, MD 20892, J & J Biotechnology Center, La Jolla, CA 92038, SW Foundation for Biomedical Research, San Antonio, TX 78284, and Georgetown University, Rockville, MD 20852

African green monkey (AGM) sera were screened for antibodies (Ab) to $SIV_{mac\ 251}$ by indirect immunofluorescence (IFA) and with an SIV env peptide ELISA. Positive sera were analyzed by western blot (WB) using 3 strains of SIV as well as HIV-1 and HIV-2. Sera were screened for Ab to STLV-1 by IFA using HTLV-1 MT-2 cells. Positive sera were analyzed by HTLV-1 WB. The results are summarized below. Groups A and B were wild-caught in Kenya and Ethiopia, Group C was wild caught in Barbados and St. Kitts, and group D was from a captive breeding colony.

| | | | BLEED | PERCENT OF AGM SERA POSITIVE IN EACH GROUP BY INDICATED ASSAY | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GROUP | N | ORIGIN | DATES | SIV IFA | SIV ELISA | WB agm155 | WB agm ver-1 | WB mac251 | WB HIV-1 | WB HIV-2 | HTLV-1 |
| A | 26 | AFRICA | 1957-62 | 42 | 35 | 38 | 38 | 38 | 19 | 31 | 46 |
| B | 39 | AFRICA | 1980-85 | 49 | 44 | 46 | 46 | 44 | 23 | 46 | 31 |
| C | 24 | CARRIBEAN | 1980-85 | 0 | 0 | ND | ND | ND | ND | ND | 0 |
| D | 34 | U.S. COLONY | 1988 | 47 | 18 | 47 | 47 | 32 | 29 | 20 | 56 |

Groups A, B, and D had variable SIV WB patterns crossreactivity with HIV-1 and HIV-2 gag and to lesser extent, pol proteins. Groups A and B showed strong crossreactivity with HIV-2 env proteins (gp120-160), whereas group D showed only HIV-2 gag crossreactivity, low or absent levels of Ab to the SIV env peptide, and a lower frequency of Ab to $SIV_{mac\ 251}$ by WB. Groups A, B, and D all displayed similar patterns of reactivity to HTLV-1 proteins. No differences were observed between dually (SIV and STLV-1) and singly (SIV or STLV-1) infected AGM with respect to their serologic profiles to either virus. SIV and STLV-1 have been prevalent in African AGM for 30 years and are absent from Caribbean AGM. These results indicate that Ab responses to SIV in AGM were variable both within and between the groups studied and likely reflect antigenic variability among naturally occurring SIV isolates.

**L 318** **SIV-INFECTED MACAQUES HARBOR MUTIPLE PROVIRAL GENOTYPES.**
Vanessa M. Hirsch and Philip R. Johnson. Department of Microbiology, Georgetown University, Rockville, Md. 20852

We previously observed that growth of SIV in human cells selects for mutants with a stop codon in the cytoplasmic domain of the *env* gene. To further examine the effects of tissue culture passage on SIV genotypes, we analyzed proviral DNA in tissues from SIV-infected macaques. Genomic DNA from tissues of 3 $SIV_{sm}$ infected rhesus macaques were analyzed by southern blots. These animals had died as a consequence of immunosupression and opportunistic infections. Provirus was readily detectable in most tissues including spleen, lymph nodes, liver, kidney, lung, salivary gland, and intestine. Most of the proviral DNA was unintegrated (linear and circular). Restriction site variants were present at varying levels in most tissues. In one animal, a variant form was equimolar to the major proviral form observed in the other two animals.

We next examined proviral forms in DNA of isolates derived by cocultivation of splenocytes with CEM cells in culture. Southern blots revealed the presence of a single proviral form. Thus, the multiple variants observed in tissue samples were absent in tissue culture isolates. To more precisely quantitate these observations, we amplified (by PCR) and cloned the transmembrane portion of the *env* gene from samples derived from one animal. Clones were obtained directly from splenic tissue and from CEM cells infected by cocultivation with splenocytes. Comparison of nucleotide and deduced amino acid sequences confirmed the southern blot findings: proviruses in CEM cells were monolithic (3 identical clones), whereas multiple variants were present in tissue (4 distinct clones). Therefore, viruses isolated in tissue culture do not represent the complete spectrum of genotypes within an infected animal since many genotypes may not be recovered by tissue culture propagation. Additional tissue-specific clones have been derived and are currently being analyzed.

**L 319** MURINE AIDS: Effect of a rHu Interferon Alpha-B/D hybrid (IFN) Against Herpes Simplex Virus Superinfection.

H.K. Hochkeppel and R.M. Cozens, Pharmaceutical Division, CIBA-GEIGY Ltd, K-125.3.11, CH-4002 Basel, Switzerland.

The lack of convenient and suitable animal models for AIDS is hampering not only the search for agents against HIV but also compounds which may be of value in the treatment of the opportunistic superinfections characteristic of AIDS. We have found the murine model of AIDS to be a convenient model for evaluating the activity of potential antiviral agents against herpes simplex virus infection in mice with a retroviral-induced immunosuppression. Sixty days after infection with LP-BM5 virus C57BL/6 mice are susceptible to challenge with HSV-1 at a dose to which immunocompetent mice are fully resistant. Three doses of IFN (5 x $10^7$/kg) given shortly after infection with HSV-1, and 24 and 48 hours later were sufficient to protect 60-70% of the mice from the challenge. A single dose given shortly after the infection was also effective but afforded only 40% protection. The muramyltripeptide, MTP-PE, also afforded protection in this model. We are presently investigating the efficacy of combinations of these two agents in the model. As previouly reported results demonstrated an effect of IFN alone, and particularly in combination with AZT, against the LP-BM5 retrovirus itself, it may be expected that IFN, possibly in combination with other agents, could be of advantage in the treatment of AIDS, particularly in view of the apparent role played by HSV in the development of AIDS.

**L 320** Novel Endogenous Sequences Related to HIV-1 Marc S. Horwitz, Michael Boyce-Jacino and Anthony J. Faras, Dept. of Microbiology and Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455.

Although many families of human endogenous retroviral elements have been described, currently none have shown homology to human immunodeficiency viruses. Under relaxed stringency hybridization conditions, Southern blots of normal human, chimpanzee, and rhesus monkey genomic lymphocyte DNA were probed with a subclone of HIV-1 representing *env* sequences. All three species showed detectable banding patterns. At least four bands ( 9.5, 7.0, 4.4, and 3.2 kb) were detected in Pst I digested human DNA under conditions allowing for 30% base mismatch. This *env* probe was used to screen two human genomic lambda libraries under reduced hybridization conditions and one clone from each was isolated and characterized. Preliminary sequencing of these endogenous HIV-1-related sequences, EHS-1 and 2, has revealed regions of 70% homology to different portions of HIV-1 *env*. EHS-1 and 2 are distinct in that they do not hybridize to each other, but are conserved among primates in that both are detectable from human to rhesus monkey DNA under high stringency hybridization. Sequence comparisons to HIV, other viral, and cellular sequences will be presented.

**L 321** **MOLECULAR CLONES OF SIV: BIOLOGIC PROPERTIES AND GENETIC DRIFT IN EXPERIMENTALLY-INFECTED MACAQUES.** Philip R. Johnson[1], Simoy Goldstein[2], and Vanessa M. Hirsch[1]. [1]Department of Microbiology, Georgetown University and [2]Laboratory of Infectious Diseases, NIAID/NIH, Rockville, Md.

We have previously described the derivation of infectious proviral molecular clones of SIV of sooty mangabeys (SIVsm) and SIV of African green monkeys (SIVagm). Virus stocks generated after transfection of these clones into permissive cells in culture were inoculated into SIV-seronegative rhesus macaques, pig-tailed macaques, and African green monkeys. Cloned SIVsm infected rhesus and pig-tailed macaques (8 of 8), but only 1 of 7 African green monkeys. SIVagm (155-4) infected African green monkeys and pig-tailed macaques (5 of 5), but not rhesus macaques (0 of 2). Ten months after inoculation, all of the primates infected by cloned SIV were healthy. However, 2 rhesus infected with molecularly cloned SIVsm have shown marked decreases in absolute numbers of CD4+ circulating lymphocytes. Thus, molecular clones of SIV can induce immunosupression in macaques and may serve as molecular models for the study of AIDS pathogenesis.

A major goal of this work is to examine genetic variation (drift) of SIV in experimentally-inoculated animals. Our approach is to analyze sequences directly from circulating PBMCs rather than sequences derived from tissue culture isolates. To this end, we have identified primers and reaction conditions that allow for the direct amplification of SIV sequences from genomic DNA of PBMC. To date, we have analyzed sequences from 4 macaques infected with a molecular clone of SIVsm (smH-3 or smH-4). Two separate regions of the genome have been examined (a 400 bp region of pol and the entire env gp40) from samples taken 2.5 after infection. To summarize, in the macaques analyzed to date, we have found minimal or no variation from the sequence of the original inoculum. Clones derived from the 2 immunosuppressed rhesus macaques (1 year after infection) are currently being analyzed.

**L 322** ISOLATION AND CHARACTERIZATION OF HIV-1 *GAG*-SPECIFIC T CELL CLONES.

R. Paul Johnson [1], Gail Mazzara[2], Dennis Panicali[2], Robert T. Schooley[1], BD Walker[1]. [1]Infectious Disease Unit, Massachusetts General Hospital, Boston, MA 02114; [2]Applied bioTechnology, Cambridge, MA 02142. Cytotoxic T lymphocytes (CTL) specific for HIV proteins have been identified in seropositive individuals and may play a role in control of viral replication *in vivo*. Characterization of CTL responses to the relatively conserved *gag* and reverse transcriptase proteins may therefore be important in the design of a vaccine which provides broadly cross-reactive immunity. However, limited information is available regarding specific epitopes recognized by CTL. Using cloned lymphocyte lines maintained in long-term culture, our laboratory has recently identified several epitopes recognized by CD8+, class I restricted CTL specific for the HIV-1 reverse transcriptase protein. (B. Walker et al., PNAS, in press). We are now using T cell clones to identify epitopes recognized by HIV-1 *gag*-specific CTL. Peripheral blood mononuclear cells (PBMC) were obtained from HIV-1 seropositive subjects and cloned by limiting dilution in the presence of a CD3-specific monoclonal antibody as a stimulus to lymphocyte proliferation, IL-2, and irradiated PBMC from seronegative donors. Clones were tested for *gag*-specific CTL activity in a standard chromium release assay utilizing autologous and allogeneic EBV-transformed lymphoblasts infected with recombinant vaccinia viruses expressing the HIV-1 p55, p24 and p17 *gag* proteins. One HIV-1 *gag*-specific clone, 63AE21, is restricted by the class I HLA antigen Bw62 and recognizes an epitope in the p17 *gag* protein. Fine specificity of gag-specific clones will be further defined using target cells incubated with synthetic peptides corresponding to the *gag* protein.

**L 323** EFFECT OF DIETHYLCARBAMAZINE IN CAS-BR-M INOCULATED NFS/N MICE: A potential immunomodulator drug for treatment of HIV infection, Lynn W. Kitchen, M.D., Marshall University School of Medicine, Huntington, WV.

Oral administration of diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide [DEC]), an immunomodulator drug used in the prevention and treatment of the filariases, has been shown to prolong survival in newborn mice inoculated with murine leukemia virus. Six (10.7%) of 56 DEC-treated Cas-Br-M inoculated mice died at an age of less than 125 days, compared to 19 (34.5%) of 55 untreated control mice ($p<0.006$), and 0 (0%) of 14 mock-inoculated control mice. This new data is consistent with previous experiments in which improved survival and a significantly decreased rate of decline of lymphocyte counts were noted in association with long-term DEC treatment begun early in the course of infection in the case of 2 feline leukemia virus-infected cats in comparison to 2 untreated littermates. DEC is an inexpensive, orally bioavailable, and relatively nontoxic drug that concentrates in the central nervous system after administration; all available data indicates that it can be given safely during pregnancy. Additional experiments are warranted to determine whether administration of DEC may prolong survival in HIV-infected patients.

**L 324** FELINE IMMUNODEFICIENCY VIRUS: A MODEL FOR ANTIVIRAL CHEMOTHERAPY.

Marck Koolen, Herman Egberink, Henk Niphuis*, Erik De Clercq*, Jan Balzarini*, Huub Schellekens* and Marian Horzinek. Institute of Virology, State University of Utrecht, Utrecht, The Netherlands;# Rega Institute for Medical Research, Leuven, Belgium; * TNO Primate Centre, Rijswijk, The Netherlands.

Until recently, a major problem in evaluating antiretroviral compounds with proven activity against HIV in vitro was the lack of a natural immunosuppressive lentivirus infection model in animals. FIV infection of cats meets criteria of such an animal model; FIV is genetically similar to HIV, and causes a disease with a similar pathogenesis. FIV is replicating preferentially in T-lymphocytes, macrophages and neural cells. In cats the infection is characterized by an initial asymptomatic phase of several months or even years during which virus can be demonstrated. Subsequently, clinical abnormalities may develop consisting of anorexia, weight loss, stomatitis, gingivitis, rhinitis, diarrhoea, pustular dermatitis, anaemia and generalized lymphadenopathy. Ultimately, the infected animals may die of opportunistic infections. We have used the FIV infection model to evaluate anti-HIV drugs in vitro and in vivo. Acyclic nucleoside analogues had a pronounced effect on both suppressing FIV infection and clinical symptoms associated herewith, in cats.

**L 325** HIV INFECTION OF PBMC-RECONSTITUTED SCID MICE. Richard A. Koup, Ruthann M. Hesselton and John L. Sullivan, University of Massachusetts Medical Center, Program in Molecular Medicine, Department of Pediatrics, Worcester, MA 01655. We have investigated the scid mouse reconstituted with human peripheral blood mononuclear cells (PBMC) (Mosier, et al.) as an animal model of HIV-1 infection. Scid mice were reconstituted by intraperitoneal (IP) injection of 10-50 million PBMC from human donors seronegative for Epstein Barr virus. Measurable amounts of human immunoglobulin (Ig) appeared by two weeks following reconstitution. Human Ig levels ranged from 12-17 mg/ml. These scid-human chimeric mice were injected IP with HIV-1 in PHA blasts or with cell-free HIV-1 at TCIDS of $10^2$ to $10^4$. Mice infected with cell-associated virus had high levels of P24 in serum at 18 days post infection. Virus was isolated from thymus, spleen, lymph nodes, bone marrow and peripheral blood in the third week after infection. Mice infected with cell-free virus at doses greater than 100 TCID had detectable serum P24 antigen by 21 days. Virus was easily isolated from lymphoid tissues as early as three weeks post infection. Animals inoculated with 100 TCID of HIV had undetectable serum P24 levels at 21 days and 42 days and HIV was not isolated from lymphoid tissues. We conclude that PBMC-reconstituted scid mice are readily infectable with HIV-1 by injection of cell-associated or cell-free virus. More virus is required per animal infectious dose than per TCID when virus inoculation is by the intraperitoneal route.

**L 326** LACK OF HOST CONTROL OF SIVagm EXPRESSION IN THE NATURAL HOST; THE AFRICAN GREEN MONKEY (AGM). Reinhard Kurth, Stephen Norley, Albrecht Werner, Michael Baier, Klaus Cichutek. Paul-Ehrlich-Institut, Paul-Ehrlich-Strasse 51-59, 6070 Langen/Frankfurt, FRG

SIVagm isolated from their natural host (AGM) have been isolated and shown to possess properties characteristic of HIV. Isolates from different laboratories exhibit a novel type of intragroup divergence in that regulatory genes such as rev and tat are highly divergent, whereas structural genes such as gag and env are more conserved. SIVagm are non-pathogenic in the natural host, which is not due to efficient immunological or intracellular host control mechanisms. The anti-viral humoral and cellular immune response of AGM is less developed than in humans against HIV and intracellular lentivirus suppression is inefficient, as viremia in AGM is easily detectable. The pathogenic potential of SIVagm in heterologous species and its use in lentivirus vaccine studies is presently investigated.

**L 327** UPTAKE AND NUCLEAR LOCALIZATION OF THE HTLV-I TRANS-ACTIVATOR PROTEIN, $TAX_1$ BY EPITHELIAL AND LYMPHOID CELLS. Paul F. Lindholm, Susan J. Marriott, Scott D. Gitlin and John N. Brady, Laboratory of Molecular Virology, NCI, NIH, Bethesda, Maryland 20892

The HTLV-I $Tax_1$ protein is a transcriptional trans-activator of the HTLV-I LTR and several cellular genes including IL-2, IL-2 receptor, and GM-CSF. We have observed that $Tax_1$ is released into the media of MT4 cells, unlike p24, which is localized predominantly in the nucleus. The cellular uptake of $Tax_1$ protein was shown by the addition of purified $Tax_1$ to the growth media resulting in rapid incorporation of the protein into lymphoid and epithelial cells. Cellular fractionation studies indicated that approximately 80 percent of the incorporated $Tax_1$ is present in the nuclear fraction. $Tax_1$ was detectable as early as one hour after introduction of $Tax_1$ into the tissue culture media. Cellular incorporation of $Tax_1$ increased rapidly over the first 8 hours. Ten to 15ng of $Tax_1$ were incorporated in $2x10^5$ cells indicating an average uptake of approximately $10^3$ molecules per cell. The addition of the lysosomotrophic agent chloroquine, did not increase the level of $Tax_1$ protein uptake, suggesting that cellular uptake of $Tax_1$ occurs by a mechanism different from the uptake of the HIV trans-activator, Tat. Cellular uptake and nuclear localization of $Tax_1$ was observed with Jurkat, CV-1 and HeLa cells. We are presently studying the mechanism of $Tax_1$ endocytosis.

**L 328** PROPHYLACTIC AND THERAPEUTIC EVALUATION OF 3'-AZIDO-3'-DEOXY-THYMIDINE (AZT) IN THE FELINE LEUKEMIA VIRUS INFECTED CATS. Lawrence E. Mathes, Phyllis J. Polas, Kathleen A.Hayes, Richard Sams, Susan Johnson and Gary J. Kociba, Department of Veterinary Pathobiology, Ohio State University, Columbus, OH 43210
A comprehensive evaluation of the antiviral activity of AZT was performed in feline leukemia virus (FeLV)/cat animal model system. AZT inhibited FeLV infection of feline lymphoid cells in vitro at concentrations of ≥ 23 picomolar. The in vivo studies were designed to determine the antiviral activity of AZT treatments begun at various times relative to virus challenge. AZT treatment was initiated at 48 before challenge or 8, 24, 48 or 96 hrs after challenge. In all of these studies, AZT was administered at a dose of 60 mg/kg/day by continuous infusion, either intravenously or subcutaneously, for a period of 4 wks. AZT treatment began 48 hrs before viral challenge protected 85% of animals against FeLV viremia conversion. Treatment begun at 8, 24 or 48 hrs post-challenge protected 60%, 67% and 50% respectively. AZT pharmacokinetics, toxicity and animal immune status are described.

**L 329** COMPARISON OF TWO SUBGROUPS OF HIV-2 : PERSISTENT INFECTION OF MACAQUES WITH A CYTOPATHIC VARIANT, Jan McClure, Shiu-Lok Hu , and William R. Morton, Oncogen and Washington Regional Primate Research Center, Seattle, Washington 98121.
Seven HIV-2 isolates were compared for polypeptide composition, antigenic variance, cytopathogenicity, and growth characteristics. From this comparison we were able to identify two distinct subgroups of HIV-2. Subgroup A is represented by the HIV-2 prototype ROD. In contrast to ROD, subgroup B isolates demonstated extreme cytopathogenicity in all cell lines, readily infected macaque peripheral mononuclear cells in vitro, and have a truncated surface glycoprotein. To investigate the in vivo pathogenicity of subgroup B, we inoculated two macaques each with a representive from each subgroup. As determined by viral isolation, all animals became acutely infected; however only the animals inoculated with the subgroup B isolate have remained persistently infected 16 weeks post-inoculation. Serological, clinical, and PCR analyses are in progress.

**L 330** SEARCH FOR MURINE LENTIVIRUS MODEL FOR AIDS, Gregory Milman and Linda M. Muul, Pathogenesis Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
Animal models for AIDS are important for studies of pathogenesis, drug discovery and vaccine development. At present, simian immunodeficiency virus (SIV) infection of rhesus macaques may be the best animal model for AIDS but research in monkeys is expensive and requires sophisticated animal care facilities. Lentiviruses have been found in sheep, goats, cows, horses and cats and it is likely that they also occur in mice. A lentivirus which induces immunodeficiency in mice would provide a valuable AIDS model. Mouse genetics are well defined with numerous inbred strains and many reagents are available for identification and analysis of murine immune cell subsets. For these reasons, NIAID solicited applications for grants to identify, isolate, and characterize murine immunodeficiency viruses. Descriptions of funded research will be presented. Most groups plan to utilize PCR and serological screening to survey mice from all areas of the world. Mouse strains and/or viruses developed in these research projects will be provided to the AIDS Research and Reference Reagent Program for world-wide distribution to qualified investigators.

**L 331** ANTIRETROVIRAL EFFICACY OF 9-(2-PHOSPHONYLMETHOXYETHYL)ADENINE AND 9-(2-PHOSPHONYLMETHOXYETHYL)-2,6-DIAMINOPURINE IN MICE INFECTED WITH FRIEND LEUKEMIA VIRUS

L. Naesens*, J. Balzarini*, A. Holý**, I. Rosenberg** and E. De Clercq, *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium and **Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Praha 6, Czechoslovakia

9-(2-Phosphonylmethoxyethyl)adenine (PMEA) and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) are potent and selective inhibitors of HIV-1 and HIV-2 replication *in vitro*; PMEA and PMEDAP also inhibit Moloney murine sarcoma virus (MSV)-induced tumor formation and associated death in newborn NMRI mice (Pauwels *et al.*, Antimicrob. Agents Chemother. 32:1025-1030, 1988; Balzarini *et al.*, Proc. Natl. Acad. Sci. USA 86:332-336, 1989; Naesens *et al.*, Eur. J. Clin. Microbiol. Infect. Dis., in press, 1990). PMEA and PMEDAP were now investigated for their efficacy in inhibiting splenomegaly in adult NMRI mice infected with Friend leukemia virus (FLV). At a daily dose of 50 mg/kg/day for 10 days post infection, PMEA and PMEDAP almost completely inhibited FLV-induced splenomegaly (80% and 100% inhibition, respectively). Azidothymidine given at 100 mg/kg/day for 10 days was equally effective as PMEA at 20 mg/kg/day or PMEDAP at 5 mg/kg/day (inhibition of splenomegaly: 40%). Also, a relatively short treatment period with PMEA and PMEDAP (5 days) at a dose of 50 mg/kg/day resulted in a marked inhibition of splenomegaly (57% and 78%, respectively). The inhibitory effect of PMEA and PMEDAP on FLV-induced splenomegaly was dose-dependent and more pronounced the longer therapy was continued. Additional experiments are underway to delineate the optimal treatment schedules for both PMEA and PMEDAP.

**L 332** RECOMBINANT THYMIDINE KINASE-DEFICIENT FELINE HERPESVIRUSES AS LIVE VECTORS FOR RETROVIRUS VACCINATION IN CATS, Jack H. Nunberg[1], Georgette E. Cole[1], Sandrina S. Phipps[1], Erik A. Petrovskis[2], Leonard E. Post[2]. [1] Cetus Corporation, Emeryville, CA 94608; [2] The Upjohn Company, Kalamazoo, MI 49001.

Feline herpesvirus (FHV) is the causative agent of feline viral rhinotracheitis (FVR) in cats. Widespread vaccination using modified live and killed FHV has been successful in controlling FVR in cats. We were interested to develop this feline-specific member of the alphaherpesvirus subfamily as a recombinant virus vector with which to explore the basis of retroviral immunity in cats. Here, we describe the initial development of FHV as a vaccine vector. In our development of recombinant FHVs, we chose to use the FHV thymidine kinase (tk) gene - as an insertion site into which to insert heterologous genes and as a selective marker to allow the facile isolation of recombinant viruses. Herpesvirus tk genes are highly divergent, sharing only short regions of imperfect amino acid identity. We have used the polymerase chain reaction method of DNA amplification to increase the specificity associated with the use of short, highly degenerate oligonucleotides derived from regions of imperfect amino acid conservation. This method was successfully used to isolate the tk gene of FHV. The amino acid sequence and genomic localization of the FHV tk gene is described and compared with those of other herpesviruses. As anticipated, the FHV tk gene was shown to be dispensable for FHV growth *in vitro* and may thus be interrupted by the insertion of relevant genes from heterologous pathogens. Initially, a recombinant FHV bearing a deletion in the identified tk gene was isolated and shown to possess the expected tk⁻ phenotype. This recombinant virus grows well in culture. Future work will focus on the development of recombinant FHVs for use as viral vaccine vectors, in particular as vectors with which to study the immune response to immunogens of the two well-characterized feline retroviruses, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV).

**L 333** **FELINE IMMUNODEFICIENCY VIRUS : DEVELOPMENT OF A SMALL ANIMAL MODEL FOR LENTIVIRAL PATHOGENESIS AND SEROLOGIC EVIDENCE FOR FIV INFECTION IN NON-DOMESTIC FELIDS.** Robert A. Olmsted[1], Vanessa M. Hirsch[1], Stephen J.O'Brien[2] and Philip R. Johnson[1], [1]Dept. of Microbiology, Georgetown University, Rockville, MD 20852; [2]NCI, Frederick, MD 21701.

Our goal is the development of a small animal model to study lentiviral pathogenesis and AIDS. To date, immunosuppression and opportunistic infections have not been characteristic features of experimental FIV infections. One reason may be the use of SPF cats for experimental infections which do not harbor the known pathogens carried by common domestic cats. We have initiated a study to evaluate the common domestic cat as an alternate to SPF cats for experimental infection with FIV. Two groups received $10^{3.0}TCID_{50}$s of either FIV-Petaluma or molecular clone virus, FIV-14. A third group was mock infected. The 10 virus infected cats seroconverted by six weeks p.i. as determined by ELISA and virus was recovered from PBL cultures from nine of these animals. The negative animal is in the FIV-14 group. PCR analyses of PBL DNA are being performed to confirm infection. None of the mock infected cats has shown signs of clinical illness. However, two FIV-14 infected cats developed watery diarrhea at 5 weeks p.i. that lasted for several days. One FIV-Petaluma infected cat has developed ringworm which has persisted for several weeks. Further details of this study will be presented.

To search for naturally occurring and potentially more pathogenic isolates of FIV, we have screened 99 plasma/serum samples from wild-caught or captive panthers by western blot analysis of FIV whole virus to assay for serologic evidence of FIV infection in non-domestic felids. Interestingly, 29 of 68 (43%) wild-caught panthers from throughout the USA showed mild to strong antibody reactivity to p28, the FIV major core protein. None of the captive panthers (n=31) were positive by immunoblot assay. ELISA, FA and RIPA assays are being performed as further confirmation. Lymphocyte DNA from a majority of these animals are available and will be screened for FIV specific sequences by PCR. These data and analyses of other exotic felids will be presented.

**L 334** NEUTRALIZATION OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I (HTLV-I) WITH ANTI-PEPTIDE ANTISERA, T. Palker, R. Streilein, R. Scearce, A. McKnight[1], P. Clapham[1], R. Weiss[1] and B. Haynes. Duke University Medical Center, Durham, NC 27710. [1]The Institute of Cancer Research: Royal Cancer Hospital, Chester Beatty Laboratories, London, U.K. Twelve synthetic peptides (SP-1, 2,3,4,4A,5,6,7,8,9,10 and 11) containing hydrophilic amino acid sequences of HTLV-I gp63 envelope glycoprotein were coupled to tetanus toxoid and used to raise anti-envelope antisera in rabbits and goats. Anti-peptide antisera raised to 6 of 7 peptides (SP-1,2,3,4,4A and 6) containing hydrophilic amino acid sequences of HTLV-I gp46 external envelope immunoprecipitated affinity-purified gp46, while 5 of 7 sera raised to peptides SP-1,3,4,4A and 6 reacted with gp46 in Western blot assays. In a preliminary study, antisera were tested for the ability to neutralize the infectivity of vesicular stomatitis virus (VSV)/HTLV-I pseudotype particles. Antisera to peptides SP-2 (envelope amino acids 86-107), SP-3 (a.a. 176-189) and SP-4A (190-209) neutralized VSV/HTLV-I pseudotype particles by greater than 80% at a 1/10 dilution. Antisera from rabbits immunized with a combined peptide SP-3/4A (a.a. 176-209) also neutralized at a 1/10 dilution. All pre-immune sera and sera raised to HTLV-I gp21 transmembrane protein were negative. Antisera from 2 goats immunized with a combination of peptides SP-2 and 3/4A contained high titers of neutralizing antibodies ranging from 1/40 - 1/640 in the pseudotype neutralization assay. Antisera from 1 goat immunized with peptides SP-2, 3 and 4A also inhibited HTLV-I-induced syncytium formation (titers of 1/10 - 1/20). None of these antisera neutralized HTLV-II pseudotype particles, indicating the presence of HTLV-I specific neutralizing determinants on gp46. Identification of neutralizing regions of HTLV-I gp46 may be useful in the design of synthetic vaccines for prevention or attenuation of HTLV-I-induced disease.

**L 335** IMPAIRED CELL-FREE VIRAL TRANSMISSION OF HIV-2 **VIF** MUTANTS IN DIFFERENT CD4 POSITIVE CELL TARGETS, Jeffrey S. Parkin, John C. Kappes, Beatrice H. Hahn, and George M. Shaw, Departments of Biochemistry and Medicine, The University of Alabama at Birmingham, Birmingham, AL 35259.

Human and simian immunodeficiency viruses (HIV/SIVs) contain a conserved unique open reading frame (ORF) encoding a 23-25kD protein designated virion infectivity factor (vif; previously termed sor, A, Q, P, or ORF-1). Previous studies by Fisher et al. (1987), Strebel et al. (1987), and Luciw et al. (1987) demonstrated that the HIV-1 vif is required for efficient cell-free viral transmission in certain $CD4^+$ cell lines. In order to more thoroughly analyze the role of the vif gene product in viral replication, a series of site-directed mutants were constructed. Strong translational stop codons (TAA) were introduced throughout the $HIV\text{-}2_{ROD}$ vif ORF resulting in the production of a series of predicted truncated protein products (2.3, 4.2, 6.9, 12.8, and 17.9kD). These mutants were reintroduced into a biologically active $HIV\text{-}2_{ROD}$ proviral genome and their effects upon cell-free viral transmission quantitatively analyzed. Vif mutant proviruses displayed impaired cell-free viral transmissibility to differing extents depending on the cell target (PBL, CEM, CEMX174, H9, and SupT1). Cell determinants responsible for these differences and the specific role of vif in viral replication are under study.

**L 336** CELL TYPE DISTRIBUTION OF HTLV-1 IN PERIPHERAL BLOOD: INFECTION OF A SUBSET OF CD4+ LYMPHOCYTES BUT NOT MONOCYTES OR OTHER NON-T CELLS Jennifer H Richardson, A Edwards, J K Cruickshank, P Rudge, A G Dalgleish. Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK.

The predominant cell type to be immortalised by HTLV-1 in vivo and in vitro is the CD4+ lymphocyte but previous studies have shown that CD8+ lymphocytes, B cells and a variety of non-lymphoid cells are also susceptible to HTLV-1 infection. To establish whether cells other than CD4+ lymphocytes are targets for infection in HTLV-1 carriers we examined the phenotype of HTLV-1 infected peripheral blood cells in patients with tropical spastic paraparesis (TSP) and an asymptomatic HTLV-1 carrier. PCR was used to detect and quantitate viral DNA in sub-populations of leukocytes obtained using a combination of Ficoll separation, E rosetting and fluorescence activated cell sorting. HTLV-1 could not be detected in peripheral blood mononuclear cells thoroughly depleted of T cells (E- CD3-) nor in highly enriched populations of B cells (E-CD19+), monocytes (E- CD14+) or NK cells (E- CD16+). The E+ (T) cells were strongly positive for HTLV-1 and fractionation of this population revealed 90% to 99% of the HTLV-1 DNA to be associated with the CD4+ and CD45RO+ subsets and only 1% to 10% with the CD8+ and CD45RO- subsets. Granulocytes were usually positive for HTLV-1 but contamination of this population with T cells has not been excluded. No difference between the cell type distribution of HTLV-1 in the asymptomatic carrier and the subjects with TSP was evident. Limiting dilution PCR analysis revealed an average of one provirus per 10 to 100 CD4+ cells, a figure confirmed by Southern blot analysis of genomic DNA from TSP patients. We conclude that the virus is largely confined to the "helper inducer" (CD45RO+) subset of CD4+ lymphocytes and that a significant proportion (assuming one provirus per infected cell) of circulating CD4+ cells are infected.

**L 337** NUCLEOTIDE SEQUENCE AND ANALYSIS OF cDNA CLONES FROM AN INFECTIOUS ISOLATE OF CAEV, Mary Saltarelli,[1,2], Gilles Querat,[3], Robert Vigne[3], and Janice E. Clements[1,2], Department of Molecular Biology and Genetics[1], Division of Comparative Medicine[2], The Johns Hopkins University School of Medicine, Baltimore, MD. 21205, U.S.A. Laboratoire de Virologie, Faculte de Medecine Nord[3], 13326, Marseille Cedex 15, France. Caprine Arthritis Encephalitis Virus (CAEV), a caprine lentivirus, is the causative agent of leucoencephalitis in young goats and arthritis in the adult animal. CAEV shares many biological and molecular characteristics with human, primate and other animal lentiviruses; in particular it shares considerable homology to ovine lentiviruses such as visna virus. A complex transcriptional pattern similar to that of visna virus and HIV-1 has been seen by Northern analysis. Complete nucleotide sequence analysis of an infectious clone of this virus has revealed the presence of two small open reading frames (ORFs) between the pol and env genes. One of these ORFs is highly homologous to the visna virus tat gene and encodes a trans-activating factor. However, the CAEV Tat protein lacks a cysteine residue which is conserved in the ovine lentiviruses. Mutational analysis has been done to determine the importance of the cysteine-rich and basic domains of this protein. PCR cDNA cloning has been used to identify a third transcribed small orf. It overlaps the 3' terminus of the env gene and is related to the HIV-2 and SIV rev genes.

**L 338** RABBIT MODELS OF HTLV-I AND HIV. S. Sell, Kent Tseng and Mary Ann Hughes. Department of Pathology and Laboratory Medicine, University of Texas Medical School, Hosuton TX, 77030

Preliminary studies of infection of rabbits with either HTLV-I or HIV-1 and the effect of superinfection with T. pallidum and Shope Fibroma Virus has revealed the following:

1) The DNA of the peripheral blood lymphocytes (PBLs) of rabbits inoculated with HTLV-I infected human or rabbits cell lines can be shown to contain intregrated HIV-1 DNA within 15 weeks.

2) Antibody to HTLV-I can be demonstrated in all rabbits by 8 weeks.

3) HTLV-I infected rabbits have poor responses to cutaneous challenge with T. pallidum and demonstrate depressed mitogen responses and IL2 production.

4) HTLV-I infected rabits with prolonged or progressive primary chncres treated with penicillin to inititute healing demonstrate chancre immunity upon rechallenge.

5) Eight of 8 rabbits inoculated with HIV infected human cells demonstrated HIV DNA in their PBLs by 5 weeks after inoculation and antibody to HIV by Western blot by 23 weeks after infection.

6) All eight rabbits has antibody to p24, 3 to p28, 6 to p41 and 1 had strong reactivity to p120.

7) Three of 4 HIV-1 infected rabbits challenged with T. pallidum had delayed healing and 1 had a progressive primary lesion.

8) Four HIV-1 positive rabbits were able to reject Shope Fibroma Induced tumors.

9) Total WBC and IL2 production were generally higher than in control T. pallidum infected rabbits, but within normal limits.

**L 339** CHARACTERIZATION OF MONOCLONAL ANTIBODIES REACTIVE TO SIV GP120 AND GP32, Clotilde Thiriart[1], Catherine Collignon[1], Anne Delers[1], Françoise Bex[2], Michel De Wilde[1] and Claudine Bruck[1], Molecular and Cellular Biology Department, Smith Kline-Biologicals, B-1330 Rixensart, Belgium (1) and Laboratoire de Chimie Biologique, Université Libre de Bruxelles, 67 rue des Chevaux, B-1640 Rhode-St-Genèse, Belgium (2)

Monoclonal antibodies (mAbs) were derived from Balb/c mice immunized with a live vaccinia recombinant expressing SIV gp160 (BK28 clone from Dr. J. Mullins). The hybridoma's supernatants were screened in an ELISA using lentil lectine affinity purified recombinant SIV gp160 as antigen. Several hybridoma cell lines reactive in this assay were selected and cloned in soft agarose. The mAb's reactivity towards the different forms of the SIV envelope glycoprotein was assessed : i) in radioimmunoprecipitation assay using $^{35}$S-Methionine labelled HUT78-BK28 lysate and using lysate or supernatant of metabolically labelled vaccinia-gp160 infected cells, ii) in Western blot analysis using purified BK28 virus as antigen. The epitopes recognized by some of the mAbs were further mapped by monitoring their reactivity with 3 E. coli produced recombinant polypeptides that together cover 99 % of the envelope precursor sequence. The potential neutralizing activity of these mAbs is currently under evaluation.

**L 340** MOLECULAR CLONING OF AN EUROPEAN FIV ISOLATE. Ernst Verschoor, Joke Ederveen, Jero Calafat*, John Elder", Regina Möhring*, Marian Horzinek and Marck Koolen. Institute of Virology, State University of Utrecht, Utrecht, The Netherlands; " Scripps Clinic and Research Foundation, La Jolla, CA 92037, USA; * Zentralinstitut für Molekularbiologie, Akademie der Wissenschaften der DDR, Berlin-Buch, DDR. * National Cancer Institute, Amsterdam, The Netherlands.

Feline immunodeficiency virus is a lentivirus, widely spread in the cat population and is replicating preferentially in T-lymphocytes, macrophages and neural cells. In cats the infection is characterized by an initial asymptomatic phase of several months or even years during which virus can be demonstrated. Subsequently, a variety of clinical abnormalities may develop. Ultimately, the infected animals may die of opportunistic infections.

Recently, we have molecularly cloned the genome of a Dutch FIV isolate, FIV-113 from infected feline thymocytes using a rapid cloning strategy. Sequence comparisons with the Petaluma strain, the prototype of FIV [Olmsted et al., PNAS 86, 2448-2452 (1989)] were made and will be discussed. Crandell feline kidney cells (CRFK) used for large scale production of FIV are persistently infected with the endogenous feline retrovirus RD114. Therefore, immuno-electron microscopy and Southern blot analysis studies were performed to sort out the possibility of phenotypic mixing.

**L 341** NONCLINICAL STUDIES FOR ASSESSING THE NEONATAL TOXICITY OF DRUGS INTENDED TO TREAT LIFE-THREATENING DISEASES, Emmanuelle Voisin, Peter C. Hoyle, and Ellen Cooper, Division of Antiviral Drug Products, U.S. Food and Drug Administration, Rockville, MD 20857.

Principles for testing the potential neonatal toxicity of drugs intended to treat life-threatening diseases in newborns have not yet been established because this situation is relatively rare in drug development. An increasing number of drug developers, however, are confronted to this problem when developing treatments for HIV-infected neonates and seek preclinical guidance from the FDA Division of Antiviral Drug Products. This matter involves weighing expedited drug development with important safety issues. Nonclinical toxicity studies that may be considered focus on exposing newborn rodents from day 1 to day 36 postpartum. Postnatal observations of the animals (pup viability, physical signs, body weight, preweaning developmental evaluations) and postweaning behavioral evaluations (development of reflexes, spontaneous movements, performance tests, operant conditioning, social behavior, development of sensory function) have been employed. These tests and their limitations will be discussed. This document is provided as a guidance, not a guideline, to facilitate the development of new AIDS drugs.

**L 342** A FELINE LYMPHOID CELL LINE (FIT-1) PERMISSIVE FOR FELINE IMMUNODEFICIENCY VIRUS (FIV). K.Weijer[x], C.H.J.Siebelink[xx], I.Chu[xx], F.G.C.M.UytdeHaag[xx] and A.D.M.E. Osterhaus[xx], [x]The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands, [xx]National Institute of Public Health and Environmental Protection, P.O.Box 1, 3720 BA Bilthoven, The Netherlands.

A feline lymphoid cell line (FIT-1) was isolated from peripheral blood mononuclear cells (PBMC) of an SPF cat by stimulation with Con A and further cultivation at limiting dilutions. FIT-1 could be cultured for more than six months in the presence of human IL-2 without any further stimulation. The cell line could be infected with FIV by cocultivation of PBMC of naturally (n=8) or experimentally (n=4) infected seropositive cats. Generally, within seven days - when the first cytopathic changes including syncytia formation were observed - the presence of FIV could be demonstrated by showing FIV antigen in the culture supernatant with a FIV specific antigen capture ELISA. Continuous production of FIV could be achieved by adding uninfected cells to an infected FIT-1 culture at regular intervals. FIT-1 could also be used for infectivity titrations of FIV, and preliminary data indicate that also for the demonstration of virus neutralizing antibodies in serum samples of cats FIT-1 can be used.

**L 343** INDUCTION OF CROSS-REACTIVE HIV-1 NEUTRALIZING ANTIBODIES IN RHESUS MONKEYS FOLLOWING A COMBINED IMMUNIZATION REGIMEN WITH HIV-1-env-RECOMBINANT VACCINIA VACCINES AND A RECOMBINANT HIV-1 gp160 VACCINE, Martha A. Wells, Emily W. Carrow, Gerald V. Quinnan, Jr., Luba K. Vujcic, Bernard N. Moss, Patricia Earl, Sylvia Merli, Ruth Seemann, Wendy L. Glass, Sylvester Daniel, and Alec E. Wittek, LRR, DV, CBER, FDA and LVD, NIAID, NIH, Bethesda, MD 20892

**OBJECTIVE**: To study HIV-1 specific antibody (Ab) responses in Rhesus (Rh) monkeys immunized with a combination regimen of recombinant vaccinia viruses containing the gp160 envelope gene of HIV-1 strains IIIB or RF, followed by a recombinant $HIV1_{IIIB}$gp160 vaccine (rgp160).

**METHODS**: Groups of 4 Rh were vaccinated with either $2X10^8$ PFU of a TK- deletion mutant of vaccinia virus (VAC-TK), or recombinant vaccinia virus containing the HIV-1 IIIB (VAC-IIIB) or RF (VAC-RF) gp160 envelope gene, at weeks 0 and 9. Two Rh from each group were immunized with 50ug of $HIV\text{-}1_{IIIB}$rgp160 (Immuno AG, Vienna, Austria) in deoxycholate and alum at weeks 34 and 52. Sera were collected at intervals for measurement of HIV-1 envelope reactive Ab by Western blotting (WB) against commercial HIV-1 strips, by whole virus ELISA, by an HIV-1 syncytium inhibition microneutralization assay, and by a $^{51}Cr$ release HIV-1 antibody dependent cellular cytotoxicity (ADCC) assay.

**RESULTS**: All Rh showed primary but not secondary takes, and all Rh receiving VAC-IIIB and RF developed Ab reactive with HIV-1 envelope. Booster responses to a second dose of VAC-IIIB or RF were inconsistently seen and generally weak. The VAC-recombinants alone did not induce HIV-1 neutralizing Ab (NA) or ADCC. Following rgp160 immunization, both HIV-naive VAC-TK Rh developed Ab to HIV-1 by WB, although only 1 was ELISA positive. All 4 Rh that previously received VAC-recombinants showed an increase in HIV-1 Ab titer. Sera from 3/4 boosted Rh showed transient NA responses ranging from 1:8 to 1:32 against one or more of HIV-1 strains IIIB, RF, and MN. No sera mediated HIV-1-specific ADCC.

**CONCLUSION**: Primary immunization with either VAC-recombinant or rgp160 elicited HIV-1 binding Ab but no NA or ADCC responses. Boosting of VAC-recombinant Rh with rgp160 induced a transient cross-reactive NA response in 3/4 animals. Combination immunization regimens using live HIV-1 recombinant vaccinia viruses as the primary vaccine and an HIV-1 subunit vaccine as a booster immunogen may provide a means to generate broadly reactive HIV-1 NA responses in man.

**L 344** INTERIORIZATION OF HIV AND OTHER RETROVIRUSES BY MEGAKARYOCYTES AND PLATELETS, Dorothea Zucker-Franklin, Zhao Yao Zheng and Stephanie Seremetis, Department of Medicine, New York University School of Medicine, New York, NY 10016.

Whether megakaryocytes can take up retroviruses is of interest from several points of view. Firstly, while the megakaryocytes (MK) of patients with AIDS show structural aberrations (Am J Path 134:1295,'89) and integrated proviral sequences (PNAS: 86, 5595,'89) it is not known whether HIV-1 is subject to interiorization by mature cells of this lineage. No virus particles have been found in freshly isolated marrow or platelets of such patients. Secondly, the cells could serve as targets for retrovirus-based vectors carrying genetic information concerned with functions that are lacking in some well recognized congenital platelet disorders. To examine whether MK/platelets take up viruses electron microscopy was carried out on mouse bone marrow cells incubated with a mouse cell line infected with an amphotropic retrovirus, and normal human marrow was incubated with HIV-1 infected H9 cells for various time periods. Uptake of virus in the murine specimens was avid at all stages of megakaryocyte differentiation, whereas interiorization of HIV by human megakaryocytes and platelets was demonstrable to a lesser extent. However, since the MK is a polyploid cell whose nucleus undergoes endoreduplication, integration of exogenous genetic material may be very efficient in this cell lineage.

**L 345** HIV-1 INFECTION OF HUMAN AND CHIMPANZEE HEMATOPOIETIC PROGENITOR CELLS. Yiling Liu, Marta Grofova, Teresa Brandon, Mikulas Popovic, Jeff Rowell and Suzanne Gartner. Primate Research Institute, New Mexico State University.

Chimpanzees represent an important animal model for HIV-1 infection. These animals are susceptible to HIV-1 itself and become persistently infected following virus exposure. Clinical disease does not follow, however, the reasons being unclear. It has been suggested that, in chimpanzees, cells of the mononuclear phagocyte lineage are not susceptible to the virus. HIV-1 has been detected in bone marrow of infected chimpanzees, but the type(s) of cells infected have not been determined. We examined the susceptibility of human and chimpanzee hematopoietic progenitor cells to HIV-1 in vitro. Clonal agar assays were performed on HIV-1-exposed cell preparations. Colonies of cells were evaluated morphologically and subjected to polymerase chain reaction (PCR) analyses. In preliminary experiments, HIV-1 genome was detected in colonies of human hematopoietic progenitor cells and cocultivation of cells from these colonies with macrophages resulted in transmission of the virus. These findings confirm that human hematopoietic progenitor cells can serve as host cells for HIV-1 infection. They also suggest that HIV-1 can actively replicate in progenitor cells and that the viral progeny are infectious. Results of our studies with chimpanzee cells will also be presented.

## HIV and AIDS: Pathogenesis, Therapy and Vaccine

*Serology and Immunology; Cell Biology*

**L 400** INHIBITION OF HIV-1 IN EARLY INFECTED AND CHRONICALLY INFECTED CELLS BY ANTISENSE OLIGODEOXYNUCLEOTIDES AND THEIR PHOSPHOROTHIOATE ANALOGUES.
Sudhir Agrawal, Daisy Sun*, Prem Sarin* and Paul C. Zamecnik; Worcester Foundation for Experimental Biology, Maple Avenue, Shrewsbury, MA 01545; *Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892.
Antisense oligonucleotides, both the phosphorothioate analogues and unmodified oligomers of the same sequence, inhibit replication and expression of human immunodeficiency virus already growing in tissue cultures of MOLT-3 cells with much greater efficacy than do mismatched oligomers and homooligomers of the same length and with the same internucleotide modification. This preferential inhibitory effect is elicited in as short a time as 4-24 hour postinfection. Likewise antisense oligomers exhibit greater inhibitory effects on human immunodeficiency virus in chronically infected cells than do mismatched oligomer and homooligomers. Phosphorothioate antisense oligomers are up to a 100 times more potent than unmodified oligomers of the same sequence in these inhibitory assay. Activity of antisense phosphorothioate oligomer falls off gradually as the length of oligomer is reduced from 35 to 10. Preliminary studies of toxicity in mice and rats show these oligomers to be nontoxic up to a dose of 160 mg/kg, pointing to the potential usefulness of the antisense oligomers in the treatment of patients with AIDS and ARC.

**L 401** VACCINE DEVELOPMENT FOR AIDS THROUGH MOLECULAR SURGERY OF A HUMAN COMMON COLD VIRUS SURFACE, Gail Ferstandig Arnold[1], Yuling Li[1], R. Kevin Ryan[1], L. Alexandra Wickham[1], Wai-ming Lee[2], Roland R. Rueckert[2], and Edward Arnold[1], 1-Center for Advanced Biotechnology and Medicine (CABM) and Rutgers University Chemistry Department, 679 Hoes Lane, Piscataway, NJ 08854-5638, 2-Institute for Molecular Virology, University of Wisconsin, Madison, WI, 53706.
By combining the techniques of virology, recombinant DNA technology, interactive computer graphics, and crystallography, it should be possible to design and produce safe and effective vaccines against a wide variety of dangerous pathogens. An ideal vector for vaccine design is human rhinovirus 14 (HRV14). Human rhinoviruses are among the safest candidates to be used as viral vectors, and are known to provoke significant and long-lasting serum and nasal mucosal immune responses. The structure of HRV14 has been determined by X-ray crystallography and refined to 3 Å resolution (Rossmann et al., 1985; Arnold & Rossmann, 1988), and thus atomic representations of the virus can be visualized via computer graphics, permitting thoughtful design of "molecular surgery" and possibly enhancing the likelihood of producing successful vaccines. The detailed mapping of the immunogenic regions of HRV14 in the protein sequence (Sherry et al., 1986) and in three-dimensional space (Rossmann et al., 1985) allows one to suggest replacements for these antigens with those identified for any of a variety of pathogens. By applying recombinant DNA technology to a cDNA clone of HRV14, we are producing chimeras, at least some of which should be capable of stimulating a protective immune response.

The construction of two chimeras, an HRV14:poliovirus 3 and an HRV14:influenza hemagglutinin chimera, have been completed and immunological and crystallographic analyses are in progress. A number of HRV14 chimeras that incorporate epitopes from the HIV envelope glycoproteins are currently being constructed and results from these studies will be presented.

**L 402** ANTI-HIV ACTIVITY OF CD4-PSEUDOMONAS EXOTOXIN HYBRID PROTEIN: HIGHLY SYNERGISTIC EFFECTS WITH AZT IN CULTURES OF HUMAN T-CELL LINES AND ACTIVITIES AGAINST OTHER INFECTED CELL TYPES, Per A. Ashorn, Bernard Moss and Edward A. Berger, Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892
CD4(178)-PE40 is a genetically engineered hybrid protein consisting of the HIV gp120 binding region of CD4 linked to active domains of *Pseudomonas aeruginosa* exotoxin A. This protein has previously been shown to be a highly potent and selective agent for killing of HIV-1 infected human T-cell lines (IC50 appr. 100 pM), and to inhibit HIV-1 spread in mixtures of infected and uninfected cells. Using such a coculture system, we now report strong synergistic activity between CD4(178)-PE40 and AZT in blocking HIV spread, as assayed by protection from virus-induced cell killing and inhibition of p24 production. The former drug alone delays but does not completely prevent virus spread; the latter drug provides more extensive protection but only when maintained in culture. However, after an initial treatment period with a combination of CD4(178)-PE40 and AZT, both drugs can be removed from the culture with no evidence of viral spread during a subsequent three-week period. As for other cell types, CD4(178)-PE40 is also active on *in vitro* HIV-1 infected peripheral blood lymphocytes, markedly decreasing HIV p24 protein release to culture medium. Moreover, CD4(178)-PE40 selectively inhibits host protein synthesis and directly kills (IC50 0.5-1.0 nM) chronically HIV-1 infected human U1 monocyte/ macrophage cells, resulting in significantly reduced HIV RT and p24 production. Finally, the hybrid toxin is comparably potent on cells expressing either HIV-1 or SIV gp160 envelope glycoproteins, as judged by protein synthesis inhibition in cells infected with recombinant vaccinia virus vectors (IC50 20-40 pM).

## L 403 Synergistic Inhibition of HIV by Combined AZT and ddI Contrasts with Additive Inhibition of Normal Human Marrow Progenitor Cells

Devron R. Averett[1], Ronna E. Dornsife[1], Andrew T. Huang[3], Tim J. Panella[3], George W. Koszalka[1], Charlene Burns[1], and Marty St. Clair[2] Divisions of Experimental Therapy[1] and Virology[2], Burroughs Wellcome Co., Research Triangle Park, NC 27709, and Division of Hematology and Oncology[3], Duke University Medical Center, Durham NC 27710

AZT and ddI provide significant synergistic inhibition of HIV when combined *in vitro*. This synergy varies in degree, but is moderate in most systems tested. *In vitro* inhibition by ddI alone of granulocyte-macrophage colony forming cells (CFU-GM) and erythroid burst forming cells (BFU-E) from healthy human donors was only observed at relatively high concentrations (I50 values were >100 μM and 31± 1.2 μM for CFU-GM and BFU-E, respectively). These results are consistent with the low frequency of reported hematopoietic toxicities in clinical studies of ddI. Addition of inhibitory concentrations of ddI to serial dilutions of AZT resulted in reductions of both CFU-GM and BFU-E which were consistent with additive inhibition of these progenitor cells. Taken together, these *in vitro* data suggest that therapy with combinations of ddI and AZT at appropriately modified doses may retain clinical benefit against HIV infection while reducing adverse effects.

## L 404 2,3-EPOXY ALCOHOLS, ACIDS, AND DERIVATIVES AS ANTIRETROVIRAL AGENTS, Jeffrey J. Blumenstein, Christopher J. Michejda, and Stephen Oroszlan, Laboratories of Chemical and Physical Carcinogenesis, and Molecular Virology and Carcinogenesis, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701

The fatty acid synthesis inhibitor, cerulenin, has been reported to exhibit antiretroviral activity against Rous Sarcoma Virus, Murine Leukemia Virus, and Human Immunodefficiency Virus, although its high toxicity has limited its utility. The nature of this activity has been presumed to be the disruption of virus protein processing through the inhibition of the virally encoded protease. Structurally related compounds, 2,3 epoxy alcohols, acids, and derivatives have been synthesized and their bioactivity examined. Several of these compounds significantly inhibit synthetic HIV-2 PR while having little effect upon AMV PR (obtained from Molecular Genetics Resources, Inc.) The *in vitro* antiretroviral activity of these agents have also been examined in MuLV infected cell lines. Production of virus is significantly decreased in the presence of 20 μg/ml of *N*-((2*R-cis*)-epoxydodecanoyl)-L-proline methyl ester. Other, but not all, compounds of this class which inhibit the HIV-2 PR also inhibit MuLV virus production. Furthermore, all of the epoxides examined appear to be nontoxic at 50 μg/ml in contrast to cerulenin, which is toxic to the cultures at 5 μg/ml. The nature of the antiviral activity and its relationship to PR inhibition is examined. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with BRI.

## L 405 PLASMA MICRONUTRIENTS AND HIV-1 INFECTION, John D. Bogden, Herman Baker, Oscar Frank, George Perez, Francis Kemp, Kay Bruening, and Donald Louria, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Cellular immunity is highly dependent on vitamin and trace element nutriture. The presence of vitamin or trace element deficiencies could exacerbate the progression and/or severity of HIV infection. This study surveyed serum concentrations of 21 vitamins, electrolytes, and trace elements in subjects seropositive for HIV-1 by ELISA and confirmatory Western blot. Thirty subjects (26 males, 4 females) were recruited at a hospital clinic. Seventeen were classified as having mild or severe ARC (AIDS-related complex), 7 had AIDS, and 6 were asymptomatic. Eight had experienced weight loss of 10 pounds or more in the past 6 months. Most (93%) were anergic to skin test antigens. Percentages with above normal values include: folate - 37% and carnitine - 37%. Some subjects with above normal values for plasma vitamins reported self-supplementation, usually with large doses. Percentages of subjects with below normal plasma concentrations include: zinc - 30%, calcium - 27%, magnesium - 30%, carotenes-31%, total choline - 50%, and ascorbate - 27%. Eighty-seven percent of the subjects had at least one abnormally low value, 50% had 3 or more low values, and 23% had 5 or more low concentrations. The results suggest that one or more abnormally low concentrations of the plasma micronutrients studied here are likely to be present in the majority of HIV seropositive patients.

**L 406** AURINTRICARBOXYLIC ACID BLOCKS GP120 BINDING TO THE HUMAN IMMUNODEFICIENCY VIRUS/CD4 RECEPTOR. Terry L. Bowlin[+], Kendra K. Schroeder[+], Alan D. Cardin[+], Debbie Taylor[π], and A. Stanley Tyms[π]. [+]Merrell Dow Research Institute, Cincinnati, Ohio 45215 and [π]MRC Collaborative Center, Mill Hill, London NW7 1AD, U.K. The CD4 molecule is a transmembrane glycoprotein which binds avidly to the HIV envelope glycoprotein gp120. Aurintricarboxylic acid (ATA) has been recently shown to prevent monoclonal antibody binding to CD4 (Schols et al., PNAS 86:3322, 1989). In the present study, we have compared ATA with several other known inhibitors of HIV, including heparin and dextran sulfate for their ability to bind CD4 positive cells and block gp120 binding. A CD4 positive lymphoblastic leukemia cell line (JM) was used for these studies. Cells were preincubated (10 sec-24 hrs) with the appropriate compound, washed, and incubated with recombinant gp120 for 2 hrs. Cells were then labeled with a monoclonal antibody against gp120 and analyzed by flow cytometry. ATA (100 µg/ml), heparin (100 µg/ml), 8000 MW dextran sulfate (100 µg/ml) and 500000 MW dextran sulfate (100 µg/ml) inhibited gp120 binding to the JM cells by 78%, 15%, 26% and 22%, respectively. In contrast, other HIV inhibitors such as azidothymidine, a reverse transcriptase inhibitor, and castanospermine, a glycoprotein processing inhibitor, had no effect on gp120 binding. ATA was the most potent inhibitor of gp120 binding tested with an $IC_{50}$ of approximately 0.4 µg/ml. Similarly, ATA inhibited HIV infectivity with an $IC_{50}$ of approximately 0.4 µg/ml. The effect of ATA on gp120 binding and HIV growth in JM cells were dose-dependent. Preincubating ATA with soluble recombinant CD4 completely blocked the ability of ATA to inhibit gp120 binding to CD4 positive cells, indicating that ATA is directly binding CD4. These results suggest that blocking CD4 may provide a useful approach in HIV chemotherapy.

**L 407** PRODUCTION OF NEUTRALISING MONOCLONAL ANTIBODIES TO HIV, J.E. Boyd, A.J.Shepherd, A.Pryde, C.V.Prowse and K.James, Retrovirus Research Laboratory, Wilkie Building, Teviot Place, Edinburgh EH8 9AG.
Neutralising monoclonal antibodies (MAbs) to HIV have a number of potential applications in prophylaxis or therapy and for the identification or purification of epitopes suitable for inclusion in a vaccine. We have utilised various forms of immunogen in order to induce neutralising antibodies in mice as a preface to preparing MAbs. Peptides representing conserved sequences on env or gag with potential to induce cross-reacting neutralising antibodies were obtained and coupled to carrier protein - thyroglobulin or KLH. Recombinant env and crude extracts of cells infected with various strains of HIV-1 and HIV-2 were also used. Balb/c mice were immunised with each immunogen in adjuvant and their reponse was monitored by ELISA. Neutralising antibodies were detected using virus-infected MT-4 cells and cell death was quantitated by MTT dye uptake after 6-7 day's incubation. The effect of conjugation on the reponse to each peptide varied, but conjugation usually improved it. Neutralising antibodies were induced by some of the peptides, infected cell extracts and recombinant gp120. After fusion of immune spleen cells, the MAbs produced were screened for binding in an appropriate ELISA, while their neutralisation capacity, isotype and reactivity in a Western blot were also determined.

**L 408** IN VITRO ANTI-HIV ACTIVITY OF PYRIMIDINE AND PURINE NUCLEOSIDE COMBINATIONS, Vera Brankovan, *Roelf Datema, Kathy Tarantini, and Andrew J. Watson, Bristol-Myers-Squibb Co,; Oncogen, Seattle, WA.98121, *Virology Department, Pharmaceutical Research and Development Division, Wallingford, CT.06492-7660.
Several different classes of compounds have been studied in search of effective treatments of acquired immunodeficiency sndrome (AIDS). Among the most widely studied group are nucleoside analogs which interfere with the HIV viral replication cycle by acting as DNA chain terminators. We have investigated a large number of those compounds and have shown that d4T in combination with ddI has higher anti-HIV activity than either of the drugs alone. In this study anti-HIV activity was evaluated in CEM and MT-4 cells as well as human peripheral blood leukocytes. HIV infection was assessed by using p24gag antigen capture assay and a formazan dye (XTT) conversion assay. The results showed that d4T+ddI at 1:5 ratio have optimal antiviral effect with $ED_{50}$ values ranging from 0.3-6 uM. In addition, this drug combination was not toxic in any of the systems tested. These results further support the notion that combinations of purine and pyrimidine nucleosides such as d4T with ddI may have enhanced therapeutic relevance as far as their anti-HIV activity is concerned .

L 409 HEPARIN BINDS gp120 AND $CD_4$ DOMAINS NOT REQUIRED FOR gp120/$CD_4$ INTERACTION, Alan D. Cardin, Richard L. Jackson, Terry L. Bowlin, Debra L. Taylor and A. Stanley Tyms, Merrell Dow Research Institute, Cincinnati, OH 45215 and the MRC Collaborative Centre, London, England. $CD_4$ is the cellular receptor on T-lymphocytes and macrophages that binds the Human Immunodeficiency Virus (HIV). Heparin and other polysulfated compounds are known to inhibit viral replication, possibly by blocking the binding of the major viral envelope glycoprotein gp120 to $CD_4$. The purpose of this study was to investigate the mechanism of this inhibition by heparin. Binding of $^{125}$I-heparin to recombinant gp120 (r-gp120) or to soluble $CD_4$ ($sCD_4$) was saturable and competitively inhibited by unlabeled heparin ($IC_{50} \approx 3$ µg/mL). A sequence-specific monoclonal antibody to the immunodominant loop (residues 307-330) of gp120 (strain $HTLV\text{-}III_B$) diminished $^{125}$I-heparin binding to gp120. This same antibody neutralizes HIV-1 infectivity but does not inhibit virus attachment to cells. $OKT_4A$, a monoclonal antibody that binds $CD_4$ and blocks HIV-1 attachment to cells, did not inhibit $^{125}$I-heparin binding to $sCD_4$. Radiolabeled ($^{125}$I) gp120 showed saturable binding that was competitively inhibited by unlabeled r-gp120, blocked by the anionic dye aurin tricarboxylic acid (ATA) ($IC_{50} \approx 1$ µg/mL) and $OKT_4A$ but not by heparin (0.001 to 100 µg/mL). The effect of heparin on r-gp120 binding to cellular $CD_4$ (JM cells) was monitored by flow cytometry. Binding was inhibited >95% with ATA (100 µg/mL) and $OKT_4A$ but not significantly (<15%) by heparin (100 µg/mL) although heparin inhibited HIV-1 replication ($IC_{50} \approx 2$ µg/mL). These studies indicate that heparin interacts with domains on gp120 and $CD_4$ important for the antiretroviral action of this glycosaminoglycan that are not directly involved in gp120-$CD_4$ binding.

L 410 INFECTION OF HUMAN MONOCYTES BY DIVERSE HIV-1 STRAINS AND INHIBITION BY ANTIBODY-POSITIVE SERUM, Emily W. Carrow, Sheila A. Grace, Martha A. Wells, Luba K. Vujcic, Deborah S. Webb, Wendy L. Glass, Alec E. Wittek, and Gerald V. Quinnan, Jr., Division of Virology, CBER, FDA, Bethesda, MD 20892

We examined the tropism of 9 HIV-1 isolates for human monocytes and the modulation of infection in monocytes by sera from HIV-1-infected individuals. Elutriated peripheral blood monocytes from normal donors were cultured for 7 days in M-CSF-supplemented medium, and then inoculated with isolates of HIV-1, including 2 monocytotropic strains. For comparison of cell tropism several strains were also cultured in U-937 and MT-4 cell lines. Productive virus infection was quantitated by p24 antigen ELISA and reverse transcriptase activity in culture supernatants. For studies of serum effects, dilutions of sera from normal or HIV-infected individuals were either preincubated with $HIV\text{-}1_{IIIB}$ for various intervals or added to cells at various time points after virus infection. Although all tested strains were infectious in monocytes, distinct differences in efficiency were noted: the two strains reported to be monocytotropic grew more efficiently in monocytes than 6/7 strains passaged in lymphocytic cell lines. Of 25 HIV antibody-positive sera tested, 16 inhibited virus growth by at least 50%. The degree of inhibition correlated with neutralizing antibody titers in a syncytium inhibition assay using a lymphocytic cell line. Positive sera were inhibitory whether added prior to or after virus infection. We conclude that human monocytes are susceptible to infection by diverse HIV-1 strains. HIV-neutralizing antisera can inhibit infection of monocytes and appear to be effective even after virus has bound to cells.

L 411 THE EFFECT OF THYMOPENTIN TREATMENT ON PROGRESSION OF DISEASE AND SURROGATE MARKERS IN HIV INFECTED PATIENTS WITHOUT AIDS, Conant, Marcus A., University of California San Francisco, California and Goldstein, Gideon, Hirsch, Robert L., Meyerson, Linda A., Kremer, Alton B., Immunobiology Research Institute, Annandale, New Jersey. We conducted a double-blind placebo controlled trial to assess the efficacy of thymopentin in controlling disease progression and surrogate market changes in HIV infected patients who had not yet developed AIDS. Ninety-one (91) patients were stratified according to the presence or absence of HIV related symptoms and were randomly assigned to receive 50mg thymopentin (47) or placebo (44) three times per week subcutaneously for 24 weeks. Four placebo treated patients (10.4%, 1- Kaplan-Meier estimate) progressed to either constitutional symptoms (2) or AIDS (2) while no thymopentin treated patients (0%) had disease progression ($p=0.03$). Contrasts between levels of CD4+ cells were most striking in asymptomatic patients entering the study with CD4 counts > 400/mm$^3$; the thymopentin treated group maintained CD4 counts near entry level while the placebo group declined. The percentage of serum p24 antigen positive patients at entry was similar for the thymopentin treated (18%) and placebo treated (20%) groups; at 24 weeks this percentage was unchanged in the thymopentin treated group but had risen to 36% in the placebo group. Treatment related changes in $Beta_2$ microglobulin levels were most striking in the symptomatic group; at 24 weeks there was a mean decrease from baseline of 0.43mg/L in thymopentin treated patients ($p=0.03$, Wilcoxon signed rank test) in contrast to a decline of only 0.04 mg/L in the placebo group. No untoward effects attributable to thymopentin were observed. These promising results suggest that thymopentin can benefit patients at early stages of HIV infection.

**L 412** MONOCLONAL ANTIBODIES FOR THE CHARACTERIZATION OF HIV-1. Patricia D'Souza, Gregory Milman, Pathogenesis Branch, Division of Aids, NIAID, NIH, Rockville, Maryland 20892. Rapid progress in the isolation, cloning and sequencing of the entire viral genome has shown the remarkable propensity of HIV for genetic variation. For some viruses, such as influenza, rapid mutation is an important means of escape from neutralizing antibodies. Similar variants arise in humans infected with HIV during the course of infection. Despite the rapid mutation rate, HIV displays little variation at certain physiologically essential sites: CD4 binding, viral assembly, membrane fusion and viral uncoating inside the cell. Neutralizing antibodies directed against such a conserved region with a critical function are active against numerous clinical isolates of HIV-1 and are labeled "group-specific". In contrast, "type-specific" antibodies are those that recognize sequences which exhibit a large amount of genetic variation between the individual isolates. Type-specific monoclonal antibodies maybe valuable to identify a viral isolate, whereas group-specific antibodies can serve to identify biologically conserved epitopes. A collaborative program to identify neutralizing monoclonal antibodies to group and type specific epitopes in gp120 will be described.

**L 413** ASSESSMENT OF THE PROTECTIVE EFFICACY IN CHIMPANZEES OF YEAST-EXPRESSED HIV-1 p55 CORE PROTEIN, Emilio A. Emini, W.A. Schleif, P. Conard, J. Eichberg*, L. Schultz, K. Hoffman, G. Vlasuk, M.A. Polokoff, D. Lehman, J.A. Lewis, J. Davide, V. Larson. Merck, Sharp and Dohme Research Lab., West Point, PA 19486 and *SWFBR, San Antonio, TX 78284 Previous studies have demonstrated that the hepatitis B core antigen is capable of eliciting a protective immune response in chimpanzees against infection with the hepatitis B virus. Hence, a study was performed to ascertain the potential protective efficacy of an immune response directed against the HIV-1 core. Accordingly, the HIV-1 p55 gag core precursor protein was expressed in cells of Saccharomyces cerevisiae, purified to homogeneity and adsorbed onto alum (aluminum hydroxide). Two chimpanzees were inoculated with 100 mcg/dose of the immunogen, intra-muscularly, at 0, 4 and 24 weeks. Both animals developed good titers of anti-p55 antibodies which bound to disrupted HIV-1 virions and which, by western blot, reacted with both p24 and p17 viral core proteins. No neutralizing activity was elicited. Upon live virus challenge of one of the animals, no protection from infection was noted. Virus was isolated by co-cultivation from the animal's peripheral blood mononuclear cells and the animal eventually developed antibody to the viral gp41 transmembrane protein.

**L 414** Relative contribution of Aerosolized Pentamidine and Zidovudine in prolonging survival with HIV: The San Francisco General Hospital Cohort and the San Francisco Community Prophylaxis Trial. DAVID W. FEIGAL, GIFFORD LEOUNG, DONALD I. ABRAMS, A. BRUCE MONTGOMERY. Departments of Medicine, University of California, San Francisco and University of California, San Diego.

A historical cohort of 201 randomly sampled patients with first episode of PCP treated at San Francisco General Hospital (SFGH) from 1981 to 1985 before the use of zidovudine (ZDV) or prophylaxis was selected to plan *Pneumocystis* carinii pneumonia (PCP) prophylaxis trials. 50 patients died during treatment of the first episode and an additional 12 died during recurrent episode. Overall, acute PCP was the single most common cause of death. From this study it was estimated that a sample size of 135 per treatment arm would be needed in a prophylaxis arm to detect at least a two fold reduction in PCP recurrences over 18 months.

The SF County Community Consortium conducted a study with 237 participants (pts) with prior PCP, 55 with Kaposi's sarcoma without PCP and 116 with ARC. Pts. were randomly assigned to 30mg q two weeks, 150 mg q two weeks or 300 mg q 4 weeks. 52% were taking ZDV. The study was terminated after analysis of results showed a 3.9 fold reduction in PCP recurrences between the 300mg and 30mg treatment arms. The effects of ZDV and 300 mg AP were multiplicative with an 7.7 fold reduction in those who took both. At termination of the study, 160 deaths had occurred, 19 due to acute PCP. There was not a difference between treatment arms in deaths due to PCP. For all-cause mortality there was a two fold reduction in PCP episodes in participants who had ever taken zidovudine vs. never ZDV

The epidemiology of PCP prior to prophylaxis and ZDV shows that 80% of PCP associated deaths occur with first episodes. With first episode case fatality of 25%, prevention of PCP prolongs life. The majority of pts in the SF CCC trial had had an episode of PCP. On prophylaxis only 12% of deaths were due to breakthrough episodes on PCP. ZDV had an additional effect in the reduction of all-cause mortality. Aerosolized pentamidine alone was more effective than ZDV alone in preventing PCP. However, the maximum benefit for primary prophylaxis would be the combination of both. All-cause mortality in secondary prophylaxis benefited from ZDV.

**L 415** IMMUNE RESPONSE OF CHIMPANZEES TO ACTIVE IMMUNIZATION AGAINST HIV-1.

Marc Girard, Michel Kaczorek[1], Abraham Pinter[2], Peter Nara[3], Françoise Barré-Sinoussi[4], Marie-Paule Kiény[5], Elizabeth Muchmore[6], Micael Yagello, Jean-Claude Gluckman[7], and Patricia Fultz[8]. 1Pasteur Vaccins, Marnes-la-Coquette, France, 2Public Health Research Institute, New York, NY, 3NCI, Frederick, MD, 4Pasteur Institute, Paris, 5Transgène, Strasbourg, France, 6LEMSIP, NYU School of Medicine, NY, 7Hôpital de la Pitié, Paris, France, and 8Yerkes Primate Research Center, Atlanta, GA.

The objective of this study was to determine if protection against an infectious HIV challenge could be obtained in chimpanzees by active immunization. One animal was successively immunized with (1) formalin and betapropiolactone-inactivated whole virus, (2) purified gp160env, and (3) a KLH-coupled synthetic oligopeptide identical to the HIV BRU-specific major neutralization epitope (hypervariable loop V3). A second animal was immunized with (1) live recombinant vaccinia virus (VV) expressing HIV BRU gp160env and p25gag, (2) purified gp160env, p18gag, p27nef and p23vif, and (3) the same KLH-coupled oligopeptide as above. All immunogens except live recombinant VV were formulated in the Syntex Thr-MDP adjuvant. Both chimpanzees developed sustained neutralizing antibody (Ab) titers and were challenged by IV injection of $\geqslant$ 100 TCID50 of the HIV IIIB virus stock from the NCI (a gift from Larry Arthur). Seroconversion and anamnestic Ab responses were not detected up to 12 weeks after challenge. Attempts to isolate virus from and PCR analysis of PBMC were negative through 6 weeks after challenge. Whether protection against infection was achieved, however, will not be known until the animals have been monitored for at least 1 year.

**L 416** **NON-INFECTIOUS HIV-1 AND MuLV NUCLEOCAPSID MUTANTS DEFICIENT IN GENOMIC RNA,**

Robert J.Gorelick[1], Stephen M. Nigida, Jr.[2], Larry O. Arthur[2], Louis E. Henderson[2] and Alan Rein[1]. [1]Laboratory of Molecular Virology and Carcinogenesis, BRI-Basic Research Program, [2]Biological Products Laboratory, Program Resources Inc., NCI-Frederick Cancer Research Facility, Frederick, MD 21701-1013.

All retroviruses contain, in the nucleocapsid domain of the *gag* protein, at least one copy of the sequence Cys-$X_2$-Cys-$X_4$-His-$X_4$-Cys which is involved in the recognition of genomic RNA. We have generated several point and deletion mutations in this motif in HIV-1 and Moloney MuLV and these virus particles appear to contain the normal complement of viral proteins as determined by a number of criteria. These mutant virions are however, deficient in genomic RNA. No infectivity could be detected in the mutant HIV-1 particles under conditions that yielded $10^5$ infectious units/mL in the wild type. Similar results were obtained in the MuLV mutants [Gorelick et al. (1988) *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 8420]. HIV-1 mutants of this type may have important applications including non-hazardous reagents for research, immunogens in vaccine and immunotherapy studies, and diagnostic reagents. In addition, the extreme conservation of the cysteine array among retroviruses may present possibilities for developing universal diagnostic reagents while the exquisite sensitivity of the virus to changes in the array suggest that the array is a potential target for the development of antiviral drugs. This work is sponsored in part by the NCI, DHHS, under contract NO. NO1-CO-74101 with BRI, NO. NO1-CO-74102 with PRI, and NIH, Individual National Research Service Award NO. 5 F32 AIO 7684-02 (awarded to Robert J. Gorelick).

**L 417** THE GROWTH, INFECTIVITY, AND NEUTRALIZATION OF HYBRID HIV CONTAINING HETEROLOGOUS PRINCIPAL NEUTRALIZATION DETERMINANTS.

Gary S. Gray[1], Ray Grimaila[1], Barb Clark[1], Greg LaRosa[1], Barbara Potts[1], Scott Putney[1], Emilio Emini[2], Tom Matthews[3], Dani Bolognesi[3], and Kent Weinhold[3]. [1]Repligen Corporation, Cambridge MA, USA, [2]Merck Sharp and Dohme Research Laboratories, West Point PA, USA, and [3]Duke University Medical School, Durham, NC,USA.

We have prepared recombinant HIV proviral clones that contain a segment of the HIV envelope which is known to represent the principal neutralizing epitope of HIV. These proviral clones were constructed using a proviral vector modified by the addition of a series of translationally silent restriction sites surrounding the neutralization epitope of the HIV envelope. The neutralization epitope was replaced with DNA fragments derived by polymerase chain reaction (PCR) amplification of a series of HIV isolates to yield clones which differed only in this region. Proviral DNAs were purified on CsCl gradients and used to transfect a CD4 positive cell line. The ability of the transfected proviral DNAs to replicate and to produce live virus was determined. Results on the growth, infectivity, and neutralization of recombinant HIV proviruses will be presented.

**L 418** DISULFIDE BOND ASSIGNMENT AND CHARACTERIZATION OF N-LINKED GLYCOSYLATION SITES IN RECOMBINANT HIV-1 TYPE IIIB GP120 PRODUCED IN CHO CELLS, Timothy J. Gregory, Cordelia K. Leonard, Lavon Riddle, James R. Thomas, Reed J. Harris and Michael W. Spellman, Genentech, Inc., 460 Pt. San Bruno Blvd., So. San Francisco, CA 94080. The complete primary structure of recombinant gp120 (rgp120) from the IIIb isolate of Human Immunodeficiency Virus Type I expressed in Chinese hamster ovary cells has been determined. Enzymatic cleavage of rgp120 and reverse-phase high performance liquid chromatography were used to confirm the primary structure of the molecule, to assign intrachain disulfide bonds and to characterize sites of N-glycosylation. Peptides were identified by amino acid analysis and/or N-terminal sequencing. All of the peptides identified were consistent with the primary structure predicted from the cDNA sequence. Mature gp120 contains 9 disulfide bonds. The combined results of tryptic mapping analysis and further selective cleavage with PNGase F, Endoproteinase ASP-N and S. Aureus V8 protease permitted the assignment of all nine intrachain disulfide bonds of rgp120. Mature gp120 contains 24 potential sites for N-linked glycosylation. Tryptic mapping of enzymatically deglycosylated rgp120 was used in conjunction with Edman degradation and fast atom bombardment-mass spectrometry of individually treated peptides to determine which of the 24 potential N-glycosylation sites are glycosylated. The data presented indicate that all 24 potential glycosylation sites of gp120 are glycosylated, that 13 of the glycosylation sites contain primarily complex-type oligosaccharide structures, and that 11 glycosylation sites contain primarily high mannose-type and/or hybrid-type oligosaccharide structures.

**L 419 SELECTIVE LOSS OF T-CELL FUNCTIONS IN DIFFERENT STAGES OF HIV INFECTION.** **R.A.Gruters, C.J.M. Van Noesel, F.G.Terpstra, R.A.W. Van Lier and F.Miedema. Central Lab. Netherlands Red Cross Blood Transfusion Service and Lab. for Exp. and Clin. Immunol. University of Amsterdam.**

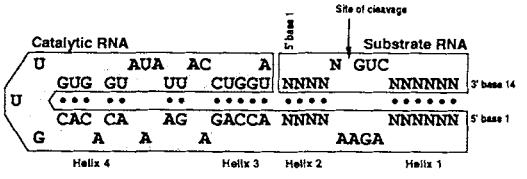
**Qualitative defects in T-cell reactivity can be observed in HIV-infected men even before depletion of $CD4^+$ T cells occurs. We present functional and phenotypic evidence that T-cell unresponsiveness in early infection is due to selective loss of $CD29^+$ T-memory cells. Like normal naive $CD29^-$ T cells, T cells in early HIV infection have a diminished response to anti-CD3 Mab, due to a lack of IL-2 production, that can be enhanced by costimulation with anti-CD28 Mab or by adding IL-2. Anti-CD3 induced CTL generation is normal. T-cell unresponsiveness is not due to a failure in early signal-transduction events ($[Ca^{2+}]_i$ increase and PKC activation). FACS analysis and cell-separation experiments demonstrated the loss of responsive $CD29^+$ T cells. In progression to AIDS also naive $CD29^-$ T cell numbers are decreased, anti-CD3 induced proliferation is further affected and CTL generation becomes severely decreased, which can be restored by IL-2. These findings show immune dysfunction early in infection which may have implications for our understanding of the pathogenesis of AIDS.**

**L 420** A NOVEL APPROACH FOR THE INACTIVATION OF AIDS VIRUS BY PREACTIVATED PHOTOACTIVE COMPOUNDS. K.S. Gulliya[1], T.C. Chanh[2], J.T.Newman[1], S. Pervaiz[1], J.L. Matthews[1]. Baylor University Medical Center, Baylor Research Foundation, Dallas, TX 75246[1]; Southwest Foundation for Biomedical Research, San Antonio, TX 78284[2].

Exposure of photoactive compounds to light prior to their use in biological targets (preactivation) resulted in the formation of new compounds that retained their target specificity and toxicity without further need for light energy. An index dye, merocyanine 540 (MC540), was activated by exposure to 514 nm laser light. Cell-free herpes simplex virus (HSV-1) and human immunodeficiency virus (HIV-1) were treated with the light-exposed (preactivated) dye. All experiments reported here were carried out in the dark. Results showed that preactivated MC540 (120 μg/mL to 200 μg/mL) was effective in neutralizing 99.99% of cell-free HSV-1 and HIV-1, whereas minimal cytotoxicity was observed with normal human peripheral blood mononuclear cells in the dark. Treatment of HIV-1 infected HUT-78 T-cells with preactivated MC540 (200 μg/mL) resulted in the survival of these cells, whereas all untreated cells were killed. Moreover, preactivated MC540 had no obvious toxic effects in DBA/2 mice and pigs treated with intravenous doses of 200 mg/kg and 20 mg/kg, respectively. A comparative analysis of the preactivated and native MC540 disclosed significant differences in high-performance liquid chromatography (HPLC) elution profiles, nuclear magnetic resonance (NMR), and fast atom bombardment data, leading us to conclude that a new class of compound(s) with target specificity and cytotoxicity has been generated. Preactivated MC540 was stable for >30 days at -135°C without significant loss in biological activity. The mechanism of selective toxicity appears to be other than the reactive oxygen species generated at excited states.

Supported by grants from Strategic Defense Initiative MFEL program, the Leukemia Association of North Central Texas and the Cell Biology Fund of the Baylor Research Foundation.

**L 421** A NEW CATALYTIC RNA CAPABLE OF CLEAVING HETEROLOGOUS RNA SEQUENCES: THE 'HAIRPIN' MOTIF, Arnold Hampel and Richard Tritz, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115. We have identified and characterized a new catalytic RNA [Hampel and Tritz Biochemistry, 28, 4929 (1989)] which is capable of being engineered to cleave heterologous RNA at a specific site [Hampel et al. NAR in press (1990)] and we have used this catalytic RNA to cleave two RNA sequences found in HIV-1. The complex between the catalytic RNA and substrate RNA has a 'hairpin' type of two dimensional structure. Mutagenesis shows the four helices shown between the 50 base catalytic RNA and 14 base substrate RNA exist. The only requirement for the substrate RNA is the GUC shown. When catalytic RNA is added to substrate RNA having a GUC such that base pairing is maintained in helices 1 and 2, cleavage will occur in the substrate just before the 'G' base. The efficiency of this reaction is partially dependent on the sequence itself and can be optimized by changing the length of helix #1 sequence. The best catalysis we have had, Km=0.03μM and kcat=7/min at 37C, pH 7.5 and low salt, is with a short substrate RNA having the target sequence of UGUCAGUCCUGUUUUUUU where the catalytic RNA base pairs with the bases flanking the AGUC. Sequences found in HIV-1, AGAGCGUCGGUAUUAA in the *gag* region and UGGGUGUCGACAUA in the *tat* region were cleaved, but less efficiently. For certain substrate sequences, this appears to be the most efficient catalytic RNA known for conditions near physiological.




**L 422 PRODUCTION OF NONINFECTIOUS HIV VIRIONS IN GENETICALLY ENGINEERED COS CELLS**, Joel R. Haynes, Benjamin Rovinski, Fei-long Yao, Jie Ma, Shie Xian Cao, and Michel H. Klein, Connaught Centre for Biotechnology Research, 1755 Steeles Ave. West, Willowdale, Ontario M2R 3T4, Canada. In an attempt to produce a safe, noninfectious preparation of HIV virions as a candidate vaccine for AIDS, we engineered COS cells to produce HIV protein products and determined whether or not these proteins would assemble into virions. These experiments involved the isolation of a fragment of the HIV provirus containing the coding sequences for all proteins except nef, but lacking those sequence elements responsible for viral genome replication, and insertion of this fragment into eukaryotic expression vectors. In expression studies all of the major HIV products were expressed and particulate reverse transcriptase activity was released into the medium. Furthermore, this activity banded on sucrose density gradients at a density consistent with intact retrovirus particles and co-sedimented with multiple HIV antigens as determined by Western blot analysis. These results demonstrate the production of noninfectious HIV virion particles which may form the basis of a safe, whole viral condidate vaccine against HIV.

**L 423** PHOSPHOLIPID DERIVATIVES OF DIDEOXYTHIMIDINE (ddT) EXHIBIT GREATLY INCREASED POTENCY AGAINST REPLICATION OF HIV IN CD4-HELA CELLS, Karl Y. Hostetler, Henk van den Bosch, Bert van Wijk, Louise M. Stuhmiller and Douglas D. Richman, University of California San Diego, the VA Medical Center, San Diego, CA, Vical Inc., San Diego, CA and the CBLE, Rijksuniversiteit Utrecht, Utrecht, the Netherlands. Zidovudine (AZT) and other dideoxynucleosides such as ddC, ddA and ddI inhibit HIV replication after conversion to their triphosphates by interfering with HIV reverse transcriptase. These agents may be regarded as prodrugs since they must be converted to their active forms by anabolic phosphorylation catalyzed by cellular enzymes. In contrast to other dideoxynucleosides, ddT is not very effective in inhibiting HIV replication in vitro because it is poorly phosphorylated by cellular thymidine kinase and has therefore not been developed as a candidate drug (for HIV infection). ddT diphosphate diglyceride (ddTDPDG) and phosphatidylddT (pddT), liponucleotide prodrugs of ddT, were synthesized. Liposomes were prepared with phosphatidylcholine/phosphatidylglycerol/cholesterol/ddT liponucleotide in a molar ratio of (50/ 10/30/10) and added to HIV-infected CD4-HeLa (HT4-6C) cells. A lipid control without ddT liponucleotide had no effect on plaque formation. Free ddT reduced plaque formation by 50% at 100 uM. However, pddT and ddTDPDG caused a similar degree of inhibition at 25 and 1 uM, representing 4- and 100-fold increase in activity, respectively. ddTDPDG shows an especially marked increase in antiretroviral activity which may be due to its metabolism to ddT diphosphate by cellular enzymes, bypassing slow phosphorylation by thymidine kinase. pddT and ddTDPDG may be targeted to macrophages in vivo and may be useful clinically in treating this important reservoir of HIV infection.

**L 424** **PRECLINICAL RESEARCH AND DEVELOPMENT OF NEW AIDS DRUGS: REGULATORY REQUIREMENTS AND CURRENT STRATEGIES.** **Peter C. Hoyle, Emmanuelle Voisin, and Ellen C. Cooper, Food and Drug Administration, Division of Antiviral Drug Products, Rockville, MD.** The preclinical research and development of new AIDS drugs has required drug developers to optimize their development strategies to get promising new drugs into the clinic as quickly and efficiently as possible. This demands the careful and expert coordination of the different disciplines associated with drug development: chemistry, manufacturing, and quality controls, microbiology, pharmacology, pharmacokinetics, toxicology, and a clinical trial design. Additionally, each development program requires a considerable amount of improvisation to meet the specific characteristics of the drug. Common problems observed in the AIDS drug development community during the last two years will be discussed, as well as suggestions for improving development strategies.

**L 425** MULTIPLE MUTATIONS IN HIV REVERSE TRANSCRIPTASE CONFER RESISTANCE TO ZIDOVUDINE (AZT). Sharon D.Kemp, Paul Kellam and Brendan A.Larder, Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.
HIV isolates with reduced sensitivity to AZT from individuals with AIDS or ARC were studied to determine the genetic basis of their resistance. Most were sequential isolates obtained at the initiation of and during therapy. Comparative nucleotide sequence analysis of the reverse transcriptase (RT) coding region from 5 pairs of sensitive and resistant isolates identified 3 predicted amino acid substitutions common to all the resistant strains (Asp67 → Asn, Lys70 → Arg, Thr215 → Phe or Tyr) plus a fourth in 3 isolates (Lys219 → Gln). Partially resistant isolates had combinations of these 4 changes. An infectious molecular clone constructed with these 4 mutations in RT yielded highly resistant HIV after transfection of T-cells. To evaluate the relative contribution of the mutations to the magnitude of resistance, we have constructed HIV variants with defined combinations of mutations in RT and assessed their sensitivity to AZT. These studies allow predictions to be made about the degree of resistance of a variant, based on detection of mutations in HIV DNA. Therefore, it is now possible to develop rapid assays to determine AZT resistance by polymerase chain reaction (PCR) analysis of virus nucleic acid.

**L 426** A STRUCTURAL PERSPECTIVE OF THE PUTATIVE CD4 BINDING SITE ON HIV. Thomas Kieber-Emmons, Thomas Ryskamp, Alice S. Whalley, David B. Weiner* and W. John W. Morrow, IDEC Pharmaceuticals Corp., La Jolla, CA 92037 and *Wistar Institute of Philadelphia, Philadelphia, PA 19104
We have defined a possible topographical structure for the putative CD4 binding site of gp120 based upon studies of molecular mimicry by anti-receptor/anti-idiotypic antibodies. Sequence homology with the immunoglobulin superfamily suggests that the putative CD4 binding domain may span amino acids 384-456 of gp120, exhibiting localized folding characteristics similar to antibody Vh-Vl reverse turn regions. Molecular modelling of the 413-456 amino acid tract indicates that a disulfide bond can be formed by the cysteine residues at positions 418 and 445. The model indicates that the residues 421-438 define a central turn region of the domain that may be surface exposed for interaction with CD4. Synthetic peptides spanning 421-438 from several HIV isolates were shown to block syncytia between infected (multi-isolate) and uninfected cells. The anti- (421-438) peptide antibodies are shown to bind to gp120 in radioimmune precipitations (RIPS). These studies suggest that the CD4 binding site is accessible to antibodies and that the interaction of gp120 with CD4 involves structural recognition principles analogous to those involved in antigen-antibody binding.

L 427 DECREASED "IN VITRO" SUSCEPTIBILITY TO ZIDOVUDINE OF HIV ISOLATES OBTAINED FROM AIDS PATIENTS. S.A. Land, G.M. Treloar, D.A. McPhee, C.J. Birch, R.R. Doherty, D. Cooper and I.D. Gust. Virology Department and NHMRC Special Unit for AIDS Virology, Fairfield Hospital, Victoria, Australia and AIDS Epidemiology and Clinical Research, Sydney, Australia.
The nucleoside analogue zidovudine inhibits replication of HIV-1 'in vitro' and 'in vivo'. Used in the treatment of patients with AIDS it reduced mortality and morbidity. Isolates of HIV, obtained before and after the patients received zidovudine therapy, were tested for susceptibility to the drug 'in vitro'. Isolates collected after therapy showed decreased susceptibility to zidovudine as assessed by replication in the HTLV-1 transformed cell line MT-2, and the production of RT activity by infected mononuclear leucocytes. Further, pre-therapy isolates were sensitive to a range of zidovudine concentrations when 100% inhibition endpoint was used. In contrast, paired isolates from several untreated individuals were equally susceptible to a narrow range of zidovudine concentrations. Site directed mutagenesis of the viral RT has been shown to reduce the inhibitory effect of zidovudine, however the actual mechanism that the virus utilizes remains unknown. Further investigation of a large group of patients is required to assess the clinical significance of this decreased susceptibility of HIV to zidovudine but these results highlight the need to monitor changes in HIV that can occur with drug therapy.

L 428 DETECTION OF MUTATIONS CONFERRING ZIDOVUDINE (AZT) RESISTANCE BY PCR ANALYSIS OF HIV DNA. Brendan A.Larder and Paul Kellam, Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K.
Current methods for assessing sensitivity of HIV to AZT involve lengthy culture procedures including virus isolation from peripheral blood lymphocytes (PBLs). Furthermore, the procedures we use allow isolation of virus suitable for accurate testing from only 30% of individuals. Thus, a rapid assay that could be applied to large numbers of individuals would be of considerable value and assist in establishing the clinical significance of AZT resistance. We have recently shown that highly specific mutations in HIV reverse transcriptase (RT) confer AZT resistance. Taking advantage of the predictable nature of these mutations, we have used the polymerase chain reaction (PCR) as the basis of rapid sensitivity assessment. Thus, point mutations in RT critical for resistance (in codon 70 and 215) were detected by PCR analysis of DNA from HIV infected cells. Analysis of 17 HIV isolates by PCR gave results in agreement with nucleotide sequence data. In some cases mixed populations of wild-type and mutant were identified by PCR at the same residue in single isolates. The PCR assay has been adapted to enable direct mutational analysis of DNA from non-cultured PBLs. Mutations associated with AZT resistance have been identified in such samples from individuals with AIDS treated for prolonged periods. Therefore, this assay will be useful for rapid detection of resistance and circumvent the need for virus culture procedures currently used to assess sensitivity.

L 429 ANTIBODY-TARGETED LIPOSOMES CONTAINING OLIGODEOXY-RIBONUCLEOTIDE SEQUENCES COMPLEMENTARY TO VIRAL RNA SELECTIVELY INHIBIT VIRAL REPLICATION,
Lee Leserman, Jean-Paul Leonetti*, Patrick Machy, Geneviève Degols*, and Bernard Lebleu*. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille CEDEX 9 France, and *Laboratoire de Biochimie des Protéines, USTL, 34060 Montpellier CEDEX France.
Murine L929 cells incubated with antibody-targeted liposomes containing 15-mer oligodeoxyribonucleotide sequences complementary to the 5' end region of the mRNA encoding the N-protein of vesicular stomatitis virus became less permissive for multiplication of that virus (up to 2 logs of reduction of viral multiplication). Protection was not seen for liposomes containing a sequence complementary to the 5' end of c-myc proto-oncogene mRNA targeted by the same antibody, or for VSV liposomes targeted by an antibody not binding the cells. Antibody-bearing liposomes containing complementary sequences thus have double specificity: a cell selected by the antibody on the liposome and mRNA in the cell complementary to the liposomal oligomer. Non-encapsulated oligomers are nuclease sensitive and usually must be administrated at high concentrations. Oligomers encapsulated in liposomes resist DNAse, and are active in amounts one to two orders of magnitude less than for those reported for unencapsulated oligomer sequences. Extension to oligomers complementary to HIV regulatory genes is under investigation.

**L 430** DIRECT DETECTION OF HIV-I IN DNA OF URINE PELLETS FROM HIV-I SEROPOSITIVE PERSONS. Jianjun Li, Michael Mirabile, Peizhang Tao, Yaoxing Huang, Yao Gi Huang, Yunzhen Cao, Bernard Poiesz*, and Alvin E. Friedman-Kien, Department of Microbiology, NYU Medical Center, New York and *Division of Hematology and Oncology, State University of New York, Syracuse, New York.

Specific IgG antibodies to HIV-I have been detected in the urine of HIV-I seropositive persons. We identified HIV-I DNA sequences in fresh urine cell pellets and from their corresponding peripheral blood mononuclear cells (PBMC) in 42 HIV-I seropositive and 8 seronegative homosexual men using a polymerase chain reaction (PCR) test with HIV-1 gag primer. 22 samples from healthy heterosexual men served as controls. The results are: 1) 30/32 (93.8%) PBMC and 29/42 (69%) urine pellet samples were PCR-DNA positive respectively, but none in the 22 controls; 2) only 6/29 (21.7%) PBMC DNA positive samples were found to be HIV-I p24 positive, and none of the 9 asymptomatic seropositive serum samples were found to be p24 positive while 6/7 and 5/11 samples were PCR-DNA positive in the corresponding PMBC and urine cell pellet samples; and 3) one PBMC and urine cell pellet sample from a HIV-I seronegative homosexual male was found to be PCR-DNA positive. Our findings suggest that the PCR technique combined with the ELISA test for antibodies to HIV-1 in urine could potentially lead to the development of a noninvasive test for diagnosing HIV-I infection.

| Group | Number | Antibodies to HIV-I(+) | p24 antigen(+) | PBMC | Urine pellet |
|---|---|---|---|---|---|
| ARC | 11 | 11 | 2/10 | 11/11 | 11/11 |
| AIDS-EKS | 19 | 19 | 3/14 | 12/13 | 12/19 |
| AIDS-01 | 1 | 1 | 0 | 1 | 1 |
| AH* | 11 | 11 | 0/9 | 6/7 | 5/11 |
| Total | 42(100%) | 42(100L%) | 5(15.2%) | 30(93.8%) | 29(69%) |

*Asymptomatic homosexuals

**L 431** DENATURATION ABOLISHES CD4 MEDIATED IMMUNOSUPPRESSIVE ACTIVITY, BUT NOT ANTIGENICITY OF gp120, Fabrizio Manca, J Habeshaw and G Dalgleish, Department of Immunology, S.Martino Hospital, 16132 Genoa, Italy and Department of Immunol. Medicine, CRC, Harrow, U.K.

HIV gp120 inhibits antigen dependent T cell activation by binding to CD4, an accessory structure used by T cells to facilitate interaction with MHC on antigen presenting cells (APC). Heat denaturation abolishes immunosuppressive activity (i.e. CD4 binding) but spares antigenicity of gp120. This was demonstrated by using a gp120 specific human T cell line generated by repeated stimulation of T cells with gp120 pulsed APC. When the established line was stimulated with gp120 pulsed APC, the proliferative response was 20.7 cpm x $10^{-3}$, over a bkg of 3.1. In the presence of native gp120, proliferation dropped to 3.7. Heat denaturated gp120 did not inhibit the response to pulsed APC (23.3). When gp120 was added directly to cultures with T cells and APC, proliferation was 3.4 with native gp120 and 21.5 with denatured gp120.

Administration of native and denatured gp120 resulted in immunosuppression (5.1), indicating that native inhibitory gp120 is functionally dominant over denatured antigenic gp120.

These data show that the physico-chemical status of gp120 dictates its immunosuppressive or its antigenic activity as defined in this functional assay and suggest that denatured rather than native gp120 could be used for vaccination aimed at inducing selectively a gp120 specific response of T cells.

**L 432** USE OF PEPTIDE-PHOSPHOLIPID COMPLEXES TO INDUCE ANTIBODIES TO GP 160 OF HIV. Raphael J. Mannino, Susan Gould-Fogerite, Leslie E. Eisele and Gail Goodman-Snitkoff. Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208

Understanding how to present specific, defined epitopes to the immune system in order to induce both humoral and cell mediated immune responses is a major challenge in the design of modern vaccines. The approach we have taken has been to attempt to determine minimal essential components required to construct a well defined immunogenic composite. Synthetic peptides, representing immunologically interesting epitopes from gp 160 of HIV, are provided with a hydrophobic tail through crosslinking to a phospholipid and then assembled into a peptide-phospholipid composite. We have found that an immunogenic mixture is comprised of two essential components: 1. Each composite must contain peptides representing both B-cell determinants and T-helper (Th) determinants presented either contiguously, i.e. part of the same synthetic peptide, or on individual peptides. 2. To be immunogenic these peptides must be covalently coupled to phospholipid and inoculated as a peptide- phospholipid conjugate. No other carriers or adjuvants are required to induce an immune response. Additionally, trace amounts of a detergent extract of the envelope of Sendai virus to act as a "generic" provider of Th-cell determinants can be included in the composites . The specific peptides used for these studies describe amino acids 307-322, 476-518, 494-518 of gp 120 and 585-615 of gp 41. Following immunization with these preparations, antibody titers increase with each subsequent inoculation and the antibody produced cross-reacts with native protein on ELISA and was able to precipitate strain specific gp 160 in a radioimmunoassay. Additional parameters being studied include dose of immunogen, route of immunization, the nature of the crosslinker used to couple the peptides to phospholipid, the phospholipid composition and the optimal time for boosting immunizations. The ability of peptide-phospholipid composites to induce antigen specific cytolytic T lymphocytes is also under investigation.

L 433 PILOT PHASE I STUDY USING ZIDOVUDINE IN ASSOCIATION WITH A 10 DAY COURSE OF ANTI-CD4 mAb, Claude E. Mawas, Daniel Olive, Marc Lopez, Catherine Tamalet, Catherine Dhiver and Jean-Albert Gastaut, INSERM U.119 and Institut Paoli-Calmettes, 13009 Marseille, France.
On the basis of experimental evidences demonstrating that monoclonal antibody (mAb) 13B8.2, a workshop qualified anti-CD4 mAb, could (i) inhibit in vitro syncitium formation as well as in vitro HIV infection of CD4+ T cells ; (ii) deliver negative signals to T cells, thus preventing T cell activation and viral replication ; (iii) contribute to CD4+ T cells clearance by its Fc portion and (iv) induce an immune response by the patient, contributing potentially to an anti-idiotypic response of interest for the control of the immune parameters of the disease, a Phase I study associating AZT and a 10 day course of anti-CD4 mAb was performed in 7 AIDS patients (CDC group 4). The treatment was well tolerated. mAb dosage and schedule were adjusted on the basis of circulating CD4+ cells and mAb pharmacokinetics; immunological and virological parameters were also monitored. One patient presented a transient increment in CD4+ T cells associated with augmented T cell functions, the suppression of p24 in the serum and a negative RT assay. A second patient had a steady increment of CD4+ T cells after completion of the treatment, with a transient decrease of p24 in the serum 5 days after completion of the anti-CD4 protocol.

**L 434 TEMPORARY IMPROVEMENT OF T-CELL FUNCTION IN LONG-TERM ZIDOVUDINE TREATED ASYMPTOMATIC HIV-INFECTED INDIVIDUALS. F.Miedema (1), R.A.Gruters (1), F.G.Terpstra (1), J.M.A.Lange (2) M.T.L.Roos (1), F. de Wolf, J.W.Mulder,(3) and P.T.A.Schellekens (1,2), 1.Centr.Lab of the Netherlands Red Cross Blood Transfusion Service,Lab for Exp and Clin Immunology, 2.Dept Internal Med, Academic Medical Centre,University of Amsterdam 3.Department of Infectious Diseases, Municipal Health Service Amsterdam,The Netherlands.**

**In a prospective cohort study in HIV infected men (n=300) anti-CD3 Mab induced T-cell reactivity was found to be decreased to 60 % of the normal magnitude already 6 months after seroconversion. In individuals who progressed to AIDS, anti-CD3 MAb reactivity further declined and was virtually absent at least 14 months before diagnosis, whereas it remained constant in stable asymptomatics. Low anti-CD3-induced T-cell responsiveness and low CD4 cell counts were independent prognostic markers for progression to AIDS. We investigated the effect of long-term (2-year) zidovudine treatment on these immunological parameters in a group of 9 asymptomatic p24 antigenemic men, 4 of whom progressed to AIDS. A group of 10 untreated HIV-infected men, 5 of whom progressed to AIDS was studied as a control. In all persons T-cell responsiveness had improved 6 months after the start of zidovudine treatment, however, $CD4^+$ T-cell numbers were not persistently elevated and restoration of T-cell responsiveness was only of short duration. At intake, both treated and non-treated progressors and non-progressors, did not differ for $CD4^+$ T-cell numbers. In contrast, 3 years before diagnosis, progressors did not respond to anti-CD3 Mab stimulation. Our results show that zidovudine treatment in the asymptomatic phase of HIV infection does not result in a sustained improvement of T-cell function. Differences in clinical course among zidovudine treated asymptomatics may be caused by heterogeneity of this group with respect to T-cell functional capacity at intake.**

L 435 DESIGN AND INTERPRETATION OF NEUTRALIZATION ASSAYS FOR THE EVALUATION OF POTENTIAL ANTI-HIV REAGENTS, W. John W. Morrow, Mai-Lan Nguyen and Alice S. Whalley, IDEC Pharmaceuticals Corp., La Jolla, CA 92037
Although the current literature describes several types of neutralization assays, no universally accepted standard has been adopted. In our laboratory, we are developing several tests for screening antibodies and synthetic peptides with therapeutic potential for the control of HIV infection. As it is important that the reagents are capable of broad, group-specific neutralization, multiple HIV isolates were employed in these evaluations. To optimize the sensitivity of the assays, a panel of $CD4^+$ cell lines was used to determine the tropism of each isolate. In addition, the growth kinetics of these isolates was studied in relation to the input dose of virus. In terms of measuring virus infection, the syncytial assay was most efficient with the $HIV_{HTLVIIIB}$ isolate using VB as the target cell line. Other HIV isolates, such as LAV1 and SF2, do not readily form multinucleated giant cells, and required different target cell lines for syncytial formation. The most sensitive determination of HIV infection was achieved using an ELISA to determine both intra- and extracellular levels of p24 *gag* antigen. Other endpoint determinations of levels of infection including nucleic acid hybridization and estimations of cellular metabolism were tested, but none achieved the sensitivity of the p24 assay. We conclude that the most practical methods for evaluating candidate products are measurement of syncytial inhibition and reduction of infection as determined by p24 *gag* antigen synthesis.

**L 436** THE ROLE OF GLYCOSYLATION IN THE INTERACTION OF RECOMBINANT CD4 AND HIV ENVELOPE PROTEINS FROM BACULOVIRUS INFECTED INSECT CELLS, Cheryl L.I. Murphy, Michael Lennick, Sophie Lehar, Gerald Beltz, Dante J. Marciani, and Elihu Young, Cambridge Bioscience Corp., 365 Plantation St., Worcester, MA. 01605. We have expressed three different HIV-1 envelope derived recombinant proteins and the full length CD4 polypeptide in insect cells in an effort to evaluate their vaccine and therapeutic potential for AIDS. DNA constructs encoding CD4, gp120, gp160 and gp160delta (full length gp160 minus the transmembrane and cytoplasmic region of gp41) were cloned into a derivative of the baculovirus expression vector pVL941 and transfected into insect cells along with wild type baculovirus DNA. Recombinant viruses were plaque purified and protein expression was determined by Western blotting with appropriate antisera. Radioimmunoprecipitation of detergent extracts of the recombinant HIV proteins showed that for each construct two major bands specifically precipitate with HIV-1 positive serum. These bands correspond to glycosylated and nonglycosylated versions of the HIV proteins as determined by $^{3}$H-mannose labeling and tunicamycin treatment of infected cells. As a measure of biological activity, we tested the ability of recombinant gp120, gp160delta and gp160 to bind to mammalian and baculovirus CD4 proteins as a function of co-precipitation in a radioimmunoprecipitation assay. Our results show clearly that CD4 and the glycosylated versions of recombinant gp120, gp160delta or gp160 specifically associated with one another in this analysis. The gp120 or gp160delta proteins from tunicamycin treated cultures did immunoprecipitate with anti-HIV-1 antiserum but did not interact with CD4 in the radioimmunoprecipitation assay. We conclude that baculovirus derived proteins CD4 and gp120, gp160delta and gp160 are biologically active and possess native structural elements and that glycosylation of these HIV envelope proteins is important for interaction with CD4.

**L 437 DEVELOPMENT OF A 3-DIMENSIONAL MODEL OF THE HUMAN IMMUNODEFICIENCY VIRUS REVERSE TRANSCRIPTASE, Lakshmi S. Narasimhan and Gerald M. Maggiora, Computational Chemistry, The Upjohn Company, Kalamazoo, Michigan 49001**

Based on an analysis of the sequences of RTs of 15 retroviruses from diverse species, a consensus sequence was obtained for RTs. This consensus sequence showed weak homology with the sequence of the polymerase portion of the Klenow fragment of E. Coli DNA polymerase I, the structure of which has been studied using X-ray crystallographic methods by T. Steitz et al. The 3-D coordinates for the alpha carbon trace of the Klenow fragment are available in the Brookhaven Protein Data Bank and it is the only polymerase for which 3-dimensional structural information is available to date. The alpha carbon trace of the polymerase subunit of the Klenow fragment is used as a starting point for the development of an HIV RTase model.

The secondary structure of the reverse transcriptase class of enzymes was predicted using a combination of the indices from Chou & Fasman, Garnier & Robson, Kyte & Doolittle hydropathy index and Karplus & Shultz flexibility index, averaging the indices amongst the homologous sequences. The predicted secondary structure and structural motifs in reverse transcriptases uncovered through a study of enzymes performing similar sub functions in conjunction with the knowledge of the biochemistry of HIV RT and the known structure and biochemistry of the Klenow fragment have led to the identification of the regions that are common to these enzymes, and a portion of the Klenow fragment structure has been identified to form the basis of further development of the HIV RT model. The features of the current model, and the rationale for its development are presented.

**L 438** ANTIVIRAL ACTIVITY AND MODE OF ACTION OF (-)-CARBOVIR, A POTENT INHIBITOR OF HIV-1 REPLICATION IN VITRO, Charles R. Penn, Jonathan A.V. Coates, Hermia T. Figueiredo, Helen J. Inggall, Clara L.P. Marr, David C. Orr, Janet M. Cameron, Department of Virology, Glaxo Group Research Ltd, Greenford, Middlesex. (-)-Carbocyclic 2'3'-didehydro-2', 3'-dideoxy guanosine (Carbovir, GR 90352) inhibits HIV-1 induced cytopathic effects in the human T-lymphocyte cell lines MT-4, C8166 and JM ($ID_{50}$ of 0.31, 0.12 and 0.13µg/ml respectively). Cytotoxicity was not observed in these cell lines nor in CEM and U937 cells at 50µg/ml. Carbovir was metabolised to 5'-mono, di and triphosphate in CEM cells.

The 5'-triphosphate of Carbovir was a potent inhibitor of the HIV reverse transcriptase, comparable to AZT triphosphate, when (rC)(dG) and influenza vRNA respectively were used as templates. The apparent Ki's for the triphosphate in these assay systems were determined as 50nM and 1.7µM respectively. The inhibition was shown to be competitive with respect to dGTP.

The mode of action of Carbovir is therefore presumed to be inhibition of HIV reverse transcriptase by Carbovir 5'-triphosphate.

**L 439** THE EFFECT OF PHARMACOLOGIC AGENTS ON HIV-1 VARIATION IN VITRO, Seth H. Pincus, Laboratory of Microbial Structure and Function, NIAID, Rocky Mountain Laboratories, Hamilton, MT 59840

We have selected HIV-infected tissue culture cells with three different pharmacologic agents to study the effects of these agents on HIV-variability. The three agents were AZT, interferon-α (IFN-α), and an antibody based immunotoxin. Two sets of variants arose upon treating H9/HTLV-IIIB cells with the immunotoxin. The first set secreted a morphologically normal, but non-infectious virus. In these variants, the gp160 was truncated at amino acid 687 by a single base deletion at this codon leading to loss of protein anchorage in the cell membrane. This unstable "gp145" was not properly cleaved, but could bind to CD4. Such viruses, which are close in structure to wild type virus but non-infectious, may be candidates for an "attenuated" virus vaccine. The second set of variants escaping the immunotoxin did not express virus but had an intact provirus in its genome. Selection of persistently infected H9/NL4-3 cells in high doses of AZT (5-10 μM) leads to variants that do not produce HIV and are resistant to the non-specific effects of AZT at doses as high as 100 μM (parental cells have 50% inhibition of $^{35}$S-Met incorporation at 1-3 μM). AZT resistant variants are as susceptible as parental cells to the cytotoxic effects of methotrexate, actinomycin-D, and G418, demonstrating that this is not multi-drug resistance. Treatment of cells in IFN-α at doses as high as 20,000 μ/ml led to no alteration in the function of the cells or virus. These studies demonstrate that pharmacologic agents can select for HIV-variants at two levels: the virus itself and the cell harboring the virus.

**L 440** CHARACTERIZATION OF SERUM ANTIBODIES IN PATIENTS IMMUNIZED WITH HIV GP160. Victoria Polonis, Cheryl Hooven-Lewis, Gayle Smith,* Charles Davis, Linda Bean, Donald Burke, Robert Redfield, Walter Reed Retrovirus Research Group, Washington, D.C. *MicroGeneSys, Inc., West Haven, Connecticut. The clinical relevance of HIV-specific anti-envelope antibody response as well as the ability to alter this response have not been clearly defined. The objective of this study was to quantitate and characterize the humoral immune response in seropositive individuals immunized with HIV gp160 envelope protein. A group of 14 patients in early stage of HIV infection were immunized with baculovirus-expressed recombinant gp160. Patient serum samples were analyzed post-immunization for antibodies directed against gp160 as well as p24 and p66 RT. The methods employed include ELISA, Western blot, and protein dot blots. While there was no change in serum antibody levels against p24 and p66, distinct alterations in total and epitope-specific anti-gp160 antibodies have been quantitated in four of eight patients studied to date. In certain patients a significant increase in antibody levels against four specific epitopes within gp120 have been detected. Two of these epitopes include regions implicated in type-specific and group-specific virus neutralization. Using the CEM-SS syncitial focus assay, neutralizing antibody titers pre- and post-immunization have been studied. A rise in neutralizing antibody titers against several viruses to include IIIB, RF, MN, HXB3, and HXB2D have been detected in immunized patients. Research directed towards the purification of epitope-specific antibodies and characterization of the biological function(s) of these antibodies is in progress.

**L 441** AMPHOTERICIN B IN THE TREATMENT AND PREVENTION OF AIDS: SELECTIVE REDUCTION OF CYTOTOXICITY BY COMPLEXING TO LIPIDS, D.R. Pontani, B. Wolf, D. Zuberbuehler, O.J. Plescia, C. Schaffner and S.I. Shahied, N.J. State Dept. of Health, Trenton, N.J. and Waksman Institute of Microbiology, Piscataway, N.J..

Lipid bound formulations of amphotericin B were prepared by the method of Janoff et al., using DMPC and DMPG with varying concentration of amphotericin B (5, 33 and 50 mole%). The 33 mole% was the least toxic of these preparations and appreciably less toxic than amphotericin B itself (AmB) based on the lysis of sheep red blood cells and viability of lymphocytes in culture. These preparations were tested for their antifungal activity against Candida albicans in vitro, and showed no reduction in their antifungal activity. These same preparations were tested for anti-HIV-1 activity in vitro using H9 and Molt-3 cells as targets for virus infection with AZT as a control. AmB was fully active at 5-10 μg/ml as previously reported. In contrast to their antifungal activity complexing with lipids reduced anti HIV-1 activity dependent upon the relative concentration of AmB to lipid. Generally the 33 mole% lipid preparation retained sufficient activity to warrant consideration. These results show promise in developing formulations of AmB that retain sufficient antifungal and antiviral activity with its cytotoxicity reduced to a tolerable level so that it can be used effectively in the therapy of AIDS.

**L 442** PEPTIDES SPANNING THE CDR-3-HOMOLOGOUS DOMAIN OF THE CD4 ANTIGEN [CD4(81-101)] SPECIFICALLY INHIBIT INFECTION AND SYNCYTIUM FORMATION INDUCED BY HIV-1 AND SIV, D. M. Rausch, K. M. Hwang, M. Padgett, V. S. Kalyanaraman, P. L. Nara, D. Buck, F. Celada, J. D. Lifson and L. E. Eiden, LCB, NIMH, Bethesda, MD 20892, Genelabs, Inc., Redwood City, CA 94063, Bionetics Res. Inc., Rockville, MD, LTCB, NCI, Frederick MD, 21701, Hospital for Joint Diseases, Orthopaedic Inst., 301 E. 17th St., NY, NY, 10003, Becton-Dickinson Monoclonal Ctr., San Jose, CA 95131. Side-chain derivatized CD4(81-92) peptides inhibit HIV-induced cell fusion, and HIV-1 infection of susceptible cells in vitro at micromolar concentrations. Anti-viral activity is sequence and derivatized-residue dependent. Peptide potency to block HIV infection/fusion is paralleled by potency to block gp120 binding to CD4 in both cell and cell-free binding assays. Cyclic derivatives of these peptides are significantly more potent than their open-chain congeners. Extension of the peptide by eight amino acids into the CDR-3-homologous region of CD4 eliminates the need for multiple derivatization of the molecule. These data support the hypothesis that CD4(81-92)-derived peptides inhibit HIV-1 and SIV infectivity and cytopathic effect in CD4-positive cells by mimicking the region of CD4 that binds gp120, and acting as a competitive 'antireceptor' for the binding of the immunodeficiency envelope glycoprotein to cell-surface CD4. Several anti-CD4 monoclonal antibodies that bind CDR-3-derived peptides inhibit HIV-1 infection and HIV-1-induced cell fusion, providing further support for the primary involvement of the CDR-3-homologous domain of CD4 in binding to gp120. Several unique properties of CD4-derived peptides merit their further consideration as potential anti-HIV antiviral therapeutic agents. These are 1) ease of synthesis, 2) potential for developing more potent analogs by further structural modification based on the predicted three-dimensional conformation of the CDR-3 domain in the CD4 holoreceptor molecule 3) ability to inhibit viral transmission in already infected cell cultures (virostatic activity) and 4) inhibition of cytopathicity of several fresh HIV-1 isolates, including at least two isolates which are relatively refractory to inhibition by CDR-2-derived anti-CD4 monoclonal antibodies.

**L 443** REGULATION OF AZIDOTHYMIDINE-5'-MONOPHOSPHATE EFFLUX FROM HUMAN LYMPHOID CELLS. Brian L. Robbins, Michele Connelly, and Arnold Fridland, Department of Pharmacology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38101
Upon treatment with 1 μM AZT human lymphoid cells CCRF-CEM initially accumulated AZT mono-, di-, and triphosphate to peak concentrations of 68, 0.84 and 1.95 μM, respectively, by 4 h which then decreased markedly with time. During this time period, AZTMP was also excreted by the cells into the medium. This excretion of AZTMP was found to be temperature sensitive and inhibited by various agents. Agents which reduce intracellular ATP levels such as sodium azide inhibited AZTMP excretion. This excretion of AZTMP was also inhibited by the nucleoside transport inhibitor dipyridamole but not by 4-nitrobenzylthioinosine. Dipyridamole treatment caused a significant increase in the retention of AZTTP in CCRF-CEM cells and decreased the cytotoxicity of AZT. Significant amounts of endogenous phosphorylated metabolites (AMP, ADP, ATP, GTP, UTP) were not released from the cells under these conditions. These results suggest the existence of a process that can mediate the excretion of AZTMP across human lymphoid cells and which may be subject to regulation. Supported in part by Grants AI27652 and CA43296 from the National Institutes of Health and National Cancer Institute Center Support (CORE) Grant P30 CA21765 and American Lebanese Syrian Associated Charities.

**L 444** CHARACTERIZATION OF HIV-1 VARIANTS CAPABLE OF REPLICATING IN PRESENCE OF HIGH CONCENTRATIONS OF ZIDOVUDINE (AZT), Ronald Rooke, Michel Tremblay and Mark A. Wainberg, Department of Microbiology, McGill University, Montreal, Canada H3T 1E2
Of 48 patients in a Canadian AZT trial, we found that 9 were viremic after at least 30 weeks of therapy for HIV-1 able to infect lymphocytes in the presence of 10 μM AZT. The Ki's for AZT-triphosphate were compared between these AZT-resistant variants and wild-type isolates obtained from the same patients prior to initiation of therapy. No significant differences were noted, even though the Vmax of the AZT-resistant viral strains was consistently higher than those of wild-type isolates. Four of these variants were studied for cross-resistance to other drugs [dideoxycytosine (ddC), didehydro-dideoxythymidine (d4T), dideoxyinosine (ddI) and deoxythiacytidine a novel compound in which a sulfur atom has replaced the 3'carbon of the ribose also called BCH-189]. One of the AZT-resistant variants was able to infect MT-4 cells in the presence of 10 μM d4T; none of the others showed cross-resistance. No significant differences were observed between these various viruses in terms of ratios between reverse transcriptase activity and p24 Ag and/or $TCID_{50}$ in each case. The *pol* genes of each variant and parental control have been amplified by PCR and are now being sequenced. Finally, we were interested in determining whether AZT-resistant viruses might also be present in non-treated patients. We were able to select for such a strain by repeated passages of an initial clinical isolate in low concentrations of AZT (0.5 μg/ml). This suggests that AZT-resistant viruses might be present in the original viral pool of infected patients and are selected for by the presence of AZT.

**L 445** RIBOZYMES AS POTENTIAL ANTI-HIV AGENTS Nava Sarver, A. Hampel*, E. Cantin**, P. Chang**, M. Johnston, and J. Rossi**, Developmental Therapeutics Branch, Division of AIDS, NIAID, Bethesda, MD; *Plant Molecular Biology Center, Norther Illinois University, DeKalb, IL 60115; **City of Hope, Duarte, CA 91010.
Certain RNA molecules, named ribozymes, possess enzymatic, self cleaving activity. The cleavage reaction is catalytic and no energy source is required. Two distinct ribozymes motifs, hammerhead (Uhlenbeck (1987) Nature 328:596), and hairpin (Hampel and Tritz (1989) Biochem. 28:4929) were identified in plant RNA pathogens. These ribozymes differ in their secondary (and possibly tertiary) structures. The present study demonstrates precise cleavage of human immunodeficiency virus (HIV-1) sequences in a cell free system using both the hairpin and hammerhead ribozyme structures. In addition to the cell-free studies, human cells stably expressing a hammerhead ribozyme targeted to HIV-1 gag transcripts have been constructed. When these cells are challenged with HIV-1, a substantial reduction the level of HIV-1 gag RNA relative to that in non-ribozyme expressing cells, is observed. The reduction in gag RNA is reflected in a reduction in p24 antigen. [Sarver, et al, Science (in press)]. These results suggest the merit in developing ribozymes as therapeutic agents against human pathogens such as HIV-1.

**L 446** A REGION OF CD4 DISTINCT FROM THE gp120 BINDING SITE IMPORTANT IN HIV INFECTIVITY AND FUSION. Quentin J. Sattentau*, Donald Healey*, Marilyn M. Moore**, Pila Estess***, David Buck*** and Peter C.L. Beverley** . * Academic Department of G-U Medicine, University College and Middlesex School of Medicine, Cleveland Street, London W1, UK. ** HTIG, Imperial Cancer Research Fund, 91 Riding House Street, London W1, UK. *** Becton Dickinson Monoclonal Center, Mountain View, CA., USA. The binding of gp120 to CD4 is an essential first step in HIV infection of CD4+ cells, and is probably followed by fusion of the virus and cell membranes. Although much is now known about the initial stages of the gp120/CD4 interaction, events subsequent to virus binding are little understood. Monoclonal antibodies (mAbs) reactive with CD4/V1 are highly efficient at inhibiting binding of gp120 to CD4, and hence prevent infection and syncytium formation, whereas mAbs binding to more C terminal regions are at best poorly able to inhibit gp120 binding and subsequent events. Using two new mAbs we have defined a region of CD4 distinct from the gp120 binding site which is important in virus infection and fusion of CD4+ cells. The binding of gp120 to CD4 is not inhibited by these mAbs, and under certain conditions they increase binding. Despite this, these mAbs potently inhibit HIV infection and syncytium formation at low concentration. Using recombinant human-mouse chimaeric CD4 molecules, we have localized the region of CD4 which interacts with these mAbs to residues 119-194, approximating to the second domain and second joining region. These findings demonstrate for the first time that a region of CD4 distinct from the gp120 binding site is important in events subsequent to HIV binding which lead to infection and cell fusion.

**L 447** SYNERGISTIC INHIBITION OF HIV REPLICATION BY CARBOVIR AND AZIDOTHYMIDINE, Marilyn S. Smith[1], Stephen G. Carter[2], Joseph S. Pagano[1], Lineberger Cancer Research Center[1], University of North Carolina, Chapel Hill, NC 27599, and GLAXO, Inc.[2], Research Triangle Park, NC 27709. A combination of two nucleoside analogues, carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine (Carbovir) and 3'-azido-3'-deoxythymidine (AZT) was tested for synergy in the inhibition of human immunodeficiency virus-type 1 (HIV-1) replication in T-cells. Virus replication was assayed by reverse transcriptase activity. We performed experiments in established T-cell lines, C3 and Jurkat. In C3 cells, using constant ratios of 1:1.5 and 1:5, the mutually nonexclusive combination indices (CI) (8 CIs) averaged 0.74 (range 0.54 to 0.97). In Jurkat cells, using constant ratios of 1:1 and 1:5, the CIs (10 CIs) averaged 0.61 (range 0.37 to 0.76). Results from 3 experiments using normal donor peripheral blood mononuclear cells will be presented. In the 3 PBMC experiments, the average $EC_{50}$ for Carbovir was 0.1 uM, and for AZT, the $EC_{50}$ was 4 nM, based on RT data. Combination indices of <1, 1, and >1 indicate synergy, additive effect, and antagonism, respectively. These values were determined using the Chou and Chou computer software. The correlation coefficients for the median-effect plots were >0.95 for these experiments. Thus, we find significant synergy between these compounds in two human T-cell lines.

**L 448** HIV CLINICAL ISOLATES EXPRESSING ALTERED SENSITIVITY TO RETROVIR
Marty H. St. Clair[1], Diane M. King[1], Brian Edlin[2], Michael Bach[3], Fredrick Siegal[4], VA Cooperative Study Group , John Bartlett[5], Hetty A. Waskin[5], Gareth Tudor-Williams[1], [1]Wellcome Research Laboratories, Research Triangle Park, NC 27709; [2]Centers for Disease Control, Atlanta, GA 30333; [3]Main Medical Center, Portland, ME 04102; [4]Long Island Jewish Hospital, New Hyde Park, NY 11042; [5]Duke University Medical Center, Durham, NC 27705. Clinical HIV isolates from 61 HIV-positive patients who had been on RETROVIR therapy for greater than 1 year were amplified in peripheral blood lymphocytes (PBLs) and analyzed for sensitivity to RETROVIR. Drug sensitivity was determined by two or more assays per isolate in PBLs. RT was measured ten days post-infection and $IC_{50}$s were determined. The mean RETROVIR $IC_{50}$ (N=5) for those isolates obtained from patients with AIDS who have never received RETROVIR therapy was 0.03 uM (0.01-0.6 uM). The isolates obtained from people with AIDS who have received RETROVIR therapy for greater than one year possessed a mean $IC_{50}$ (n=6) of 7.0 uM (1.5-13.9 uM). Only 2 of 25 coded isolates obtained from the VA Cooperative Study #298 (early ARC) exhibited RETROVIR $IC_{50}$s of 1.0 uM or greater (1.0 and 1.4 uM). The remaining 23 isolates had a mean $IC_{50}$ of 0.2 uM RETROVIR (0.01-0.8 uM). Four of 25 isolates obtained from ACTG 019 (also still coded) had an average $IC_{50}$ of 1.7 uM (1.2-2.9 uM), while the remaining 21 isolates had a mean $IC_{50}$ of 0.22 uM RETROVIR (0.02-0.92 uM).

**L 449** DECREASE IN OPPORTUNISTIC INFECTIONS (OIs) IN ARC/PRE-ARC PATIENTS (T4=60-300) RECEIVING AMPLIGEN FORMULATED IN GLASS BOTTLES COMPARED TO PLACEBO,
D.R. Strayer[1], I. Brodsky[1], E. Pequignot[1], S.M. Miller[2], R.S. Schulof[3], G.L. Simon[3], R. Suhadolnik[4], N. Reichenbach[4], K. Strauss[1], D. Gillespie[1], and W.A. Carter[1]. [1]Hahnemann Univ., Phila., PA; [2]Baylor Univ., Houston, TX; [3]George Wash. Univ., WDC.; [4]Temple Univ., Phila., PA. 20 ARC/pre-ARC patients (pts) (mean T4 = 196/µl) received 100-200 mg Ampligen (Amp) IV twice weekly for 4.5-36 mos. One of the 20 pts (5%) developed an OI after 494 days. The frequency of OIs was compared to a similar cohort of ARC/pre-ARC pts (mean T4 = 198) who received placebo (pla) in a multicenter study of Amp (AMP101). Amp increased time to OIs, ($p<.05$, Wilcoxon, one-sided) compared to pla (13/144 or 9%, mean time to OI = 78 days). There was no significant difference in baseline mean T4, % p24 positivity (Amp = 50%, pla = 48%) or the % of pts with ARC symptoms (Amp = 60%, pla = 61%). Glass bottle Amp decreased p24 ($<.05$) and increased T4 cell number ($P<.05$) compared to pla. In contrast similar ARC/pre-ARC pts receiving Amp formulated in plastic IV bags developed OIs at a rate no different than pla. Physicochemical studies to showed that the plastic bag formulation yielded a drug with a higher sedimentation value (more open structure, $p<.05$), shorter RNase resistant cores (lower helicity, $p<.002$) and reduced ability to activate 2'-5' oligoadenylate synthetase ($p<.001$). These studies suggest that the similar progression rates to AIDS in the pla and Amp arms in the AMP101 study may have been related to changes in the formulation of Amp used in the randomized study compared to the Amp formulation used in the initial pilot study. Clinical, immunological/virological effects and safety of Amp formulated in glass bottles continue to suggest Amp is active and safe in ARC/pre-ARC.

**L 450** ANALYSIS OF THE gp120 BINDING SITE ON CD4.
R. Sweet, J. Arthos[1], Q. Sattentau[2], K. Deen, M. Chaikin, A. Shatzman, P. Maddon[3], R. Axel[4], A. Truneh and M. Rosenberg. Smith Kline & French Labs , King of Prussia, PA; [1]Univ. of Pa., Philadelphia, PA; [2]Middlesex Hospital, London, UK; [3]Progenics, Tarrytown, NY; [4]Columbia Univ., NY, NY.

Soluble CD4 proteins, consisting of all or portions of the extracellular region of human CD4, are potent inhibitors of HIV but have no effect on normal T-cell function in vitro. The combination of these properties has led to clinical trials with soluble CD4 to assess its efficacy in the treatment of AIDS. Current efforts on derivatives of soluble CD4 require a knowledge of the location of the gp120 binding site and its structural determinants. Through the expression of truncated or chimeric proteins, we and others determined that the binding site resided within the first excellular domain, V1. Through the creation of substitution mutants within V1 and quantitative assay of their affinity for gp120, we further localized this site to residues 41-55, a region similarly defined in several other mutagenesis studies. The V1 domain shares sequence homology with Ig $V_K$ and, in this context, the gp120 binding site overlaps the region which aligns with CDR2 in the antibody chain. We obtained indirect evidence for a $V_K$-like structure for the V1 domain through epitope mapping of >50 anti-CD4 mAbs: epitopes occurred in regions of predicted exposed loops and apparently discontinuous epitopes involved residues predicted to lie close in space. Recent C-terminal truncations of the V1 domain further support this structure.

**L 451** A SYNTHETIC HUMAN IMMUNODEFICIENCY VIRUS PROTEASE INHIBITOR WITH POTENT ANTIVIRAL ACTIVITY ARRESTS HIV-LIKE PARTICLE MATURATION, Tarpley, W.G., T.J. McQuade, A.G. Tomasselli, V. Karacostas, B. Moss, T.K. Sawyer, and R.L. Heinrikson, Research Laboratories, The Upjohn Company, Kalamazoo, MI and National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

A synthetic peptidemimetic substrate of the HIV-1 protease with a nonhydrolyzable pseudodipeptidyl insert at the protease clevage site was prepared. The peptide (U-81749) is a potent inhibitor of recombinant human immunodeficiency virus type 1 (HIV-1) protease in vitro (Ki of 70 nM) and HIV-1 replication in human peripheral blood lymphocytes ($IC_{50}$ of 0.1-1 μM). Moreover, 10 μM concentrations of U-81749 significantly inhibited proteolysis of the HIV-1 gag polyprotein (Pr55) to the mature viral structural proteins Pr24 and Pr17 in cells infected with a recombinant vaccinia virus expressing the HIV-1 gag-pol genes. The HIV-1 like particles released from inhibitor-treated cells contained almost exclusively Pr55 and other gag precursors, but not Pr24. Incubation of HIV-like particles recovered from drug-treated cultures in drug-free medium indicated that inhibition of Pr55 proteolysis was at least partially reversible, suggesting U-81749 was present within the particles. Our data indicate that synthetic compounds targeting the HIV-1 protease can exhibit potent anti-HIV effects in mammalian cells and emphasizes that protease is an attractive target for anti-HIV drugs.

**L 452** IMPROVED ADJUVANTS FOR ENHANCEMENT OF IMMUNITY TO RECOMBINANT HIV-1 gp120 ENVELOPE ANTIGENS, Paula Traquina, Keith W. Higgins, Nancy L. Haigwood, Peggy Wentworth, Paul Skiles, Jorg W. Eichberg, D. Rick Lee, Gary Ott, Dino Dina, Kathelyn S. Steimer, and Gary Van Nest, Chiron Corporation, 4560 Horton Street, Emeryville, California, 94608. In order for an anti-viral subunit vaccine to be effective, it is essential to (1) identify the appropriate viral proteins to use as immunogens, (2) be able to elicit high levels of immunity with such antigens and (3) induce sufficient immunologic memory to resist a subsequent viral challenge. Thus, the development of improved adjuvants for enhancing immunity to soluble protein immunogens is critical in any subunit vaccine strategy. To date, the only adjuvant approved for human use is aluminum hydroxide (alum). We have been developing alternative adjuvant formulations consisting of muramyl peptide derivatives in metabolizable oil emulsions. Comparisons of the efficacy of these formulations with alum for enhancing immunity to recombinant HIV-1 gp120 antigens (both non-glycosylated denatured and fully glycosylated native gp120 versions) have been carried out in various animal species including guinea pigs, goats, and baboons. While most adjuvant formulations were effective in guinea pigs (and also mice and rabbits), the properties of the emulsion dramatically influenced the efficacy of oil based adjuvants in larger animals such as goats and baboons. Several of these new adjuvant formulations were at least ten fold more effective than standard alum formulations in enhancing anti-gp120 antibody responses. This increase in antibody titers as assayed by ELISA, was paralleled by an increase in the titers of antibodies capable of neutralizing HIV-SF2 virus in vitro. Lymphocyte proliferation assays also point to an increase in T-helper cell immunity when these improved adjuvant formulations were utilized.

**L 453** APPLICATION OF RIBOZYMES AND GENE THERAPY TO THE TREATMENT OF AIDS,

Marc VASSEUR and Luc d'AURIOL, Laboratoire de Virologie Moléculaire and GENSET SA UFR de Biochimie, Hall des Biotechnologies, Université of Paris 7, 2 place Jussieu, 75005 PARIS, FRANCE.

Antisense oligonucleotides and ribozymes offer a rational approach for the design of new anti-viral molecules with very high specificity. We present here the results of a ribozyme-based gene technology for the treatment of AIDS. To investigate the potential of ribozymes to inhibit HIV development, we have synthezised genes encoding ribozymes directed against various HIV mRNA. The ribozymes transcribed from T7 polymerase promotors function *in vitro* and in nuclear extracts of lymphocytes and macrophages derived cell lines. Direct synthesis of ribozymal RNA by mean of automated oligoribonucleotide synthesis was also tested and theses synthetic molecules were proved to be efficient in *in vitro* model systems. For *in vivo* analysis, sequences encoding ribozymes directed against *tat* and/or the 5'-leader/*gag* region were inserted into retroviral murine vectors. Gene transfert into human T lymphocyte cell lines by these vectors is a very efficient process. The inhibition of HIV-1 expression in cells expressing anti-HIV ribozymes was monitored by counting syncitia, by the analysis of viral antigen expression and the measurement of cell viability. Our results show that retroviral vectors are proper tools for the delivery of mRNA targeted efficient molecules inhibitory to HIV into human lymphocytes cells. Comparisons of the relative efficiencies of antisense oligonucleotides and ribozymes approaches for the inhibition of HIV expression in cultured cells will be presented and discussed.

**L 454** **Investigations on the capacity of autologous and heterologous particle antigens as carriers for HIV epitopes**

Albrecht von Brunn, Ralf Wagner, Holger Fließbach, Peter Gütter°) and Hans Wolf

Max von Pettenkofer Institut, Pettenkoferstraße 9a, 8000 München 2, FRG; °) Charite, Humboldt Universität, Ostberlin, GDR

Current strategies for the development of HIV vaccines are primarily based on the envelope protein gp120. However, immunity to gp120 is possibly accompanied by adverse effects such as the induction of antibodies which enhance viral infection. Terefore it might be desirable to select only those regions of the molecule known to induce neutralizing antibodies or to be involved in cell to cell interaction.

Ideally, such sequences should be coupled to carrier molecules presenting the epitopes at high density and with an intrinsic adjuvant potential. We are investigating the capacity of an autologous structural molecule, the HIV gag protein, and of hepatitis B virus antigens to form hybrid particulate structures carrying env epitopes. We have constructed procaryotic (E. coli) and eukaryotic vectors (Vaccinia, Baculovirus) for the expression of the hybrid genes. Biophysical and biochemical characterization of the hybrid proteins with respect to particle formation are under way.

**L 455** INHIBITION OF HIV-1 REPLICATION BY POKEWEED ANTIVIRAL PROTEIN (PAP) TARGETED TO $CD4^+$ CELLS BY CONJUGATION WITH MONOCLONAL ANTIBODIES, Joyce Zarling[1], Patricia Moran[1], Joan Sias[1], Dorothea Myers[3], Virginia Kuebelbeck[3], Jeffrey Ledbetter[1], Celsa Spina[2], Douglas Richman[2], and Fatih Uckun[3], [1]Oncogen, Seattle, WA 98121, [2]Veterans Administration Hospital, San Diego, CA 92161, and [3]University of Minnesota, Minneapolis, MN 55455.

Pokeweed antiviral protein (PAP) is a 30 kDa protein isolated from Phytolacca americana that was previously shown to inhibit replication of certain plant and mammalian viruses. We have found that PAP inhibits replication of a retrovirus, HIV-1, in fresh human $CD4^+$ T cells with an inhibitory dose 50 ($ID_{50}$) of approximately $5 \times 10^{-3}$ pM but PAP inhibits proliferation of uninfected $CD4^+$ T cells by only 10% at $1 \times 10^{-5}$ pM. PAP has an in vivo half-life of only 10-20 min whereas PAP conjugated to mAb has a half-life of 8-16 hours. Furthermore, internalization of PAP-mAb conjugates by receptor mediated endocytosis yields a more efficient delivery of PAP through the cell membrane. Thus, PAP-mAb conjugates reactive with CD4 and CD7 antigens expressed on $CD4^+$ T cells were prepared and evaluated for inhibiting HIV-1 production. The $ID_{50}$ of PAP-anti-CD7 and PAP anti-CD4 for inhibiting HIV-1 production is approximately 5 pM whereas the $ID_{50}$ of these PAP-mAb conjugates for proliferation of uninfected $CD4^+$ cells and bone marrow progenitor cells is approximately 100 and 1000 times greater, respectively. PAP-anti-CD7 and PAP anti-CD4 conjugates are approximately 500 times more effective at inhibiting HIV-1 replication than is non-conjugated PAP. PAP-mAb conjugates, which target PAP to cells susceptible to infection by HIV, thus show potential for treating HIV-1 infected humans.

## *Vaccine and Treatment*

**L 500** RAPID DEVELOPMENT OF ISOLATE-SPECIFIC NEUTRALIZING ANTIBODIES AFTER PRIMARY HIV-1 INFECTION AND CONSEQUENT EMERGENCE OF VIRUS VARIANTS WHICH RESIST NEUTRALIZATION BY AUTOLOGOUS SERA. Jan Albert[1,2], Bengt Abrahamsson[1], and Eva Maria Fenyö[2]. 1) Department of Virology, National Bacteriological Laboratory, 2) Department of Virology, Karolinska Institute, S-105 21 Stockholm, Sweden.

Objective: To study the kinetics of appearance and specificity of HIV-1 neutralizing antibodies and the role of these antibodies in the emergence of new virus variants.
Methods: HIV-1 was isolated from four individuals during symptomatic primary HIV-1 infection and repeatedly thereafter and tested in neutralization assay against autologous sera collected in parallel. Relevant regions of the env gene have been PCR amplified and will be sequenced directly by a new solid phase method.
Results: Our patients developed isolate-specific low-titer neutralizing antibodies within - 4 weeks and the titers to the first isolates increased with time. In three patients we could document the emergence of virus variants with reduced sensitivity to neutralization by autologous, but not heterologous, sera. These virus variants were, however, not resistant to neutralization per se, since they were readily neutralized by the positive control serum. Our patients did not develop antibodies capable of neutralizing the new virus variants during the observation period. The emergence of the neutralization resistan virus variants does not seem to be directly correlated in time with a rapid deterioration of the clinical status of the patient. It is, however, likely that the emergence of virus variants which the patient fails to neutralize in the long run contributes to disease progression. DNA sequencing is under way and the results will be presented at the symposia

*Abstract Withdrawn*

**L 502** IMMUNOPATHOLOGY OF HIV INFECTIONS IN CNS AND LYMPH NODES.
**Peter Biberfeld***, Carlo Parravicini**, Anna-Lena Petrén*, Marianne Ekman*, 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 effector role of CD8+ cells in the immunopathology of HIV associated lesions is suggested.

**L 503** MOLECULAR CHARACTERIZATION OF NON-INFECTIOUS, *ENV*-DEPLETED VIRUS STRUCTURES PRODUCED BY CLONED U-937 CELLS: INVOLVEMENT OF CD4-*ENV* INTRACELLULAR COMPLEXES,
Stephan Bour, François Boulerice, Romas Geleziunas and Mark A. Wainberg, Jewish General Hospital, Montreal, Canada H3T 1E2
Ultradilution cloning of HIV-1 chronically infected U-937 cells gave rise to a clone named UHC15.7 which produces non-infectious progeny virus. Northern and western blot analysis of this clone showed that all viral mRNA species and proteins were made. However, although *env* proteins were normally synthesized in these cells, they were not expressed at the cell membrane. Virus-like particles could be seen budding from these cells by electron microscopy. They were reverse transcriptase positive but of atypical appearance and devoid of gp120 and gp41, as studied by western blot assay. Coprecipitation of CD4-*env* using OKT4 M.Ab showed that the UHC15.7 *env* proteins retained their capacity to bind to the cellular receptor and to form intracellular CD4-*env* complexes. We next asked whether the UHC15.7 phenotype could be due to a change in CD4/*env* ratio. Solid phase radioimmunoassays were performed using M.Ab against CD4, gp160, or gp120. The results showed a decrease in the *env* protein intracellular pool, when compared with wild-type infected U-937 cells. Moreover, no variation in the CD4 pool was observed. The CD4-*env* ratio calculated in UHC15.7 is close to 1. We thus suggest that the formation of CD4-*env* intracellular complexes involved all the available *env* proteins and is responsible for *env* surface depletion. More information on the mutation responsible for the downmodulation of *env* protein expression will be obtained by sequencing the UHC15.7 *env* gene.

**L 504** A MONOCLONAL ANTIBODY TO HIV-1 MEDIATING CELLULAR CYTOTOXICITY (ADCC) AND NEUTRALIZATION. Per A. Broliden[1,2,3], K. Ljunggren[2,3], J. Hinkula[1], B. Mäkitalo [1], E. Norrby[2], L. Åkerblom[4] and Britta Wahren[1]. Dept. of Virology, National Bacteriological Laboratory 1. Dept. of Virology ,2. Dept. of Immunology 3, Karolinska Institute, Stockholm, Dept. of Veterinary Micrbiology, Biomedical Center, Uppsala, Sweden 4.
Objective: To identify regions and sequences important for ADCC and neutralization.
Methods: Murine monoclonal antibodies (MAbs) were raised against HIV-gp120. ADCC and virus neutralization were performed. Solid phase synthesized 15 aminoacid (a.a.) peptides with an overlapping sequence of 10 a.a. representing the complete region of the envelope (env) protein based on the HTLV-IIIB sequence were used as antigens in ELISA-assays. Fine mapping was performed using 8-mer peptides sequentially overlapping with seven, and sets of peptides with sequential deletions or substitutions.
Results: One MAb P4/D10 was found to mediate high ADCC and virus neutralization. Five other MAbs with a similar epitopic reactivity did not show any ADCC activity but had a virus neutralizing capacity. The reactivity was located to a conserved sequence within the hypervariable region between a.a. 296-331. The essential aminoacids could be limited to four, GPGR, which is an highly conserved sequence. A high reactivity was also seen against peptides representing other HIV-1 isolates which contained GPGR.
Conclusion: An epitope important for ADCC was identified in the major neutralizing region. A relation between these functional activities was found by the MAb showing both ADCC and neutralizing capacity. The highly conserved sequence of the epitope indicates a broad functional activity to different HIV-1 isolates. Ability to select for functional activities by selecting regions of interest is of importance for the development of a vaccine against HIV.

**L 505** HIV-1 GLYCOPROTEINS GP120 AND GP160 INHIBIT THE CD3/T CELL ANTIGEN RECEPTOR PHOSPHOINOSITIDE TRANSDUCTION PATHWAY, Daniel Céfaï, Patrice Debré, Michel Kaczorek, Thierry Idziorek and Georges Bismuth, Laboratoire d'Immunologie Cellulaire et Tissulaire, CERVI, GH Pitié-Salpétrière, Paris, France. The interference of the recombinant HIV-1 glycoproteins gp160 and gp120 with the CD3/T cell antigen receptor (TcR)-mediated activation process has been investigated in a CD4+ antigen-specific human T cell clone. Both glycoproteins clearly impair the T lymphocyte proliferation induced in an accessory cell-free system by various CD3-specific monoclonal antibodies (MAb) or by a MAb specific for the TcR $\alpha$ chain (up to 80% inhibition). Addition of soluble CD4 could prevent the gps-induced inhibition of T cell proliferation. Biochemical studies further demonstrate that a 16 hour exposure of T cells to both glycoproteins specifically inhibits the CD3/TcR phospholipase C transduction pathway, without affecting the CD3 and CD2 cell surface expression. Thus, inositol phosphates production, phosphatidic acid turn-over and $[Ca^{2+}]_i$ increase induced by CD3/TcR-specific MAbs are decreased in treated cells. Similar results are not observed for short term exposure of the clone (< 2 h). We also found that gp160 and gp120 have no effect on the CD2-mediated activation pathway. Taken together, these results show that soluble HIV-1 glycoproteins may directly impair the CD3/TcR-mediated activation process in uninfected T cells via the CD4 molecule independently of any accessory cell-T lymphocyte interaction.

**L 506** TRANSACTIVATION-BASED ASSAYS FOR THE DETECTION OF INFECTIOUS HIV

**Sunil Chada, Lisa Laube, Jack Barber, John Warner, Doug Jolly and Daniel St. Louis.**
**Viagene Inc., San Diego CA 92121.**

The detection and quantification of infectious human immunodeficiency virus (HIV) will be important for monitoring blood products, patients given various therapeutic treatments and screening antiviral drugs. We have developed human CD4$^+$ indicator cell lines containing reporter genes under the control of HIV regulatory elements. The reporter genes utilized code for beta-galactosidase or a secreted form of alkaline phosphatase. The genes are linked to the HIV LTR and the "CRS-CAR" sequences. In the absence of HIV these genes are not expressed, however upon exposure to infectious HIV, reporter gene expression is activated. The beta-gal HIV indicator cell line, employed to titer infectious HIV, has exhibited 20-50 fold greater sensitivity for HIV detection compared to the syncytia formation assay. These cell lines have also been used to detect HIV neutralizing antibody as well as to screen the effectiveness of antiviral drugs.

**L 507** IN SITU/IN VIVO DETECTION OF EPITOPE SPECIFICITY OF ANTIBODY FORMING CELLS AND IMMUNE COMPLEXES DIRECTED AGAINST HUMAN IMMUNODEFICIENCY VIRUS.
Eric Claassen, Jon D. Laman, Koen Gerritse & Wim J. A. Boersma. Dept. Immunology, TNO Medical Biological Laboratory, POB 45, 2280 AA Rijswijk, The Netherlands.

Using recombinant HIV-1 gp120 and synthetic peptides in a mouse model, we have shown that it is possible to detect epitope specificity of anti-HIV antibody forming cells (AFC) and immune complexes in vivo. Mice were immunized with these antigens and cryostat sections of lymph nodes and spleen were incubated with the antigen coupled to Alkaline Phosphatase (AP). Subsequent staining revealed blue stained B-cells producing antigen specific antibody and immune complexes localised in lymphoid follicles containing such antibodies. The application of synthetic peptides in conjugates allows the determination of epitope specificity of the antibodies. Typically, 30 - 200 AFC were found per spleen or lymph node section. Double staining with antigen-AP and polyclonal rabbit anti-mouse isotype HRP (horse radish peroxidase) allowed simultaneous detection of antigen/epitope specificity and isotype of locally present antibody. Double staining results in 1. blue cells: AFC binding the conjugate with antibody of unknown isotype 2. red cells: producing the antibody of the isotype under investigation 3. violet cells: AFC binding the conjugate with antibody of the isotype under investigation. This method offers great potential for studies of both patient and animal tissues with regard to: 1) Kinetics of AFC against neutralising epitopes in lymphoid tissues. 2) The involvement of immune complexes in the destruction of follicular dendritic cells (FDC, cf; Laman et al., AIDS, 3:543, 1989). 3) The efficacy of candidate vaccines to induce local (mucosal) immunity. 4) The production of auto-antibodies by antigenic mimicry of HIV proteins. 5) Possible involvement of EBV and CMV in AIDS. 6) Specificity of B-cell infiltrates in lymph nodes.

**L 508** PHAGOCYTOSIS OF CANDIDA ALBICANS BY HIV-1 INFECTED MONOCYTE-MACROPHAGES, Suzanne Crowe*, Anne Colvin*, ELizabeth Neate*, Katherine Silburn*, Secondo Sonza*, Nicholas Vardaxis#, *Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Melbourne, Australia, 3078, #Phillip Institute of Technology, Melbourne, Australia, 3083 Objective: To determine the effect of HIV-1 infection on the ability of monocyte-macrophages (MØ) to phagocytose Candida albicans. Methods: MØ were isolated from buffy coats by density gradient centrifugation and glass adherence. Cells (5-20 days in culture) were infected with HIV-DV at a MOI of 0.1. After 7 days, infected and uninfected MØ were incubated with opsonised C. albicans (labelled with propydium iodide) for 1-24 hours. The proportion of MØ containing C. albicans and the number of organisms per MØ were determined by FACS analysis, light microscopy and con-focal laser microscopy. HIV infection was assessed by p24 monoclonal antibody staining and FACS analysis, kinetic RNA slot blot analyses and supernatent HIV antigen quantitation (Abbott HTLV III EIA). Results: A mean of 81% of uninfected MØ (n=6) and 58% of HIV-infected MØ (n=6) from same donors, and of same age in culture, were found to phagocytose C. albicans by FACS analysis. These results were concordant with values obtained by light microscopy. Optimal time of incubation for phagocytosis was 5 hours. Approximately 36% of infected MØ contained HIV p24 antigen by FACS analysis. Supernatent p24 antigen analysis and HIV RNA slot blot confirmed productive infection. Con-focal laser microscopy defined the intracellular localisation of C. albicans. Kinetic studies to correlate surface Fc receptor expression and phagocytic capacity are in progress. Conclusion: HIV infected MØ phagocytose C. albicans less efficiently than uninfected MØ of same donor and age in culture.

**L 509** A Pertussis Toxin Sensitive GTP-Binding Protein Is Associated With CD4 And Transduces HIV-1 gp120-Initiated Signalling. W. Cruikshank, T. Ryan, J. Rubins, B. Dickey, D. Center, and H. Kornfeld, Pulmonary Center, Boston Univ. Sch. Med., Boston, MA. 02118.

Stimulation of resting CD4+ lymphocytes by HIV-1 gp120 results in increased cellular migration and expression of IL-2 receptors. We investigated whether the signalling induced by gp120, which results in a rise in intracellular calcium and generation of inositoltrisphosphate (IP3), were transduced by a CD4-associated GTP-binding protein (G-protein). Human CD4+ lymphocytes were incubated with either pertussis toxin (PT)(500ng/ml) or cholera toxin (CT) (1ug/ml) for three hrs, then stimulated with gp120 ($10^{-8}$M). PT, but not CT, inhibited the calcium flux from 265nM $Ca_i$ for untreated cells to 135nM for treated cells, and inhibited motility from 192% control cell migration to 115%. To demonstrate coupling between CD4 and a PT-sensitive G-protein, we assessed gp120-induced changes in GTP S binding and GTPase enzyme activity in lymphocyte membranes. [35S]-labelled GTP S binding to purified plasma membranes was increased by 40% at 10 min. in the presence of gp120. Gp120 stimulation also increased GTPase activity, measured by release of [32P] from [ -32P]GTP, 60% (162pM/mg protein/min as compared with 100pM/mg protein/min for control cells) over the first 10 min. Both gp120-stimulated [35S]-GTP S binding and [ -32P]GTPase activity were inhibited by pretreatment of lymphocyte membranes with PT. These results indicate that cellular functions observed following gp120 stimulation involve signal tranduction through a CD4 coupled to a PT-sensitive G-protein.

**L 510** TAT PROTEIN OF HIV-1 STIMULATES GROWTH OF AIDS-KAPOSI'S SARCOMA-DERIVED CELLS, Barbara Ensoli, Giovanni Barillari, S. Zaki Salahuddin, Robert C. Gallo and Flossie Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Recent evidence suggests a direct etiological correlation between HIV-1 infection and the appearance of Kaposi's sarcoma (KS) (1-4). Supernatants from HIV-1 infected cells promote growth of cells derived from KS lesions of AIDS patients (AIDS-KS cells) (1) and the HIV-1 tat gene, introduced into the germ line of mice, induces skin lesion closely resembling KS (4). Here we report that 1) the Tat protein of HIV-1 is released from both tat-transfected COS-1 cells and HIV-1 acutely infected H9 cells into the cell culture supernatant; 2) these supernatants specifically promote growth of AIDS-KS-derived cells; 3) the growth-promoting effect is inhibited by anti-Tat antibodies; and 4) recombinant Tat proteins expressed in different systems have the same growth-promoting properties as the Tat containing supernatants. These results indicate that the tat gene product of HIV-1 released by infected cells has a direct stimulatory effect on the growth of AIDS-KS-derived cells. We suggest that this effect may be fundamental to the development of KS in HIV-1 infected individuals.

References: (1) Nakamura, S. et al. Science 242:426, 1988; (2) Salahuddin, S.Z. et al. ibid 242:430, 1988; (3) Ensoli, B. et al. ibid 243:223, 1989; (4) Vogel, J. et al. Nature 335:606, 1988.

**L 511** DETECTION OF HIV-1 SPECIFIC ANTIBODIES BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), IMMUNOFLUORESCENCE (IFA), WESTERN BLOT (WB), AND RADIOIMMUNOPRECIPITATION (RIPA) IN THE URINE OF SEROPOSITIVE PERSONS. Alvin E. Friedman-Kien, Michael Mirabile, Yaoxing Huang and Yunzhen Cao, Department of Microbiology, NYU Medical Center, New York.

One hundred serum and corresponding urine samples from 57 patients with ARC or AIDS and 43 asymptomatic HIV-seropositive individuals (AHI) were tested for antibodies to HIV-1 by ELISA and WB assays, as well as by IFA and RIPA. The results are shown in the table. Antibodies to HIV-1 were detected in urine specimens of HIV-1 infected individuals which were also shown to be band reactive to HIV-1 gp160 by WB (100%) and RIPA (95%; 44/46). Although the WB and RIPA techniques have the advantage of high specificity for the detection of specific viral antibodies, these tests are labor-intensive and expensive making them impractical for rapid screening of large numbers of specimens. Our results show that the ELISA is a highly sensitive method for detecting antibodies to HIV-1 in urine samples from HIV-1 infected individuals and is almost comparable to serum testing.

TABLE

| | ELISA | | IFA | | WB | | RIPA | |
|---|---|---|---|---|---|---|---|---|
| Group | Serum | Urine* | Serum | Urine** | Serum | Urine* | Serum | Urine** |
| AIDS-KS | 39/39 | 36/39 | 37/39 | 32/39 | 39/39 | 29/39 | 23/25 | 15/25 |
| AIDS-OI | 10/10 | 8/10 | 10/10 | 10/10 | 10/10 | 5/10 | 7/7 | 6/7 |
| ARC | 8/8 | 7/8 | 8/8 | 8/8 | 8/8 | 7/8 | 3/3 | 3/3 |
| AHI | 43/43 | 40/43 | 40/43 | 38/43 | 43/43 | 34/43 | 11/11 | 10/11 |
| Total | 100/100 (100.0) | 91/100 (91.0) | 95/100 (95.0) | 88/100 (88.0) | 100/100 (100.0) | 75/100 (75.0) | 44/46 (95.7) | 34/46 (73.9) |

*Unconcentrated
**Concentrated 200X

**L 512** RAPID DETECTION OF EARLY HIV-1 REPLICATION IN CELL CULTURES, Sharon J. Geyer, Thomas E. Simms and Jay S. Epstein, Division of Blood and Blood Products, CBER, FDA, Bethesda, MD 20892.

Three markers for HIV-1 were assessed for rapid detection of early viral replication in T-cell cultures. H-9 cells were exposed to cell-free virus ($10^4$-$10^5$ I.U.) for 1h and cultured for 19d. H-9 cells and culture fluids were cryopreserved at various times after infection. Supernatants and H-9 cell extracts were tested for p24 antigen expression (Coulter) and reverse transcriptase (RT) activity (rapid microassay) without prior virus concentration. Samples were also tested for TAT expression in a 3 day culture assay for infectious virus with the indicator T cell line, H-938, containing HIV-1 LTR CAT (Felber and Pavlakis, Sci. 239:184, 1988). Results showed that the three markers signalled HIV replication in nearly parallel fashion and appeared earlier in cell extracts (2-5d) than in culture supernatants (3-5d). The signals produced in the LTR-CAT indicator assay were greatly amplified, suggesting a basis for the development of a quantitative virus co-culture assay. These results demonstrate that rapid, sensitive assays for RT, antigen and TAT expression are feasible and comparable for routine monitoring of HIV cell cultures.

**L 513** Neutralization Loop Variability in HIV-1 Islolates from the Kinshasa Area

H.-G. Guo, D. Zagury, D. Waters, J. Fitzgibbon, D. Dubin, F. Wong-Staal, G. Myers, R.C. Gallo, and M. Reitz

We analyzed a central portion of the env gene of a number of isolates of HIV-1 collected in 1987 from Kinshasa, Zaire and its surrounding regions. The env gene sequences were generated by PCR and included the PB1 loop, a region which is frequently recognized by type specific neutralizing antibodies in hyperimmune sera, and downstream sequences. 20 of the 32 amino acids within the loop were invariant among seven isolates, compared to 12 and 8 consensus amino acids among six North American and six previous Zairean isolates previously analyzed and collected at various times and locations. The PB1 loop was more conserved in general than the region immediately downstream, suggesting functional constraints on change in this region. The data suggest that in a given area within a limited time span, the PB1 area variability is more limited than apparent from previously available sequence data.

**L 514** DELAYED NEUTRALIZING ANTIBODY RESPONSE TO CLINICAL ISOLATES OF HIV, Carl Veith Hanson, Leta Crawford, and Haynes W. Sheppard, California Department of Health Services, Berkeley, CA 94704

Levels of patient antibody which neutralize HIV in vitro often have little prognostic significance. Most such neutralization studies employ HIV strains adapted to growth in continuous cell lines. We have found that neutralizing antibody (Nt Ab) titers depend on the type of host cell in which virus isolates are grown. HTLV-$III_B$ and at least one local clinical isolate grown in H-9 cells were neutralized by dilutions of patient sera up to 1000-fold higher than dilutions required to neutralize the same viruses grown in human PBMCs. Using 11 PBMC-grown HIV isolates and the corresponding patient sera in a micro-plaque reduction assay, cross-neutralization titers ranged from <1:10 up to 1:686. Under these conditions, none of the 11 sera were able to neutralize the corresponding virus isolated from the same blood sample and 40% of these contemporaneous sera enhanced expression of the virus. Analysis of sequential monthly serum samples from an individual patient showed a gradual development of neutralizing capacity to each isolate starting 1 to 3 months subsequent to the isolation date. In one patient, Nt Ab to a particular isolate failed to develop at all in 9 subsequent monthly samples. Our data support a model similar to that implicated in Visna virus infection, in which antibody responses often lag behind rapid virus phenotype changes in vivo.

**L 515** FAILURE TO DETECT EITHER VIRUS OR CELLS FROM AN HIV(+) MOTHER IN HER FETUS: A CASE REPORT. Richard C. Hard, Jr., Medical College of Virginia/VCU, Richmond, VA 23298

There has been a rapid, continuing increase in the numbers of pediatric AIDS cases because of the increasing numbers of infected mothers. It is not known whether the virus is passed from mother to child as free virions or via infected cells, when infection occurs and why only about 40% of the babies of HIV(+) mothers are infected .

The death of an HIV(+) mother and her fetus in the 22nd week of gestation provided the opportunity to test the hypothesis that the virus is passed via infected maternal cells. Serum of the mother contained p24 antigen and antibodies (Ab) to HIV. Serum and cerebrospinal fluid of the fetus contained Ab but no antigen. Cells from lymph nodes were free of virus assayed after amplification by the PCR. Use of polymorphic DNA probes to test for materno-fetal chimerism failed to reveal the presence of maternal cells in the nodes of the fetus.

In summary, neither HIV or maternal cells were detected in the fetus of an HIV(+) mother. This indicates that free virus did not pass the placenta in the first 22 weeks of gestation in this case.

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**L 516** HIV-1 ENTRY INTO GLIAL CELLS IS NOT MEDIATED BY CD4 BUT IS EFFICIENT
J.M. Harouse, M. A. Laughlin, B. Godfrey, H. Friedman, F. Gonzalez-Scarano

A number of CD4-negative glial cell lines can be infected with HIV-1. This infection cannot be blocked with anti-CD4 monoclonals like Leu3a or OKT4a, nor by the use of soluble CD4 preparations and it is normally only detectable by co-cultivation of the infected glial culture with highly susceptible CD4 positive cells. It has not been determined whether glial infection proceeds via a specific alternate receptor or by non-specific viral fusion at the plasma membrane. To characterize glial infection (U373) we have taken two approaches: (1) we transfected a glial cell line with a CD4 construct and determined the characteristics of HIV-1 infection in it and (2) we used a viral internalization assay based on the intracellular detection of $p24^{gag}$ after a short period of incubation of virus and cells. The results indicate that glial cells produce low levels of virus after infection even when they express a functional CD4 molecule. This would suggest that latency is determined by factors other than the efficiency of entry. Furthermore, the results of internalization assays indicate that there is significant viral uptake into intracellular compartments of glial cells after 30 min exposure at 36°C. Preliminary results indicate that this viral entry is mediated by a protease-sensitive molecule at the cell surface.

**L 517** A PUTATIVE HIV TM RECEPTOR ON THE CELL SURFACE IS IDENTIFIED THROUGH THE USE OF A SYNTHETIC PEPTIDE. Lee A. Henderson, Nasar M. Qureshi, David H. Coy and Robert F. Garry, Departments of Pathology, Medicine and Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.

A specific TM sequence, denoted CS3, inhibits T cell activation in vitro and antibody specific to CS3 is associated with the absence of AIDS related disease in HIV seropositive patients. CS3, when conjugated to human serum albumin (HSA) and labelled with fluorescein, bound specifically to $CD4^+$ cell lines and human T cells, B cells and mononuclear cells. Crosslinking of CS3-HSA to its receptor on RH9 cells revealed a putative subunit size of approximately 44 Kd. Incubation of RH9 cells, a CD4 cell line, with CS3-HSA prior to addition of HIV prevented HIV mediated cell lysis. These results suggest that the interaction of the CS3 region of HIV TM with a specific cell surface receptor may be required for HIV mediated cell lysis. The biological response to CS3 was also investigated to extended prior observations. Incubation of PBMC with CS3-HSA for 24-72 hrs prior to activation with mitogen (PHA) resulted in a progressive decline in the ability of mitogen to stimulate incorporation of $^3$H-thymidine. Furthermore, even at low doses of CS3 (10 ng/ml), CS3-HSA initially enhanced anti-CD3 induced intracellular calcium mobilization and $^3$H-thymidine incorporation, but the peak response of proliferation was significantly reduced. The biological significance of interaction of HIV TM with its receptor portends several avenues of approach for therapeutic treatment and vaccine development.

**L 518** QUANTITATION OF HIV-1 RNA IN SERUM AND CORRELATION WITH DISEASE STATUS USING THE POLYMERASE CHAIN REACTION, Mark Holodniy, David A. Katzenstein, Sohini Sengupta, Alice Wang*, Clayton Casipit*, David H. Schwartz, Mike Konrad*, Eric Groves* and Thomas C. Merigan, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA. 94305, *Cetus Corporation, Emeryville, CA 94608.

The amount of HIV-1 present in serum may be a potential marker in HIV related disease. A method that detects and quantifies HIV-1 viral RNA in serum is presented. To detect HIV-1 RNA, sera was extracted by a guanadinium thiocyanate method, reverse transcribed with MLV reverse transcriptase and amplified by the polymerase chain reaction using a gag gene primer pair(SK38/39) including a biotin labelled upstream primer. The biotinylated PCR product was liquid hybridized to a horseradish peroxidase conjugated probe,bound to avidin, and quantitated from the optical density of a colorimetric reaction.

Reverse transcription and amplification of known amounts of gag gene RNA and infectious $HIV_{IIIB}$ virus yielded a log-linear relationship between optical density and $10^2$ and $10^5$ copies of gag RNA and $TCID_{50}$ of virus respectively. No HIV viral RNA was detected in the serum of 5 seronegative healthy controls. In HIV infected patients who were not receiving therapy, serum HIV-1 RNA was detected in 0/5 asymptomatic, 4/5 ARC and 4/5 AIDS patients with copy numbers ranging from $10^2$-$10^5$/200ul of serum. Ultracentrifugation of patient sera revealed detectable signal in pellets,but not supernatant, indicating that signal is attributable to viral RNA. In addition, extracted material was directly amplified for the presence of viral DNA and gave no detectable signal.

We have demonstrated that HIV-1 viral RNA can be detected and quantitated in patient serum over a four log range. An RNA gag gene sequence was used to quantitate viral copy number. In addition, a nonisotopic enzyme-linked affinity assay in a microtiter plate system allows easy PCR product detection and quantitation. Quantitation of HIV-1 viral RNA in serum by PCR may be a useful marker for disease progression or monitoring antiviral therapy.

**L 519** MONOCYTE PHENOTYPE MARKERS IN HIV-INFECTED INTRAVENOUS DRUG USERS, C. Robert Horsburgh Jr., Bonnie M. Jones, Robert S. Klein, Katherine Davenney, Gerald Friedland, Janet A. K. Nicholson, Centers for Disease Control, Atlanta GA 30333 and Montefiore Medical Center, Bronx NY 10467.

Intravenous drug users (IVDU) with HIV infection show increased susceptibility to bacterial pathogens as well as defects in cell-mediated immunity. We investigated monocyte phenotypic markers in IVDU to evaluate immunologic competance in antigen processing and cell-mediated responses. Subjects were 21 HIV-IVDU, 15 asymptomatic HIV+IVDU, 11 IVDU with ARC or AIDS, and 10 HIV- non-drug-using controls. Monocytes were identified by light scatter and 3-color immunofluorescence. Mean % positive cells were:

| Monocyte Phenotype | Controls | HIV- | HIV+ | ARC/AIDS |
|---|---|---|---|---|
| CD14+(LeuM3+) | 77 | 51 | 57 | 53 |
| HLA Class I+ | 100 | 99 | 99 | 99 |
| HLA-DR+ | 58 | 74 | 85 | 80 |
| HLA-DQ+ (Leu10+) | 44 | 41 | 49 | 51 |
| HLA-DQ+DR+ | 39 | 40 | 49 | 50 |
| CD14+ HLA-DR+ | 41 | 43 | 53 | 46 |
| CD14+ HLA-DQ+ | 30 | 18 | 30 | 29 |
| CD4+ (Leu3a+) | 42 | 66 | 44 | 52 |

All IVDU had increased HLA-DR+ monocytes compared to controls (p=.001); other differences were not significant. We conclude that alterations in monocyte antigen expression do not correlate with clinical stage of HIV infection in IVDU.

**L 520** IN VIVO INTERACTION OF HIV gp160 WITH ITS RECEPTOR, CD4: A MECHANISM FOR THE RECEPTOR DOWN-REGULATION, M. Abdul Jabbar and Debi P Nayak, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.The envelope glycoprotein of human immunodeficiency virus (HIV) plays a major role in the down-regulation of its receptor, CD4. Using a transient-expression system, we investigated the interaction of HIV envelope glycoprotein with CD4 during their movement through the intracellular membrane traffic. In singly transfected cells, the envelope glycoprotein, gp160 was synthesized, glycosylated and localized predominantly in the endoplasmic reticulum (ER). Only, a minor fraction of gp160 was proteolytically cleaved producing gp120 and gp41 and the gp120 was secreted into the medium. The CD4 molecule, on the other hand, was glycosylated and transported efficiently to the cell surface. However, when gp160 and CD4 were expressed together in the same cell, gp160 formed a specific intracellular complex with CD4 blocking the intracellular transport as well as the productive delivery of CD4 to the plasma membrane. The specific gp160: CD4 complex was localized predominantly in the ER undergoing primarily high mannose oligosaccharide modifications. The present studies provide a biochemical basis for the down-regulation of CD4 molecule in HIV-infected cells.

**L 521** THE GP120 BINDING SITE ON THE CD4 PROTEIN IS A COMPOSITE SURFACE. Bradford A. Jameson and Dorothee Wernicke, Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140. Recently it has been shown that expression of the first 104 amino acids of the CD4 protein are sufficient to account for the high affinity interaction with the gp120 protein of HIV. Site-directed mutagenesis has further implicated a small stretch of amino acids, residues 45-55, as a direct contact point for the gp120 protein. We have now modeled the first 104 amino acids of the CD4 protein and used this structure as a template for designing conformationally-restricted synthetic peptides. All of the linear peptides synthesized from the 45-54 region have failed to show any biological activity at any concentration tested, however, the conformationally designed peptides display concentration-dependent blocking of virus binding to CD4 positive cells. This constitutes the first direct evidence of the involvement of this site in virus binding. We have also synthesized conformational analogs of the CD4 domain 84-101 that display conformation- and concentration-dependent inhibition of virus binding. Finally, we have synthesized a linear peptide (residues 23-41) that blocks virus-induced syncytium formation, but not virus binding. The three regions from which these biologically active peptides were synthesized are analogous to the complimentarity determining regions of an antibody (CDR1, CDR2 and CDR3). We now have evidence suggesting that these three CD4 domains (residues 25-30, 42-54 and 85-98) fold together to form a continuous surface on the face of the CD4 protein and that the contact between gp120 and CD4 is directly analogous to an antibody-antigen contact.

L 522 ABROGATION OF IgG2 RESPONSE TO gp-41 CORRELATES WITH CLINICAL MANIFESTATION OF HIV INFECTION. Renu B. Lal, Ibrahim M. Heiba, Rita R. Dhawan, Peter I. Perine, Division of Tropical Public Health, Uniformed Services University of Public Health Sciences, Bethesda, MD.
To analyze differential antibody responsiveness of potential pathogenetic significance, sera from 66 patients with HIV-1 infections at various Walter-Reed stages (WR) of the disease were analyzed by Western blot analysis to determine the subclass distribution of HIV antibodies. IgG1 was the major subclass reactive with env, pol and gag antigens, IgG2 and IgG3 were almost equally represented in the response to gag gene products and IgG4 showed minimal reactivity to p24 antigen in all HIV infected patients regardless of their clinical presentation. In contrast, there was a progressive loss of IgG2 anti-gp41 as patients progressed from WR 1 & 2 (88%) to WR 5 & 6 (5%). IgG2 response to a recombinant gp120/41, however, remained unchanged suggesting that the lack of IgG2 response may be associated with loss of responsiveness to the carbohydrate epitopes on gp41. Indeed, parallel measurements of IgG antibody responses to group A carbohydrate showed that the antibody responsiveness to the cell wall component of Streptococcus pyogenes also declines with HIV-disease progression, without affecting antibody responses to Haemophillus influenzae type b polysaccharide and phosphocholine. Since antibody responses to group A carbohydrate with its N-acetyl D-glucosamine (GlcNAc) determinant were abrogated as HIV-disease progressed, GlcNAc may be one of the antigenic determinants on gp41 which may play a critical role in some of the pathologic events of HIV infection.

L 523 PATIENTS WITH ARC AND AIDS HAVE A PROGRESSIVE DEFECT IN TISSUE FACTOR EXPRESSION IN MONOCYTES, Janet L. Lathey*, Jan M. Agosti**, and Stephen A. Spector*, *Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093, **University of Washington, Seattle, WA 98195
Monocytes/macrophages are important in regulating immune and coagulation systems. Such cells can be infected with human immunodeficiency virus (HIV) and infection of the host may alter the regulation of modulating factors. Thus, tumor necrosis factor (TNFα), interleukin 1-ß (IL-1ß) and tissue factor (TF) were evaluated in monocytes from HIV seropositive patients. RNA was extracted from monocytes with or without LPS stimulation, and hybridized with cDNA probes. Steady state levels of TNFα, IL-1ß, and TF mRNA were low to undetectable in all monocytes before LPS stimulation. In contrast, LPS induced levels of TF mRNA were depressed in patients' monocytes, while TNFα and IL-1ß were "normal." Induced TF mRNA was reduced 66% in monocytes from AIDS patients, followed by a 20% reduction in ARC patients, as compared to TF mRNA in monocytes from HIV seronegative individuals. The defect in TF expression did not correlate with the presence of HIV antigen as measured by the Abbott HIV p24 antigen assay. However, there was a significant correlation between $T_4$ cell number and TF expression. Thus, TF may provide an effective surrogate marker for monitoring the progression of HIV disease by signaling an alteration in monocyte functions.

L 524 DEVELOPMENT OF A RETROVIRAL VECTOR SYSTEM BASED ON HIV, A. Lever[1], J. Richardson[1], H. Gottlinger, W.A. Haseltine, J. Sodroski, [1]St. George's Hospital Medical School, London, SW17 ORE, UK, [2]Dana Farber Cancer Institute, Boston, MA 02115, USA. A mutant of HIV with a deletion in a non coding region has been described which produces morphologically normal virion particles but is deficient at packaging its own genomic viral RNA (Lever et al, J. Virol., 1989, 63; 4805). This mutant forms the basis of a packaging system for retroviral vectors based on HIV. Vectors expressing a variety of prokaryotic and eukaryotic genes have been constructed and data will be presented on the packaging efficiency, transmissibility and expression of the vector sequences in target cells after packaging by this system.

L 525 COMPARATIVE THERAPEUTIC EFFECTS OF AZT AND LIPOSOMAL AZT MONOPHOSPHATE IN MURINE RETROVIRUS-INDUCED IMMUNODEFICIENCY DISEASE, J.L. Li, Philip L. Felgner, Douglas D. Richman and Karl Y. Hostetler. Department of Virology, Vical Inc., San Diego, CA, 92121, the VA Medical Center, San Diego, CA and the Departments of Pathology and Medicine, University of California, San Diego, La Jolla, CA 92093. C57BL/6 mice infected with LP-BM5 murine leukemia virus develop a disease with many features of AIDS that has been referred to as MAIDS and has been shown to be responsive to treatment with zidovudine (AZT). Treatment of mice with 50 mg/kg twice daily by intraperitoneal injection prolonged survival and 5 mg/kg b.i.d. was less effective. To direct larger amounts of drug to macrophages which are heavily infected with LP-BM5, we encapsulated [$^3$H]AZT monophosphate in liposomes (L-AZT-MP). Tissue and plasma levels of L-AZT-MP were manyfold higher than those observed after AZT administration in a equimolar dose. Tissues rich in monocyte/macrophages retained the most drug. Tissue levels of L-AZT-MP were substantial 24 to 48 hours after a single dose of 10 mg/kg while AZT produced substantial tissue levels for only 4 to 6 hours. Four hours after infection with LP-BM5 AZT (100, 31.6 or 10 mg/kg/day in divided bid doses), L-AZT-MP (10, 3.9, 1 and 0.39 mg/kg/day, single dose every other day) or controls were injected ip. After 23 weeks, the largest number of surviving mice was in the 10 and 3.9 mg/kg/ day L-AZT-MP group (86 and 79%); in the 100 and 31.6 mg/kg/ day AZT group survival was slightly lower (75 and 71%) while the empty liposome control group had the lowest survival (64%). These data suggest that q.o.d. L-AZT-MP treatment was about 10 times more effective than treatment b.i.d. with AZT. L-AZT-MP may be useful as a treatment for the macrophage reservoir of AIDS.

L 526 CONSERVED N-GLYCOSYLATION SITES AROUND THE CD4-BINDING SITE OF HIV-1 ARE IMPORTANT FOR HIGH AFFINITY RECEPTOR INTERACTION, Dirk Lindemann, Jan Mous and Luc Dirckx, Central Research Units, F.Hoffmann-La Roche, Ltd., CH-4002 Basel, Switzerland. The interaction of the exterior envelope glycoprotein gp120 of the human immunodeficiency virus type 1 (HIV-1) with its receptor CD4 represents the initial step in the process of infection and accounts for HIV's tropism and cytopathic effect. Gp120 is a heavily glycosylated protein, comprising more than 20 potential N-glycosylation sites. However, the function of the numerous N-glycans of gp120 is largely unknown and the possible role of N-glycosylation in binding to CD4 still controversial. To address this question, we mutated different conserved N-glycosylation site Asn-residues in the vicinity of the putative CD4-binding site to Gln, as single mutations or in combinations. As a control, env genes were constructed having Cys 425 or Cys 452 mutated to Ser. Authentic and mutant gp120 proteins were produced using the Baculovirus expression system. All mutant proteins were produced and secreted (except Cys 425->Ser) at similar levels and could be immunoprecipitated with an HIV$^+$-serum. In addition all single glycosylation mutants retained the full capacity to bind CD4, a triple mutant however with reduced affinity. On the other hand, the two Cys->Ser mutants were no longer able to interact with soluble CD4. To evaluate the "in vivo" consequences of the env mutations, different gp120 mutant genes were introduced in an infectious proviral DNA clone. Upon transfection of MT-2 cells, the authentic HIV-1 clone induced virus production reaching a maximal value after 5-6 days. In the case of the triple glycosylation mutant, comparable virus production was obtained but with a delay of about 10 days.The Cys->Ser mutants failed to elict measurable virus production.

L 527 DENDRITIC CELL DEPLETION AND DYSFUNCTION IN HIV INFECTION, Steven E. Macatonia, Steven Patterson, Richard Lau*, Anthony J. Pinching * and Stella C. Knight. Antigen Presentation Research Group, MRC Clinical Research Centre, Watford Road, Harrow, UK, HA1 3UJ. *St Mary's Hospital Medical School, London WC1 PG.

Immune responses in resting T-cells are initiated by bone-marrow derived dendritic cells (DC)[1]. These antigen presenting cells, which occur in small numbers in most tissues trap antigens, travel to lymph nodes and induce clustering and activation of T-cells. Normal DC are susceptible to infection with HIV in vitro[2]. To study the relationship between HIV and DC in patients, and the relevance to the pathogenesis of disease, DC were isolated from the blood of individuals across the spectrum of HIV infection, the numbers of DC, presence of HIV genome and antigen presenting function was examined. Infection, depletion and impaired antigen presenting function of DC occur early in HIV infection in individuals in CDC group II (asymptomatic). These defects precede both the appearance of symptoms and changes in T-cell numbers and may be instrumental in the development of AIDS. Furthermore, since DC numbers and function differ at different stages of disease, monitoring these may contribute to the clinical assessment and lead to new therapeutic approaches.

1. Macatonia, Taylor, Knight & Askonas, J. exp. Med. (1989) 169: 1255
2. Patterson & Knight, J. Gen. Virol. (1987) 68: 1171

**L 528** HLA ANTIGEN FREQUENCIES IN PATIENTS WITH HIV-1 RELATED KAPOSI'S SARCOMA (KS), Dean L. Mann, Mary O'Donnell, William A. Blattner, and James J. Goedert, Immunogenetic and Viral Epidemiology Sections, National Cancer Institute, Bethesda, MD, Frederick, MD 21701.

Studies are being conducted to assess the possible role of genetic factors in HIV-1 related disease progression and outcome. In this pursuit HLA antigen typing (HLA-A, B, C, DR, DQ) was performed on cells from 278 caucasian homosexual men, 44 of whom were diagnosed as having KS, 50 with opportunistic infections (OI), 14 with KS and OI, 83 individuals who were HIV-I seropositive-disease free and 87 HIV-1 seronegative. The HLA antigen frequencies that were found in each of the groups were compared and analyzed for statistically significant differences by chi square test. Antigen frequencies in the total cohort did not differ from the reported results of an international study for North America caucasians indicating that the gene pool in the populations at risk was representative of the general population. Higher frequencies of HLA-B35, -C4, DR1, and DQ1 were found in the patients with KS compared the frequencies found in the cohort of HIV-1 seropositive disease free and/or HIV-1 seronegative individuals. In comparing the frequencies found the KS group with the frequencies found in patients with OI, HLA antigens C4, DR14 and DR 53 were significantly higher and B8, C5 and DR3 lower. The results suggest that the genes and/or gene products of the major histocompatibility complex influence disease outcome in HIV-1 infected homosexual men.

**L 529** HIV-1-SPECIFIC CTL RESPONSES IN LONG-TERM ASYMPTOMATIC SEROPOSITIVE INDIVIDUALS. Alison C. Mawle, Mitchell R. Ridgeway, Marie-Paule Kieny and Alan R. Lifson. Immunology Branch, Centers for Disease Control, Atlanta, Ga. 30333, Department of Public Health. San Francisco, Ca. and Transgene, Strasbourg, France. It has long been considered that the major component of the immune system responsible for controlling persistent viral infections is the presence of virus-specific CTL. We have studied the CTL response to the HIV-1 antigens env, gag and nef in long-term survivors from the San Francisco cohort who have been seropositive and completely asymptomatic for an average of eight years. This group was compared with a group of AIDS patients from the same cohort. Two types of response were seen in the asymptomatic group. CTL directed against env were antigen-specific but not MHC-restricted. However, CTL directed against gag were both antigen-specific and MHC-restricted. No CTL response against nef has been observed so far. In contrast, none of the patients with AIDS had any detectable CTL. We have also looked at ADCC and NK responses in these men. No differences were seen between asymptomatics and AIDS patients in either response. These data suggest that the presence of MHC-restricted CTL is important in controlling the progression to symptomatic disease.

**L 530** CELL PATHOGENESIS OF NEURONAL, NON-SYNCYTIUM INDUCING HIV-1 ISOLATES, Dale A. McPhee, Karen M. Coates, and Elizabeth V. Neate, Special Unit for AIDS Virology, Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Melbourne, AUSTRALIA.

Selected isolates of human immunodeficiency virus type 1 have shown unique or unusual properties 'in vitro'. Three non-syncytium inducing (NSI) isolates derived from cerebral spinal fluid or brain, from patients with advanced disease and neurological disorders, caused little or no cytopathic effects (cpe) when cultured in peripheral blood or CD4+ lymphocytes with one replicating to $10^{5.5}$ $TCID_{50}$/ml. In contrast 3 syncytium inducing (SI) isolates caused typical cpe. The SI isolates all down regulated surface expression of CD4, with or without an accompanying decrease in cell viability, whereas there was little or no reduction for all NSI isolates tested. How the virus was affecting surface expression of CD4 is not clear. The presence of nef antibodies was detected by Western blot analysis in 2/3 patients where NSI isolations were made but not in patients from whom SI isolates were obtained. Interestingly although virus was isolated from the CNS with these patients it was not recovered from peripheral blood lymphocytes. Thus, the replication strategy of the NSI isolates studied does not involve down regulation of CD4 or cell death and may involve an altered nef gene product.

**L 531** USE OF AN INDIVIDUAL VIABLE CELL FLUOROGENIC ASSAY (FACS-GAL) TO STUDY HIV GENE REGULATION AND ASSAY FOR INFECTIOUS VIRIONS.
Monty A. Montano, Mario Roederer, Steve N. Fiering, Garry P. Nolan, and Leonard A. Herzenberg. Department of Genetics, Stanford University, Stanford, CA.

We have previously developed a sensitive, versatile reporter gene assay capable of measuring gene expression in individual cells (Nolan et al, *Proc. Natl. Acad. Sci USA.* **85**, 2603-7, 1988). The technique, termed FACS-Gal, is based on the measurement of the generation of fluorescence by the enzymatic conversion of fluorescein digalactoside to fluorescein by exogenously introduced LacZ and subsequent production of β-Galactosidase.

In order to develop a sensitive assay for infectious HIV particles, we constructed a fusion gene composed of the HIV promoter/enhancer regions (LTR) driving the LacZ coding sequence. The expression of LacZ by this construct is activated 20 to 40-fold by the presence of the HIV-encoded transactivating protein TAT. Ideally, using cells expressing the HIV-LacZ construct, we should be able to distinguish individual cells infected and producing the TAT protein from those lacking the protein.

We have generated a series of cell lines in which the HIV-LacZ construct is stably integrated. The activity of LacZ as measured both *in vitro* in cell extracts and *in vivo* using FACS-Gal increases either upon the infection of an engineered retrovirus which constitutively produces TAT or upon infection with a cultured HIV strain. This demonstrates that the assay is potentially useful in quantitating HIV particles in clinical samples.

**L 532** DEMONSTRATION OF ANTIEPITOPIC ANTIBODIES IN SERONEGATIVE CHILDREN BORN TO HIV-I INFECTED MOTHERS. Viviana Moschese, Maurizio Alimandi, Valter Lombardi, Carlo Fundaro, PierAngelo Tovo, Britta Wahren and Paolo Rossi. Dept. of Immunology, Karolinska Institute and Natl. Bact. Lab., Stockholm, Sweden; Dept. of Pediatrics, II Univ. of Rome and Turin, Italy.

OBJECTIVE. To investigate antibodies reacting with selected linear peptides in sera from sero negative children born to HIV-I infected mothers (SENC) for evidence of HIV-I infection.

MATERIALS AND METHODS: Sera from 48 SENC (age range 9-84 mo.) were analysed by peptide ELISA against a panel of env and gag peptides. Sera from 15 HIV-I positive and non at risk healthy children were also studied as positive and negative controls, respectively.

RESULTS: 18 of 48 (38%) sera from SENC were reactive with one to ten peptides (median 4.2). In the peptide positive SENC sera the distribution of antipeptide reactivity was diversified though higher to gag peptides. No reaction to any peptide was found in sera from the negative control population. 5 of 6 symptomatic SENC showed antipeptide antibodies, too.

CONCLUSIONS: These data suggest that persistent HIV-I infection is not an uncommon event in SENC. Accordingly, peptide ELISA might be a useful diagnostic tool for early detection of HIV-I infection.

**L 533** HIV GAG SPECIFIC CYTOTOXIC T LYMPHOCYTES: SPECIFICITY AND FUNCTION, Douglas F. Nixon, Frances Gotch, Stephane Huet, Nick Alp, Les Borysiewicz and Andrew J. McMichael, Molecular Immunology Group, Institute of Molecular Medicine, Univ. of Oxford, Oxford, U.K., OX3 9DU and Department of Medicine, Univ. of Cambridge, Cambridge, U.K.

Cytotoxic T lymphocytes (CTL) recognise viral proteins presented as processed fragments bound to, and restricted by, HLA class 1 molecules on the surface of the infected cells. We have identified a peptide epitope from HIV gag (265-279) that is restricted by HLA-B27. This peptide shows sequence homology with another B27 restricted epitope from influenza A nucleoprotein (s. Huet et al. Unpublished). CTL recognition of hybrids of these two peptides have been tested both in the influenza A and HIV systems. We have looked for further epitopes in HIV-gag and have identified an epitope restricted by HLA-B8 (255-269) Further peptides from gag are being ivestigated for recognition and HLA restriction. The CTL response in some seropositive individuals can be demonstrated without secondary in vitro stimulation. One donor, 007, shows a strong CTL response to the B27 restricted gag peptide in fresh PBL. We have determined the peptide CTL precursor frequency in the PBL of this patient. The frequency is 1 in $10^4$, but even this high frequency cannot easily explain the high levels of lytic activity demonstrable in fresh PBL. One possible explanation is that in this patient CTL memory cells may be terminally differentiating into effector cells.

**L 534** DETECTION OF ANTI HIV-1 SECRETORY IgA ANTIBODIES IN URINES OF HIV-1 INFECTED CHILDREN. Shlomit Orgad, Ze'ev Trainin, Myron Essex and Arie Rubinstein. Dept. of Cancer Biology, Harvard School of Public Health, Boston Ma. 02115. Dept. of Pediatrics Albert Einstein College of Medicine, Bronx N.Y. N.Y. 10461. We have studied urine samples from 15 children with ARC and AIDS, aged 2.5 to 10 years. The urines were passed through chain specific anti-human Immunoglobulins (IG's) and anti-secretory component columns and the eluates were concentrated 6-8 times. Secretory IgA, non-secretory IgA and IgG were recovered from the samples of all 15 patients. Non-secretory IgA and IgG were eluted from 12 of 15 urine samples. The equivalent of 0.2 mg protein from each sample were tested by ELISA for specific antibody (Ab) reactivity against an HIV-1 gp 120 recombinant protein. Sixty six percent of the samples containing secretory IgA tested positive, whereas only 33.3% of the non-secretory IgA samples were positive. Six samples of patients uninfected with HIV-1 who had various kidney disease - served as controls. All had relatively high levels of SIgA, but none was positive for specific Ab for HIV-1. The implications of these tests might contribute to the understanding and early detection of pediatric AIDS.

**L 535** HIV INFECTION OF PERIPHERAL BLOOD ANTIGEN PRESENTING DENDRITIC CELLS (DC). S. Patterson, J. Gross, P. Bedford and S. Knight, Division of Immunology, Clinical Research Centre, Harrow, Middx. HA1 3UJ. UK. Peripheral blood mononuclear cells were enriched for the presence of low density DC and then examined by immunogold electron microscopy. Two populations of cells, termed type I and type II DC, were observed. They lacked markers for monocytes/macrophages, T, B and NK cells but expressed MHC class II antigens. Type I DC had features corresponding with those of classical DC with an irregular surface and long, often branched projections. Type II DC were characterised by large expanses of cytoplasm devoid of organelles and with a smoother cell surface and only few processes. The presence of cells with a morphology intermediate between these two suggest that types I and II DC are related. Both DC types express low levels of CD4 and a low proportion of type I cells express high levels of CD4. Overnight incubation with interferon gamma can increase CD4 expression on type I but not on type II DC. Both populations of DC are susceptible to *in vitro* HIV infection but in 7 separate experiments a greater percentage of type II cells were observed to be infected. A combined immunolabelling *in situ* hybridization technique was employed to examine DC preparations infected *in vitro* for the presence of viral RNA and DNA. At 5 and 10 days post-infection a greater percentage of DC were positive for DNA than for RNA whilst for persistently infected H9 cells the number of DNA and RNA labelled cells was similar. This suggests that DC may become latently infected or synthesise little virus. DC may thus provide a site in which HIV can persist and escape immune surveillance.

**L 536** MODELING THE INTERACTION OF THE IMMUNE SYSTEM WITH HIV, Alan S. Perelson, Theoretical Biology and Biophysics, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

I present a quantitative model of the interactions between HIV and the immune system that involves free HIV; uninfected, latently infected, and actively infected $CD4^+$ T cells; macrophage/monocytes; and syncytia. Using reasonable guesses for parameter values, I show that this model can account for some of the puzzling features of AIDS: the long latent period, the almost complete absence of free virus particles, the low frequency of infected T4 cells, and the slow T cell depletion seen during the course of the disease. Further, the model suggests why the latent period may be significantly shorter in children than in adults.

**L 537** DIFFERENTIAL REPRESENTATION OF CD8 T CELL SUBSETS IN THE PROGRESSION OF HIV INFECTION, Susan Plaeger-Marshall, Lance E. Hultin, Mary Ann Hausner, Chen-Cheng Chou, Ronald T. Mitsuyasu and Janis V. Giorgi, Departments of Pediatrics and Medicine, UCLA School of Medicine, Los Angeles, CA 90024 The activation and expansion of CD8 T cells in HIV infection has been well documented. We have examined the detailed phenotype of CD8 cells from individuals at different stages of infection with HIV using 3-color immunofluorescence analysis by flow cytometry. In normal individuals, most CD8 T-cells are HLA-DR$^-$ and CD38$^-$ (OKT10, Leu17)$^-$. At the time of seroconversion, a large number of CD8 T-cells with the activated phenotype of HLA-DR$^+$CD38$^+$ appear. Cells with this phenotype may include cytotoxic T-lymphocytes directed against HIV antigens. A role for these CD8 T-cells in the CD4 cell decline around the time of seroconversion is possible. In asymptomatic seropositives whose CD4 levels are stable, there are substantial numbers of HLA-DR$^+$ CD8 cells that are CD38$^-$. The association with stable HIV disease suggests these CD8 cells could be protective. These cells are also present in long-term AIDS survivors but are rare in advanced AIDS. We are engaged in experiments to test whether cells with this phenotype are responsible for controlling viral replication in activated HIV-infected CD4 cells. In AIDS, despite an overall increase in CD8 T-cells, there are almost none that are HLA-DR$^-$CD38$^-$, the phenotype that is most prevalent in normal individuals. There are also very few with the HLA-DR$^+$CD38$^-$ phenotype. Instead there are a large number of CD38$^+$ CD8 cells, some of which express HLA-DR. It is not clear whether these cells represent activated or immature CD8 T-cells, or if they play a role in HIV pathogenesis.

**L 538** **AIDS KAPOSI'S SARCOMA-DERIVED CELLS PRODUCE AND RESPOND TO INTERLEUKIN-6 (IL-6)**, Ahmad R. Rezai, Steven A. Miles, Jesus F. Salazar-Gonzalez, Meta Vander Meyden, Ronald T. Mitsuyasu, Tetsuya Taga, Toshio Hirano, Tadamitsu Kishimoto, and Otoniel Martínez-Maza, Departments of Obstetrics & Gynecology and Microbiology & Immunology, Division of Hematology-Oncology and the UCLA AIDS Center, UCLA School of Medicine, Los Angeles, CA 90024, and Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan. Cell lines derived from Kaposi's sarcoma lesions of patients with AIDS (AIDS-KS cells) produce various cytokines, including endothelial cell growth factor (ECGF), interleukin 1$\beta$ (IL-1$\beta$), and basic fibroblast growth factor (BFGF). Because exposure of human monocytes to HIV results in increased levels of IL-6 gene expression and IL-6 secretion, and because endothelial cells produce IL-6, we examined IL-6 expression and response in an AIDS-KS-derived cell line and IL-6 expression in an AIDS-KS biopsy. The AIDS-KS cell line (N521J, kind gift of Dr. S. Nakamura) constitutively secreted large amounts of immunoreactive and biologically active IL-6. Both IL-6 and IL-6 receptor (IL-6-R) mRNA were detected in these cells. The functional activity of the IL-6-R was demonstrated by examining cellular proliferation of the AIDS-KS cells following exposure to recombinant human IL-6 (rhIL-6); after exposure of N521J cells to > 10 units/ml rhIL-6, cellular proliferation was seen to increase. We confirmed that AIDS-KS cells produced IL-6 *in vivo* by isolating total RNA from KS lesions and from uninvolved skin, taken from an AIDS patient, and by examining IL-6 expression by immunoperoxidase staining. We detected immunoreactive IL-6 in both the KS lesions and in uninvolved skin, with a > 10-fold elevation in IL-6 mRNA seen in the KS lesion, when compared to uninvolved skin. These results indicate that both IL-6 and IL-6-R are produced by AIDS-KS cells and suggest that IL-6 may be a growth factor for AIDS-KS cells.

**L 539** **CO-AMPLIFICATION OF HIV-1 ENV AND REV GENE EXPRESSION IN CHO CELLS,** Bill Zeck, Arlene Manelli, Sushil Devare, and Terry Robins, Department of Human Retroviruses, Abbott Laboratories, Abbott Park, Illinois, 60064

We have constructed a single eukaryotic expression vector, pCMV/rev, that is capable of expressing both the HIV-1 env and rev genes. This vector contains, in addition to other sequences, the immediate early promoter of Cytomegalovirus (CMV) and an intact fragment of the 3′ region of the HTLV-IIIB provirus containing the rev and env coding regions. We have demonstrated in both transient transfection experiments using COS cells and in stably transfected CHO cells that both viral proteins are expressed from this vector. We believe that this vector mimics expression from HIV-1 in that it expresses the rev protein from a spliced mRNA and the env protein from the same unspliced mRNA.

We have derived stable transfectants in CHO cells by co-transfection of pCMV/rev with the pSV-DHFR plasmid. Cells containing amplified copies of the vector DNA were selected using stepwise increases in the concentration of methotrexate (MTX). These cells were shown to express high levels of both the rev and env proteins. Furthermore, the gp160 protein expressed in CHO cells was processed to yield both gp120 and gp41. Analysis of the supernatant media from these CHO cells indicates that the gp120 protein, but not gp160 or gp41, is "secreted" into the media.

**L 540** TWO IMMUNODOMINANT DOMAINS WITHIN THE HIV-1 GP41 TRANSMEMBRANE GLYCOPROTEIN BIND INFECTION-ENHANCING ANTIBODIES, W. Edward Robinson, Jr.[1], M. Gorny[2], D. Lake[3], W. Mitchell[1], S. Zolla-Pazner[2,4], and E. Hersh[3]. [1]Dept. of Pathology, Vanderbilt University, Nashville, TN 37232;, [2]Dept. of Pathology, New York University, New York, NY 10016; [3]Dept. of Medicine, University of Arizona, Tucson, AZ 85719; [4]Veteran's Administration Medical Center, New York, NY 10010.

Previous studies have demonstrated that antibodies to HIV-1 can enhance HIV-1 infection *in vitro* by two mechanisms: the first requires cells bearing complement receptors (C'-ADE) while the second requires cells bearing Fc receptors (FcR-ADE). In this study, we identify four human monoclonal antibodies (huMAb) to HIV-1 which enhance HIV-1 infection *in vitro* and map them to two immunodominant domains of the HIV-1 transmembrane glycoprotein, gp41. Four of eighteen huMAb enhance HIV-1 infection *in vitro* by a complement-dependent mechanism. Enhanced infections were characterized by increased reverse transcriptase release, cytopathic effect, and number of HIV-1 antigen positive cells. These huMAb bound to the pENV9 polypeptide fragment of HIV-1 and were demonstrated to be immunodominant in serum competition assays. The four huMAb map to two distinct domains of gp41 using peptide ELISA. These same peptides compete with the monoclonal antibodies to block enhanced infections *in vitro*. Further, the peptides block much of the enhancement of HIV-1 infection *in vitro* mediated by human polyclonal anti-HIV serum. The results suggest that enhancing antibodies bind to a few, discreet epitopes in the HIV envelope and that deletion of such epitopes from current and future candidate vaccines might increase vaccine efficacy.

**L 541** **AIDS-KAPOSI'S SARCOMA-DERIVED CELL CULTURES: AN IN VITRO MODEL FOR A PARACRINE TUMOR.** Willi Kurt Roth, Sabine Werner, Peter Hans Hofschneider, Max-Planck-Institut für Biochemie, am Klopferspitz 18a, 8033 Martinsried.

Cultivated Kaposi's sarcoma (KS)-derived cells express endothelial cell markers indicating an endothelial origin. KS cells have a limited lifespan, are invasive through basement and connective tissue membranes, but do not form colonies in soft agar or tumors in nude mice. Despite these low-malignant growth properties they reveal an enhanced proliferation rate in 0.5% FCS in comparison to nontransformed cells. Therefore we investigated their autocrine growth factor synthesis and their dependence on externally supplied growth factors. In northern blotting we found synthesis of growth factors like aFGF, bFGF, FGF-5, PDGF-A and TGFα at a similar extent as in fibroblasts. By investigating the mitogenic activity of conditioned KS cell culture supernatants on KS cells we probably detected an as yet unknown growth factor which may explain their limited autocrine growth in 0.5% FCS. On the other hand, we found KS cells to be much more dependent on platelet-derived growth factor (PDGF) than control fibroblasts, although there is no difference to fibroblasts with respect to autocrine PDGF and PDGF-receptor synthesis, PDGF receptor binding, signal transduction and mitogenicity of different PDGF homodimers. However, investigating the mitogenic activity of epidermal growth factor (EGF) on KS cells and fibroblasts we found a significant lower growth stimulation in KS cells which may, besides other growth limiting effects, account for the observed PDGF dependence. On basis of these data we present a KS-tumor model in which KS (spindle) cells, endothelial cells, macrophages and platelets are involved in a paracrine and autocrine stimulatory circle.

**L 542** NOVEL FLOW CYTOMETRIC METHOD TO MONITOR CELL FUSION BETWEEN HIV-INFECTED AND UNINFECTED $CD4^+$ CELLS

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Fusion (syncytium formation) between HUT-78 cells, persistently infected with human immunodeficiency virus type 1 (HIV-1), and uninfected CD4-bearing MOLT-4 or CEM cells results in a rapid destruction of the MOLT-4 or CEM cells. This syncytium formation is due to the interaction between the gp120 glycoprotein expressed by the persistently HIV-1-infected HUT-78 cells and the CD4 receptor present on MOLT-4 or CEM cells. A flow cytometric method has been developed to separate the infected (HUT-78) from the uninfected (MOLT-4, CEM) cell populations. This method is based on a modified DNA staining protocol which very clearly shows the differences in DNA content between HUT-78 cells, on the one hand, and MOLT-4 or CEM cells, on the other hand. Using this novel flow cytometric method we have demonstrated that those compounds (i.e. sulfated polysaccharides, aurintricarboxylic acid) that interact with gp120 (of the HIV-infected cells) or CD4 (of the uninfected cells) suppress syncytium formation, and concomitant destruction of the $CD4^+$ cells.

**L 543** STRUCTURE/FUNCTION STUDIES OF HIV-1, Tatsuo Shioda, Deborah Seto, Jay A. Levy and Cecilia Cheng-Mayer, Cancer Research Institute, Department of Medicine, University of California San Francisco, San Francisco, Califronia 94143-0128

HIV-1 isolates vary in their ability to infect different cell types, to replicate to high titers, and to cause cytopathic changes in the infected cells. Some of these in vitro biologic properties correlate with pathogenicity of the virus in vivo. To map viral determinants of the different biologic properties, recombinant viruses were generated between biologically active molecular clones of HIV-1 isolates that show differences in T-cell or macrophage tropism, replication kinetics and cytopathogenicity. The isolates studied are: HIV-1$_{SF2}$, a T-cell tropic isolate; HIV-1$_{SF162}$, a macrophage-tropic isolate that cannot replicate in established T cell lines; and HIV-1$_{SF13}$, an isolate recovered from the same individual as HIV-1 SF2, but five months later when the patient had advanced in disease state. HIV-1$_{SF13}$ is a fast-growing, highly cytopathic virus, and displays a wide host range. Results from our studies showed that host range tropism and cytopathology are determined by the envelope gene. Specifically, a 1.8 kb EcoRI/SspI fragment containing 455 amino acid residues of the N-terminus of gp120 appear to control infection of established T-cell lines. Similar genetic exchanges have been made to examine macrophage-tropism. Furthermore, site-directed mutagenesis studies are in progress to determine the specific amino acid changes that affect the different biologic properties of HIV-1.

**L 544** ISOLATION OF HIV FROM CEREBROSPINAL FLUID (CSF) AND THE DEVELOPMENT OF NEUROPSYCHIATRIC SYMPTOMS, Stephen A. Spector[1], J. Allen McCutchan[2], J. Hampton Atkinson[3] and Igor Grant[3], Depts of Pediatrics[1], Medicine[2] and Psychiatry[3], University of California, San Diego, La Jolla, CA 92093.

Thirty-one homosexual men without clinically apparent AIDS dementia (22 HIV+, 17 CDC II/III and 5 CDC IV; and 9 HIV-) were longitudinally evaluated for CNS disease with an extensive battery of neuropsychological tests. At least 1 of 2 CSF cultures obtained 1 yr apart contained HIV in 16(73%) of 22 HIV+ pts (2/5 CDC IV and 14/17 CDC II/III) vs 0/9 HIV- controls. Of the 3 pts with 2 CSFs HIV culture pos, 1 had impairment at entry and the 2 others had worsening neurological function on repeat testing. 2 patients with moderate impairment on enrollment have had neg CSF cultures. Isolation of HIV from CSF correlated with a CSF pleocytosis (wbc/mm$^3$ mean $\pm$ S.D.: pos: 16.1 $\pm$ 14.6 vs neg: 6.6 $\pm$ 2.0; p=.01). HIV p24 antigen was not detected in any CSF specimens. Peripheral blood $T_4$ cells in HIV+ pts did not correlate with CSF cultures pos for HIV (mean 501/mm$^3$ in culture pos vs 469/mm$^3$ in culture neg). These findings indicate that most infected pts have HIV in their CSF and that the ability to culture HIV is related to the number of CSF mononuclear cells. Also, although repeated isolation of HIV from CSF may be associated with the development of CNS disease, pts frequently have virus present in the CSF without AIDS or dementia, and some pts with HIV-related CNS disease may not have HIV cultured from their CSF.

**L 545** EFFECT OF HIV-1 INFECTION OF NEONATAL MACROPHAGES. Andrea R. Sperduto, Irvin S. Y. Chen and Yvonne J. Bryson. Department of Pediatric Infectious Diseases. UCLA School of Medicine. Los Angeles. CA. 90230

Perinatal Human Immunodeficiency Virus type 1 (HIV-1) infection has a more rapid fatal course than HIV-1 disease seen in adults or older children. The fetus and neonate are particularly susceptible to severe and fatal disseminated illness (including viral and bacterial infections) which may be partially explained by an immature immune system. The macrophage has become a focus of attention as a reservoir of HIV-1 infection in adults and can be infected in vitro by only certain strains of macrophage-tropic HIV-1. Previous studies in our laboratory and others have suggested that the newborn macrophages are functionally immature and thus more susceptible to viral replication of Herpes Simplex Virus (HSV). The relative susceptibility of the neonatal macrophage to HIV-1 infection as well as other fetal cells has not been previously investigated. In a series of 3 separate experiments, our data reveal that one HIV-1 strain, HIV-1(JR-FL), isolated from the brain of an adult patient JR with acquired immunodeficiency syndrome (AIDS), can infect neonatal/cord macrophages more readily than adult cells. In addition, a second viral isolate, HIV-1 (JR-CSF) derived from cerebrospinal fluid of patient JR, infected neonatal/cord macrophages more efficiently than adult cells as well. HIV-1(JR-CSF) was initially thought to be non-macrophage-tropic in adult cells. Further studies are underway investigating the mechanism by which neonatal macrophages are more permissive to infection with HIV-1 than adult macrophages.

**L 546** CLONAL ANALYSIS OF CHANGES IN BIOLOGICAL PHENOTYPE OF SEQUENTIAL HIV ISOLATES FROM SEROCONVERTING AND SEROPOSITIVE INDIVIDUALS.

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We have previously shown that syncytium-inducing (SI) HIV isolates with a broad host range are associated with rapid CD4+ T-cell depletion and progression to AIDS. It is not clear, however, whether SI isolates are present from seroconversion onwards or emerge in the course of HIV infection. We analyzed the biological phenotype of sequential HIV isolates of 14 seropositive individuals of whom the moment of seroconversion was documented. SI isolates were observed in 4 of these individuals. In all four individuals a moment of conversion from non-syncytium-inducing (NSI) to SI variants could be pointed out, and only NSI variants could be detected early after seroconversion. The presence of SI variants at seroconversion was demonstrated in one individual. In this person disappearance of the SI variant 6 weeks after seroconversion was observed. Since HIV isolates consist of multiple clones, we proceeded to investigate the relation between the biological phenotype of an HIV isolate and the clones it is composed of. Multiple clonal or oligoclonal viral isolates were obtained from 12 individuals. From individuals that in bulk isolation yielded SI isolates, both SI and NSI clonal isolates could be recovered. In contrast, only NSI clones were recovered from individuals with bulk NSI isolates, indicating that the biological phenotype of an HIV isolate is determined by the most virulent clone present.
The appearance of SI variant clones only in the course of HIV infection and their suppression early after seroconversion suggests that immune surveillance plays a critical role in maintainig the asymptomatic status.

**L 547** MUTATIONAL ANALYSIS OF THE RNASE H DOMAIN OF HIV-1 REVERSE TRANSCRIPTASE, EFFECT OF MUTATIONS ON VIRUS INFECTIVITY.

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Carboxyterminal truncated mutants of recombinant HIV-1 reverse transcriptase have previously been prepared and analysed for RT function (Tisdale et al., 1988). This analysis revealed a region within the C-terminus domain between amino acids 530-540 where increasing truncation produced a marked reduction in RT activity. This region contains only 1 highly conserved amino acid, $His_{539}$ which we have mutated by site-directed mutagenesis to Asp or Asn. These two mutants plus four truncated mutants -17, -19, -21 and -130 amino acids have been analysed for both RT and RNase H activity in situ on activated gels and by filter assays.
From this analysis two mutants were selected for further study, ($His_{539} \rightarrow$ Asn and -19 truncation) which had high level RT activity in crude E.coli extracts but which on purification showed some reductions in RT stability compared to wild type, and reduced RNase H function, with the -19 truncation showing negligible RNase H activity. These two mutants were also prepared in the infectious proviral HIV clone HXB2-D and their infectious potential assessed after transfection of mutant proviral DNA into different T cell lines (MT2, MT4, CEM). Both of these mutant viruses produced after transfection had detectable RT activity comparable to controls but were found to be non-infectious. Thus it appears that the carboxyterminus of HIV-1 RT where the RNase H resides and the highly conserved residue $His_{539}$ are essential for HIV replication.

Ref. Tisdale, M., et al. J.Virol 62, 3662-3667. 1988.

**L 548** EARLY DEVELOPMENT OF ANTI-HIV IgG.

Britta Wahren[a], Per Anders Broliden[a,b], Jerzy Trojnar[c] and Hans Wigzell[d], [a]Dept. of Virology, National Bacteriological Laboratory, S-105 21 Stockholm; [b]Dept. of Virology, Karolinska Institute, S-105 21 Stockholm; [c]Ferring AB, P.O.Box 30561, S-200 62 Malmö; [d]Dept. of Immunology, Karolinska Institute, Stockholm, Sweden.

A critical event during HIV infection is the binding of virion ligand protein to the cellular receptor. By modification of the natural amino acid sequence of a part of the CD4 binding region, we discovered a peptide to which the majority of both HIV-1 and HIV-2 infected persons have a strong seroreactivity. Several peptides synthesized according to the linear sequence of HIV-1 (strain $HTLV\text{-}III_B$) were assayed for seroreactivity in ELISA. The strongest and most frequent reactivity was seen with the peptide JB-8p. When the resulting JB-8p peptide was used with HIV-1 seropositive sera, 93% of the patients showed a clear seroreactivity. The figure for HIV-2 infected persons was 73%. Anti-JB-8p antibodies developed in primary HIV-1 infection. The conformational change imposed on the peptide JB-8p revealed and exposed stretches recognized as immunogens in the patient, but not well discovered by linear peptides. This peptide, which represent parts of the CD4 binding region of gp120 should be considered for vaccine attempts.

**L 549** HIV-1 GLYCOSYLATION MUTANTS WITH ALTERED CYTOPATHOGENIC PROPERTIES
Andrzej Wasiak, Sheryl A. Haggerty, Michael P. Dempsey, Trevor L. Stanwick, and Mario Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha NE 68105. The external envelope glycoprotein of HIV-1 is heavily glycosylated. N-linked glycosylation appears to play an important role in HIV-1 envelope processing and in dictating conformation of the envelope glycoprotein. Given the importance of envelope conformation on interaction with the cellular receptor for HIV (CD4), it is plausible that alterations in glycosylation affect envelope conformation and subsequently HIV-1/CD4 interaction. We have identified and cloned naturally occurring HIV-1 mutants lacking highly conserved N-linked glycosylation sites. The construction of viral hybrids indicates that the noncytopathic phenotype maps to gp120 and can be accounted for by a loss in highly conserved N-linked glycosylation sites. Site directed mutagenesis of HXB2 envelope glycoprotein was used to remove highly conserved N-linked glycosylation sites. HXB2 glycosylation mutants were markedly attenuated in their ability to induce cytocidal effects within infected host cells, while immunoprecipitation analysis revealed marked differences in relative stability of gp120. The results indicate that N-linked glycosylation, through its role in maintaining envelope conformation is important for the cytopathogenic properties of HIV-1. Thus, *in vivo*, mutation and sequence diversity may generate novel phenotypes of HIV-1 which play a role in the cytopathogenesis of AIDS.

**L 550** CELL-MEDIATED CYTOLYTIC REACTIVITIES AGAINST EPITOPES CONTAINED WITHIN THE V3 REGION OF HIV-1 GP120,
Kent J. Weinhold, Holly L. Hedrick, Cynthia A. Place and Mark W. Sebastian, Department of Surgery, Duke University Medical Center, Durham, NC 27710.
The V3 loop of HIV-1 gp120, encompassing amino acid residues 303-338 (Ratner numbering system), represents an immunodominant hypervariable region containing at least two B-cell epitopes which are responsible for eliciting 'isolate specific' neutralizing antibodies. In a series of experiments, we have attempted to identify similar domains within V3 which can serve as T-cell epitopes for human cytolytic T lymphocytes (CTL) either elicited in cultures of peripheral blood mononuclear cells (PBMC) from seronegative individuals or measured directly from PBMC of HIV-1 infected patients. Peptide analogues of the V3 region of 3 different prototypic HIV-1 isolates ($III_B$, RF, and MN) were found to be highly stimulatory in lymphocyte cultures, eliciting isolate specific blastogenic reactivities. T-cell clones derived from these blast cultures recognized one of three separate but overlapping epitopes, all of which include the crown of the V3 loop comprised of the amino acid sequence G-P-G-R. Approximately 40% of the T-cell clones exhibited cytolytic activity against peptide coated autologous B-lymphocyte cell lines (BLCL). All such CTL were CD4+ and lysed targets in an MHC class II restricted manner. Lastly, anti-V3 cytolytic reactivities were documented in the PBMC of HIV-1 infected patients. Lysis was MHC class I restricted and mediated by classical CD8+ killer cells. The fine specificity of these CTL was similar to that seen with in vitro derived CD4+ CTL from seronegative donor lymphocyte cultures. These studies suggest a high degree of T-cell immunogenicity for regions contained in the V3 hypervariable domain of gp120 - an essential element in future vaccine strategies based on these determinants.

**L 551** HIV-1 EXPRESSION IN HEARTS OF AIDS PATIENTS: HIV-1 RNA IS LOCALIZED TO AREAS OF CLINICAL AND PATHOLOGIC INVOLVEMENT.
B. Weiser, D. La Neve, P. Campbell, A. Cohen, J. Fuhrer, and H. Burger, SUNY Stony Brook, Stony Brook, NY.
Various cardiac abnormalities occur commonly in patients with AIDS, and in most, the etiology of the heart disease is unknown. To investigate HIV-1 pathogenesis in infected individuals and ask if HIV-1 expression has a direct role in the development of cardiac disease, we used *in situ* hybridization to examine hearts from 3 AIDS patients with cardiac disease. In all 3 hearts, we detected HIV-1 expression and localized this expression to mononuclear inflammatory cells in areas of clinical and pathologic involvement. In 2 patients with pericardial effusion and pericarditis, HIV-1 was localized to the pericardium. In one with recurrent ventricular arrhythmias, by contrast, HIV-1 was found in focal mononuclear cell infiltrates in the septal myocardium. Neither opportunistic pathogens nor tumor were found in these tissues. These data suggest a role for HIV-1-infected mononuclear inflammatory cells locally in the development of cardiac disease in AIDS patients and add to the body of evidence that these cells play a role in the pathogenesis of HIV-1-related disease.

L 552 IDENTIFICATION OF A RECOMBINANT POLYPEPTIDE FOR USE IN AN HIV-2 IMMUNOASSAY, Annelie Wilde, James Storey, Debbie Van Reit, Chung-ho Hung, Lauri Tang, Gerald A. Beltz and Elihu Young, Cambridge BioScience Corporation, 365 Plantation Street, Worcester, MA 01605. Four contiguous non-overlapping restriction fragments covering 95% of the HIV-2 envelope coding region were cloned into an E. coli expression vector. All four recombinant clones expressed protein at high level in E. coli. Only one of the E. coli expressed proteins was capable of detecting HIV-2 positive sera from a panel of well characterized W. African sera. This peptide (K3) represents 43% of the total sequence of the HIV-2 envelope, consisting of the C-terminal one-third of the external glycoprotein and the N-terminal one-half of the transmembrane glycoprotein. After isolation to greater than 95% purity from inclusion body preparations, K3 was used to construct an experimental ELISA for HIV-2. In preliminary studies this immunoassay was able to detect all HIV-2 positive sera in our panel.
Thus we have identified and expressed a region of the HIV-2 envelope gene that will be useful for the diagnosis of HIV-2 infection.