PREVENTION AND TREATMENT OF AIDS

Organizer: Dani Bolognesi

January 23 - 30, 1994; Hilton Head Island, South Carolina

Sponsored by SmithKline Beecham Pharmaceuticals and Specialty Laboratories, Inc.

Plenary Sessions *Page*

Poster Sessions

Prevention and Treatment of AIDS

Overview Lectures I

J 001 HOST FACTORS IN THE IMMUNOPATHOGENESIS OF HIV DISEASE, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

The establishment and progression of HIV disease is a complex multifactorial and multiphasic process. We and others have previously established that the lymphoid tissue serves as a major reservoir of viral burden and replication throughout the usually prolonged course of HIV disease. Virus is trapped extracellularly in the follicular dendritic cells of the germinal centers of the lymphoid organs. Infection exists simultaneously in a latent as well as actively expressing form with the latter increasing as disease progresses. Host factors are being increasingly recognized as contributing to the progressive deterioration of the immune system. These include: 1) the gradual dissolution of the microenvironment of the lymphoid organs; 2) aberrant immune activation and its role in the propagation of HIV disease; 3) cytokine expression in the lymphoid tissue milieu; and 4) host response to superantigens and its impact on immune system activation. These and other concepts in the immunopathogenic mechanisms of HIV disease will be discussed together with therapeutic strategies derived from an understanding of these diverse immunopathogenic events.

J 002 NEW APPROACHES FOR INTERFERING WITH HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND FOR KAPOSI'S SARCOMA, Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Progress in antisense and gene therapy targeting specific human immunodeficiency virus regions will be summarized, and two additional and novel approaches for inhibiting human immunodeficiency virus replication will be discussed. Concepts on the pathogenesis of Kaposi's sarcoma based on results from *in vitro* and *in vivo* model systems will also be summarized with suggestions for new forms of therapy for this tumor.

Overview Lectures II

J 003 **UNDERSTANDING HIV VARIATION THROUGH ANALOGIES, Gerald Myers, Theoretical Biology and Biophysics Los Alamos National Laboratory, Los Alamos N.M. 87545.**

"Biochemists can agree with naturalists that every nucleotide position has a unique history, as does every atom of gas. But, they also recognize that the universal gas law (PV = nRT) was not discovered by detailed analysis of the behavior of individual atoms. Bringing together molecular biology and natural history in the search for general laws of evolution requires, as many naturalists now recognize, a willingness to transcend 'microscopic' analysis."

-- Allan Wilson, 1987

HIV Entry

J 004 DOMAINS OF RETROVIRAL GLYCOPROTEINS INVOLVED IN OLIGOMER ASSEMBLY AND FUSION. Eric Hunter*, Helene Bernstein*, John Dubay*, David Einfeld*, Simon Tucker*, Carl Wild† and Tom Matthews†, University of Alabama at Birmingham, Birmingham, AL 35294 and †Duke University, Durham, NC 27710.

The glycoprotein complex of HIV mediates viral genome entry into target cells through binding its receptor (CD4), and a process of membrane fusion in which the lipid envelope of the virus fuses with the bounding membrane of the cell. The molecular events that occur following receptor binding which result in membrane fusion are poorly understood, and the regions of the glycoprotein that function to initiate the fusion process have not been well characterized.

Retroviral glycoproteins exist as oligomeric structures in virions and we have now shown in the avian sarcoma virus system that the TM protein expressed alone can efficiently oligomerize and be transported to the cell surface. In a previous mutagenic analysis of the HIV-1 TM protein (gp41), we showed (Dubay et al. J. Virol. 66: 4748) that mutations within a region (a.a. 555-591), defined previously as a leucine zipper and implicated in oligomer formation, had no detectable effect on the gp160 oligomerization process, but could interfere with HIV glycoprotein-mediated fusion. Substitution of a leucine or valine for a highly conserved isoleucine at position 573 yielded wild-type glycoproteins capable of inducing fusion and mediating infection. In contrast, substitution of serine, glycine, proline, aspartic acid or glutamic acid completely abolished biological activity; and an alanine substitution yielded an intermediate phenotype.

Peptides corresponding to the leucine zipper domain (a.a. 558-595) show a propensity to form higher order coiled-coil interactions and have the ability to block HIV-1 induced cell fusion and virus infectivity (Wild et al. PNAS 89:10537). We show here that substitution of alanine, serine and proline for isoleucine-573 in these peptides reduced both their capacity to form higher order structures and their ability to block virus fusion and entry. The melting temperatures of the coiled-coil structures decreased in a consistent fashion with the loss of fusion inhibition and consistent with the effects observed on glycoprotein biological function *in vivo*. As an alternative way to examine the role of the leucine-zipper region in HIV glycoprotein function we have expressed residues 538-593 as a fusion protein with protein-A from the bacterial expression vector pRIT2T. Size exclusion chromatography of the fusion protein containing the wild-type *env* sequence indicated the bulk of the protein was in an oligomeric form, in contrast to the native protein-A molecule which appeared to remain monomeric. Both chromatography and analytical centrifugation indicate that the oligomer mediated by this region of gp41 is larger than a dimer. Expression of the alanine, aspartic acid and proline mutations described above, as well as deletions, within the leucine zipper domain yielded fusion proteins that showed a decreasing propensity to oligomerize. The results of both peptide and fusion protein studies suggest that if the leucine zipper plays a role in glycoprotein oligomerization, its propensity to form a stable coiled-coil is not the major force driving that interaction. Nevertheless, the correlation between the capacity of the glycoprotein to induce cell fusion and this region to form stable multimers, suggests that a some point in the fusion process the formation of a coiled-coiled structure is critical.

J 005 GENE VACCINES, A NEW APPROACH TO IMMUNIZATION, H.L. Robinson[1], E.F. Fynan[1], S. Lu[1], J.R. Haynes[2], and R.G.Webster[3], [1]University of Massachusetts Medical Center, Worcester, MA 01655; [2]Agracetus, Inc., Middleton, WI; [3]St. Jude Children's Research Hospital, Memphis, TN

Early studies using DNA-expression vectors for vaccination have had highly promising results. Vaccination is accomplished by the uptake and expression of the antigen encoded by the DNA. DNA-encoded antigens are expressed in their native forms and can access both MHC class I and class II dependent immune responses. Our vaccination trials have used influenza and immunodeficiency virus models. In the influenza studies, vaccination and a single boost have been with plasmid DNAs that express the influenza virus hemagglutinin glycoprotein. Challenge has been via the nares with an influenza virus with a hemagglutinin glycoprotein of the same subtype as expressed by the DNA. These studies have demonstrated that:

(1). Chickens, mice and ferrets can be protected against influenza virus challenge by vaccination with HA-expressing DNAs.

(2). Parenteral as well as mucosal routes of DNA inoculation can provide protective immunity for influenza virus challenges.

(3). Vaccination by gene gun-delivery of DNA to the epidermis is 100 to 1000 fold more efficient at raising protective immunity than inoculations in saline.

(4). DNA inoculations can be used to raise neutralizing antibodies for HIV-1.

HIV Gene Expression and Variation

J 006 ROLE AND MECHANISM OF ACTION OF THE EARLY GENE PRODUCTS OF HIV-1, Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.

Early after infection, HIV-1 expresses a set of three viral proteins that are essential for efficient viral replication and pathogenesis *in vivo*. Both Tat and Rev are nuclear regulatory proteins that act through structured RNA target sites termed, respectively, TAR and RRE. Tat is known to activate transcription directed by the HIV-1 LTR while Rev is required for the nucleocytoplasmic transport, and hence translation, of RNAs that encode the late, structural proteins of HIV-1. Nef is a cytoplasmic, myristolyated protein that efficiently and specifically down-regulates the cell-surface expression of CD4, the glycoprotein receptor for HIV-1 infection. This activity renders these cells refractory to superinfection and may facilitate the release of progeny virions by the infected cell. Recent data relevant to the role and mechanism of action of these three critical HIV-1 proteins will be presented.

J 007 MOLECULAR EVOLUTION OF WEST AFRICAN PRIMATE LENTIVIRUSES, Feng Gao[1], Ling Yue[1], Jennifer Jin[1], Huxiong Hui[1], David L. Robertson[2], Patricia N. Fultz[1], Paul M. Sharp[2], George M. Shaw[1], and Beatrice H. Hahn[1], [1]Departments of Medicine and Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, and [2]Department of Genetics, Trinity College, Dublin, Ireland.

Elucidation of the origin of human immunodeficiency viruses is fundamental to our understanding of AIDS pathogenesis and the mechanisms responsible for their recent epidemic spread. To determine the phylogenetic relationships of human and non-human primate lentiviruses in West Africa, we used PCR and lambda phage cloning methodologies to characterize geographically-diverse HIV-2 strains from humans, SIV_{SM} viruses from captive mangabeys, and SIV_{AGM} isolates from wild-caught West Africa green monkeys. Sequence analysis of subgenomic fragments (*gag, pol, env*, LTR), as well as full-length proviruses (HIV-2/7312A, HIV-2/F0784, SIV_{AGM}SAB384), revealed several important findings:

1. HIV-2 and SIV_{SM} viruses comprise a single, highly diverse group of primate lentiviruses. Analogous to HIV-1, this group can be subdivided into at least five distinct sequence subtypes (A-E), one of which contains both HIV-2 and SIV_{SM} strains. Sequence analysis of complete HIV-2 proviruses indicates the existence of intergenic recombinants, likely reflecting recent double infection of humans with viruses from more than one sequence subtype. Finally, *in vitro* biological characterization indicates varying properties of members of the different sequence subtypes, ranging from high level cytopathicity for subtype A viruses to lack of growth in tissue culture for some subtype C and E viruses.
2. West African SIV_{AGM} viruses are characterized by a mosaic genome. In phylogenetic trees derived from protein sequences representing the 3' half of their genome (i.e., the carboxy-terminal half of *pol, env* and *nef*), they cluster with other SIV_{AGM} viruses in as high as 100% of bootstrap samples. By contrast, in trees derived from the 5' part of their genome (i.e., *gag*, and the amino-terminal half of *pol*), West African green monkey viruses cluster with the SIV_{SM}/HIV-2 group of viruses in as many as 99% of bootstrap samples. Finally, West African SIV_{AGM} viruses contain a duplicated TAR sequence, a feature so far only identified in HIV-2/SIV_{SM} viruses.

Taken together, these findings confirm the simian origin of HIV-2, demonstrate simian/human and simian/simian virus transmissions, and provide molecular evidence for coinfection of individuals with more than one virus subtype. These factors contribute to the evolutionary complexity of primate lentiviruses.

HIV Pathogenesis I

J 008 BIOLOGICAL AND MOLECULAR FEATURES OF HIV-1 PATHOGENESIS, Cecilia Cheng-Mayer, Leonidas Stamatatos, Atsushi Koito, and Earl Sawai, Cancer Research Institute, University of California, San Francisco, CA 94143-0128

We and others have previously reported that the V3 loop of envelope gp120 contains a major determinant for certain HIV-1 biological properties that appear to correlate with its pathogenicity in the host. These include viral infectivity, syncytium induction, and infection of primary macrophages. However, regions of gp120 outside of the V3 loop can also affect these biological properties. To examine the mechanisms by which the V3 loop and other regions of gp120 regulate these properties, structure/function and immunochemical analyses of envelope gp120s were conducted.

We observed that the overall conformation of gp120 plays an important role in determining these biological properties of HIV-1, but that the V3 loop structure is pivotal. With regard to viral infectivity and syncytium induction, we found that amino acid changes both within and outside (e.g., the C2 region) of the V3 loop can alter its conformation, and hence affect its function. For tissue tropism, an interaction of the V3 loop with other regions (e.g., V1/V2 domain) can confer on gp120, a structure imparting efficient infection of primary macrophages. Thus, the gp120 structures of T-cell line-tropic and macrophage-tropic strains appear to be different. The influence of this difference on virus tropism could be reflected by the types of conformational changes that the external glycoprotein undergoes upon binding to its receptor to mediate viral entry into different cell types.

Another gene product of HIV-1 that has been implicated to play a role in AIDS pathogenesis is Nef. To understand the mechanism by which Nef mediates its effect(s), co-immunoprecipitation studies were conducted to identify its potential cellular targets. Results show that Nef specifically interacts with a serine kinase which phosphorylates substrates of 68 and 72 kDa. This Nef-associated kinase activity is not blocked by inhibitors of protein kinase C or protein kinase A. Further characterizations of these cellular targets of Nef should reveal its function.

J 009 NIAID HIV/AIDS PATHOGENESIS RESEARCH AGENDA. Margaret I. Johnston, and John M. Killen, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

Understanding the mechanisms that underlie HIV pathogenesis is critical to the identification and rapid development of effective vaccine and therapeutic strategies. The National Institute of Allergy and Infectious Diseases has lead responsibility among the Institutes of the National Institutes of Health for the support of biomedical research on: (1) fundamental aspects of HIV disease and its sequela, (2) the discovery and development of therapies for HIV infection and its complications, and (3) the design and development of an effective HIV vaccine. The National Institute of Allergy and Infectious Disease has developed an HIV/AIDS Research Agenda which comprehensively documents the Institute's major scientific programs, priorities, and plans for each of five broad scientific areas: (1) pathogenesis; (2) epidemiology and natural history; (3) therapeutics research and development; (4) vaccine research and development; and (5) pediatric disease. The Agenda is intended to serve as a focus for scientific planning, program management and evaluation, and communication about the scope and nature of the NIAID's efforts. The Agenda has been developed with the assistance and advice of many colleagues in the scientific, industrial, patient and activists communities. It is intended to be a fluid document and that yearly revisions will involve similar participation.

Critical scientific gaps in HIV pathogenesis discussed in the Agenda include understanding the molecular interactions involved in HIV reproduction and regulation; the events at the cell and organ system level that characterize the course of HIV infection; the role and mechanism of action of viral genotypic and phenotypic determinants, of host factors, and of co-factors on the course of infection; the direct and indirect mechanisms of HIV-mediated immune dysfunction; how animal models can best be utilized to understand HIV-mediated pathogenesis; and the mechanisms of sexual/mucosal transmission of HIV. Ongoing and upcoming activities to focus research efforts on critical gaps in HIV pathogenesis will be overviewed. These will emphasize research on: the "what, how much, where and when" of HIV transmission and disease progression; identification and mechanism of action of host factors and responses that influence susceptibility to infection or disease; mechanism(s) of HIV-mediated immune dysfunction operative in vivo.

J 010 VIRUS-HOST INTERACTIONS AND IMMUNE RESPONSES IN HIV INFECTION

Hanneke Schuitemaker, Michèl Klein, Angélique van 't Wout, Linde Meyaard, Marijke Th. Roos, Peter Schellekens, and Frank Miedema, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

HIV-1 isolates show variation in biological properties. Emergence of SI variants in an individual usually precedes accelerated CD4 cell decline whereas in stable asymptomatic individuals, low frequencies of predominantly macrophage-tropic NSI HIV-1 clones are present. Clonal virus isolation from homosexual and mother-to-child transmission pairs revealed a selective expansion of macrophage-tropic virus variants in the recipient, irrespective the presence of SI HIV variants in the donor.

HIV-1 isolates differ in their capacity to replicate in monocyte derived macrophages (MDM). Moreover, in a series of donor macrophages, evidence was obtained for differential susceptibility for HIV-1. Restricted virus replication was either determined at entry or during reverse transcription. Upon inoculation of MDM with HIV-1, virus could be rescued from only a minor cell fraction (2-10%). A comparable small fraction of MDM had proliferative capacity as could be demonstrated by incorporation of BrdUrd. Moreover, PCR analysis on FACS sorted BrdUrd+ and BrdUrd- cell fractions demonstrated the presence of proviral DNA only in this BrdUrd+ fraction.

In the face of predominantly monocytotropic HIV-1 variants, Th1 cellular immunity, by analysis of large panels of T-cell clones generated from asymptomatic individuals, was lost preceding progression to AIDS. In a four-year prospective cohort study indeed this loss of T cell reactivity was an independent predictive marker for disease progression. In two long term (> 8 years) asymptomatic (LTA) individuals with relatively stable CD4 cell counts and low viral load, T cell reactivity was preserved, accompanied by persistent high frequencies of class I restricted gag specific CTL. In these LTA, a high percentage of both CD4+ and CD8+ cells was programmed for death, indicative for immune activation.

These data support the idea that continuous immune control of virus replication may contribute to maintenance of the asymptomatic state.

HIV Pathogenesis II

J 011 HIV GENE EXPRESSION AND PATHOGENESIS, Ashley T. Haase, Department of Microbiology, University of Minnesota, Minneapolis, MN

HIV gene expression covers a spectrum and the low and high ends of the spectrum are correlated respectively with covert infections or productive infection and disease. With the goal of understanding regulation of gene expression *in vivo*, we have developed *in situ* hybridization methods that will distinguish in individual cells the balance between the *tat* and *rev* regulatory gene transcripts and mRNA encoding the *gag* gene product. I will describe in a cellular model of viral latency and activation how subtle changes in the concentrations of the *tat* and *rev* transcripts trigger productive infection.

J 012 MECHANISM OF CD8+ CELL SUPPRESSION OF HIV REPLICATION, Jay A. Levy, Carl E. Mackewicz, Edward Barker, David Blackbourn, Cancer Research Institute, and Department of Medicine, University of California, San Francisco, CA, 94143

CD8+ cells from HIV infected individuals have shown the ability to suppress HIV replication in infected CD4+ lymphocytes. This antiviral response is strongest in healthy individuals, particularly long-term survivors of HIV infection. Over time, the CD8+ cell antiviral activity decreases concomitant with development of disease. The mechanism involved in this antiviral activity appears to be in part a novel cytokine that suppresses virus at the transcription level. Its possible effects on HIV prior to integration are under study. The factor produced by CD8+ cells has a low molecular weight, is pH and temperature stable, and does not affect CD4+ cell activation or replication. The extent of the CD8+ cell antiviral response is influenced by various cytokines, particularly those produced by the Th1 subset of CD4+ lymphocytes. IL-2 enhances CD8+ cell activity, whereas IL-10 (a Th2-type cytokine) suppresses CD8+ cell response.

This CD8+ cell antiviral activity is not detected in uninfected individuals when evaluated by the ability of CD8+ cells to suppress acute virus infection of normal CD4+ lymphocytes. Nevertheless, using this assay, CD8+ cells from some high-risk uninfected individuals and uninfected infants of HIV-positive mothers have shown antiviral activity against HIV. These results suggest exposure of the individual to virus or viral antigens without established infection. Clones of CD8+ cells with antiviral activity have been derived and compared with CD8+ clones that do not show this activity. These cell clones should be helpful in the eventual characterization of the antiviral factor and the mechanisms involved in CD8+ cell suppression of HIV replication.

J 013 QUANTITATIVE ANALYSIS OF HIV-1 REPLICATION IN ACUTE AND CHRONIC INFECTION AND FOLLOWING TREATMENT WITH NOVEL ANTIVIRAL REGIMENS, George M. Shaw[1], Michael S. Saag[1], John C. Kappes[1], Beatrice H. Hahn[1], Emilio A. Emini[2], David D. Ho[3], Michael Piatak, Jr.[4], and Jeffrey D. Lifson[4], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294; [2]Merck, Sharp & Dohme Research Laboratory, West Point, PA 19486; [3]Aaron Diamond AIDS Research Center, New York, NY 10016; and [4]Genelabs Technologies Inc., Redwood City, CA 94063.

The rapidity with which drug resistant HIV-1 variants evolve *in vivo* (Saag *et al., NEJM,* Oct. 1993, in press) and the demonstration of circulating virions in plasma at all stages of infection (Piatak *et al., Science* 259:1749, 1993) implicate ongoing viral replication as a primary determinant of HIV-1 pathogenesis. In order to understand more fully the relation between HIV-1 replication, viral natural history, and the biological effects of novel antiviral therapies, we utilized a complementary set of virologic assays to quantify virus expression in patients at all stages of infection, many of whom were then treated with novel classes of antivirals. Patients receiving either no treatment, treatment with nucleoside or non-nucleoside RT inhibitors, "triple-combination" nucleoside/non-nucleoside therapy, or a novel HIV protease inhibitor were evaluated by quantitative competitive PCR (QC-PCR), branched chain DNA (bDNA) amplification, regular and immune complex-dissociated p24 antigen, and infectious plasma virus titers. While the sensitivities and dynamic ranges of the respective assays differed, there was a significant correlation among all assays in changes observed in viral load (correlation coefficients generally 0.7 to 0.9). The magnitude of change in virologic markers ranged from 2-fold to greater than 1000-fold, depending on the drug or drug combination. In instances where viral drug resistance developed, virologic markers were generally predictive of this occurrence. The results of these studies suggest that complementary quantitative assays of viral replication can provide important insight into viral replication patterns *in vivo* and the relative activity and duration of effect of antiviral drugs and drug combinations. These findings are important both to the rapid assessment of antiviral therapies and to the evaluation of vaccine efficacy in subjects with "breakthrough" infection.

HIV Immune Responses (Humoral)

J 014 GENETIC VARIATION AND PHENOTYPIC CHARACTERISTICS OF HIV-1 STRAINS AT THE MOMENT OF PRIMARY HIV-1 INFECTION: RELATIONSHIP TO RATE OF CD4+ CELL DECLINE AND TIME TO AIDS DIAGNOSIS, Jaap Goudsmit[1], Suzanne Jurriaans[1], Marion Cornelissen[1], Elly Baan[1], Gerrit-Jan Weverling[2], Bob van Gemen[3], Maarten Koot[4], Roel Coutinho[5], Hanneke Schuitemaker[4], and Carla Kuiken[1], [1]Human Retrovirus Laboratory, AMC, Amsterdam, [2]NATEC, Amsterdam, [3]Organon Teknika, Boxtel, [4]Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, [5]Municipal Health Service, Amsterdam, the Netherlands.

The acquired immune deficiency syndrome (AIDS) occurs in HIV-1 infected individuals after a variable symptom-free period, ranging from six months to decades. This may be explained in three ways:

1 the risk at HIV-1 seroconversion to develop AIDS is generally high, but particular virus-host interactions decrease that risk
2 the risk at HIV-1 seroconversion to develop AIDS is low for some individuals and high for others, depending on the genotype and phenotype of the virus
3 the risk at HIV-1 seroconversion to develop AIDS is generally low, but changes in genotype and phenotype of the virus increase that risk.

To differentiate between these options, 42 acutely infected individuals were studied at the moment of seroconversion. Twenty developed AIDS within a median follow-up time from seroconversion to AIDS of 1,200 days and 22 remained symptom-free within a median follow-up time from seroconversion on of 2,400 days. Variation in genotype was defined as variation in the V3 regions and variation in phenotype was defined as variation in MT2 tropism, V3 determinants of SI phenotype and number of HIV-1 particle-associated genomic RNA. All 42 seroconverters at the moment of seroconversion carried NSI viruses. Eight progressors and six non-progressors showed a conversion to the SI phenotype during the symptom-free period. At seroconversion neither the number of virus particles, as assessed by genomic RNA copy number, nor the CD4+ cell count separated the progressors from the non-progressors. Each of 16 direct sequences of the 276 bp region, including the V3 loop coding domain obtained from seroconverters progressing subsequently to AIDS, was equidistant to the consensus of the progressors and the consensus of the non-progressors sequences. The same was true for the 19 direct sequences obtained from seroconverters not progressing to AIDS. Three years after seroconversion, the number of HIV-1 particles in serum differed significantly between progressors and non-progressors, as did the CD4+ cell count. The progressors showed a mean CD4+ cell decline of 39% in the first three years of HIV-1 infection ($p=0.0004$) and the non-progressors of 18% ($p=0.008$), indicating a CD4+ cell decline in both groups. The progressors showed a mean RNA copy number increase of 2% ($p=0.6$) in the first three years of HIV-1 infection, while the non-progressors showed a mean RNA copy number decrease of 16% ($p=0.0007$). The RNA changes were independent of the stability of the NSI phenotype. These results indicate that HIV-1 production reaches the same level at seroconversion in all individuals with primary infection. V3 loops of viruses causing HIV-1 infection in future progressors cannot be distinguished genetically from that of non-progressors. Virus production appears to decrease subsequently in non-progressors while remaining high in progressors during a period of steady decline of CD4+ cells in both groups. Apparently particular host-virus interactions decrease the risk to develop AIDS in some HIV-1 infected individuals. A risk that appears to be universally high and linked to HIV-1 infection in general.

J 015 HIV AND SIV COMPLEMENT-MEDIATED INFECTION-ENHANCING ANTIBODIES: NEW EPITOPES AND CORRELATION IN SIV/MACAQUE STUDIES, David C. Montefiori,[1] Shiu-Lok Hu,[2], Keith A. Reiman,[3] Norman L. Letvin,[3] Marshall R. Posner,[4] Lisa Cavacini,[4] Mark G. Lewis,[5] Muthiah D. Daniel[3] and Ronald C. Desrosiers,[3] [1]Duke University Medical Center, Durham, NC 27710, [2]Bristol-Myers Squibb, Seattle WA 98121, [3]RERPRC, Harvard Medical School, Southborough, MA 01772, [4]New England Deaconess Hospital, Boston, MA 02215, [5]Henry M. Jackson Foundation, Rockville, MD 20892.

Antibodies that enhance HIV-1 and SIV infection in vitro in the presence of complement are commonly detected in sera from humans and macaques, respectively, following infection or vaccination. Previous studies have identified two naturally occurring enhancing epitopes in the highly conserved amino acids 586-620 and 644-663 of the HIV-1 transmembrane gp41 (Robinson et al., J. Virol., 64:5301-5305, 1990; ibid. 65:4169-4176, 1991). From a vaccine study in macaques immunized with peptide fusion proteins, we have identified similar epitopes in the corresponding regions of the SIV transmembrane gp32. In addition, a complement-mediated, infection-enhancing human monoclonal antibody to the HIV-1 gp120 was identified. The role of infection-enhancing antibodies in HIV-1 and SIV pathogenesis and their potential impact on AIDS vaccine development remain mostly unknown. Previous studies from this laboratory showed a lack of correlation of enhancing antibodies with vaccine failure in the SIV/macaque model. However, the validity of these results are now in question owing to the contribution of anticell immune responses to vaccine protection in the majority of animals studied. In order to re-address the clinical correlation of enhancing antibodies in the SIV/macaque model, we are currently evaluating sera from live-attenuated and subunit-immunized animals where anticell antibodies are not induced, and vaccine protection and failures have been observed. We are also evaluating the presence of these antibodies with respect to virus load and ELISA and neutralizing antibodies following acute SIV infection in macaques.

J 016 ROLES OF CONFORMATIONAL AND GLYCAN-DEPENDENT EPITOPES OF HIV-1 gp120 IN VIRUS NEUTRALIZATION.
Abraham Pinter[1], S. Kayman[1], Z. Wu[1] W. Honnen[1], H. Chen,[1] J. McKeating[2], C. Shotten[3], S. Warrier[1]and S. Tilley.[1] [1]Public Health Research Institute,, New York, N.Y. 10016; [2]Reading University, Reading, U.K.;, [3]Natl. Inst. for Biol. Standards & Control, Hertfordshire, U.K.

The hypervariable V1/V2 domain of HIV-1 gp120 is a highly conformational and heavily glycosylated region. Mutational studies have established that these sequences are required for function of gp120, and strongly neutralizing monoclonal antibodies (MAbs) have been identified that bind to this region. In order to facilitate the characterization of neutralization epitopes in these domains, a glycoprotein fusion vector based on the murine leukemia virus *env* gene was used to express the native V1/V2 domain of multiple strains of HIV-1. Most of the anti-V2 MAbs were fairly type-specific, but several reacted with more than one strain. Three classes of neutralization targets were detected in the HXB2 V2 domain by these studies. One group of antibodies reacted with denatured protein and synthetic peptides corresponding to the N-terminus of the V2 loop; these were recognizing continuous epitopes that were independent of glycosylation. A second group of antibodies did not bind to synthetic peptides or denatured molecules, but did react with deglycosylated fusion proteins. These epitopes were conformational but not glycan-dependent. A potently neutralizing chimp MAb was affected by deglycosylation but not by disulfide reduction, indicating that it was seeing a glycan-dependent, continuous epitope. The role of specific N-linked glycans in the formation of this epitope will be discussed, as will the structural requirements for the conformational epitopes. Sera from a number of HIV-1-infected chimpanzees and a fraction of HIV-1-infected humans recognized V1/V2 fusion proteins prepared from a broad range of viral isolates. These reactivities are not competed by synthetic V2 peptides, indicating that they require discontinuous or glycosylated structures. These results indicate that conserved epitopes exist in the V1/V2 domain that are immunogenic in infected individuals and that may function as neutralization targets. Further structural characteristics of the epitopes seen by these sera will be described.

J 017 PROTECTIVE HUMORAL IMMUNITY TO HIV-1, Susan Zolla-Pazner[1,2], Miroslaw K. Gorny[2], Constance Williams[1], Thomas VanCott[3], Sherri Burda[1], Suman Laal[1,2], Aby Buchbinder[1,2], Scott Koenig[4], Anthony Conley[5], and Emilio Emini[5], [1]New York Veterans Affairs Medical Center, New York, NY 10010, [2]New York University Medical Center, New York, NY 10016, [3]Walter Reed Army Institute of Research, Rockville, MD 20850, [4]MedImmune, Inc., Gaithersburg, MD 20878 and [5]Merck Research Labs, West Point, PA 19486.

Polyclonal anti-HIV immunoglobulin preparations and monoclonal antibodies (mAbs) to various regions of HIV-1 gp120 have been shown to neutralize HIV-1 *in vitro* and to protect against viral infection *in vivo*. In order to identify epitopes for inclusion into vaccines that will induce the most potent neutralizing Abs and to identify mAbs that can be most effectively used in passive immunization, we studied the ability of various human mAbs, alone and in combinations, to neutralize laboratory and primary isolates of HIV-1. When various human mAbs were tested for their neutralizing ability against laboratory isolates, anti-V3 mAbs were the most potent. Anti-CD4 binding domain (CD4bd) mAbs were approximately one order of magnitude less potent and anti-C-terminus mAbs and a human anti-V2 mAb were unable to neutralize at concentrations up to 100 μg/ml. The neutralizing ability of the anti-V3 mAbs correlated with their dissociation rate constants (k_{off}) but not with their association rate constants (k_{on}). One mAb, 447-D, directed against G P G R at the tip of the V3 loop of Type B HIV-1 was more broadly cross-reactive with laboratory strains of Type B HIV-1 than was any anti-CD4bd mAb tested. In infectivity reduction assays, 447-D neutralized all 21 primary isolates tested, including one which contained G P G Q at the tip of the V3 loop. Neutralization was achieved in all cases using 100 μg/ml of mAb; some primary isolates could be neutralized with as little as 10 μg/ml. Finally, when used together, some combinations of mAbs acted in synergy. Thus, combinations of 447-D and several anti-CD4bd mAbs acted in synergy to neutralize HIV-IIIB. Similarly, synergy was noted with some combinations of anti-CD4bd and anti-C-terminus mAbs--this being the first example of synergy between a neutralizing and non-neutralizing anti-HIV mAb combination. Synergy was also noted with the combined use of two different anti-CD4bd mAbs. However, when an extremely potent anti-V3 mAb was used, e.g., mAb 694/98-D vs. HIV-IIIB, addition of various other mAbs could not enhance its neutralizing potency. The data confirm that (1) anti-V3 mAbs are the most potently neutralizing anti-HIV Abs and demonstrate that (2) they can be extremely cross-reactive with primary isolates. (3) Use of certain mAbs in combination sometimes results in synergy although (4) the activity of the most potent anti-V3 mAbs cannot be augmented by other mAbs.

HIV Immune Responses (Cellular)

J 018 FACTORS REGULATING THE INDUCTION OF CYTOTOXIC T LYMPHOCYTES TO HIV ANTIGENS, Jay A. Berzofsky[1], Mutsunori Shirai[1], Margaret A. Marshall[1], Jeffrey K. Actor[2], R. Mark L. Buller[3], C. David Pendleton[1], Jeffrey D. Ahlers[1], Toshiyuki Takeshita[1], Mark Newman[4], Marika Kullberg[1], and Alan Sher[2], [1]Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892, [2]Laboratory of Parasitic Disease, NIAID, NIH, Bethesda, [3]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, and [4]Cambridge Biotech, Worcester, MA.

We have explored the role of $CD4^+$ helper T cells and the cytokines they produce in the induction and the regulation of the $CD8^+$ cytotoxic T lymphocyte (CTL) response to HIV antigens in mice. First, several vaccine constructs were made by synthesizing as single peptides different clusters of helper T-cell epitopes followed by the P18 segment of HIV-1 IIIB V3 loop of gp160, which is both a neutralizing antibody epitope and a CTL epitope in both mice and humans. These were tested in multiple strains of mice by immunization in QS21, a saponin adjuvant that does not require sequestration of the antigen in an emulsion. We found that the clusters of helper epitopes were necessary for induction of P18-specific $CD8^+$ CTL, compared to P18 alone. The effect was not a trivial one of peptide stability or conformation, because the different helper clusters had different rank order of potency in strains of different class II MHC type. Moreover, help was not observed if the helper cluster peptide was simply mixed with P18 in QS21. Thus, covalent linkage was necessary to produce help for CTL. Although cognate help for B cells is well established, and help for CTL is known to be more effective if the CTL and helper epitopes are on the same cell in an allogeneic response, this is the first demonstration of a requirement for covalent linkage between helper and CTL epitope in a molecular construct. These results may help in HIV vaccine design.

Second, we have found that infection with the helminth parasite *Schistosoma mansoni* affects the Th1/Th2 cytokine balance not only to schistosome antigens, but also to other antigens encountered during the patent stage of the infection, including soluble proteins in adjuvant and HIV gp160 expressed during infection with a recombinant vaccinia virus. Strikingly, the $CD8^+$ CTL response to HIV gp160 was also completely suppressed in these mice, and the clearance of recombinant vaccinia virus was delayed. We have now found that the failure to induce gp160-specific CTL during schistosome infection is due to active inhibition by a $Thy\text{-}1^+$, surface Ig-negative cell, and is mediated by a soluble factor(s). We are currently further characterizing this cell and its factor(s). These results suggest that the prevailing Th1-like/Th2-like cytokine balance at the time of viral infection markedly affects the host $CD8^+$ CTL response to the virus. These findings have implications for the course of HIV infection in areas where prior infection with prevalent parasites or other infectious agents could influence the cytokine balance, for the differential impact of vaccination of individuals infected with such parasites compared to uninfected vaccinees, and for the strategies to alter cytokine balance to influence the outcome of infection or vaccination.

HIV Vaccines

J 019 PRIMARY ISOLATE NEUTRALIZING ACTIVITY OF HUMAN ANTIBODIES DIRECTED TO RECOMBINANT, NATIVE HIV-SF2 GP120 (rgp120$_{SF2}$) Kathelyn S. Steimer, Doreen Sakamoto, Yi De Sun, David West, Jürg Baenziger, and Faruk Sinangil. The Biocine Company/Chiron Corporation, Emeryville, California 94608

Serum specimens from human volunteers immunized with four doses of rgp120$_{SF2}$ in MF59 adjuvant, either with or without the muramyl tripeptide MTP-PE, were tested for their ability to neutralize laboratory and clinical isolates of HIV-1. Sera from 27/28 (96%) of the immunized volunteers neutralized HIV-SF2, the isolate from which the recombinant antigen originated, and a majority (64%) of these sera also cross-neutralized the closely related laboratory isolate HIV-MN in T cell line (HUT-78) based neutralization assays. Sera from the five volunteers with highest titer of HIV-SF2 neutralizing activity following four immunizations were tested for neutralization of a panel of six independent primary U.S. isolates of HIV-1, including several isolates with central V3 regions homologous to the U.S./European consensus sequence, in PBMC-based neutralization assays. At a 1/10 serum dilution, there was no evidence of neutralization of any of these six primary isolates by these five sera. In contrast, these six isolates were all neutralized by at least three of the five high titer sera collected from asymptomatic HIV-1 infected individuals, assayed at the same time. In addition, four of the five vaccinee sera were able to neutralize the homologous isolate HIV-SF2 in a PBMC-based neutralization assay, albeit at titers at least 10-50 fold lower than in T cell line based neutralization assays. To determine whether the failure to cross-neutralize primary isolates was because of low sensitivity of the assay, rgp120$_{SF2}$-specific antibodies were purified from pooled vaccinee plasma and added to the assay at titers approximately 20-fold higher than could be added with a 1/10 serum dilution. Again, no cross-neutralization of these six clinical isolates was observed. In parallel with the purification of rgp120$_{SF2}$-specific antibodies from vaccinee sera, rgp120$_{SF2}$-specific antibodies were also purified from two independent pools of plasma from asymptomatic HIV-1 infected individuals. In contrast to the vaccinee rgp120$_{SF2}$-specific antibodies, rgp120$_{SF2}$-specific antibodies purified from HIV-1 infected individuals were able to cross-neutralize these six primary isolates. These results suggest that the antibody response induced when humans are immunized with four doses of rgp120$_{SF2}$ in MF59 adjuvant is qualitatively different than the antibody response induced in the context of an active HIV-1 infection. The impact of these findings on future efficacy trials of this candidate vaccine remains to be determined.

J 020 CHARACTERIZATION OF VIRUS NEUTRALIZING ANTIBODIES FROM FIV-VACCINATED CATS. B.A. Torres[1], A.Varela[1], S. Okada[2], R. Pu[2], H.M. Johnson[1], and J.K. Yamamoto[2] Department of Microbiology & Cell Science[1] and Department of Comparative Experimental Pathology[2], University of Florida, Gainesville, FL 32611.

The genome of the feline immunodeficiency virus (FIV) contains an open reading frame (ORF) that overlaps into the 3'long terminal repeat. This ORF (designated here as ORF4) encoded for a 71 amino acid protein which has been reported to possess rev-like activity. Four overlapping peptide were synthesized corresponding to the entire length of the putative ORF4 protein derived from the FIV-Petaluma strain. Pooled sera from cats immunized with inactivated virus vaccine (previously shown to be protective *in vivo*) showed high reactivity to ORF4(1-30), little reactivity to ORF4(21-55) and no reactivity to the C-terminal peptides. A similar pattern of reactivity was seen with pooled sera from cats immunized with inactivated infected cell vaccine, although the reactivity was weaker. Sera from a specific pathogen free cat did not recognize any of the peptides. The reactivity of *pooled antisera from* FIV-infected cats drawn at different times postinfection was tested. Reactivity to all peptides peaked at 10 weeks postinfection, followed by a rapid decline to a plateau. Interestingly, a second peak of reactivity to ORF4(21-55), ORF4(31-65) and ORF4(51-71) occurred late in infection. However, no peak was seen against ORF4(1-30) and, in fact, reactivity seemed to be decreasing. Peptide immunoaffinity columns were produced to isolate anti-peptide antibodies. Partially purified pooled antisera containing a high titer of virus neutralizing (VN) antibodies was passed over the peptide columns and tested for residual VN titers. High VN activity was seen in the fraction eluted from the ORF4(1-30) column, whereas reduced VN activity was found in the fraction that did not bind to the column, as compared to unfractionated sera. Fractions eluted from the other peptide columns did not have VN activity, with the VN activity residing in the fractions that did not bind to the columns. VN antibodies seem to recognize the N-terminal portion of ORF4(1-30), since a 9 amino acid overlap occurs between that peptide and ORF4(21-55). Immunoblot analysis of pelleted FIV showed two strong protein band (8.9 and 17.8 K) and one faint band (21 K) that were recognized by ORF4(1-30)-reactive antibodies. Whole serum recognized numerous proteins including the proteins recognized by the ORF4(1-30)-reactive antibodies. ORF4(1-30)-reactive antibodies did not recognize proteins of a MW corresponding to whole FIV envelope or to envelope peptides. The mechanism by which ORF4(1-30)-reactive antibodies neutralized FIV infectivity remains to be elucidated. However, these results suggest that this protein is expressed *in vivo* and that it may be important in the pathogenesis of FIV.

HIV Vaccine Clinical Trials

J 021 EVALUATION OF CANDIDATE HIV-1 VACCINES IN PHASE I/II TRIALS, Patricia E. Fast, Mary Clare Walker, Nzeera Ketter, Alan M. Schultz and the AIDS Vaccine Clinical Trials Network, NIAID, Bethesda MD 20850

Worldwide, more than 25 Phase I trials of AIDS vaccine candidates have been initiated in volunteers who are not infected with HIV; fourteen of these trials have been supported by NIAID. The vaccine candidates appear to be safe, reactions have been those expected with immunization and the vaccines have not caused loss of CD4 cells or decline of immune competence.

The recombinant envelope vaccines tested to date have induced antibody responses in a dose-dependent manner; the antibody declines wanes within months. Immunologic memory is long-lasting but does not necessarily continue to increase after the third injection. The mammalian-produced rgp120 vaccines tested to date have induced type-specific neutralizing and syncytium-inhibiting antibodies in most recipients, some to high titers. Cross-reactivity of these antibodies is limited. Priming with vaccinia-rgp160 increases the likelihood that gp160 produced in insect cells will induce neutralizing antibodies and the combined regimen induces circulating precursors of CD8 cytotoxic T lymphocytes about half of the volunteers. A new protocol will compare different recombinant proteins as boosts after the vaccinia-gp160 priming.

A peptide vaccine (V3-MAPS) is presently under evaluation. Two comparative adjuvant trials will compares Biocine rgp120 formulated with alum, liposomes, monophosphoryl lipid A, SAF with and without MDP, MF59 with and without MTP-PE or Genentech gp120 formulated with alum, QS21 or both). New protocols involving immunization of HIV-infected pregnant women, newborn infants of HIV-infected pregnant women, and HIV+ infants/children. A Phase II protocol comparing subjects at higher risk of HIV infection through sexual or injection drug use is also under way.

J 022 COMBINED ANTIGENS REGIMEN FOR THE VACCINATION AGAINST HIV, S.A. Plotkin[1], J.L Excler[1], G. Pialoux[2], D. Salmon[3], D. Sicard[3], T. Matthews[4], Y. Rivière[2], B. Autran[5], J.C Gluckman[5], [1]Pasteur Mérieux Connaught, Marnes-la-Coquette, France, [2]Hôpital Pasteur, Paris, France, [3] Hôpital Cochin, Paris, France, [4]Duke University, Durham, North Carolina, [5]:Hôpital Pitié-Salpétrière, Paris, France.

Two prime boost-protocols were studied in human clinical trials. One protocol involved priming with canarypox vector containing the gp160 gene followed by boosting with soluble gp160. The second protocol involved priming with soluble gp160 followed by boosting with linear V3 peptide. Except for the canarypox, volunteers received the antigens together with alum or IFA adjuvant. The vaccination was well-tolerated. Nearly all volunteers developed neutralizing antibodies, highest in those who received gp160/V3 adjuvanted in IFA. CD8 mediated CTL was induced in about half of those who were in the canarypox gp160/gp160 protocol, whereas the gp160/V3 protocol elicited low levels of non-specific cytotoxicity.

Therapy of HIV Disease

J 023 CLINICAL TRIALS OF INHIBITORS OF THE HIV-1 PROTEASE, Michael S. Saag[1], Kathleen E. Squires[1], Hedy Teppler[2], Roger Pomerantz[2], Scott Waldman[2], Thorir Bjornsson[2], George M. Shaw[1], John Kappes[1], Emilio Emini[3], and Paul Deutsch[3], [1]Department of Medicine, The University of Alabama at Birmingham, Birmingham, AL, [2]Department of Medicine, Jefferson Medical College, Philadelphia, PA and [3]Merck Research Laboratories, West Point, PA.

Inhibition of HIV-1 protease activity represents a promising approach to suppression of HIV-1 *in vivo*. Although several candidate agents are in development, only a few exhibit antiviral activity in the nanomolar range and achieve acceptable serum concentrations when given orally. We have recently studied one such agent, L-735,524. In phase I clinical trials, the drug was generally well tolerated. The $t_{1/2\beta}$ was 1 to 1.5 hours. Despite the relatively short half-life, the mean six hour post-dose plasma concentration after 10 days of administration at 400 mg every six hours was approximately 200 nM. The IC_{95} for virus inhibition in cell culture ($\leq$ 100 nM) was exceeded throughout the dosing interval in most patients when administered at 400 mg every six hours. Initial clinical studies of *in vivo* antiviral activity are in progress. Protease inhibitors such as L-735,524 may represent a significant advance in our ability to suppress HIV-1 replication in patients.

New Approaches

J 024 MOLECULAR GENETIC INTERVENTIONS FOR AIDS, Gary J. Nabel, M.D., Ph.D., The University of Michigan, Ann Arbor, MI 48109-0650

The immune system confers protection against a variety of infectious pathogens. Several cell types participate in the recognition and lysis of virus, and appropriate immune stimulation provides therapeutic effects. For example, foreign major histocompatibility complex (MHC) proteins serve as a potent stimulus to the immune system, and we have previously introduced a foreign MHC gene directly into malignant tumors in vivo in an effort to stimulate tumor rejection. In contrast to traditional approaches to induce immunity by cell-mediated gene transfer, the recombinant gene was introduced directly in vivo. Expression of the foreign class I gene induced a cytotoxic T cell to other antigens present on unmodified tumor cells, and caused complete tumor regression in many cases. Direct gene transfer in vivo can therefore induce cell-mediated immunity against specific gene products which provides effective immunotherapy for malignancy and can be applied to the treatment of HIV and opportunistic infections in humans. Based on these and other studies, a phase I/II human trial using direct gene transfer for patients with melanoma has begun. Additional studies have been undertaken to develop gene therapy protocols for AIDS utilizing a transdominant inhibitory form of Rev. HIV infection can be inhibited in susceptible T leukemia cells and human peripheral blood lymphocytes stably expressing a transdominant Rev protein, in the absence of deleterious effects on T cell function. Such a strategy could provide a therapeutic effect in the T lymphocytes of AIDS patients. A human clinical protocol has been approved by the RAC in an effort to determine whether this gene product prolongs the survival of T cells in individuals infected with HIV.

J 025 MOLECULAR BIOLOGY OF HIV, Flossie Wong-Staal, Ph.D., Departments of Medicine and Biology, UCSD, La Jolla, CA 92093

The genetic organization of the human immunodeficiency virus (HIV) is far more complex than the usual gag-pol-env containing type-C viruses. At least six additional genes have been identified. Many of these genes are dispensable for virus replication in vitro, but may increase the efficiency of infection and/or virus production and further may determine cellular tropism and pathogenicity in vivo. Therefore, there is ample opportunity to study novel mechanisms of virus-host interactions in this system. For example, the nef early gene product down-regulates CD4, providing a rapid mechanism for establishing interference. It also dissociates interaction of lck and CD4, thereby increasing the sensitivity of the infected cells to activation by non-specific signals, a process which may in turn activate virus production. The vif gene product appears to compensate for the function of some cellular gene product which may not be expressed in crucial targets for HIV, such as primary T-lymphocytes and monocytes. Of even greater importance, the regulatory genes tat and rev have been shown to be essential for virus replication and their mechanisms of action are also better understood. Tat and rev are early genes, i.e. they are expressed prior to the production of virus structural proteins and most accessory proteins. The temporal switch from early to late gene expression is mediated by Rev, which operates at the level of mRNA processing and transport. One scenario for HIV latency is that there is a subthreshold level of Rev activity. Although the precise mechanisms of action of Tat and Rev have not been elucidated, our current knowledge already points to some obvious avenues to pursue. One important theme to emerge from the study of HIV regulation is the importance of RNA-Protein interactions, not only between Tat and Rev and their respective targets, TAR and RRE, but also cellular proteins that bind to the same RNA targets. These components form large functional complexes, the disruption of which would halt virus production. The use of RNA decoys, transdominant mutant proteins all aim at interfering such complex formation. In addition, anti-sense and catalytic RNA (ribozymes) molecules can attack multiple steps in the virus replication cycle. A new emerging technology for treatment of AIDS is gene therapy, i.e. to introduce an inhibitory gene into the target or precursor cells to confer resistance to HIV infection. As the technology of gene therapy is refined, the prospect of treatment of AIDS using these approaches would also improve.

Late Abstract

RECOMBINANT NEUTRALIZING HUMAN ANTIBODIES TO HIV-1, Dennis R. Burton[1], Carlos F. Barbas III[1], James M. Binley[1], Henrik Ditzel[1], Michael Hendry[2], H. Clifford Lane[3], Robert Walker[3], John P. Moore[4], Peter M. Nara[5], Erling Norrby[6], Markus Thali[7] and Joseph Sodroski[8], [1] The Scripps Research Institute, La Jolla, [2] California Institute of Public Health, Berkeley, [3] NIAID, Bethesda, [4] Aaron Diamond AIDS Research Cer [illegible], New York, [5] National Cancer Institute, Frederick, [6] Karolinska Institute, Stockholm, [7] Dana Farber Cancer Institute, Boston.

Large panels of human antibodies to HIV-1 have been generated from libraries displayed on the surface of phage. The library donors were primarily long term asymptomatic HIV-1 seropositive individuals. Fab fragments reacting with the CD4 binding site, a site overlapping but distinct from the CD4 binding site, the V3 loop and several distinct epitopes on gp41 have been characterized. A number of Fab fragments from each category have been identified which show potent neutralizing activity against laboratory strains of HIV-1 in a number of assays. The most effective neutralizing Fab to the CD4 binding site, Fab b12, has been converted to a whole antibody. This IgG1 is found to neutralize MN and LAI strains of HIV-1 about 100 to 1000-fold more effectively, under comparable conditions, than a number of other CD4 site antibodies described in the literature and compared in the NIH Antibody Serology Project. Furthermore, the antibody is found to neutralize more than 50% of field isolates in a plaque assay. Engineering of Fab b12 by a mutagenesis/selection strategy has been carried out to improve the neutralization potency by more than an order of magnitude. Recent advances in developing new specificities, improving existing antibodies and understanding the molecular basis for potent neutralization will be presented.

HIV Entry; HIV Gene Expression and Variation

J 100 EVALUATION OF A PUTATIVE TRANSMISSION EVENT BY SEQUENCING SINGLE MOLECULES OF HIV-1 DNA OBTAINED DIRECTLY FROM PERIPHERAL BLOOD, Catherine Arnold, Jonathan P. Clewley and Peter Balfe, Virus Reference Division, CPHL, 61 Colindale Avenue, London NW9 5HT, U.K. and Division of Virology, UCLMS, 46 Cleveland Street, London W1P 6DB, U.K.

HIV-1 shows considerable sequence diversity both between and within individuals, mainly due to the high error rate of the viral reverse transcriptase. Heterogeneity is not uniform throughout the genome, being at its greatest in the external glycoprotein (gp120) region of the envelope gene.

Previous studies have shown that there is less sequence divergence in the genomes of virus from individuals infected from the same source than in the genomes of unconnected groups. This observation was the basis of several recent studies concerning transmission of the virus. Our study of possible HIV transmission differed from previous investigations in that it used limit dilution then PCR to amplify several single gp120 molecules from proviral DNA obtained from infected individuals. These samples were then sequenced using an automated DNA sequencer (ABi 373A).

The sequences obtained were compared with sequences from the same region in the Los Alamos database and their relatedness analysed by various software packages. Two software packages were used to determine the degree of relatedness of the intra and inter-patient sequences. ClustalV was used to align the sequences, and the Phylip package was then used for subsequent analysis. All of the results indicate that the HIV strains infecting the two individuals are not related.

J 101 CELLULAR FACTORS ASSOCIATED WITH SELECTIVE FUSOGENIC ACTIVITIES OF HIV-1 ENVS FOR SPECIFIC CD4+ CELL TYPES. Christopher C. Broder, Ofer Nussbaum, and Edward A. Berger. National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, 20892

The ability of the HIV-1 env/CD4 interaction to promote membrane fusion depends on additional features of the CD4+ cell. We are studying this specificity using vaccinia vectors to express CD4 and env on separate cell populations; cell fusion is quantitated by a newly developed assay based on the activation of a reporter gene (*lacZ*) selectively in fused cells by vaccinia-encoded phage T7 RNA polymerase. One form of fusion selectivity is the ability of the human CD4 molecule to mediate fusion when present on a variety of human cell types, but not on animal cell types. We have used the *lacZ* gene activation assay to confirm our previous finding that animal cells expressing human CD4 are rendered fusion-competent by formation of transient hybrids with human cells; this result suggests the presence of a human-specific accessory fusion factor(s). Preliminary results indicate that this component can be functionally transferred from membrane vesicles to animal cells using PEG. A second form of selectivity is the distinct tropism of different HIV-1 isolates for specific CD4+ human cell types (primary macrophages vs. T-cell lines); the determinants for this selectivity reside within env. To study this cell-type specificity, we have prepared a battery of vaccinia-based env constructs from HIV-1 isolates with different cell-type tropisms. We have found that envs from macrophage-tropic isolates mediate fusion preferentially with CD4+ primary macrophages compared to CD4+ cell lines; envs from T-cell line-tropic isolates show the opposite fusion selectivity. These results provide direct evidence that the macrophage vs. T-cell line tropisms of different HIV-1 isolates are associated with highly selective fusogenic properties of the corresponding envs. We are performing experiments to determine whether this cell-type fusion selectivity is determined by corresponding cell type-specific accessory fusion factors.

J 102 ENTRY OF HIV INTO CD4+ CELLS REQUIRES A T-CELL ACTIVATION ANTIGEN, CD26, C. Callebaut, B. Krust, E. Jacotot and A.G. Hovanessian, Unité de Virologie et Immunologie Cellulaire (UA CNRS 1157), Institut Pasteur, 28, rue du Dr. Roux 75015 Paris France.

The CD4 molecule is essential for binding HIV particles to target permissive cells but by itself, is not sufficient for efficient viral entry and infection. This and the potential requirement for the cleavage of the third hypervariable domain (the V3 loop) in the surface glycoprotein of HIV have suggested the involvement of a cell surface protease besides the CD4 molecule in the mechanism of HIV entry.

Here we provide evidence to indicate that this coreceptor which may interact and cleave the V3 loop is dipeptidyl peptidase IV (DPP IV) also referred to as CD26. DPP IV is an integral serine-protease that binds collagen and adenosine deaminase, and is characterized by a catalytic activity specific to proline residues, cleaving synthetic peptides with motifs GP, RP, KP, AP. In the V3 loop, the RP motif is conserved for HIV-1, HIV-2 and related simian isolates whereas the GP motif is more than 90 % conserved among HIV-1 isolates. Consequently, entry of HIV-1 LAI into T lymphoblastoid CEM, Jurkatt and MOLT4 cells, and into monocytoid U937 cells, is 80-90 % inhibited, either by a specific monoclonal antibody against DPP-IV or a specific peptide inhibitor of this protease. Other peptides containing the GP, RP or KP motif can also inhibit both viral entry and enzyme activity. Interestingly, such inhibitory agents also blocked infection of CEM cells by HIV-2 EHO which is completely unrelated to HIV-1 LAI, thus indicating that the requirement of DPP IV for viral entry is a general phenomenon for different isolates of HIV-1 and HIV-2. The observation that peptide inhibitors do not affect the binding of HIV surface glycoprotein to CD4 expressing cells, suggested that the inhibitory mechanism is a post-binding event. These observations provide the potential for the development of new and potent inhibitors of HIV infection.

J 103 FUSION FROM WITHOUT : SYNCYTIA INDUCED BY HIV-1 PARTICLES IN ABSENCE OF VIRUS REPLICATION.

François Clavel and Pierre Charneau
Unité d'Oncologie Virale, Département SIDA et Rétrovirus, Institut Pasteur, Paris.

Fusion from without is the process through which particles of some enveloped viruses can direct fusion of target cells in the absence of viral replication. We have demonstrated that HIV particles can efficiently promote fusion from without. Using HeLa-CD4 cells carrying a Tat-inducible lacZ gene, syncytia were observed as early as 4 hours after exposure to HIV particles, before HIV gene expression could be detected. Efficient syncytium formation could be obtained when cells were treated by AZT, which blocked HIV replication and gene expression but did not affect cell-cell fusion. Fusion was also observed when the indicator cells cells were exposed to particles of a replication-defective HIV integrase mutant. Fusion from without by HIV particles could be blocked by a monoclonal antibody specific for the V3 loop of the HIV-1 envelope glycoprotein, and by soluble CD4, but only at high concentrations (40µg/ml for soluble CD4, and 50µg/ml for the V3 loop-specific monoclonal antibody). Recent reports have emphasized the high concentration of HIV particles found in the lymphoid tissues of HIV-infected patients even during the asymptomatic phase of the infection. These particles appear to be trapped in the lymphoid organs by dendritic cells. Fairly large amounts of particles can also be detected in the peripheral blood, the majority of these particles being replication-defective. Therefore, fusion from without, which can involve cells that do not actively replicate HIV and can be directed by replication-defective particles, could participate in the pathogenicity of the CD4 cell depletion that characterizes HIV infection.

J 104 INDUCED DISSOCIATION OF HIV-1 gp120 FROM gp41 BY HEAVY METAL CHELATORS, Sandra Demaria, Shermaine A. Tilley, Abraham Pinter and Yuri Bushkin, Public Health Research Institute, New York, NY 10016.

The HIV-1 envelope protein is synthesized in the endoplasmic reticulum as a 160 kD precursor which is transported to the Golgi and then cleaved into two subunits, gp120 and gp41. These glycoproteins are expressed on the surface of virally infected cells as noncovalently associated oligomers of transmembrane gp41 and extracellular gp120. While genetic analysis has indicated that some of the amino acids in the C1 and C5 regions of gp120 and certain residues in the extracellular domain of gp41 are important for gp120/gp41 association, the dynamics of the interaction between these envelope proteins remain poorly understood. We have explored the possibility that specific heavy metals are involved in gp120/gp41 association by treating HIV-1-infected cells with 1,10-phenanthroline (1,10-phe) and derivatives of 1,10-phe that chelate zinc and some other heavy metals. Treatment of CEM-NKR cells chronically infected with HIV-1_{IIIB} with 1,10-phe induced a 30 to 60% decrease in the expression of three different gp120 epitopes, as detected by staining with mAbs and flow cytometric analysis. One of these epitopes overlaps the CD4-binding site, while the other two are located in the V2 and V3 regions. In contrast, the binding of a mAb recognizing gp41 to these cells was increased by approximately 50% upon treatment with 1,10-phe, and by 300% upon treatment with a cell-impermeable analog of 1,10-phe, 4,7-diphenyl-1,10-phenanthroline disulfonic acid. Biochemical analysis of supernatants from radioiodinated cells confirmed that treatment with 1,10-phe results in dissociation of gp120 from gp41 and its subsequent release into the medium. Incubation of cells with EDTA, a potent chelator of Ca^{2+} and Mg^{2+}, did not induce the release of gp120, indicating that the effect of 1,10-phe was metal-specific. These data suggest a possible role for heavy metals, such as zinc, in the association between gp120 and gp41. Further studies are in progress to elucidate the mechanism(s) for this effect.

J 105EXAMINATION OF HIV-1 CA PROTEIN USING SPECTRAL ANALYSIS AND LIMITED TRYPTIC DIGESTION AS PROBES OF PROTEIN STRUCTURE, L.S.Ehrlich[1], B.Agresta[1], C.A.Gelfand[2], J.Jentoft[2] and C.A. Carter[1], [1]Dept.of Microbiology, S.U.N.Y, Stony Brook, N.Y. 11794; [2]Dept.of Biochemistry, Case Western Reserve U., Cleveland, OH, 44106-4935.

The secondary structure of recombinant capsid protein (CA) and CA protein isolated from mature virions were evaluated using circular dichroic (CD) spectral analysis. The spectra of the two proteins showed that their backbone configurations are identical indicating that recombinant CA protein can accurately reflect interactions of authentic viral CA protein. Our studies show that recombinant CA proteins self-associate into non-random dodecamers comprised of two distinct types of hexamers. These hexamers displayed differential susceptibility to trypsin which is attributable to subunit packing. Proteolysis generated relatively large N- and C-terminal CA fragments which strongly suggests that the protein is organized into two structural domains separated by an exposed region. We have shown that, as was reported for CA proteins of avian and murine retroviruses, recombinant HIV-1 CA proteins can be chemically crosslinked with DTSSP. In this study, we show that the DTSSP-crosslinkable interface is in the trypsin-susceptible CA hexamer. A mutation in a highly conserved region of CA altered protein sensitivity to these reagents and displayed an assembly-defective phenotype (see poster of Ebbets-Reed,D, et.al.). Thus, while the physiological significance of the CA oligomers remains to be determined, they allow analysis of protein-protein interactions that may be critical to virus assembly.

J 106 G TO A HYPERMUTATION IN HIV-2, Feng Gao[1], Ling Yue[1], Robert J. Biggar[2], George M. Shaw[1], and Beatrice H. Hahn[1], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294.

Clustered G to A hypermutation is a characteristic feature of HIV and may influence viral pathogenicity *in vivo*. We have recently identified an HIV-2 infected individual who harbored an unusual number of defective viral genomes due to extensive G to A hypermutation. G to A changes accounted for 66-87% of all nucleotide substitutions, were found in blood samples obtained four months apart, and resulted in sequence differences among individual *gag*, *pol* and *env* clones of up to 11%. To begin to elucidate which mechanisms are responsible for the generation of G to A changes *in vivo* (mutant reverse transcriptase; specific properties of the viral RNA template; cellular factors) we PCR amplified complete RT genes directly from primary PBMC DNA and analyzed them functionally. Transient prokaryotic expression identified five of 27 clones to contain a functional RT gene. To study whether F0784 RT was particularly error-prone, we used the prokaryotically expressed enzymes for RNA and DNA sequence analysis. No differences in misincorporation frequencies were observed between the F0784 RT and similarly analyzed RT enzymes from HIV-2_{ROD} and HIV-1_{HXB2}. These data suggest that G to A hypermutation in patient F0784 is likely not the result of a mutant reverse transcriptase. Studies of chimeric viruses are underway to determine misincorporation rates of F0784 RT in the context of a replication competent provirus and in naturally infected target cells.

J 107 The HIV epidemic in India: Close homology among HIV-1 and HIV-2 strains suggests a recent spread from a single common ancestor for each of these viruses

Manuel Grez, Ursula Dietrich, Peter Balfe*,Hagen von Briesen, Eric L. Delwart§, James I. Mullins§, and HelgaRübsamen-Waigmann. Chemotherapeutisches Forschungsinstitut, Georg-Speyer-Haus, 60596 Frankfurt/M 70,FRG; §: Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305-5402, USA; &: University College London Medical School, Department of Medical Microbiology, London W1P 6DB, England

DNA sequences encoding the surface envelope glycoprotein of the human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) were amplified by the polymerase chain reaction (PCR) from peripheral blood mononuclear cells obtained from serologically defined HIV-1/-2 mixed infections from Bombay, India. HIV-1 specific PCR products were obtained in 7 out of 7 randomly chosen double reactive samples while HIV-2-specific sequences were detected in 5/7 samples (71%). DNA sequence analysis showed that the HIV-1 gp120 coding sequences were closely related to each other (nucleotide sequence divergence between 3.1% and 6.8%). Phylogenetic tree analysis placed the Indian strains to the C subtype of HIV-1 with closest similarity to sequences originating from Central and South Africa. The HIV-2 sequences were also closely related to each other with an overall sequence divergence between 5.6% and 10.5%. The low level of nucleotide divergence among Indian HIV-1 and HIV-2 sequences suggests a fairly recent introduction of each virus into this population from a single point of entry in each case. The HIV-2 sequences reported here represent the first description of Asian HIV-2 strains and confirm the serological pattern in India detected previously, indicating that a substantial spread of HIV-2, together with HIV-1, has appeared outside Africa in a hitherto naive population for HIV. These findings have major implications for future vaccine and therapy development.

J 108 V3 BINDING PROTEINS OF U937 CELLS,

Toshio Hattori*, Shido Kawase*†, Toshio Murakami*, Takashi Uchiyama*, *Laboratory of AIDS Immunol., Institute for Virus Research, Sakyo-ku, Kyoto, 606, Japan and †Research & Development Center, UNITIKA Ltd., Uji, Kyoto, 611, Japan

The synthetic V3 loop peptides interact with V3 bonding protein (V3BP), the cell surface molecule of mature T4 cells (Molt-4 cells). V3BP is apparently a tetramer of 32-33kDa proteins. The binding study indicated that both macrophages and U937 cells bind biotinylated-V3 loop peptides more efficiently than do normal T or T4 cell line cells (BBA 1181,155, '93). Therefore, we attempted to isolate and characterize V3BP of U937 cells.

The molecular weight of V3BP was defined by precipitating the proteins using peptide-conjugated beads from ^{125}I-labeled U937 cells. The experiment gave the definitive band whose Mr is around 33 kDa. Fifty billions cells were used to isolate the membrane fraction of both U937 and Molt-4 cells by a dextran gradient. The plasma membranes was applied to the peptide-coupled affinity column chromatographies, V3-MN- followed by V3-BH10-column. The purified V3BP from both cells gave the identical bands, whose Mr were 32 and 33 kDa acording to SDS-PAGE. It was also seen that the amount of V3BP of U937 cells was much higher than that of Molt-4 cells. We therfore think that V3BP may play a more important roll on the infection of HIV-1 into macrophages. Further analyses of V3BP may elucidate the mechanism of infection of HIV-1 and lead to new prophylactic strategies for AIDS.

J 109 SYNTHETIC PEPTIDE FROM HIV-1 GP41 INHIBITING HIV-1 INFECTION

Shibo Jiang, Kang Lin, Nathan Strick and A. Robert Neurath
New York Blood Center, New York, NY 10021

Studies on interdomain interactions involved in assembly of some enveloped RNA viruses suggested that peptides from virus envelope glycoproteins may have antiviral activity. We recently demonstrated that a synthetic peptide from HIV-1-IIIB gp41 region (amino acid residues 637-666: EWDREINNYTSLIHSLIEESQNQQEKNEQE *GGC*) effectively inhibited p24 production, cytopathogenic effect (CPE), and cell fusion induced by an array of HIV-1 strains, including IIIB; MN; RF; SF2; V32, a IIIB variant resistant to neutralization by antibodies directed against the IIIB V3 loop of gp120; and those derived from clinical isolates differing in sensitivity to AZT. The peptide (637-666) selectively binds to the fusion domain at the N-terminus of gp41, suggesting that inhibition of HIV-1 infection is caused by blocking fusion of HIV-1 with cells or of infected cells with uninfected cells. Since this peptide has antiviral activity against both homologous and heterologous HIV-1 isolates, and has no detectable cytotoxicity, it offers a novel approach to chemotherapy and prophylaxis of AIDS.

J 110 FUNCTIONAL ROLE OF THE V1/V2 REGION OF HIV-1 ENVELOPE GP120 IN INFECTION OF PRIMARY MACROPHAGES AND SCD4 NEUTRALIZATION. Atsushi Koito, Jay A. Levy, and Cecilia Cheng-Mayer, Cancer Research Institute, Department of Medicine, UCSF, San Francisco, CA 94143.

We have examined the influence of the V1/V2 region of the HIV-1 gp120 on certain biological properties of the virus. We observed that on the genomic background of the T cell line-tropic strain HIV-1SF2mc, both the V1 and V2 domains of the macrophage-tropic strain HIV-1SF162mc, in addition to the required V3 domain are necessary to attain full macrophage tropism. Furthermore, the V2 domain confers sCD4 neutralization resistance and stable gp120/gp41 association to HIV-1 isolates that are initially sensitive to sCD4 neutralization and exhibit an unstable gp120/gp41 association. Structural studies of envelope gp120s of the parental HIV-1SF2mc and HIV-1SF162mc indicate that their conformation is strain-specific. The functional role of the V1/V2 domain in infection of primary macrophages and in sCD4 neutralization resistance is to interact with the V3 domain and confer on the envelope gp120, a conformation that is more characteristic of the macrophage-tropic strain. The structural conformation of envelope gp120, therefore, plays an important role in determining HIV-1 tissue tropism and sensitivity to sCD4 neutralization.

The effect of specific features within the V1/V2 domain, e.g. the relative importance of the N-linked glycosylation sites, on the function of the gp120 will be also presented.

J 111 DIFFERENTIAL EFFECTS OF A MONOCLONAL ANTIBODY TO THE gp120-CD4 COMPLEX ON HIV-INDUCED SYNCYTIUM FORMATION AND VIRAL INFECTIVITY, Krystyna Konopka[a], Elizabeth Pretzer[a], Egilde Seravalli[b], Franco Celada[b] and Nejat Düzgüneş[a,c], [a]Department of Microbiology, University of the Pacific, School of Dentistry, San Francisco, CA 94115; [b]Institute for Molecular Immunology, Hospital for Joint Diseases, New York, New York 10003; and [c]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

A murine monoclonal antibody raised against the soluble rCD4/rgp120 complex (mAb F-55-91) was found previously to inhibit syncytium formation without inhibiting the interaction of CD4 with gp120, and its binding site was localized within the first two domains (D1/D2) of CD4 (F. Celada *et al.*, (1990) *J. Exp. Med.* **172**, 1143-1150). We investigated whether this antibody inhibited the infectivity of HIV-1 in the CD$^+$ T-cell lines, A3.01, Sup-T1, and H9. We also examined the effect of the antibody on syncytium formation between these cells and chronically infected H9 cells. The effect of mAb on HIV-1 infectivity was very limited with A3.01 and Sup-T1 cells, although it inhibited syncytium formation between A3.01 or Sup-T1 and H9/HTLV-IIIB cells. In contrast, the mAb inhibited significantly the infectivity of HIV-1 in H9 cells, but the mAb also inhibited syncytium formation between H9 and chronically infected H9 cells to a greater extent than in the case of the other cell lines. Our results indicate that cellular systems used for syncytium assays differ in their susceptibility to inhibitory antibodies. In the A3.01 and Sup-T1 cell systems, the differences in the ability of the mAb to block viral entry or syncytium formation raise the possibility that the interaction of gp120/gp41 with cell membrane CD4 may be different in cell-cell and virus-cell membrane fusion.

This work was supported by grants AI-25534 and AI-32399 from the National Institute of Allergy and Infectious Diseases, and by grant R91 UP002 from the California Universitywide AIDS Research Program. Additional support was provided by an Aaron Diamond Foundation grant (F. Celada).

J 112 MODULATION OF THE PATHWAY BY WHICH MACROPHAGES TAKE UP HIV-1 DNA FROM APOPTOTIC INFECTED T CELLS AND REGENERATE INFECTIOUS VIRIONS. Richard S. Kornbluth and Douglas D. Richman, Dept. of Medicine and Pathology, Univ. Calif. San Diego, and the San Diego V. A. Medical Center, La Jolla, CA 92093-0679.

CEM T cells infected with the lymphotropic strain HIV-LAI (LAI) undergo apoptotic cell death (J. Clin. Invest. 87:1710, 1991). This process generates cellular debris containing degraded cellular DNA but intact HIV DNA. Scavenging macrophages avidly take up this debris and we have proposed that they become infected by the viral DNA it contains.

To test this hypothesis, we fed apoptotic debris containing radiolabelled DNA to macrophages and analyzed the DNA that survived intracellular digestion. Whereas almost all of the input DNA apoptotic ladder was destroyed inside of the macrophages, a discrete high m.w. form of radiolabelled DNA remained. This suggests that the DNA in the infected apoptotic debris may follow an intracellular pathway which evades hydrolysis.

The macrophages that take up this debris proceed to release infectious virions. Whereas AZT and soluble CD4 effectively blocked infection by HIV-1 BaL virions in macrophages, these agents were at best only partly effective in macrophages infected with debris containing LAI DNA. Conversely, rabbit polyclonal antibody produced against uninfected CEM cells had little effect on BaL infection but completely prevented infection by HIV-infected CEM debris. Pretreatment of macrophages with TNF-α had no effect on BaL replication but markedly enhanced the production of p24 in debris infected cultures (5-20X). These data suggest a distinct cell biology of a non-virion, DNA-mediated pathway for the infection of macrophages by apoptotic debris derived from HIV-1-infected cells.

J 113 ANALYSIS OF GENETIC DIVERSITY IN MATERNALLY-TRANSMITTED SIV-INFECTED MACAQUE NEONATES, Angela Martin-Amedee, Billie Davison-Fairburn, Anita Trichel, Richard Harrison, and Michael Murphey-Corb. Tulane Regional Primate Research Center, Covington, LA 70433.

We have previously reported the development of a non-human primate model for materal-fetal transmission of HIV using macaques infected with SIV/DeltaB670. Three female macaques were inoculated prior to conception or during pregnancy and have given birth to vaginally-delivered SIV-positive infants. A comparison of the disease status during pregnancy and the placental pathology found in these mothers and several other SIV infected macaques has shown that anemia/thrombocytopenia, immunosupression and placental lesions appear to be involved in fetal infection.

To extend this study, we have characterized the genetic diversity in both maternal and neonate viruses. The genetic variants were identified by sequences in hypervariable regions of the SIV envelope gene. These sequences were determined directly from the infected tissue by polymerase chain reaction amplification of this region followed by cloning and dideoxy sequencing.

The females, as expected, had a swarm of genetically distinct variants. Viral clones derived from the spleen, lymph node, thymus, and PBL of the infected neonates represented a subset of the maternal genotypes. In one mother:baby pair, the neonate variants did not reflect those expressed in the mother at delivery.

The selective transfer of genetic variants to the neonate from a swarm of genetically distinct forms in the mother suggests that certain phenotypes (e.g. the ability to replicate in certain cell types) may be another factor promoting infection of the fetus.

J 114 HIV-1 GENOMIC RNA VARIATION IN THE ENV AND GAG GENE FOLLOWING PERINATAL TRANSMISSION. Mulder-Kampinga GA[1], Simonon A[4], Kuiken C[1], Scherpbier HJ[2], Boer K[3], van de Perre P[4], Goudsmit J[1]. Department of Virology[1], Department of Neonatology[2], Department of Obstetrics and Gynecology[3], Academic Medical Center, Amsterdam, The Netherlands. AIDS Reference Laboratory, Kigali, Rwanda[4]

Objective: To investigate the genetic relationship between the virus population in HIV-1 infected mothers and their infants.
Materials and Methods: Sequentially collected serum samples of a mother and her intra-uterine infected child and the first seropositive samples of 4 mother-child pairs from Rwanda, seroconverted 3 to 18 months after delivery, were studied. cDNA was amplified by nested PCR with primers specific for the V3 and p17 encoding region. Ten to 22 clones were sequenced. For 2 of the 4 mother-child pairs from Rwanda, only direct sequencing was performed.
Results: As previously observed for the V3 region (G.A. Mulder-Kampinga et al., J Gen Virol, 1993; 74: 1747-1756), the gag sequence populations observed in the maternal samples collected during the first trimester of pregnancy and at delivery were more heterogeneous than in the cord blood sample (mean intrasample nucleotide variation of 0.7, 0.9 % and 0.4%, respectively), but variation was less than that observed in the V3 region (2.4, 4.2%, and 0.7%, respectively). In both maternal samples one particular gag variant predominated, which was identical to a minority (14%) of the child's sequence population. The predominant variant in the child's sample was not found in the maternal samples and only a minority (6%) of the maternal clones contained the substitution typical for this variant.
In 3 African mother-child pairs, mother and child had an homogeneous clonal (intrasample variation of 0.6%) or direct sequence, as well as an identical consensus or direct sequence. We have studied variation in the gag region in one mother-child pair. The variation in the gag region was less than in the V3 region (0.4% and 0.3% in maternal and child's sample, respectively). However, in the maternal sample, but not in the child's sample, in the gag region an identical substitution was observed in several clones, while in the V3 region only at random variation was observed. In the remaining mother-child pair, both the mother as well as the child harbored a more heterogeneous V3 population with mean intrasample variation of 1.4% and 2.2% respectively and with a mean distance between the maternal and infants consensus sequence of 2.2%.
Conclusions: Infection in the intra-uterine infected child is established by minor maternal variants. The results of the gag sequences suggests that perinatal infection is initiated by a limited number of virions. The transmission of a predominant maternal variant by mothers recently infected and harboring viruses with an homogeneous V3 sequence population, is not surprising since these viruses have "proved" to be transmittable and favored to replicate in the genetic background of the mother which is closely related to that of the child. The heterogeneous an different V3 population observed in one African mother and her child, is probably the result of a fast evolution, resulting in replacement of the original virus population present at the time of transmission.

J 115 NESTED PCR AMPLIFICATION OF PBMC-DERIVED, FULL-LENGTH gp120-gp41 SEQUENCES FROM PRIMARY AND T CELL LINE-ADAPTED ISOLATES OF HIV-1. Daniel S. Nam, Tuofu Zhu, Lin Qi Zhang, Yunzhen Cao, John P. Moore, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY.

Primary isolates of HIV-1, taken from infected patients, exhibit markedly different biologic properties versus laboratory strains (i.e., primary isolates adapted for growth in immortalized T-cell lines). The method reported herein permits the sensitive amplification of large HIV-1 fragments from primary and T-cell cultured samples. A 12-hour, multi-enzyme (i.e., 10) digest, phenol/chloroform purification, ethanol precipitation, and serial dilution of genomic DNA--prior to nested PCR--give routine amplification products spanning in excess of 3.5 Kb. Full-length gp120-gp41 sequences are achieved in a nested PCR format with Hot Tub Polymerase.

We examined two HIV-1 isolates, P17 and C17, for which DNA sequence data indicate four amino acid changes (3 in gp120, 1 in gp41) going from primary to T-cell line cultured derivatives. These two isolates are also notable for the observation that P17, a primary isolate, is highly resistant to CD4 neutralization, whereas C17, its T-cell cultured counterpart, is highly sensitive to CD4 neutralization. We isolated 7 P17 and 8 C17 ssDNA clones with full-length envelopes. Construction of chimeric viruses which contain the complete envelopes with these amino acid changes intact or modified by site-directed mutagenesis is being approached for subsequent functional studies. Using these chimeric viruses, we hope to understand the mechanism of HIV-1 resistance to soluble CD4 and possibly, neutralizing antibodies.

J 116 GP120 STRUCTURE/ FUNCTION STUDIES USING ENZYME-LINKED IMMUNOSORBENT ASSAYS AND MONOCLONAL ANTIBODIES TO THE V3, C2 and CD4 BINDING DOMAIN, Oanh Nguyen, Leonidas Stamatatos and Cecilia Cheng-Mayer, Cancer Research Institute, UCSF, San Francisco, CA 94143-0128. SF2 is a slow replicating, non-syncytium-forming HIV isolate, while SF13 is a faster replicating and more cytopathic variant, isolated from the same patient as SF2 at a later time during HIV infection. We previously reported, based on studies of recombinant and mutant viruses, that the major determinants for viral infectivity and syncytia induction of these isolates mapped to an 159 amino acid (aa) region of gp120, encompassing the V3 loop. Amino acid changes both within the V3 and C2 domains of this region of gp120 can affect these biological properties. We hypothesized that these properties are controlled by the overall structure of gp120, which in turn is affected by interactions between the V3 loop and the C2 region. To evaluate this possibility, we examined by immunochemical approaches the conformation of wild-type and mutant gp120s. The extent of binding of mAbs to the V3 and CD4 binding domains of wild type and mutant gp120s was quantitated by ELISA. Our results indicate that Ab binding to the CD4 binding domain is similar among the different gp120s regardless of whether the mutations are located within the V3 or C2 region. This observation suggests that mutations within these two regions of gp120 do not affect the structure of the CD4 binding domain, and that the binding affinity of the viruses to $CD4^+$ cells is most likely not responsible for differences in infectivity and syncytia forming abilities. However, the binding of mAbs to the V3 loop was altered by mutations introduced within the V3 and C2 regions, suggesting that these mutations affect the conformation of the V3 loop. Such changes could then be responsible for the different biological properties observed. Currently we are investigating by similar immunochemical techniques whether mutations within the V3 loop can affect the structure of the C2 or other regions of gp120.

J 117 SELECTION OF SOLUBLE CD4 (sCD4)-SENSITIVE HIV-1 VIRIONS FROM A sCD4-RESISTANT PRIMARY ISOLATE POPULATION BY PASSAGE THROUGH THE C8166 CELL LINE, Sherry L. Orloff, Claudiu I. Bandea, Gregg M. Orloff, and J. Steven McDougal. Division of HIV/AIDS, Centers for Disease Control, Atlanta, GA 30333.

Fresh HIV-1 isolates obtained from infected people tend to be relatively resistant to the neutralizing/inactivating effects of soluble CD4 (sCD4) compared to laboratory-adapted strains of HIV-1. Primary isolates also tend to readily infect macrophages and normal peripheral blood lymphocytes (PBL) but not continuous T cell lines. Both macrophage tropism and the sCD4 resistant phenotype have been mapped to the V3 region of the envelope glycoprotein gp120 using chimeric virus constructs. The infectious titer of two primary isolates rose progressively with serial passage in PBL (20 passages), but the virus stocks maintained their sCD4-resistant phenotype. However, when passed twice through the continuous T cell line C8166, the emerging virus stock had become sensitive to sCD4. The post-C8166 passage stock did not revert to the sCD4-resistant phenotype by further passage in PBL. Pre- and post-C8166 stocks were biologically cloned by limiting dilution in PBL, recloned, and the clones were tested for sCD4 sensitivity. Thirteen of 16 pre-C8166 clones were sCD4 resistant and 3 were sensitive. Six of 11 post-C8166 clones were sCD4 sensitive and 5 were resistant. Sequence analysis of the clones has been completed for the V3 region of gp120 and is pending for the remainder of the env gene. The data indicate that the V3 region is not the only determinant of sCD4 sensitivity in that some clones with resistant and sensitive phenotypes were found to share the same V3 sequence.

J 118 ANTIGENIC PEPTIDE DISTRIBUTION IN THE GP120 SEQUENCES OF DIVERGENT AFRICAN AND AMERICAN HIV-1 SUBTYPES. **Gary Pestano[1], Alex Spira[2], Karlene Hosford[1], Janice Riley[1], Lawrence Brown[3], Alfred Prince[4] and William Boto[1]. [1]Dept. of Biology, The City College of New York, NY, 10031, [2] Aron Diamond AIDS Research Center, NY 10016, [3] Addiction Research and Treatment Corporation, NY 11201, and [4] NY Blood Center, 10021.**

The envelope glycoprotein of HIV-1 is immunogenic and is a primary focus for vaccine design. The extreme variability noted for the ENV gene in the viral subtypes has thus presented a major obstacle to the development of broadly efficacious vaccines. To assess a possible effect of sequence divergence on the distribution of linear antigenic epitopes, we have analyzed amino acid sequences for gp120 encoded in an American derived subtype R01p, and in two recently described Ugandan HIV-1 isolates, UG06c and UG23c. R01p represents a consensus sequence of PCR clones amplified directly from PBLs recovered from one patient. The region examined extends from the C2 to the V5 domain of gp120. SURFACE PLOT and PEPTIDE STRUCTURE computer analyses were conducted to determine the surface probability profiles of high potential antigenic sites. These data were compared to those from similar analyses of the reference isolates, MN and HXB2R. Analogous antigenic sites were identified in all the isolates tested. The epitope encompassing V5 was apparently the most conserved. Many of the residues previously associated with CD4 binding and broad neutralizing activity were also identified in the linear antigenic sites. Synthetic peptides corresponding to analogous antigenic sites in the isolates were tested in ELISA for reactivity with serum samples obtained from patients in Uganda. Neutralizing antibodies that effectively cross-react with UG06c, UG23c, MN and HXB2R have been previously described in infected individuals from Uganda and New York. These observations suggest that the analysis of potential antigenic sites may yield information of critical value in the formulation of broadly reactive candidate immunogens for divergent HIV-1 subtypes.

J 119 IDENTIFICATION OF AN HIV-1 PROVIRAL GENOME RECOMBINANT BETWEEN SUBTYPE B AND F IN PBMC OBTAINED FROM AN INDIVIDUAL IN BRAZIL, E Sabino, E Shpaer, M Morgado, V Bongertz, S Cavalcante, B Galvao-Castro, J Mullins, RM Hendry, A Mayer. Irwin Meml Blood Ctrs, San Francisco CA USA (ES, AM); Inst Adolfo Lutz, Sao Paulo, Brazil (ES); Inst Oswaldo Cruz, Rio de Janeiro, Brazil (MM, VB, SC); Stanford Univ, Palo Alto CA USA (ES, JM); LASP CP Gonalo Moniz, Ba, Brazil (BG-C); CA Dept Health Svcs, Berkeley CA USA (RMH).

We characterized the V3 and flanking regions of the HIV-1 env gene from 28 Brazilian PBMC samples by sequencing PCR product. Of these 28 individuals, we found that 26 were infected with subtype B, and 1 with subtype F, HIV-1. One sample (RJ IO1) yielded a segment of the C2 region (about 120 nucleotides homologous to positions 6993-7113 of HXB2) that shared with the subgroup F sequence 14 nucleotides (7 of them silent) that are different from all B subgroup sequences. In contrast, this RJ IO1 C2 segment had only 5 nucleotide differences from the F subgroup sequence. Phylogenetic bootstrap analysis of this 120 nucleotide fragment of RJ IO1 and 6 HIV-1 subgroups confirmed that this C2 region of the RJ IO1 sequence belongs to subgroup F, while analysis of the surrounding regions of the env gene showed these regions of RJ IO1 to belong to subgroup B. This indicates the recombinational origin of the RJ IO1 sequence. RJ IO1 sequences from 2 separate PCR reactions both showed the same recombined region, indicating that the observed recombination did not occur during PCR but was rather present in at least 2 proviral genomes in the RJ IO1 PBMC sample. This finding suggests that double infection in vivo can occur with HIV-1 of different subtypes, and that recombination between these 2 subtypes can occur in infected individuals. Further investigation of this unusual recombinant HIV-1 will include analysis of viruses in the individual who infected the patient from whom sample RJ IO1 was obtained.

J 120 COMPARISON OF V3 LOOP EPITOPE MAPPING BY DIFFERENT METHODS. Stephen J. Seligman[1], Karen Sokolowski[2], and Albert T. Profy[2]. 1 SUNY/Health Science Center, Brooklyn, NY 11203; 2 Repligen Corporation, Cambridge, MA 02139.

Various methods have been proposed for the mapping of epitopes. However, criteria for distinguishing among the results of the different methods are unclear. Serial deletion mapping, a method in which competition ELISA assays with series of competing antigens deleted first at the N-terminus and then at the C-terminus, was used to map the epitope recognized by antibody R/V3-59.1, a murine monoclonal antibody raised against a cyclic synthetic peptide corresponding to the V3 loop sequence of HIV-1 MN. Using this method, the epitope was operationally defined as RKRIHIGPGRAFY(T), appreciably longer than the epitope defined by amino acid substitution in a direct ELISA assay, GPGRAF (White-Scharf et al, Virology 192, 197, 1993). In comparison, in a recently determined X-ray crystal structure of the antigen-antibody complex, IGPGRAF was in contact with the FAb fragment (Ghiara et al, submitted). The longer sequence peptide defined by serial deletion mapping was associated with almost 1000 fold greater affinity than the structurally-defined epitope suggesting that the additional amino acids affect the conformation of the contact residues. Consequently the increased size may be functionally significant. Methods of identifying epitopes that maximize antibody activity need to be considered both in studies of pathogenesis and in the design of subunit vaccines.

J 121 ANALYSIS OF TWO CRITICAL DETERMINANTS OF GP41 THAT INFLUENCE HIV-1 FUSION. Diane C. Shugars, Carl T. Wild, Teresa K. Greenwell and Thomas J. Matthews, Center for AIDS Research, Duke Univ. Medical Center, Durham, NC 27710.

Virus-induced membrane fusion is a crucial step in the entry of HIV into susceptible cells. The fusion event is mediated primarily through the transmembrane glycoprotein gp41, following initial interactions between the surface glycoprotein gp120 and CD4. Using synthetic peptides, we have identified two regions within the extra-cellular domain of gp41 that contribute to virus-cell fusion (Proc. Natl. Acad. Sci. USA 89: 10537-10541, 1992; AIDS Human Retroviruses, in press). These peptides correspond to amino acid residues 558-595 (DP-107) and 643-681 (DP-178) of $gp160_{HIV\text{-}1\ LAI}$. Each peptide potently inhibited virus-mediated cell-cell fusion and cell-free infection with several HIV-1 prototypic and primary isolates. To further characterize their physical and biological properties, we have expressed these regions as fragments fused to the C-terminus of the maltose binding protein. The fusion proteins were expressed in bacteria and purified via amylose affinity chromatography. The oligomeric nature of these fusion proteins were characterized by a variety of biophysical techniques, including circular dichroism, FPLC, seive chromatography and analytical centrifugation. The fusion proteins were also evaluated for their ability to inhibit virus-mediated cell-cell fusion and infectivity. These studies provide additional insights into the structural and functional determinants of HIV-1 fusion mediated through gp41. Supported by NIH Grants RO1AI30411 and T32MH15177.

J 122 HIV-1 INFECTION IN PERIPHERAL BLOOD MONOCYTES AND T CELL SUBSETS OF NEONATES AND INFANTS INFECTED BY MATERNAL TRANSMISSION, **John W. Sleasman and Maureen M. Goodenow, Department of Pediatrics, Division of Immunology, Department of Pathology, and the Center for Mammalian Genetics, University of Florida College of Medicine, Gainesville, FL 32610**

Preferential HIV-1 infection of different subsets of peripheral blood mononuclear cells (PBMC) may contribute to more rapid disease progression, a characteristic of infants and neonates infected by maternal transmission. To address this possibility, PBMC from neonates and infants were fractionated using monoclonal antibodies and magnetic microspheres into $CD14^+$ monocytes and $CD4^+$ T cells expressing either CD45RO or CD45RA isoforms of the leukocyte common antigen. Purity of the cell populations was greater than 95% as assessed by flow cytometry. Purity of the $CD14^+$ monopcytes was greater than 99.9%, as determined by an absence of detectable rearrangements in T cell receptor genes using PCR. To detect HIV-1 sequences in cell populations, primers flanking an 800-bp region of *gag/pol* were used to amplify ten-fold serial dilutions of DNA in triplicate reactions. Following gel electrophoresis and Southern blotting, amplified products were analyzed by hybridization of radiolabelled, double-stranded DNA probes. Both CD45RO and CD45RA subpopulations of peripheral T cells were HIV-1 infected in neonates and infants, similar to T lymphocytes from HIV-1 infected adults. Because approximately 90% of T lymphocytes in infants express the CD45RA isoform, this subpopulation of $CD4^+$ T cells represents the predominant HIV-infected T lymphocyte in neonates. In contrast to adults and older children, $CD14^+$ monocytes in the peripheral blood of neonates and infants contain HIV-1 sequences. It is possible that the differences between infants and adults reflect mode of transmission or stages of immunological development. Alternatively, the differences could reflect the acute stage of HIV-1 infection in newborns.

J 123 TRUNCATION OF THE CYTOPLASMIC DOMAIN OF THE SIV ENVELOPE GLYCOPROTEIN ALTERS THE CONFORMATION OF THE EXTERNAL DOMAIN AND INCREASES FUSION ACTIVITY.

Claus Peter Spies*, G.D. Ritter*, M.J. Mulligan*@ and R.W. Compans# Depts. of Microbiology* and Medicine@, Univ. of Alabama at Birmingham, Birmingham, AL 35294 and Dept. of Microbiology and Immunology, #Emory University, Atlanta, GA 30322.

Continuous passage of simian immunodeficiency virus (SIV) in human cell lines has previously been shown to select for the appearance of viruses with envelope glycoproteins whose long cytoplasmic tail of more than 150 amino acids was deleted to less than 20 amino acids. It has also been further shown that viruses with such truncated envelope proteins were attenuated and, unlike the wild-type virus, did not lead to a clinical AIDS-like disease in infected monkeys. We have expressed the glycoproteins of the full-length viral clone SIVmac239 and a truncated form of SIVmac239 using a vaccinia expression system. Our results show that truncation of the cytoplasmic domain of the SIVmac239 envelope glycoprotein enhanced its ability to induce cell fusion in a variety of cell lines. To investigate the basis for the observed differences in cell fusion activity, we examined the expression of the full-length and truncated SIV glycoprotein complexes on cell surfaces. Using a biotinylation assay, we found that under conditions where the full-length TM protein could not be detected on cell surfaces, the truncated TM protein was detected efficiently. In contrast, a cell surface iodination procedure detected the full-length as well as the truncated TM proteins on cell surfaces. No difference between the full-length and truncated proteins was observed in the detection of the SU proteins in the biotinylation assay. We also observed that the truncated TM protein formed more stable SDS-resistant oligomers than the full-length TM protein. These findings indicate that truncation of the cytoplasmic tail of the SIVmac239 envelope glycoprotein affects the conformation of the external domain of the TM protein on the cell surface, even though the two proteins have no differences in the amino acid sequences of their external domains. This altered conformation could play a role in the enhanced fusion activity of the truncated SIV glycoprotein.

J 124 HIV-CELL TROPISM AND sCD4 NEUTRALIZATION ARE REGULATED BY DIFFERENT MECHANISMS, Leonidas Stamatatos Albrecht Werner, and Cecilia Cheng-Mayer, Cancer Research Institute, UCSF, San Francisco, CA 94143-0128. We are examining how the structure of the env gp120, in particular the V3 loop, affects the biological properties of HIV. Recombinant and mutant viruses were constructed and the: tropism, susceptibility to sCD4 neutralization and binding to $CD4^+$ cel's were investigated. The following table summarizes our current results.

Isolate	V3 loop sequence	Tropism	sCD4 Neut. (ID_{50} µg/ml)	KA# to HUT-78
SF2	iYigpgrafHTtgRiigdirKa	T	0.3	19
R19*	-T-------YA--D------Q-	mϕ	10	2.5
Mu1	-T-------YA--D------Q-	mϕ	0.2	15
Mu3	---------YA-- D--------	mϕ	0.6	20
Mu8	---------- D--------	T	0.3	10
SF162	-T-------YA--D------Q-	mϕ	>10	4

Mutant viruses were generated on the background of SF2. # KA: affinity constant values ($x10^{10}$ M^{-1}). * A 160 amino acid (AA) StuI/MstII region of the gp120 (encompassing the V3 loop) of SF2 (T-cell line tropic) was substituted by that of SF162 [macrophage tropic (mϕ)]. Replacement of the StuI/MstII region (R19), the V3 loop (Mu1), three AA within the V3 loop (Mu3) of SF2 by those of SF162 resulted in mϕ viruses. sCD4 neutralization assays indicated that SF2 was more than 100 fold more sensitive to neutralization than the mϕ viruses (SF162 and R19). However, certain macrophage tropic isolates (Mu1 and Mu3), showed similar sensitivity to sCD4 neutralization as SF2, suggesting that tropism and sCD4 neutralization are regulated by different mechanisms. Measurements of the affinity constant (KA) of the various virions towards cellular CD4, indicated that isolates with high affinity (SF2, Mu1, Mu3 and Mu8) are susceptible to sCD4 neutralization. Therefore, it appears that while sCD4 neutralization is related to the binding affinity, tropism is not (Mu1 and Mu3). Experiments designed to quantitate the amounts of nucleocapsid entering into T cells during HIV-cell incubations indicated that post-binding events can regulate the efficiency of entry and subsequently control the cell tropism of HIV.

J 125 MOLECULAR DYNAMIC SIMULATION INDICATE CONFORMATIONAL CHANGES IN Asn^{52} CD4 MUTANT, Zoltán Székely, András Perczel, Adorján Aszalós*, József Molnár and Botond Penke, Department of Medical Chemistry, Albert Szent-Györgyi Medical University, Dóm tér 8. Szeged, H-6720 Hungary, *Food and Drug Administration, Washington D.C. 20204
The normal physiological functions of the CD4 membrane protein in T4 cells are its interaction with MHC class II molecules and delivery of the $p56^{lck}$ kinase to the T-cell receptor. It also serves as primary receptor of the envelope glycoprotein, gp120. Inhibition of CD4-gp120 recognition has been thought as a therapeutic way to stop the infection cascade. One of the most important requirement for such inhibitor molecules is that they should not affect MHC II binding of the CD4 molecule. Binding studies with gp120 and mutations introduced in the CD4 molecule pin-pointed the biologically important epitopes in both of these molecule. These studies and X-ray crystallographic data permitted the formation of a three-dimensional computer model for the CD4 molecule. The $Asn^{52} \rightarrow Asp^{52}$ mutation of CD4 was studied by Teshima et al. (Biochemistry 30:3916, 1991) and was shown reduced capacity for gp120 binding, but with potential preserved structural motif for MHC II binding. We have studied this mutation induced changes on Lys^{46}, which amino acid essential for gp120 binding, by molecular dynamics simulation. Simulation data indicate significant conformational and electronic changes around Lys^{46}. These changes are associated with the reduced binding ability of this mutated CD4 molecule for gp120. These and other computational data dealing with altered gp120 binding properties of the CD4 molecule will be presented.

J 126 INTRA- AND INTERPATIENT SEQUENCE VARIATION IN THE V1 AND V2 HYPERVARIABLE REGIONS OF HIV-1 gp120 Ning Wang, Tuofu Zhu, Yunzhen Cao and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY.
The V1 and V2 regions of HIV-1 gp120 contain complex functional determinants for syncytium inducing capacity, cytotropism and immune recognition. To investigate the associations between genetic diversity in V1 and V2 domains and functional differences in HIV-1, we have analyzed genetic diversity in V1 and V2 domains in 50 HIV-1 isolates from North America, Australia and China. Proviral sequences of uncultured peripheral blood mononuclear cells were amplified by PCR and directly sequenced. It is apparent that sequences in V1 and V2 evolved independently. The V1 domain is more variable than V2 domain, but there is a high proportion of amino-acid substitutions in V2 as well as considerable length variation in a region adjacent to C2. In addition, in several patients, we have been examining the V1 and V2 sequence changes longitudinally. The correlation between genetic variation in V1 and V2 with HIV-1 biological properties and clinical status will be presented and discussed.

J 127 SEROTYPES OF HIV-1 : DEFINITION, DISTRIBUTION AND RELATIONSHIP TO NEUTRALIZATION. Jonathan Weber, Pontiano Kaleebu, Simon Beddows, David Callow and Rachanee Cheingsong-Popov. Dept of Communicable Diseases, St Mary's Hospital Medical School, Praed Street, London W2 1NY, UK.

HIV-1 V3 variants which are immunologically distinct from the typical North American HIV-1 MN strain were identified from the Former Soviet Union, Thailand, and Brazil. The selection and identification of HIV-1 variants were based on pattern of antibody binding to MN V3 peptide in studied subjects. We have investigated the distribution of the HIV-1 variants within these countries using V3 peptides generated from consensus sequences of such variants. There is a close correlation between V3 serotype and sequence data.

The HIV-1 V3 "**ROSTOV/ELYSTA**" variant circulating in the south of Russia is common among children who acquired HIV-1 via parenteral infection. HIV-1 subtype E was identified from HIV-1 infected subjects from Thailand with negative or low antibody binding to MN peptide. HIV-1 ThaiB subtype is associated with high titre of antibody binding to MN and is prevalent among IV drug users.

HIV-1 serotype A is prevalent in Rwanda whereas, both A and D serotypes are prevalent in Uganda. There is a correlation between the Thai HIV-1 V3 serotypes B and E and neutralization of sero-type specific primary isolates by pools of type-specific human antisera. Preliminary data from Uganda between A and D serotypes is still equivocal. Further neutralization serotypings will be presented.

J 128 STUDIES OF HIV-1-MEDIATED MEMBRANE FUSION IN STABLE GP160 EXPRESSING CELL LINES AND USING FLUORESCENT DYES, Carol D. Weiss and Judith M. White. Dept. of Pharmacology, University of California, San Francisco, CA 94143-0450

We are studying several aspects of HIV-1 gp160-mediated fusion by following transfer of fluorescent dyes in stable cell lines that express wild-type and GPI-anchored gp160 (Weiss and White, J. Virol., in press). Using a cytoplasmic dye marker, we monitor fusion activity even in the absence of syncytia. Using a lipid dye to mark the plasma membrane, we dissociate fusion involving cytoplasmic mixing from fusion limited to membrane mixing only (hemi-fusion). Preliminary results indicate that GPI-anchored gp160 mediates mixing between plasma membranes without allowing cytoplasmic contents to mix. Other results using a variety of gp160s and target cells will also be presented.

J 129 A SYNTHETIC PEPTIDE FROM HIV-1 gp41 IS A POTENT INHIBITOR OF VIRUS MEDIATED CELL CELL FUSION. Carl Wild, Teresa Greenwell, and Thomas Matthews, Department of Surgery, Duke University Medical Center, Durham, NC 27710.

Recently, a number of reports have appeared in the literature attributing biological activity to synthetic peptides sharing primary sequence homology with the envelope glycoproteins of HIV-1. Our lab has reported that a synthetic peptide based on the leucuine zipper region of HIV-1 gp41 (DP-107) exhibited both stable solution structure and anti-viral activity. As part of our continuing effort to delineate structure/function relationships within the HIV-1 envelope we have synthesized a second series of gp41 peptides. The prototype of this series, designated DP-178, corresponds to residues 643-678 of the HIV-1 LAI isolate. In our fusion assay this material exhibited unprecedented anti-viral activity with an IC_{50} of approximately 1.7ng/ml (.38nM). Although the site of gp41 from which the peptide is derived is fairly conserved among HIV-1 isolates, there exists some sequence variation within the amino terminal region. However, this limited variability does not seem to affect the anti-viral activity of DP-178 with respect to divergent HIV-1 isolates. The inhibitory activity of DP-178 appears to be virus specific and unrelated to cytotoxic or cytostatic effects and at present we are in the process of determining the extent of this activity. We anticipate that these studies will shed new light on the events associated with virus/cell interaction which are critical for membrane fusion and viral infection.

HIV Pathogenesis I & II

J 200 **PROTEIN-PROTEIN INTERACTIONS IN HIV-1 GAG MUTANTS** B. Agresta, G. Zybarth, and C.A. Carter, Dept. of Microbiology, SUNY, Stony Brook, N.Y. 11794.

Assembly of the Human Immunodeficiency Virus (HIV) involves formation of a transitional non-infectious immature particle that is characterized by a distinct morphology of the assembled Gag proteins. Maturation follows proteolytic processing of the various domains in Gag and the formation of the viral core structure. Our laboratory is engaged in several projects aimed at elucidating protein-protein interactions involved in the assembly of both immature and mature structures. To identify interacting regions in Gag, a test system based on reconstitution of the activity of the yeast GAL4 transcriptional activator is being used (Fields and Song Nature 340:245-246). In this assay, protein-protein interaction is indicated by GAL4 promoter-dependent expression of beta-galactosidase. We and others (Luban et. al. J. Virol 66:5157-5160.) have obtained signals reflecting dimerization of the Gag polyprotein. To determine which domains are involved in this interaction, mutant Gag proteins truncated at the N- or C-terminal domains, or both, were tested. The truncated proteins had the ability to dimerize almost as efficiently as the wild-type Gag protein. The capsid domain alone, however, did not give a dimerization signal. Our results indicate that the regions involved in Gag dimerization do not lie in a single domain; rather they are located throughout the Gag protein.

J 201 **Role of HIV-1 Non-structural Genes *in vivo***
Grace. M. Aldrovandi, Beth D. Jamieson, Lianying Gao, Greg Bristol, Lillian Block, Jerome A. Zack.

Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA .

We have recently developed the SCID-hu mouse as an *in vivo* model in which to study HIV-I induced pathology (Aldrovandi, et al Nature, 363, 732-736, 1993). This animal model is constructed by surgical implantation of human fetal thymus and liver into mice which genetically lack an immune system. These thymus/liver implants form a cojoint organ which, histologically and immunologically, resembles human thymus. When directly infected with various strains of HIV-1 these Thy/Liv implants show severe depletion of all CD4 bearing cells and histologic changes similar to those described in the thymuses of HIV-1 infected persons. In this system high viral load correlates with thymocyte depletion. Recent studies have demonstrated that *nef* mutants replicate more slowly in this system and have attenuated pathogenic properties (see abstract by Jamieson et al). We have constructed a series of HIV *nef* mutants and are assaying them in this system in order to localize the region responsible for this replicative difference. Additionally, we are currently testing deletion mutants of *vif, vpr,* and *vpu* in SCID-hu mice to determine the effects of these mutations on pathogenicity . These studies will not only assist in determining the *in vivo* role of these genes, but may also be useful in the development of attenuated HIV strains for potential use in vaccines.

J 202 CYSTEINE RESIDUES IN THE CYTOPLASMIC TAIL OF CD4 REQUIRED FOR ASSOCIATION WITH $p56^{lck}$ ARE NOT REQUIRED FOR DOWN-REGULATION BY HIV-1 NEF, Stephen J.Anderson and J. Victor Garcia, Dept. of Virology & Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38101.

HIV-1 Nef down-regulates surface expression of both murine and human CD4. We recently reported that the cytoplasmic tail of CD4 is required for down-regulation by Nef, raising the possibility that Nef competes with $p56^{lck}$ for binding to CD4. Thus, we have used a series of murine CD4 mutants to map the region of the cytoplasmic tail of CD4 required for down-regulation by Nef. These CD4 mutants were previously characterized for their ability to associate with $p56^{lck}$ (Turner et al., Cell 60:755-65, 1990). Mutants T1, T2, and T3 are truncation mutants lacking the C-terminal 34, 23, and 10 amino acids of the cytoplasmic tail of murine CD4, respectively. In addition, we tested the mutant MCA1/2, in which the two cysteine residues critical for $p56^{lck}$ association were substituted with alanines, and D412, which has a deletion of 18 amino acids within the membrane-proximal portion of the cytoplasmic tail. Of these, only CD4-T3 was able to associate with $p56^{lck}$. NIH3T3TK$^-$ cells expressing the CD4 mutants were transduced with a retroviral vector conferring Nef expression, and analyzed for surface CD4 levels by FACS, and for the presence of Nef by Western blotting. Of the CD4 mutants described above, only T3 and MCA1/2 showed significant down-regulation by Nef. All other CD4 mutants were unaffected despite similar levels of Nef expression in all the cells. Thus, a nearly full-length cytoplasmic tail is required for the down-regulation of CD4 by Nef. However, the cysteine residues which are critical for the association of CD4 with $p56^{lck}$ are not required. These results indicate that while the domain of CD4 required for its down-regulation by Nef may overlap the $p56^{lck}$ binding domain, the specific residues involved in the interaction between Nef and CD4 are not the same as those required for $p56^{lck}$-CD4 association. Re-distribution of $p56^{lck}$ in T cells expressing Nef could have a significant effect on the utilization of signal transduction pathways in response to surface receptor-mediated stimulation.

J 203 HUMAN FETAL ASTROCYTES AS A MODEL OF HIV-1 LATENCY AND REACTIVATION IN THE CENTRAL NERVOUS SYSTEM, Atwood, W.J., Tornatore, C.S., Conant, K.E., Traub, R., and E.O. Major, National Institute of Neurological Disorder and Stroke, National Institutes of Health, Bethesda, MD 20892

One third of all vertically transmitted pediatric AIDS patients develop neurological complications associated with HIV-1 infection in the central nervous system (CNS). The predominant cell types infected with HIV-1 in the CNS are macrophages and microglial cells. Recently we have demonstrated HIV-1 infected astrocytes in autopsy tissue from pediatric AIDS patients with well documented HIV-1 associated dementia. In vitro, HIV-1 infection of human fetal astrocytes results in an initial burst of virus production which is followed by a long term persistent phase during which neither virus nor viral mRNA can be detected. This demonstrated latency in vitro has been proposed to account for the difficulty in detecting HIV-1 infected astrocytes in vivo. Treatment of the persistently infected cells with TNF-α and Il-1β results in reactivation of HIV-1 gene expression which leads to further virus production. To examine the molecular basis for the induction of HIV-1 gene expression in human fetal astrocytes we transfected the cells with a molecular construct containing the reporter gene chloramphenicol acetyl-transferase (CAT) under the control of the HIV-1 5'-LTR. Treatment of the transfected cells with TNF-α and Il-1β resulted in a 5.6 and 4.4 fold increase in CAT activity over untreated cells. In addition treatment of human fetal astrocytes with TNF-α resulted in the induction of NFkB (p50/p65) in these cells. These results suggest that reactivation of HIV-1 gene expression in human fetal astrocytes may proceed in a similar manner to reactivation of HIV-1 in cells derived from the immune system.

J 204 Characterization of genetic and biologic features of HIV-1 in vertical transmission.

Velpandi,A., Ugen,K.E., Fernandes,L., Goedert,J.J*., Williams,W.V. and Weiner,D.B. Department of Pathology, IBAMM, University of Pennsylvania, Philadelphia, PA-19104; * NCI, Bethesda, MD.

To study the evolution of genomic diversity of HIV-1 in vertical transmission, genomic DNA was isolated from the peripheral blood lymphocytes of two infected women with different transmission status. The samples were collected during the third trimester of their pregnancy. Sixty four clones (43 from the transmitter and 21 from the non-transmitter) were sequenced in a portion of envelope gene including the principal neutralization domain (PND). The sequence analysis reveals that the transmitter has a single major population and limited number of sub groups. In contrast the sequence analysis in the non-transmitter demonstrates that half of the clones (10/21) are 100% identical. The intra patient variation in transmitter and in non-transmitter viral sequences is 12 and 36% respectively, where as the interpatient variation is 23%. The molecular differences observed should impact on the immunological repertoire. We there in analyzed the sera from the maternal samples against a variety of V3 peptides from known HIV-1 isolates and observed that (i) both the transmitter and non-transmitter sera showed more binding towards the V3 peptides based on SF-2 and MN sequences; (ii) the vertical transmission of HIV-1 is correlated with the absence of high maternal antibodies to the PND (Transmitter); (iii) the high affinity binding of the sera with SF-2 and MN V3 peptides are well in correlation with the sequence analysis data, which shows that the V3 sequences from both patients are closely related to SF-2(85%) and decreases in the order of MN(81%), IIIB(79%) and Z6(64%). The biologic characteristics of these viruses were analyzed by infecting a number of established T-tropic and M-tropic cell lines and the results show that the viral isolate from transmitter infect a number of T-cell lines as cell free virus where as the non-transmitter virus does not infect even the primary lymphocytes by cocultivation. These results have important implications for understanding the establishment of infection and transmission of HIV-1 from mother to infant.

J 205 RESISTANCE TO AIDS IN CHIMPANZEES IS CORELATED WITH THE LACK OF GP120-INDUCED ANERGY AND PRIMING FOR APOPTOSIS. Nirmal Kumar Banda, Annegret Dunlap, William Satterfield and Terri Helman Finkel, NJCIRM, Denver, CO. 80206

Human Immunodeficiency Virus type -1 (HIV-1) infects both chimpanzees and humans, but while humans develop Acquired Immunodeficiency Syndrome (AIDS) associated with qualitative and quantitative defects in $CD4^+$ T lymphocytes, chimpanzees resist progression to disease. Our previous findings showed that crosslinking of bound gp120 on $CD4^+$ T lymphocytes followed by stimulation through the antigen receptor results in activation-dependent cell death known as apoptosis or programmed cell death. It is possible that this mechanism is responsible for the progressive CD4 cell elimination in HIV-1 infected patients. We asked whether such a mechanism is operational in chimpanzees, since HIV-1 infection in chimps is neither followed by elimination of $CD4^+$ T cells nor results in an AIDS like disease. Specifically we predicted that while $CD4^+$ T cells from HIV negative humans undergo apoptosis inresponse to CD4 ligation and subsequent activation, cells from HIV negative chimps would not. Our studies in chimps show that lack of apoptosis and anergy may indeed protect chimpanzees from AIDS like disease. Here we report that chimpanzee $CD4^+$ T cells, in contrast to humans, do not undergo apoptosis or becomes anergic after they are primed either by anti-CD4 antibody or recombinant gp120 and subsequently activated through the antigen receptor. We have also compared the signal transduction mechanisms used by chimpanzee and human T cells, specifically $P56^{lck}$ activation and intracellular $[Ca2^+]i$ mobilization in order to understand the biochemical basis for the differential response to CD4 ligation and subsequent activation. Thus, it is quite possible that chimpanzees escape AIDS- like disease due to the absence of apoptosis and cellular anergy of $CD4^+$ T cells seen in human AIDS.

J 206 CHIMERIC SIVs CARRYING THE U3 REGION AND NEF GENE OF HIV: TOOLS FOR THE *IN VIVO* ANALYSIS OF HIV LTR AND NEF FUNCTION, Peter Barbosa and Mark B. Feinberg, Gladstone Institute of Virology and Immunology, PO Box 419100, San Francisco, CA 94141

The role of nef in the HIV life cycle and viral pathogenesis remains controversial, but it appears to be critical for the development of HIV and SIV disease. The *nef* gene of the pathogenic clone SIVmac239 has been shown to be required for disease development in rhesus monkeys. It has been demonstrated that the nef protein product of both HIV and SIV down regulates cell surface expression of CD4. HIV nef promotes viral replication in primary cells by enhancing the infectivity of viral particles. In order to study the role of HIV nef in a context that would allow *in vivo* analysis, we have generated a series of chimeric constructs which contain the HIV *nef* gene and the U3 regulatory region, in the context of the pathogenic molecular clone SIVmac239. Three constructs have been generated: 1- SIVmac239 containing the sequence for SIV *nef* in which 3 point mutations have been introduced, causing 2 premature transcriptional stop codons and destroying the natural AUG initiation codon for the SIV *nef* gene, 2- SIVmac239 in which the region spanning the SIV *nef* gene and the U3 regulatory region containing the NF-kB and SP1 binding sites have been substituted for the respective region from HIV, and, 3- An identical construct as #2 above, in which the NF-kB sites have been destroyed. Viral stocks from these constructs were generated by electroporation of CEMx174 cells, and a kinetic analysis of viral replication was performed. It has been shown that the first two constructs above show similar kinetics as SIVmm239 when analyzed in human PBLs, CEM and CEMx174 cells. The NF-kB mutant virus displays significantly delayed replication kinetics in cells known to express substantial levels of activated NF-kB. The viral constructs described above represent a system that will allow *in vivo* analysis of the role of the HIV LTR and nef gene product in viral pathogenesis, the analysis of the *in vivo* activity of diverse naturally occurring *nef* alleles and the functional evaluation of site directed mutants of the HIV LTR and *nef* gene.

J 207 HIV-2 INFECTION OF BABOONS: AN ANIMAL MODEL FOR VACCINE DEVELOPMENT AND STUDIES OF HIV PATHOGENESIS. Susan W. Barnett, Krishna Murthy*, David J. Blackbourn, and Jay A. Levy. Cancer Research Institute, School of Medicine, University of California, San Francisco, CA, 94143, and *Southwest Foundation for Biomedical Research, San Antonio, Texas 78228.

Baboons were inoculated intravenously with approximately 5000 human $TCID_{50}$ of two different HIV-2 strains (UC2 and UC14) which were previously found to grow efficiently *in vitro* in baboon PBMC. In the first experiment, 4/4 animals inoculated with UC2 seroconverted within two weeks, and three of these continue to exhibit an HIV-2 specific antibody response for almost two years. Western blot analysis revealed the presence of antibodies to all the major viral proteins in these animals. Virus was recovered from the PBMC of all four animals at two weeks, and from two of the animals continuously thereafter, and from a third animal, intermittently thereafter. All infected animals demonstrated lymphadenopathy which was associated with a mixed cellular hyperplasia when lymph node biopsies were examined. This condition lasted for 20 to 28 weeks in these animals. In a second experiment, 3/3 baboons inoculated with the UC14 strain seroconverted at 2-4 weeks and continue to show high antibody titers and Western blot reactivity to all viral proteins. These UC14-infected animals exhibited positive virus cultures from week two and then intermittently for at least eight months. Two of these baboons have shown evidence of plasma viremia, and one of them is exhibiting a decline in CD4+ T-cells after eight months. A continuous loss of CD4+ lymphocytes also has been observed after three years in one additional baboon which has been persistently infected with UC2 for six years. Neutralizing antibodies to the autologous strains were found, and CD8+ cell-mediated antiviral activity also was detected in these animals. HIV-2 infection of baboons provides a good animal model for the study of HIV persistence and possibly, pathogenesis. This model will be helpful in the future for testing antiviral and vaccine strategies.

J 208 IDENTIFICATION OF MIXED HIV-1 AND HIV-2 INFECTIONS IN SOUTHERN INDIA, Padma Baskar, Ratna Rao, Thomas Quinn, James E.K. Hildreth and Robert C. Bollinger, Department of Pharmacology and Molecular Sciences and Department of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Sera from HIV positive patients were analyzed by ELISA and Western blot. The results indicated that 24/50 were reactive to HIV-1 while 1/50 were reactive to HIV-2. Moreover 3/50 were reactive to both HIV-1 and HIV-2. To genetically define the HIV-1 strain within the population, we analyzed peripheral blood mononuclear cells from selected HIV-1 seropositive patients for the presence of HIV-1 proviral DNA using the nested polymerase chain reaction. Different PND primer sets were used for the amplification and detection of viral sequences from the envelope regions. Our analysis of PCR amplified envelope suggests that the HIV sequence from these patients are homologous to North American strains. This is in contrast to previous reports which suggest that strains from India are highly divergent from North American and African strains. Studies are underway to determine the strains of HIV-2. Implications of these studies in the development of vaccine strategies will be discussed.

J 209 Targeting of HIV-1 Nef to the Plasma Membrane induces Activation of T Cells, Andreas S. Baur[1], Earl T. Sawai[2], Cecilia Cheng-Mayer[2] and B. Matija Peterlin[1]. [1]Howard Hughes Medical Institute, Departments of Medicine, Microbiology and Immunology, University of California, San Francisco; [2]Cancer Research Institute, University of California, San Francisco.

To study the function of the nef gene product of HIV-1 we created a fusion protein between the extracellular and transmembrane domains of CD8 and the full-length Nef protein of HIV-1SF2. This chimeric protein was expressed stably in Jurkat cells. All T cell clones obtained expressed the CD8Nef chimeric protein predominantely intracellularly. Upon signaling through the T cell receptor these cells showed a strong inhibition of the tyrosine kinase pathway and the Ca-flux. In order to study effects of Nef when targeted to the plasma membrane where it is required for its function, cells with a high surface exprssion of CD8Nef were obtained by FACS sorting. High levels of the hybrid CD8-Nef protein, but not of the truncated CD8 alone, at the plasma membrane of these cells led to T cell activation as defined by the induction of tyrosine phosphorylation and expression of CD69 and IL-2R. Furthermore, as prolonged T cell activation leads to cell death in Jurkat cells most the cells selected for high surface expression of CD8Nef died within 3 weeks in culture. After 4-6 weeks however a cell clone grew out with mutated *nef* sequences resulting in a truncated Nef protein. These truncations closely resemble those observed with some HIV-1 and SIV strains after passage in T cell lines. Thus, expression of Nef, anchored at the inner plasma membrane appears to activate T cells.

J 210 THE MODULATORY ROLE OF DEFECTIVE PARTICLES IN THE PROCESS OF HIV-1 REPLICATION, Richard Bernier and Michel Tremblay, Infectiologie, Centre de Recherche du CHUL, Département de Microbiologie, Université Laval, Québec, Canada, G1V 4G2

Studies with RNA viruses have indicated that defective particles can modulate *in vitro* replication of fully-infectious viruses. The goal of this study was to evaluate whether defective viruses could influence replication of infectious HIV-1 particles *in vitro*.

In order to determine if such a phenomenon is occuring with HIV-1, a CD4-positive T-cell line (Jurkat E6-1) was transfected with pHXB2-D, an infectious molecular clone of HIV-1. At days 4, 7, 10 and 14 post-transfection fully-infectious viruses released into culture supernatant were harvested. Thereafter, we have co-infected a lymphoid T-cell line, as well as PHA-stimulated peripheral blood mononuclear cells (PBMC's) isolated from healthy donors, with infectious viruses generated by transfected cells and with various amounts of defective HIV-1 particles. Such defective particles consist of viruses devoid of both mature reverse transcriptase (p66) and of integrase (p33) proteins. The extent of virus replication was monitored by measuring reverse transcriptase activity into culture supernatant.

Co-infection experiments using both infectious and defective virus particles led to a significant inhibition (98%) of virus replication. A single addition of defective particles was sufficient to induce such marked decrease in virus production.

Results from these experiments have demonstrated that defective particles can negatively affect replication of infectious viruses. We have further determined that this effect is neither associated with a modulation of CD4 nor with a diminished cellular proliferation. These data may reveal great clinical implications, considering the fact that numerous studies have demonstrated a high frequency of defective genomes in HIV-1 infected individuals.

J 211 MONITORING LYMPHOMAGENESIS AND EBRV ASSOCIATION IN SIV IMMUNOSUPPRESSED MONKEYS.

Peter Biberfeld*, Ephata E. Kaaya*, Su-ling Li*, Hans Feichtinger#, Susanna Rezikyan*, Marianne Ekman*, Ingemar Ernberg**, Per Putkonen@, Gunnel Biberfeld@., *Immunopathology Lab., Karolinska Institute, **Department of Virology and @Department of Immunology, Swedish Institute for Infectious Disease Control, Stockholm, Sweden, and #Institute of Pathology, Univ. of Innsbruck, Austria.

Objectives: To monitor development of lymphomagenic clones and EBV-infection in SIV immunosuppressed cynomolgus monkeys (Macaca fascicularis).

Material and Methods: Monkeys which after infection with SIVsm (SMM3) developed malignant lymphomas (21/51) were studied for clinical signs of immunodeficiency and biopsies examined for B-cell clones and EBRV association by immunohistopathology, PCR, Southern blot (SB), in situ hybridization, electron microscopy and cell culture.

Results and Conclusions. Lymphoma induction time varied from 5 to 46 months. All monkeys with lymphoma were markedly immunosuppressed. Most lymphomas were primarily extranodal including CNS, of high-grade malignant histology, of B-cell origin and associated with a B-lymphotropic herpes virus related to human EBV ($EBRV_{Mf}$). $EBRV_{Mf}$ showed considerable restriction map homology with EBV in the EBNA1 and 2 regions but less within the IR1, the EBER and the ori-p regions. By in situ hybridization for EBER-RNA, EBRV associated lymphoma cells could be detected before clinical lymphoma manifestation which was also indicated by results of DNA-PAGE of the VDJ immunoglobulin heavy chain region in individual lymphoma clones.

These observations indicate a marked similarity of lymphomas in SIV immunosuppressed monkeys with human AIDS-lymphomas associated with EBV and the possibility of early diagnosis of incipient AIDS-lymphomas.

Supported by Swedish Medical Research Council, SAREC, ICSC World Laboratory project MCD2/2, and Royal Swedish Academy of Sciences.

J 212 PCR CLONING OF FELINE IMMUNODEFICIENCY VIRUS (FIV) SUBGENOMIC FRAGMENTS CONTAINING FIV ENVELOPE (*ENV*) GENES FROM TISSUES OF FIV-INFECTED CATS, L. Bigornia, N.C. Pedersen and E.E. Sparger, Department of Medicine, School of Veterinary Medicine, University of California at Davis, Davis, CA 95616

Nested-primer polymerase chain reaction (PCR) was used to molecularly clone a 3.4 kb fragment of feline immunodeficiency virus (FIV) proviral DNA from tissues of FIV-Petaluma^{PBMC}-infected cats. This subgenomic fragment consists of the *env* gene, the 3" half of *vif*, *orf-2* and *orf-L*. The *env*-containing fragments were amplified from the lymph node and PBMC of asymptomatic, chronically infected, CD4 T-cell-depleted cats 5000 and 2429, respectively, and thymus and tonsil of acutely infected, viremic cats 5127 and 5126 (also CD4 T-cell-depleted), respectively. These fragments were cloned into plasmid vectors using restriction enzyme sites contained within the PCR primers and DNA fragment. Plasmid clone 39 contains the *env* fragment isolated from a lymph node taken from a cat (#5000) chronically infected with Petaluma^{PBMC}-FIV, whereas clone 117 contains an *env* fragment isolated from tonsil harvested from a cat (#5127) during the primary stage of infection with Petaluma^{PBMC}-FIV. Preliminary nucleotide and amino acid sequence within the hypervariable region-4 of *env* indicate that clone 39 is unique yet shares some similarities to FIV-Petaluma clone FIV-14 (derived from FIV-Petaluma^{CrFK}), FIV-PPR (FIV-pPPR) and clone 19K1, an FIV isolate from the Netherlands. Nucleotide and amino acid sequence within the hypervariable regions of these *env* genes from tissues harvested from acutely and chronically Petaluma^{PBMC}-FIV-infected cats will be compared. These clones will be used to construct recombinant viruses with other molecular clones of FIV and used in pathogenesis studies to examine *env* determinants of CD4 T-cell depletion in FIV-infected cats.

J 213 IDENTIFICATION OF SPECIFIC BEL-1 RESIDUES INVOLVED IN TRANSCRIPTIONAL ACTIVATION USING A NOVEL MUTAGENESIS SELECTION SCHEME IN YEAST, Wade S. Blair, Hal P. Bogerd, and Bryan R. Cullen, Howard Hughes Medical Institute, Duke University, Durham, NC 27710

Human foamy virus (HFV) like other complex retroviruses, including Human immunodeficiency virus type 1 (HIV-1) and Human T-cell leukemia virus type 1 (HTLV-1), encodes a potent transcriptional *trans*-activator (Bel-1) of its cognate long terminal repeat (LTR) promoter element. As with other transcriptional activators, Bel-1 sequences have been delineated into functional domains which direct DNA target sequence specificity or regulate transcriptional activation potential. Sequences encoding transcriptional activation potential correspond to the carboxy-terminal region of the Bel-1 protein and exhibit transcriptional activation function in *Saccharomyces cerevisiae* when targeted to the upstream activating sequences (UAS_G) of the GAL1 promoter by fusion to the GAL4 DNA binding domain. Although Bel-1 activation domain(s) have been identified, specific residues involved in transcriptional activation have not. We demonstrate that expression from a strong promoter of the Bel-1 activation domain (residues 260 - 300) fused in frame to the GAL4 DNA binding domain in yeast results in host-cell growth toxicity which is dependent on Bel-1 transcriptional activation function. We have utilized this transcriptional activation domain-dependent yeast cell toxicity to develop a novel screen for mutants of the Bel-1 activation domain. Using such a scheme, we have isolated several point mutations in the Bel-1 activation domain, which result in the reduction or loss of Bel-1 activation potential in both yeast and mammalian cells. This selection scheme, which harnesses the powerful genetic tools available in the yeast system to identify specific residues important for transcriptional activation in mammalian cells, should have general utility.

J 214 EXPRESSION OF COSTIMULATORY MOLECULE CD28 ON T CELLS IN HIV-1 INFECTION, Jan E. Brinchmann, Jan H. Dobloug, Bernt H. Heger, Lise L. Haaheim, Mette Sannes and Torstein Egeland, Institute of Transplantation Immunology, Rikshospitalet National Hospital, Department of Infectious Diseases and Life Insurance Companies' Institute of Medical Statistics, Ullevål Hospital, Oslo, Norway.

In order to study the functional integrity of T cells from HIV-1 infected individuals, $CD4^+$ and $CD8^+$ T cells were examined for proliferation and secretion of IL-2 in response to staphylococcal superantigens and antibodies to CD3 and the $\alpha\beta$T cell receptor. A functional defect was observed within the $CD8^+$, but not within the $CD4^+$ T cells from HIV infected individuals. Within $CD8^+$ T cells, proliferation and secretion of IL-2 was restricted to the cells expressing the costimulatory molecule CD28. In patients with advanced immunodeficiency, however, evidence of a functional defect was found also within the $CD28^+$ $CD8^+$ T cells.

In a cross-sectional study involving 73 HIV-1 infected individuals and 15 seronegative controls, a highly significant inverse correlation was observed between expression of CD28 on both $CD8^+$ and $CD4^+$ T cells and clinical stage. The absolute number of $CD28^+$ $CD8^+$ T cells were similar for seronegative controls and asymptomatic HIV-1 infected individuals (CDC II/III), but greatly reduced in patients with HIV related disease (CDC IV). Thus, the expansion of $CD8^+$ T cells observed in the majority of HIV-1 infected individuals could be explained entirely by an expansion of $CD28^-$ $CD8^+$ T cells. A highly significant correlation was observed between the absolute number of $CD28^+$ $CD8^+$ T cells and the absolute $CD4^+$ T cell count.

The results suggest that regulation of expression of CD28 plays an important part in the immunopathogenesis of AIDS.

J 215 PATHOGENESIS OF SYNCYTIUM-INDUCING AND NON-SYNCYTIUM-INDUCING HIV-1 ISOLATES AND MOLECULAR CLONES IN SCID-hu MICE David Camerini, Beth D. Jamieson, Jerome A. Zack and Irvin S.Y. Chen, Departments of Microbiology & Immunology and Medicine, UCLA School of Medicine, Los Angeles, California 90024

We are using the severe combined immunodeficient mouse, implanted with human fetal thymus and liver (SCID-hu mouse), to characterize the pathogenesis of syncytium inducing (SI) and non-syncytium-inducing (NSI) isolates of HIV-1. This model system recapitulates acute HIV-1 infection of the thymus in patients. The pathogenesis of sequential SI and NSI HIV-1 patient isolates, obtained from Dr. David Ho (Connor, R. et al, J. Virology 67,1772), and molecular clones of HIV-1 was assessed by direct injection into conjoint human thymus/liver organs established in SCID mice. Flow cytometric analysis indicated that all 15 thymus/liver implants infected with the SI molecular clone, $HIV\text{-}1_{NL4\text{-}3}$, showed severe depletion of the predominant CD4+, CD8+, CD45RO+ thymocyte subset, while only 4 of 13 implants infected with the NSI molecular clone, $HIV\text{-}1_{JR\text{-}CSF}$, were depleted of this population. The mature CD4+, CD8-, CD45RA+ subset was depleted in all implants infected with $HIV\text{-}1_{NL4\text{-}3}$, and was also depleted in four of the $HIV\text{-}1_{JR\text{-}CSF}$ infected implants. Similarly, 3 of 6 thymus/liver implants infected with SI patient isolates from two patients tested showed depletion of immature and mature CD4-bearing thymocytes while only 1 of 6 implants infected with NSI isolates from the same patients were depleted of CD4+ thymocytes by six weeks post-infection. More than 10% of the remaining CD4+ cells in these patient SI isolate infected implants expressed the HIV-1 envelope glycoprotein, gp120. We are currently using quantitative PCR of HIV-1 DNA in infected implants to determine the viral load achieved in each infection. Our experimental infections of SCID-hu mice suggest a significant difference in the pathogenic potential of SI and NSI isolates of HIV-1.

J 216 BIOLOGIC CHARACTERIZATION OF HIV-1 FROM ACUTE SEROCONVERTORS. Yunzhen Cao and David D. Ho. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY.

In a cohort of acute seroconvertors with symptomatic primary infection syndrome, we have measured the viral load of HIV-1 in PBMC and plasma longitudinally using a number of quantitative techniques (p24, end-point-dilution cultures, quantitative PCR, bDNA assay, etc.). High levels of HIV-1 viremia followed by rapid decline were consistently found. The host immune responses that temporally correlate with the declining viremia are presently under investigation in our laboratory.

Each of the viral isolates obtained from acute seroconvertors has also been characterized biologically in vitro. Thus far, a homogeneous phenotype has been found. Every isolate is capable of efficient replication in macrophages but not in T-cell lines, and all isolates are non-syncytium including in MT2 cells. However, significant variations in replication kinetics have been found for the isolates. Better characterization of the early virus in acute seroconvertors is important for understanding the transmission of HIV-1.

J 217 ROLE OF NF-IL6 IN PROVIRAL ACTIVATION OF HIV-1 BY INTERLEUKIN-6

Benjamin K. Chen, Kalle Saksela, Christopher Roman, and David Baltimore, The Rockefeller University, New York, NY 10021

Elevated serum levels of interleukin 6 (IL-6) have been documented in some HIV-1 infected patients. IL-6 has also been shown to upregulate HIV production during both acute and chronic infection *in vitro*. NF-IL6 (C/EBP-β, LAP) is a bZIP-containing C/EBP family transcription factor which can be induced by IL-6 in certain cell types.

To investigate whether NF-IL6 plays a role in IL-6-mediated activation of HIV-1, we have transduced a neomycin-selectable construct encoding LIP, a dominant negatively-acting alternative translational product of rat NF-IL6 (LAP), into the U1 cell line. This cell line is a chronically HIV-infected line derived from the promonocytic cell line U937, and displays very low basal expression of HIV, yet can be induced to produce significant levels of infectious virus when exposed to cytokines such as IL-6 or TNF-α. The effects of TNF-α are believed to be mediated largely via two tandem binding sites for the transcription factor NF-κB in the HIV-1 LTR element, whereas the mechanisms of transcriptional activation by IL-6 are poorly understood.

In accordance with previous reports, when U1 cells stably expressing neomycin-resistance alone (U1/neo) were stimulated with either IL-6 or TNF-α a large increase in supernatant p24 was observed. However, when stably LIP-expressing cells (U1/LIP) were similarly treated, virus induction by IL-6 was significantly (10-fold) reduced, whereas only a very small reduction (less than 2-fold) in the viral activation by TNF-α was observed. Selective inhibition of the IL-6-mediated virus induction by the LIP protein suggests that NF-IL6 or some closely related transcription factor is necessary for IL-6 induction of HIV.

We have further explored the mechanism by which NF-IL6 may affect HIV viral production by testing the ability of NF-IL6 to transactivate HIV-1 LTR reporter gene constructs in transient cotransfection assays. We have found that overexpression of NF-IL6 in U937 cells induces a powerful (10- to 30-fold) activation of the HIV LTR. We have mapped the promoter elements necessary for this trans activation to a site which overlaps with the Sp1 binding sites. These results suggest that the Sp1 sites, in addition to being important basal promotor elements, may also mediate IL-6 induced expression of HIV.

J 218 EFFECT OF HIV-1 *NEF* DURING SINGLE CYCLE REPLICATION. *Michal Y. Chowers, Celsa A. Spina, T. Jesse Kwoh, Douglas D. Richman, and John C. Guatelli. University of California and VA Medical Center, San Diego, CA 92093-0679*

Nef has been shown recently to be important for optimal replication of HIV. Involvement of the *nef* gene in the virus replication cycle has been assessed in this study. Comparison of *nef+* and *nef-* viruses in a single replicative cycle was achieved by adding viral neutralizing Ab after high multiplicity infection of CEM cells. Nef- virus produced 3 fold lower levels of virus p24 antigen in these single cycle infections.

In a complementary experiment, CEM cells were transfected with nef+ and nef- viral DNA. Neutralizing Ab was added after transfection to prevent secondary rounds of infection. p24 production by nef+ and nef- constructs was equal 48 hours after transfection . The different result obtained by transfection in comparison to infection suggested that nef had a positive effect on early events of the viral replicative cycle. Specifically, these data implied a positive effect of nef on infectivity rather than on gene expression from an established provirus.

To study the nef effect on viral infectivity, end point titration of nef+ and nef- viruses was performed. Equal amounts of virus, based on p24 quantitation, were titered. Viruses derived from supernatants of both acutely-transfected cells and from chronically-infected cells were studied. Nef- viruses had 3 to 10 fold lower infectivity per pg of p24 compared to the parental nef+ virus.

These results suggest that *nef* plays a positive role in viral infectivity.

J 219 ENHANCED IN VITRO HIV-1 REPLICATION IN B CELLS EXPRESSING A TRANSFECTED HIV ENVELOPE GP41 IMMUNOGLOBULIN RECEPTOR, David I. Cohen, Yoshihiko Tani, Eileen Donoghue, Sandra Sharpe*, Elizabeth Boone , H. Clifford Lane, and Susan Zolla-Pazner*, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892 and *Dept. of Pathology, New York University Medical Center, New York, NY 10010.

We transfected a CD4 negative, LFA-1 negative B cell line to express the genes encoded by the human anti-gp41 monoclonal antibody, 98-6 (sIg/gp41). SIg/gp41 transfected cells acquired the ability to bind HIV gp160, but not gp120, consistent with the specificity of the monoclonal antibody. Despite binding HIV envelope, sIg/gp41 cells could not be infected by HIV-1 (reverse transcriptase (RT) < 100 cpm). These cells were not intrinsically defective for supporting HIV-1 infection, because, when directed to produce surface CD4 by CD4-encoding retroviral constructs, they gained the ability to replicate HIV-1. Interestingly, doubly transfected cells with dual expression of surface CD4 and sIg/gp41 receptors replicated HIV both better and more quickly (peak RT 15,000 cpm, day 5) than cells expressing only CD4 (peak RT 3000 cpm, day 11). This enhancement resided specifically in the sIg/gp41, because isotype-specific, anti-IgG1 antibodies directed against sIg/gp41 blocked the enhancement. These data directly establish the ability of a cell surface anti-gp41 receptor to enhance HIV-1 replication. The slower HIV growth in CD4+ cells when compared to CD4+, sIg/gp41+ cells appears similar to the delay in viral replication reported for LFA1- lymphocytes, suggesting that the anti-gp41 receptor on sIg/gp41+CD4+ cells may be playing a role similar to LFA1 in enhancing viral spread. This model should facilitate understanding the mechanisms of antibody enhancement (ADE) occurring naturally during HIV infection.

J 220 INHIBITION OF REV-DEPENDENT HIV-1 EXPRESSION BY INTERFERON-INDUCIBLE GENES

P. Constantoulakis, G. Nasioulas,* E. Afonina, J. Turpin, C. P. Cunningham,* J. Harrison, B. K. Felber,* and G. N. Pavlakis Human Retrovirus Section and Human Retrovirus Pathogenesis Group*, ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702-USA

HIV-1 mRNAs expressing structural proteins are defective due to multiple inhibitory/instability elements (INS or CRS) (1). These defects are overcome in human cells by the interaction of the Rev regulatory protein with its RNA target on the viral RNAs, named Rev responsive element (RRE). We study the cellular factors that interact with RRE and INS elements.

We have identified interferon (IFN) α- and γ-inducible cellular proteins that bind to the HIV-1 RNA, within the region of the RRE (2). One IFN-inducible cellular protein was identified as a member of the IFN-inducible 1-8 gene family by screening a human monocyte cDNA expression library with an RRE RNA probe. In transient transfection studies, the protein produced by this cDNA, named RBP9-27, inhibited the Rev-dependent expression of the HIV-1 structural proteins Gag and Env in a dose-dependent manner. Down-regulation by this protein is a posttranscriptional event, since the expression of Rev-independent genes is not affected. A second member of the 1-8 gene family (18U) inhibits HIV-1 expression to a lesser extent, while a third member (18D) is inactive. In vitro binding studies after purification of these proteins revealed that all three proteins bind to RRE RNA, albeit with different affinities. Coexpression of these proteins in human cells revealed that RBP927 and 18U cooperatively inhibit HIV-1 expression, while 18D antagonizes the function of both proteins.

We also examined whether the different members of the 1-8 gene affect expression of other lentiviruses and the HTLV family of retroviruses. It was found that both HIV-1 and HTLV-I expression was inhibited by some of these proteins. Our data show that the mechanism of function of these proteins is similar, i.e. the interference with Rev/Rex function. It is therefore suggested that RBP9-27, RBP18U and RBP18D may participate in a novel IFN-induced antiviral pathway. These studies contribute to the elucidation of the mechanisms that mediate the antiviral effects of IFNs and may lead to the further understanding of the IFN effects on HIV-1 and to the development of novel therapeutic strategies for HIV-1 infection.

Research sponsored by the National Cancer Institute, DHHS, under contract no. N01-CO-74101 with ABL.

1. S. Schwartz, *et al.*, *J Virol* 66, 7176-7182 (1992).
2. P. Constantoulakis, *et al.*, *Science* 259, 1314-1318 (1993).

J 221 DYNAMIC RELATIONSHIP OF VIROLOGIC CORRELATES OF HIV DISEASE PROGRESSION, Brian Conway, Doreen S. Ko, Marcus Shaw, Francisco Diaz-Mitoma, George A. Wells. University of Ottawa AIDS Research Group, Ottawa, CANADA K1H 8L6

To study the relationship between zidovudine (ZDV0 resistance and other virologic and clinical parameters, we identified 22 patients on ZDV therapy 5-28 (mean 16.0) months. Patients were randomized to continue ZDV or change to didanosine (DDI) therapy. Monthly evaluations of clinical status, CD4 cell counts, circulating proviral load (quantitative PCR) and ZDV resistance (PCR culture and ACTG consensus assays) were performed. At entry, patients carried ZDV resistant (15) or susceptible (7) isolates. Resistance was associated with longer prior ZDV therapy (17.2 vs 12.9 mo) and higher proviral load ($> 10^3$ copies/10^6 CD4 in 7/15 vs 1/7, $p < .05$). CD4 counts were comparable (289 vs 282 cells/μl). Low proviral loads ($< 300/10^6$ CD4) were seen in 4/10 patients with CD4 > 300 vs 1/12 others ($p < .05$). Eleven patients continued on ZDV (4 susceptible, 7 resistant). Twelve months later, those with resistant isolates had lower CD4 counts (278 vs 325) and higher proviral loads (7/7 vs 2/4 > $10^3/10^6$ CD4). In all patients, a trend towards increasing proviral load was observed, which may be linked to impending resistance and clinical deterioration in those still carrying susceptible isolates. Clinical and laboratory follow-up of these patients is ongoing. Analysis of patients on DDI is underway. In one patient, a rise in CD4 count (340 to 510) paralleled a decreasing proviral load (2500 to $200/10^6$ CD4) over 6 months. Isolates initially resistant to ZDV became susceptible. Over the next 8 months, a decreasing CD4 count (310) and rising proviral load (2800) were observed. ZDV resistance returned without known drug re-exposure. If validated, this would favor early vs late reintroduction of ZDV therapy in patients changed to DDI. This will be the subject of a clinical trial in Canada. Work supported by CANFAR, PSI and the Pharmaceutical Manufacturers Association of Canada.

J 222 APOPTOSIS IN HIV INFECTION.Mark F Cotton*, Richard DukeΦ, Mary SchleicherΦ and Terri H Finkel# Pediatric Infectious Diseases, The Children's Hospital*,Cancer CenterΦ and National Jewish Center for Immunology and Respiratory Medicine#,UCHSC, Denver, Co 80206.

Aim: The purpose of this pilot study is to define features of apoptosis by flow cytometry in adults with HIV infection. **Methods:** Stimulated (BMA-031) and unstimulated PBMC's from 3 asymptomatic adults and controls were compared. Irradiated PMBC's were a positive control. Samples were stained with Fitc-labelled CD4 and CD8 antibodies, fixed in 1% paraformaldehyde and analysed by FACSCAN. Apoptosis was identified by diminished forward scatter (size) and increased side scatter (granularity), and confirmed by morphology.

Results: % APOPTOSIS IN CD4+ CELLS

	Time zero	Day 1	Day 2	Day 3⊕
Control	1,5 (0,7)	1,9 (1,2)	5,7 (0,7)♦	2,9
Control-S		4,8 (1,7)	7,2 (1,8)	4,5
HIV	3,6 (1,7)	4,9 (2,1)	12,8 (0.9)♦	10,8
HIV-S		15,3 (7,3)	27,5 (8,3)	8,8
Irradψ		3,9 (2,7)	19,9 (5,1)	14,8

ψ500 rad; ⊕n=2; ( )=standard error of mean; ♦p=0,03.

A similar, but less dramatic increase was seen in stimulated HIV+ CD8's. Morphology tended to parrallel flow cytometry. **Conclusion:** Increased apoptosis in HIV+ CD4 lymphocytes at time zero, suggest that it occurs "in vivo". It was also greater in stimulated and unstimulated HIV+ than control cells esp on day 2. This assay may assist in the study of HIV pathogenesis.

J 223 IDENTIFICATION OF A REGION IN CD4 THAT IS NECESSARY AND SUFFICIENT FOR NEF-MEDIATED DOWN-REGULATION, Paul Crisell, Penny Davis and Bryan R. Cullen, Section of Genetics and Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.

The *nef* gene products of both HIV-1 and SIV can induce an efficient and specific post translational down regulation of the cell surface expression of the CD4 glycoprotein receptor for these viruses. Although Nef is dispensible for viral replication in culture, experiments using the SIV system demonstrate that it is essential for both efficient viral replication and pathogenesis *in vivo*. Because of the likely importance of Nef-mediated down-regulation of cell surface CD4 in the *in vivo* pathogenesis of HIV-1, we have attempted to precisely identify the regions within the CD4 molecule that are required for Nef-mediated down-regulation and to define the mechanism by which Nef effects this reduction.

Using a whole cell ELISA to measure the cell-surface level of CD4 expressed on transfected Cos cells, we show that the *nef* gene products of SIV, HIV-1$_{NL4-3}$ and primary HIV-1 isolates efficiently down-regulate cell surface CD4 expression in this heterologous cell context. We have used this assay to define a short region of CD4 which is both necessary for Nef down-regulation of CD4 and sufficient to confer Nef-mediated down-regulation on the normally non-responsive Interleukin-2 receptor alpha chain. Additional mutational analysis of this region has permitted us to identify individual amino acids within CD4 that are critical to Nef-induced down–regulation. More recently, we have used closely similar variants of CD4 that are either fully responsive or entirely refractory to Nef-mediated down-regulation in experiments designed to test whether Nef directly interacts with its protein target *in vivo*.

J 224 HIV REPLICATION IN MONOCYTES AND MACROPHAGES.

Suzanne Crowe, Secondo Sonza, Anne Maerz, Rosemary Kiernan, Melissa Churchill and John Mills. Macfarlane Burnet Centre for Medical Research, P.O. Box 254, Fairfield 3078, Victoria, Australia.

Objectives: To determine the time, post isolation, when peripheral blood monocyte/macrophages show increased susceptibility to infection with HIV and identify the stage of viral replication which is blocked in cells which are less susceptible.

Methods: Monocytes were isolated from HIV seronegative buffy coats by density gradient centrifugation and plastic adherence or elutriation. Cells were infected with HIV-1 Ba-L on Day 0,1,2 and 7 post isolation. HIV infection was quantitated by PCR using primer pairs which detect all initiated, partially completed and almost full-length cDNA transcripts, intracellular p24 quantitation by MAb staining and flow cytometric analysis or p24 antigen in supernatant by EIA. PCR was also used to detect one and two LTR species of circular HIV DNA. Integration was detected using a novel *Alu*-PCR procedure which amplifies DNA between HIV-1 and ubiquitious *Alu* repeat sequences.

Results: Freshly isolated monocytes were generally not susceptible to infection with HIV-1. At least 24 hours in culture was required before any sign of viral replication could be detected. The block appears to be prior to reverse transcription.

Conclusion: There is a block to HIV infection in freshly isolated monocytes which is overcome when cells have been cultured for 24 hours Further studies are ongoing to compare internalization of virus, as well as later steps in replication including translocation of DNA from cytoplasm to nucleus, and NFkB activity in the two populations.

J 225 QUANTITATION OF PROVIRAL DNA IN LYMPHOCYTE SUBSETS OF SIV-INFECTED MACAQUES, Gregg A. Dean, Gerhard H. Reubel and Niels C. Pedersen, Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616

The CD4 molecule has been identified as the receptor utilized by simian immunodeficiency viruses for binding and entry into susceptible cells. The lymphoid target of HIV in peripheral blood and lymph node has been shown to be the CD4+ lymphocyte. To determine the target cell phenotype of SIV infection *in vivo*, CD4+, CD8+, and CD20+ (B-cells) lymphocytes were purified to greater than 97% purity. The number of cells containing proviral DNA was determined with quantitative PCR. No proviral DNA was demonstrated in B-lymphocytes, while a large amount of provirus was found in CD4+ cells. Surprisingly, proviral DNA was also detected in CD8+ cells. A mean of 1% CD4+ cells were positive in chronically infected animals compared to 10% in acutely infected macaques. Approximately 0.1% of CD8+ cells were positive in either chronically or acutely infected macaques. No difference in proviral burden in CD4+ or CD8+ lymphocytes was observed between peripheral blood and lymph node in acutely infected animals. Virus was readily isolated by coculture from purified CD4+ and CD8+ populations. Because the CD8+ population was purified based only on the expression of the CD8 molecule, we investigated the possibility that a population of CD4+CD8+ cells was responsible for the positive PCR result. Using flow cytometric cell sorting of lymphocytes dual stained for CD4 and CD8, a CD4+CD8+ population was shown to contain a relatively large amount of proviral DNA, however, a small number of CD8+CD4- cells were also infected. The SCID-Hu model has demonstrated a strong tropism and cytopathicity of HIV for thymic CD4+CD8+ cells, but dual positive cells in peripheral blood do not represent a circulating counterpart to the thymic CD4+CD8+ cells. Circulating dual positive cells have been shown to be stimulated, mature lymphocytes that transiently express both subset molecules during the S and G2-M cell cycle phases. It is likely that CD4+CD8+ cells containing provirus were originally infected as single positive CD4 cells, however it is evident that a small percentage of CD8+ cells may be infected when transiently expressing CD4 during proliferation and become persistently infected. The significance of SIV-infected CD8+ cells, functionally and as reservoirs, remains to be examined.

J 226 THE NF-κB AND SP1 MOTIFS OF THE HIV-1 LTR FUNCTION AS NOVEL THYROID HORMONE RESPONSE ELEMENTS, Vandana Desai-Yajnik and Herbert H. Samuels, Departments of Medicine and Pharmacology, New York University Medical Center, New York, NY 10016

We report that thyroid hormone (T3) receptor (T3R) can activate the HIV-1 long terminal repeat (LTR). Purified chick T3R-α1 (cT3R-α1) binds as monomers and homodimers to a region in the LTR (-104/-75) which contains two tandem NF-κB binding sites and to a region (-80/-45) which contains three Sp1 binding sites. In contrast, human retinoic acid receptor-α (RAR-α) or mouse retinoid X receptor-β (RXR-β) do not bind to these elements. However, RXR-β binds to these elements as heterodimers with cT3R-α1 and to a lesser extent with RAR-α. Gel mobility shift assays also revealed that purified NF-κB p50/65 or p50/50 can bind to one but not both NF-κB sites simultaneously. Although the binding sites for p50/65, p50/50, and T3R, or Sp1 and T3R overlap, their binding is mutually exclusive. With RXR-β, in reaction, the major complex is the RXR-β/cT3R-α1 heterodimer. The NF-κB region of the LTR as well as the NF-κB elements from the κ light chain enhancer both function as thyroid hormone response elements (TREs) when linked to a heterologous promoter. The TREs in the HIV-1 NF-κB sites appear to be organized as a direct repeat with an 8 or 10 base pair gap between the half-sites. Mutations within the NF-κB motifs which eliminate binding of cT3R-α1 also abolish stimulation by T3, indicating that cT3R-α1 binding to the Sp1 region does not independently mediate activation by T3. The Sp1 region, however, is converted to a functionally strong TRE by the viral *tat* factor. These studies indicate that the HIV-1 LTR contains both *tat*-dependent and *tat*-independent TREs and reveal the potential for T3R to modulate other genes containing NF-κB- and Sp1-like elements. Furthermore, they indicate the importance of other transcription factors in determining whether certain T3R DNA binding sequences can function as an active TRE.

J 227 HIGH MATERNAL HIV VIRAL LOAD IS ASSOCIATED WITH INCREASED RISK OF VERTICAL TRANSMISSION

Ruth Dickover, Eileen Garratty, Pamela Boyer, Maryanne Dillon, Audra Deveikis, Margaret Keller and Yvonne Bryson. Department of Pediatrics, UCLA School of Medicine, Los Angeles, Ca 90024.

Vertical HIV transmission accounts for the vast majority of pediatric HIV infection. Factors influencing transmission to the infant may include virus load and maternal immune status. We therefore, quantitated both cell-associated (PCR and coculture) and cell-free (ICD p24 antigen and limiting dilution plasma culture) HIV in the circulation of 54 HIV infected pregnant women close to or at the time of delivery and correlated these results with infant outcome. Total CD4+ cell counts and percentages were also measured close to or at delivery. The HIV DNA copy number in 15 transmitting mothers was significantly higher than in 39 non-transmitting mothers (mean=320±118 SD vs. 45±57 SD copies/μg PBMC DNA; p<.0001). Transmitting mothers also had significantly lower CD4 counts (mean=415±220 SD vs. 648±302 SD CD4+ cells/ml; p=.02) and higher ICD p24 antigen levels (mean=246±345 SD vs. 10±23 SD pg/ml; p<.0001). Plasma viremia was more common and at higher titer in mothers who transmitted infection than in those who did not (9/13 vs. 5/26; p=.002 and mean=173±365 SD, vs. 5±20 SD, TCID/ml; p=.001). Interestingly, the five non-transmitters with detectable plasma viremia received Zidovudine over the last 6-40 weeks of their pregnancy. These results suggest that high maternal levels of infectious virus, either cell-associated or cell-free, present at the time of delivery are associated with high risk of vertical transmission.

J 228 **QUANTITATION OF HIV-1 INFECTION KINETICS IN MACROPHAGES AND PERIPHERAL BLOOD LYMPHOCYTES**, Dimiter S. Dimitrov,[1] George Englund,[2] and Malcolm A. Martin[2], [1]National Cancer Institute, [2]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

We have recently developed a novel approach to analyse HIV-1 infection kinetics in tissue culture systems and have used it: 1) to quantitate the number of infecting virions transmitted from infected to uninfected cells and 2) to determine the time needed for one cycle of infection. This approach has now been applied to infection of macrophages and peripheral blood lymphocytes (PBLs) by unpassaged primary HIV-1 isolates. While the number of infecting virions transmitted to uninfected PBLs was very similar to that for laboratory adapted strains in T cell lines (about 100 per cell per cycle), it was 10-fold lower for a spreading infection in macrophages. Interestingly, the time required to complete one cycle of infection was approximately the same for PBLs, macrophages and T cell lines (about 3 days). Infected PBLs or infected macrophages transmitted HIV-1 to uninfected macrophages or PBLs, respectively, with very high efficiency. These results demonstrate that the infection kinetics in macrophages is slower than in PBLs because of the lower number of infecting virions transmitted to uninfected cells rather than changes in the rate of virus replication.

J 229 A LONGITUDINAL STUDY OF *IN VITRO* IMMUNE RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS* IN HIV-SEROPOSITIVE SUBJECTS, Kimberly A. Fisher[1], John P. Phair[2] and Anne S. De Groot[3], [1]Brown University, Providence, RI, [2]Comprehensive AIDS Center, Northwestern University Medical School, Chicago, IL, [3]Division of Biology and Medicine, Brown University and HIV/AIDS Unit, Lemuel Shattuck Hospital, Boston, MA

Objective: To determine 1) the immunological basis for the increased incidence of tuberculosis (TB) in HIV-seropositive individuals, and 2) the kinetics of the loss of *in vitro* immune response to TB during the progression of HIV infection.

Methods: T cell proliferation and cytokine secretion in response to *in vitro* stimulation with the following *Mycobacterium tuberculosis* (Mtb) antigens will be measured: PPD, Mtb culture filtrate, and lipoarabinomannan-depleted Mtb culture filtrate. Control antigens will include PHA, tetanus toxoid, PPD, and Candida albicans. Peripheral blood mononuclear cells (PBMCs) from MACS Cohort subjects will be used for the *in vitro* assays. The study subjects will include, HIV+ PPD+, HIV+ PPD-, HIV- PPD+, and HIV- PPD- individuals. Samples were taken from each subject at several time points in the course of their HIV infection, each separated by at least 6 months.

Results: T cell response parameters to be measured will include: 1) T cell proliferation, measured by [^{3}H]-thymidine uptake, 2) IL2 and IL4 release, detected with an IL2/IL4 dependant cell line, and 3) γ-IFN secretion, measured by ELISA. In a previous cross-sectional study, the response of Mtb-naive (PPD-negative), HIV-infected subjects to Mtb antigens was shown to be suppressed, as compared to Mtb naive, HIV-seronegative controls.

Conclusion: Longitudinal studies of Mtb responsiveness in HIV-seropositive individuals may contribute to the development of appropriate immunotherapeutic interventions for Mtb in the era of HIV.

J 230 PROLONGED SURVIVAL OF IMMUNODEFICIENT RETROVIRUS INFECTED MICE (MAIDS) TREATED *IN VIVO* WITH LITHIUM. VS Gallicchio*, ML Cibull*, NK Hughes*, KF Tse*. Hematology & Oncology, Department of Clinical Sciences, Internal Medicine, Microbiology & Immunology, and Pathology, University of Kentucky Medical Center and Department of Veterans Affairs, Lexington, KY, USA.

Murine immunodeficiency virus disease (MAIDS) induced with LP-BM5 MuLV shows many similarities to human HIV-infection. The etiological agent is a defective MuLV capable of inducing disease with the aid of a helper virus. Lithium (Li) influences numerous immunohematopoietic cell types and cellular processes that involve cell proliferation and differentiation. We report here results of *in vivo* studies investigating the effect of Li in MAIDS. Virus control and Li-treated viral infected C57BL6 mice were monitored for survival and development of MAIDS pathology. Virus-infected mice were grouped to initiate Li (1 mmol) daily as follows: (1) 7-days before virus; (2) 2-days before virus; (3) at the same time as virus, and (4) 5-weeks post virus infection. Daily Li was continued during the study period. Following 36 weeks of observation, percent survival was as follows: virus controls, 0%; Li 7-days 100%; Li 2-days 90%; Li day-0 85%; and Li 5-weeks post-virus 80%. Development of lymphoma in Li-treated virus infected mice was reduced significantly as measured via ultrastructural, histopathological, and gross anatomical analysis measured by thymus involvement, lymphadenopathy and splenomegaly (gms): virus control 1.21 ± 0.21; Li 5-weeks post-virus, 0.48 ± 0.03, Li day-0, 0.001). Bone marrow and spleen derived myeloid, erythroid and megakaryocyte hematopoietic progenitors were also increased compared to virus infected controls. These studies indicate Li is an efficacious treatment in modulating MAIDS and raises important questions regarding its potential role in the pathophysiological processes associated with retroviral infections.

J 231 ENVELOPE REGIONS OF HIV-1 INVOLVED IN CELL TROPISM AND CYTOPATHICITY, Sajal K. Ghosh, Beatrice H. Hahn, and George M. Shaw, University of Alabama at Birmingham, Birmingham, AL 39294.

Isolation of syncytium-inducing (SI) HIV-1 strains frequently correlates with advanced disease or more rapid subsequent decline of $CD4^+$ cells. Identification of determinant(s) responsible for syncytium induction may provide better insight into disease pathogenesis. Previous studies, using primarily highly passaged laboratory strains of HIV-1, have suggested that the V3 and V1-V2 envelope regions are important in conferring the SI phenotype. We prepared recombinant chimeras between the monocyte-tropic nonsyncytium-inducing (NSI) virus HIV-1_{YU2} (that had been derived from uncultured human brain tissue) and two SI viruses derived from patients with either acute infection (HIV-$1_{WEAU\ 1.60}$) or end-stage AIDS (HIV-1_{SG3}). The three parental virus strains and 15 envelope chimeras were analyzed for their ability to replicate and form syncytium in human PBMCs, MT-2 cells, and CEMx174 cells. Exchange of the complete envelope genes of SI and NSI viruses conferred the SI phenotype of WEAU 1.60 and SG3 viruses to YU2. Similarly, an envelope fragment extending from the 5' terminus of V3 to the 3' terminus of gp41 of WEAU 1.60 and SG3 viruses conferred the same wild type SI phenotype to YU2. Exchange of V3 alone did not fully confer the wild type SI phenotype. However, exchange of the V4 region along with the V3 region from the SI viruses again preduced the SI phenotype in YU2. These studies suggest that the V3-V4 region of naturally-occurring HIV-1 strains may be important in determining virus tropism and pathogenic properties. In light of other studies that have identified determinants of cell tropism and cytopathicity in envelope regions 5' of V3, it is likely that the three dimensional configuration of the envelope and strain-specific combinations of envelope domains are involved in determining viral phenotype.

J 232 NEF 27, BUT NOT THE NEF 25 ISOFORM OF HIV-1 DOWN-REGULATES EXPRESSION OF CELL SURFACE RECEPTORS IMPORTANT FOR DEVELOPMENT OF NORMAL IMMUNE RESPONSE

Alison Greenway[1], Dale McPhee[1] and Ahmed Azad[2]. [1]AIDS Cellular Biology Unit, Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria, 3078, Australia. [2]Biomolecular Research Institute, Parkville, Victoria, 3052, Australia.

Continuing controversy surrounds the cellular effects of the Nef protein of HIV-1, a non-structural protein expressed by most isolates. Highly purified protein isoforms of Mw 27 kDa (Nef 27) and 25 kDa (Nef 25), produced in *E.coli* by translation from the first and second codons of HIV-1 *nef* clone pNL4.3, respectively, were introduced into cells by a sophisticated electroporation technique which uses electric field rather than electric charge to transfer macromolecules across cell membranes. Electroporation of Nef 27 reduced the expression of cell surface CD4 by 30-50%, as measured by flow cytometry, on phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells (PBMC) as well as on a variety of CD4+ve T-cell lines (MT-2, Jurkat and CEM). Reduction in CD4 was observed in all cells of the CD4+ve T-cell lines but only in the CD4+ve cells of the mixed PBMC population. Electroporation of Nef 27 into MT-2 cells and PHA-activated PBMC also reduced surface expression of IL-2 receptor (IL-2R) to background levels. Other cell surface antigens analysed such as CD2, CD7 or tansferrin receptor were not affected by the introduction of HIV-1 Nef 27. In contrast to the effects of Nef 27, electroporation of Nef 25 into cells at equivalent concentrations did not affect the surface expression of CD4 and IL-2R. These data show that the HIV-1 clone pNL4.3 Nef 27 but not the Nef 25 isoform specifically decreases expression of two cell surface receptors important for antigen recognition of MHC class II antigens and for cell proliferation. Production of Nef 27 during HIV-1 infection of cells of the immune system may contribute to immunodeficiency even in the absence of direct viral cytopathic effects.

J 233 ***IN UTERO*** **TRANSMISSION OF HIV VIA TNFα-MEDIATED DAMAGE TO THE PLACENTAL BARRIER,** Larry J. Guilbert, Jane Yui, Tom Wegmann, and Maria Garcia-Lloret. Department of Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The placental pathology that best correlates with vertical transmission of AIDS is necrotic villitis, a focal inflammation of placental villi that manifests to varying degrees as an influx of maternal T cells and monocytes, loss of the trophoblastic syncytium and associated basement membrane, hyperplasia of stromal fibroblasts and Hofbauer cells and fibrosis. We investigated the possibility that maternal HIV infection contributes to the formation of villitis. We found that monocytes strongly adhere to pure (>99.8%) trophoblast cultures in an activation and divalent cation (Mn>Mg>Ca) dependent manner. Within 48 hours of adherence, patches of pyknotic trophoblast nuclei appear beneath the monocytes, an event inhibited by anti-TNF mAb. We thus asked whether, and how, TNFα alone could kill trophoblasts. TNFα at 10ng/ml induced appearance of pyknotic nuclei followed by loss of 30% of trophoblasts from the culture. IFNγ at 100 U/mL has no effect alone, but in combination with TNFα enhances cell loss to 60% and decreases the half-maximal dose dependency of TNFα from 2.5 to 1 ng/mL. Cells detaching from TNF/IFN-treated cultures exclude the vital dye trypan blue, contain much less DNA than untreated cells and the DNA is fragmented into a nucleosome-sized ladder pattern, all of which suggest death by apoptosis. The role of TNFα in the disruption of the stromal mesenchyme was further investigated. TNFα stimulate placental fibroblast proliferation and their production of CSF-1, which in turn stimulate Hofbauer cell proliferation and their production of TNFα. These data suggest that monocytes activated to express inappropriate levels of TNFα at the maternal placental surface initiate villitis by destroying the protective trophoblast and then further develop the lesion via a TNFα - CSF-1 - TNFα positive feedback loop. We propose that maternal monocytes or T cells, either directly or indirectly activated by HIV, home to the trophoblastic syncytium and there initiate and maintain a TNFα-driven process that, unless unchecked, can lead to transient loss of the integrity of the placental blood barrier and thereby to *in utero* transmission of HIV.

J 234 A NOVEL,PCR, BASED VIRAL CAPTURE ASSAY FOR THE DETECTION OF HIV-1 IN CULTURE FLUIDS. Indira K.Hewlett,.Bharat H. Joshi, Jay S. Epstein,. Lab. Mol. Virology, DTTD, CBER, FDA, Nicholson lane Research Centre, Kensington MD-20895.

We have developed a novel and sensitive method based on PCR for detection of HIV-1 in culture and body fluid. Anti-IgG coated magnetic beads were coated with 25, 50 and 100 ng of either anti p24 and anti-gp 120/ 160 monoclonal antibodies at 4°C for 16 hours. Coated beads were mixed with different dilutions of supernatants obtained from H9 cells infected with the HIV-1 MN strain and shaken for 16 hours at 4°C to capture and concentrate virions. RNA from the antigen- antibody complexes was extracted with guanidinum thiocyanate, precipitated with isopropanol and amplified by PCR after conversion of RNA to cDNA by reverse transcriptase. Amplified products were detected with a radiolabelled probe. The sensitivity of the assay was directly proportional to the amount of antibody used to coat beads. By this method an equivalent of 10 ng of p24 antigen (approximately 10-50 copies of HIV RNA) could be detected. A panel of sera from HIV-1 infected individuals is currently being analyzed to evaluate the utility of the assay in clinical diagnosis. This assay is also being adapted for use with chemiluminescence probes.

J 235 HIV-2 EHO, A HIGHLY VIRULENT AND DIVERGENT ISOLATE THAT INDUCES SINGLE CELL KILLING BY APOPTOSIS, A.G. Hovanessian, J. Galabru, A.G. Laurent-Crawford, B. Krust, L. Montagnier and M.A. Rey-Cuillé. Department of AIDS and Retroviruses (UA CNRS 1157), Institut Pasteur, 28, rue du Dr. Roux, 75015 Paris France.

The HIV-2 EHO isolate from an Ivory Coast patient with AIDS was characterized here by its distinct biological and genetic properties. Comparative studies with the first cytopathic HIV-2 isolate (ROD) demonstrated that at equal multiplicities of infection of CD4 expressing CEM cells, the preparation of HIV-2 EHO contains 500 to 1000 fold less viral particles than that of HIV-2 ROD. Although both virus infections result in cell killing by apoptosis, syncytia formation is observed only in cell cultures infected with HIV-2 ROD. Infection of cells with HIV-2 EHO results in single cell killing by apoptosis. Consistent with this, electron micrographs illustrated the condensation of cellular chromatin in HIV-2 EHO-infected cells with intact cytoplasmic and nuclear membranes. Interestingly, monoclonal antibodies against the CD4 receptor suppress the cytopathic effect and the induction of apoptosis by both virus strains without affecting significantly virus production. These observations indicate that cell-surface interaction of envelope glycoproteins with CD4 receptor molecules is necessary for the induction of apoptosis, as we have reported in HIV-1 LAI infected cells (AIDS Res Hum. Retroviruses 9, 761-773, 1993). Nucleotide sequence analysis of the HIV-2 EHO genome revealed a significant degree of divergence of its envelope gene from that of other known HIV-2 strains. This divergence for the deduced amino acid sequence corresponding to the surface envelope glycoprotein was 26 to 30 %. These unique genetic and biological properties along with the phylogenetic analysis of the HIV-2 EHO sequence, show the heterogeneity of HIV-2 isolates in West Africa and indicate that the HIV-2 EHO isolate is a distinct prototype in the HIV-2 family.

J 236 CHARACTERIZATION OF NEF IN LONG TERM SURVIVORS OF HIV-1 INFECTION. Yaoxing Huang, and David D. Ho. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

Although SIV studies provide convincing evidence that nef is a gene important for viral virulence in vivo, the role of nef in HIV-1 infection in man remains poorly understood. To provide a basis for studies on the function of nef in HIV-1 infection, we used targeted polymerase chain reaction amplification and DNA sequencing to determine the nucleotide sequence in longitudinal samples of PBMC obtained from individuals who have remained asymptomatic with a normal CD4 cell count despite over 12 years of HIV-1 infection. To date, we have found both open-nef and truncated-nef in these samples, as well as unusual amino-acid substitutions. The capacity of these nef alleles to down regulate CD4 is now being compared with those from patients who progressed clinically. In addition, the effect of these nef genes from long-term survivors on growth properties of HIV-1 in vitro will be examined.

J 237 ORGANOTYPIC CULTURES OF THE BRAIN: AN ANIMAL MODEL OF AIDS ENCEPHALOPATHY. David L. Huso, Hong Cui, and James J. Vornov, Division of Comparative Medicine and Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21224.

Primary neurological disease is a common sequelae to HIV infection in both adults and children. The vast majority of infected cells in the lentivirus-infected CNS have been shown to be macrophages /microglia. However, the details of neuronal dys-function, neuronal damage and neurodevelopmental abnormalities associated with HIV infection remain poorly understood. These events could perhaps be better understood by developing an animal model system that 1)reflects the macrophage/microglial tropism of neuropathogenic lentiviruses in a natural host, 2)provides an easily monitored culture system that accurately represents the complexity of interactions that occur in the infected brain, and 3)allows chronic exposure to soluble substances, but exhibits reproducible CNS changes in a reasonable time span following infection. Visna virus infection of long-term organotypic cultures of the hippocampus fulfills many of these objectives. The developing hippocampus is sectioned into 400 um slices and cultured intact on microporous membranes for weeks to months. The culture conditions and stage of development best suited for hippocampal cultures have been adapted from the rat system to fetal sheep. Neurons and glia have been specifically identified in the cultures by morphology as well as by specific cellular markers. Neuronal cell death has been continuously monitored quantitatively over time at the individual cell level by uptake of propidium iodide, a fluorescent dye excluded from living cells. The cultures are susceptible to direct effects of visna virus infection and indirect effects of soluble substances released during coculture with visna virus infected PBL's. The model gives new insights into HIV pathogenesis.

J 238 ROLE OF THE HIV-1 VPU PROTEIN IN THE DOWN-REGULATION OF CD4: THE REQUIREMENT OF ANCHOR/CYTOPLASMIC DOMAINS OF CD4 FOR VPU-INDUCED PROTEOLYSIS IN THE ENDOPLASMIC RETICULUM. M. Abdul Jabbar, Nicholas U. Raja, and Martin J. Vincent, Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195

The HIV-1 proteins, gp160, Vpu, and Nef, are involved in the down-modulation of CD4 in HIV-1 infected cells. Previous experiments have demonstrated that gp160 traps CD4 in the endoplasmic reticulum, and the Vpu protein induces rapid degradation of CD4 in the ER. The Vpu protein is a transmembrane phosphoprotein which is localized in the perinuclear region of HIV-infected cells, presumably the ER or Golgi region. In the present study, we have investigated the sequence requirement for Vpu-dependent proteolysis of CD4 in the endoplasmic reticulum. To this end, we generated chimeric envelope glycoproteins having the ectodomain of HIV-1 gp160, the anchor domain of CD4, and 38, 25, 24, 18, 7, 4, and 1 aa of the CD4 cytoplasmic domain. Using the vaccinia virus-T7 RNA polymerase expression system, we analyzed the expression of chimeric proteins in the presence and absence of Vpu. In singly transfected cells, the chimeric envelope glycoproteins having 38, 24, 18 aa, and less of the CD4 cytoplasmic domain were endoproteolytically cleaved and biologically active in the fusion of HeLa $CD4^+$ cells. However, one of the chimeras having 25 aa of the CD4 cytoplasmic tail was retained in the ER using the transmembrane ER retention signal (KKTC) and hence was defective in membrane fusion. Furthermore, biochemical analyses of the coexpressing cells revealed that the Vpu protein induced degradation of the envelope glycoproteins having 38, 25, and 24 aa of the CD4 cytoplasmic tail and degradation occurred in the ER. As a consequence, the fusion-competent glycoproteins did not induce the formation of syncytia in HeLa $CD4^+$ cells expressing Vpu. However, the HIV-1 gp160 and envelope glycoprotein having the membrane proximal 18 aa or less of the CD4 cytoplasmic tail were stable and fusion-competent in cells expressing Vpu. Further analyses have revealed that the anchor domain of CD4 appears to provide the initial 'interaction motif' through which the Vpu protein could recognize sequences or structural determinants in the cytoplasmic domain of CD4. We will discuss the mechanism by which Vpu induces the proteolysis of CD4 and chimeric proteins in the ER of mammalian cells.

J 239 STANDARDIZED METHOD OF MEASUREMENT OF HIV-1 INFECTIVITY IN HUMAN PBL. M. Kanter, C. Hutto, R. Geffin and G. Scott. Department of Pediatrics, University of Miami School of Medicine. Miami, Fl., 33136.

Objective: To obtain a reliable reproducible assay to measure the infectivity of Human immunodeficiency virus (HIV-1) from patients in human peripheral blood lymphocytes(PBL).

Methods: Phytohemagglutinin stimulated PBL cultured in the presence of IL-2 were used as host cells. Virus obtained from supernatants of positive cultures, was filtered and frozen in liquid nitrogen until use. Serial 5-fold dilutions of virus stocks were used to infect $2x10^6$ lymphocytes for 1 hour at 37°C. Cells were washed and incubated in 24 well tissue culture plates at 37°C in 5% CO_2.Virus production was monitored using a p24 antigen

ELISA on days 4, 7, 10 and 14 after infection. In addition to lymphocytes from individual donors, the virus titer in pooled lymphocytes from 3 donors was tested. These pooled lymphocytes were either used fresh or were frozen and thawed at a later date to compare the susceptibility of fresh and frozen lymphocytes to infection.

Results: A total of 22 individual PBL preparations were tested. A great variability in the infectivity of these donor's lymphocytes was found. While some donor's cells could be infected very rapidly, and produce virus 4 days after infection, some others were not permissive; and in two different PBL donors, virus could not be substantiated in the culture supernatant for up to 3 weeks after infection. The variability in infectivity of different donor's PBLs could be circumvented by pooling lymphocytes from 3 different donors. When pooled lymphocytes were used, titers obtained from the same virus stock were extremely reproducible. This was true independently of which 3 random donors were used. Virus titers were very similar when fresh or frozen lymphocytes were used. One week or two months of freezing gave similar results.

Conclusion: A reproducible assay that measures HIV-1 infectivity of PBL was obtained by pooling lymphocytes from three different donors. This method could be very useful for standardization of other assays that use virus infectivity as a measurement,such as virus neutralization and sensitivity to anti-viral drugs.

J 240 CD4 DOWN-MODULATION CAN BE INDUCED BY BOTH NON-PATHOGENIC AND PATHOGNIC SIV *nef* ALLELES, Harry W. Kestler and Janelle R. Salkowitz, Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195

The *nef* gene is essential for the induction of AIDS and is necessary for maintenance of high virus loads (Kestler et al. *Cell* 65: 651-662). Deletion of 182 bases in *nef* produced a virus that was unable to sustain high loads observed in Rhesus monkeys infected with SIVmac239/*nef*-open which encodes a full-length *nef* gene. *Nef* has been shown to down-modulate CD4 from the surface of the cell and a number of investigators have proposed that this is the "pathogenic" phenotype. Here we describe experiments that address that possibility. We have tested the ability of *nef* from pathogenic and non-pathogenic SIV molecular clones to down-modulate cell surface CD4. *Nef* derived from the pathogenic molecular clone SIVmac239/*nef*-open efficiently down-modulates cell surface CD4 from the surface of lymphoid *nef* stable cell lines. Surprisingly, the allele of *nef* derived from the non-pathogenic molecular clone SIVmac239/*nef*-deletion retains the ability to down-modulate cell surface CD4. The *nef* deletion gene retains information for the first 58 amino acids of *nef* while the full-length gene codes for 265 residues. The truncated *nef* gene is expressed *in vivo*. An animal that received SIVmac239/*nef*-deletion developed a humoral immune response to *nef* despite the low virus loads and the small size of the *nef* coding region. Thus, the ability to down-modulate CD4 by *nef* while important to the life cycle of primate lentiviruses does not predict the pathogenic potential of the virus.

J 241 ENDOGENOUS CYTOKINES DRIVE HIV-1 REPLICATION IN PRIMARY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) STIMULATED WITH INTERLEUKIN-2 IN THE ABSENCE OF MITOGENS. A.L. Kinter, G. Poli, L. Fox, D. Goletti and A.S. Fauci, Laboratory of Immunoregulation,National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Cytokines, such as tumor necrosis factor (TNF) alpha, have been shown to modulate HIV expression in an autocrine manner in acutely or chronically infected primary cells and cell lines. However, these studies were conducted in culture systems that are highly selective for the growth of a single cell lineage and thus the interaction between different cell types and the cytokines they produce are difficult to delineate. In the present study we describe an in vitro culture system in which PBMC are maintained in IL-2 in the absence of prior mitogenic stimulation. IL-2-stimulated PBMC are characterized by the presence of multiple PBMC subsets and progressive T cell activation. Furthermore, upon acute HIV infection, the production of cytokines, particularly proinflammatory cytokines, peaks just prior to or concomitantly with the peak of HIV reverse transcriptase (RT) activity. In contrast, in conventional PHA blasts T cell activation and cytokine production peak prior to exposure to virus. IL-2-stimulated PBMC exhibit syncytia formation and cytopathic effects similar to what is observed in PHA blasts. In addition, IL-2-stimulated PBMC maintain a sizable adherent cell population throughout the course of infection (25-30 days). Virological data using this alternative method of PBMC infection can be summarized as follows: 1) Replication of a laboratory adapted lymphotropic (IIIB) strain occurs with comparable efficiency, whereas replication of the BAL macrophage tropic strain is significantly more efficient in IL-2-stimulated PBMC as compared to PHA blasts. 2) All primary HIV-1 isolates tested thus far replicate well in IL-2-stimulated PBMC. 3) HIV replication in IL-2-stimulated PBMC is largely dependent on the autocrine activity of multiple endogenously produced cytokines as demonstrated by the ability of anti-cytokine antibodies or antagonists to suppress viral RT activity and protein production. In particular, endogenous IL-1, TNFa and in some cases IFNg, plays an important role in the ability of IL-2-stimulated PBMC to sustain high levels of HIV replication. Thus, this system may serve as a physiologically relevant model to study the autocrine/ paracrine regulation of HIV replication by endogenous cytokines.

J 242 MUTUALLY DEPENDENT, CONCURRENT RNA AND DNA SYNTHESIS DURING EARLY PHASE HIV REPLICATION, Tuckweng Kok, Peng Li and Christopher J. Burrell, Division of Medical Virology, Institute of Medical and Veterinary Science, Adelaide 5000 South Australia

In a cell-to-cell HIV infection model it was found that there were two distinct phases of induced HIV RNA synthesis. The first phase (4h - 12h pi) was marked by a significant increase in only the genomic-length viral RNA. The second phase (24h onwards) comprised a dramatic increase in the levels of all three species of viral RNA. The first phase, but not the second phase of viral RNA induction, was abolished when unintegrated viral DNA synthesis was inhibited by AZT. When virus donor H3B cells were pre-treated with actinomycin D and mixed with untreated recipient Hut78 cells; the second phase, but not the first phase, of induced viral RNA transcription was abolished. Reverse transcription was not affected by actinomycin D. Full length linear unintegrated viral DNA was produced but viral integration was inhibited when donor and recipient cells were arrested in the G1 phase of the cell cycle by aphidicolin. Under these conditions, it was found that the second phase, but not the first phase of induced viral RNA synthesis was abolished. Taken together, these results suggest a mutually dependent concurrent viral RNA/DNA synthesis early after cell-to-cell transmission of HIV infection. The template for the first phase of viral RNA induction is likely to be the linear unintegrated viral DNA and the second phase may use newly integrated as well as proviral DNA in the donor cells as a more effective transcription template.

J 243 CHARACTERIZATION OF DEFECTIVE VIRUSES PRODUCED BY AN SIV CHRONICALLY INFECTED CELL CLONE, Edmundo N. Kraiselburd, Carlos Málaga, Manuela Beltrán, Nancy Leung and José Torres, Department of Microbiology, UPR School of Medicine, Río Piedras, Puerto Rico 00936 and University of California, Davis, CA 95616

SIV_{sm}chronically infected cultures were obtained after infection of CEMX174 cells with SIV_{sm}H3. Single cell clones were derived from these cultures and examined for the production of virus specific proteins. Western blot analysis, performed with either monoclonal or polyclonal sera, showed that one of these clones (B7) produced particles which contained envelope (gp135 and gp43), gag precursors, and gag proteins (p27, p16, and p8). However, these particles lacked detectable levels of RT, vpx, and integrase, and contained several fusion proteins which expressed viral protease antigens. These defective viruses failed to infect established CD_4+ cell lines, as well as primary cultures of macrophages and of peripheral blood lymphocytes, obtained both from humans and rhesus macaques. Lack of infection correlated with lack of viral DNA detection of PCR amplification of genomic DNA extracted from these cultures. After more than two years of "in vitro" cultivation, no infectious virus was recovered from B7 cells. These particles were shown to be immunogenic, yet they did not establish infection in rhesus macaques. Preliminary experiments suggest that B7 particles contain a defective genome. The approximately 1.6 kb deletion possibly involves the integrase, vif, vpx, and vpr genes. Thus, B7 cells produced stable, non-infectious immunogenic virus mutants, which contained env and gag proteins, but lacked detectable amounts of vpx and of enzymes required for virus replication in tissue culture and in rhesus macaques.

J 244 BASIS FOR THE APATHOGENICITY OF SIMIAN IMMUNODEFICIENCY VIRUS IN HEALTHY NATURAL HOSTS, Reinhard Kurth, Joachim Ennen, Klaus Cichutek, Michael Baier, Albrecht Werner, Stephen Norley, Paul-Ehrlich-Institut, 63225 Langen, Germany

The mechanisms of HIV- and SIV-induced pathogenicity are still at best incompletely understood. In most infections with pathogenic viruses, clinical consequences are determined by properties of both the virus and the host. Far too little experimental use has so far been made of animal models in which SIV may or may not induce SAIDS. Comparison of SIV-infected healthy natural hosts, namely Old World monkeys, with monkey species not adapted to SIV and developing SAIDS, like the rhesus macaque, may yield insight into the successful defense mechanisms of natural hosts. These studies should be complemented with thorough clinical and experimental investigations of healthy long-term survivors (LTS) of HIV-infected individuals.

In the best-studied animal model for natural infection, the SIVagm-infected African green monkeys (AGM), neither the humoral immune response, cell tropism, virus variability nor virus load appear to be the cause for apathogenicity. Instead, as also suggested by yet limited studies of LTS, it might well turn out that both a well-developed anti-viral cytotoxic T-cell response and the capacity of CD8+ lymphocytes to produce an "Immunodeficiency Virus-Suppressing Lymphokine (ISL)" contribute to the well-being of infected individuals. Investigations have been initiated to identify the ISL.

J 245 STIMULATION OF HIV REPLICATION IN MONO- NUCLEAR PHAGOCYTES BY LEUKEMIA INHIBITORY FACTOR, Anasua B. Kusarit, Shoba Broor, Baoping Zhang, Pradeep Seth, Douglas D. Richmant, Dennis A. Carson, William Wachsmant and Martin Lotz, From the Department of Medicine and Cancer Center, University of California, San Diego, La Jolla, CA 92093 and †Research Service, VA Medical Center, San Diego, CA 92161

This study examined the effects of leukemia inhibitory factor (LIF) on HIV replication in mononuclear phagocytes (MNP). LIF induced a dose-dependent increase in p24 antigen production in the chronically infected promonocytic cell line U1. The magnitude and time kinetics of the LIF effects were similar toTNF, andother cytokines likeIL-1,IL-6 also induced HIV replication in this cell line. To characterize mechanisms responsible for these LIF effects, levels of HIV mRNA, activation of the DNA binding protein NF-kB, signal transduction pathways and potential interactions with other cytokines were analyzed. LIF increased steady state levels of HIV mRNA at 2.0, 4.3 and 9.2 kB. This was detectable by 24h and persisted until 72h. The DNA binding protein NF-kB is a central mediator in cytokine activation of HIV transcription. NF-kB levels were higher in unstimulated U1 cells as compared to the parent cell line U937. In both cell lines LIF increased NF-kB activity. Induction of NF-kB and HIV replication by cytokines are at least in part dependent on reactive oxygen intermediates. The oxygen radical scavenger N-acetyl-L-cysteine but not an antagonist of nitric oxide formation inhibited LIF induced HIV replication. LIF induces the production of other cytokines in monocytes but its effects on HIV replication were not inhibited by antibodies to IL-1, TNF or IL-6. These results identify LIF as a stimulus of HIV replication. Its effects are related to increased HIV mRNA production, NF-kB activation and independent from the production of other cytokines.

J 246 DUAL INFECTION OF RHESUS MACAQUES WITH SIVmne$_{E11s}$ and SIVmac$_{239}$ Mark G. Lewis, Sharon Bellah, Kathy McKinnon, Jacob Yalley-Ogunro, Jack Greenhouse, William Elkins, Charles Brown, Yvonne Rosenberg, Suzanne Gartner, and Gerald Eddy. Henry M. Jackson Foundation 1500 E. Gude Dr. Rockville MD.

The purpose for this study was to determine if dual infection by two virulent and pathogenic SIV isolates is possible in rhesus macaques. In addition, we wanted to determine if pre-existing immunity alters the anticipated course of infection. The two isolates used in this study, SIV_{mac239} and $SIV_{mne/E11s}$, differ genetically by only 3-5% in the gag or envelope gene. Both isolates have been shown to cause disease, at different rates and intensities, in rhesus macaques. Monkeys infected with either isolate can generate cross-reacting antibodies to all of the SIV proteins. Eight rhesus macaques previously infected with $SIV_{mne/E11s}$ were used in this study. These monkeys had circulating SIV antibody of differing levels, had previously been virus isolation positive, but at the time of the second challenge were virus isolation negative. The eight monkeys, plus two controls, were challenged with either 10 ID_{50} or 1000 ID_{50} of SIV_{mac239}. Seven of the eight animals became virus isolation positive within 14 day after the 239 challenge, with 4 positive by 7 days post-challenge. All 7 had anamnestic responses to SIV antigens by 7 to 21 days PI.The remaining macaque, which had the highest levels of SIV-antibody prior to the second challenge, became virus isolation positive on week 6 PI, but remained so for only 2 weeks. Four of the eight animals became virus isolation negative by 8 weeks, while the remaining 4 were routinely isolation positive. No acute syndrome was detected in any of the animals with pre-existing immunity, whereas both control animals had detectible *antigenemia on day* 10 PI and had lymphadenopathy within two weeks PI. Preliminary analysis by RT-PCR of virus loads in the peripheral blood indicate that antibody levels prior to the second challenge correlates inversely with detectable virus signal. The PCR and restriction analysis indicated that the isolated virus 3 weeks post infection was SIV_{mac239}, but in some animals at later time points the mac239 isolate was not detected. These finding show that rhesus macaques with varying levels of pre-existing SIV immunity to $SIV_{mne/E11s}$ can be dually infected with SIV_{mac239}. The level of dual infection and pattern of virus isolation appears to be a function of the level of pre-existing SIV immunity, but this immunity is not sufficient to block infection by the second isolated.

J 247 FIALURIDINE (FIAU) TRIPHOSPHATE INHIBITS BOVINE HEPATIC DNA POLYMERASE-γ *in vitro*: POSSIBLE MECHANISM OF TOXICITY OF FIAU, William Lewis*, James F. Simpson*, Joseph M. Colacino† and Ralph R. Meyer‡ *Department of Pathology University of Cincinnati (UC) College of Medicine, ‡Department of Biology, UC College of Arts and Sciences, Cincinnati, OH 45267-0529 and †Lilly Research Laboratories, Indianapolis, IN

Hepatitis B virus (HBV) infection is prevalent in AIDS. Additionally, chronic hepatitis (CH) due to HBV infection is a liver disease that afflicts approximately one million Americans and up to 5% of the world's population. Major goals of therapy in chronic HBV infection include reduction or elimination of viral replication and halting progress of irreversible hepatocellular disease. Fialuridine [FIAU, 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouridine] recently was used in clinical trials for CH with HBV and was found to be extremely toxic to patients. Evidence pointed to a possible FIAU mitochondrial toxicity, but mechanisms were unclear. Since we showed that zidovudine (AZT) triphosphate inhibited bovine mitochondrial DNA polymerase-γ (DNA pol-γ, the enzyme responsible for mtDNA replication) at micromolar concentrations (Lewis et al., 1993 [in press]), we reasoned that similar mechanisms of DNA pol-γ inhibition may occur with nucleoside analogs like FIAU. Inhibition kinetics of purified bovine cardiac and hepatic mitochondrial DNA pol-γ were defined with FIAU triphosphate (FIAUTP). The K_m for dTTP was 0.8 ± 0.3 μM. FIAUTP incubation with DNA pol-γ *in vitro* resulted in a competitive K_i of 0.02 μM. The K_i for FIAUTP with DNA pol-γ was significantly lower than that determined for AZTTP with DNA pol-γ. Unphosphorylated FIAU inhibited DNA pol-γ minimally at relatively high concentrations (<15% inhibition at 30 μM FIAU). Biochemical findings here suggest that inhibition of mitochondrial DNA pol-γ may be integral to the pathogenesis of FIAU-induced toxicity. FIAUTP is an active toxic moiety in this *in vitro* model of FIAU toxicity.

J 248 BIOPHYSICAL, BIOCHEMICAL, AND SPECIMEN PROCESSING CONSIDERATIONS IN THE USE OF QC-PCR FOR ANALYSIS OF HIV-1 VIRAL LOAD IN PLASMA OF INFECTED PATIENTS. J. D. Lifson*, K.C. Luk*, J.C. Kappes**, M.S. Saag**, G.M. Shaw**, and M. Piatak, Jr.*, **HIV and Exploratory Research, Genelabs Technologies, Inc., Redwood City, CA 94063; **Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, 35294*

QC-PCR is an an internally controlled titration-based approach for the sensitive and accurate quantification of HIV-1 nucleic acid sequences. Among the most attractive applications of QC-PCR is its use for quantifying circulating virion-associated HIV-1 RNA in the plasma of HIV-infected patients, in both natural history and treatment intervention studies. To assess whether HIV-1 RNA quantified by QC-PCR from plasma was truly virion-associated, plasma virus was prepared on continuous 20-60% sucrose gradients, and the gradient fractions analyzed to determine specific gravity, p24 antigen content, and HIV-1 RNA content (by QC-PCR). The vast majority of p24 antigen localized to fractions corresponding to the specific gravity expected for intact virions. The QC-PCR-determined HIV-1 RNA peak was superimposable, indicating that the measured RNA is indeed virion-associated. In view of past suggestions that a fraction of circulating culturable HIV-1 is platelet associated, we also evaluated the effect of platelet depletion on the amount of HIV-1 RNA measured in plasma samples. An initial centrifugation at 200 X g was performed to remove formed cells. HIV-1 RNA levels and platelet numbers in the 200 X g supernatants were quantified. A subsequent 1,000 X g centrifugation removed on average >90% of total platelets, but did not appreciably affect QC-PCR determined HIV-1 RNA levels. When the supernatants from the 1,000 X g spins were centrifuged at 10,000 X g to remove essentially all platelets, the amount of QC-PCR determined HIV-1 RNA associated with the platelet pellets was on average 11% of the total present in the supernatant of the 200 X g spin, within the experimental variability of the QC-PCR assay. Platelet associated-virus does not appear to meaningfully influence circulating levels of plasma virus, as determined by our standard QC-PCR procedure. In a final test of the underlying principle of QC-PCR, the same HIV-1 HXB2 DNA preparation was quantitated by QC-PCR using either *gag*-based primers and a *gag* internal control template containing an 80 bp internal deletion or *env*-based primers and an *env* internal control template containing an 80 bp internal insertion. The results obtained in replicate assays (n=9) for quantification of HIV-1 DNA (5,000 copies) confirmed both the accuracy and precison of the QC-PCR method and the validity of the underlying principle of QC-PCR, with mean results of 4,766 (±8%, r.s.d.) for the *gag* reagents and 4,812 (±13%, r.s.d.) for the *env* reagents. Taken together, these results provide further support for the utility of QC-PCR for measurement of virion-associated HIV-1 RNA in plasma, in both natural history and treatment intervention studies.

J 249 DOMINANT NEGATIVE EFFECT OF REV PROVIRAL MUTANTS OF HIV-1. Little S.J., Riggs N.L., Richman D.D, Guatelli J.C. University of California and VA Medical Center, San Diego, CA 92103-6329.

During a study of mutant genomes of HIV-1 containing altered splice acceptor sequences for tat and rev, a negative transdominant effect of a rev mutant was observed. Mutation of the exon 4B splice acceptor sequence resulted in a mutant genome (4BSAM) defective for either exon 4A or exon 4B-containing RNA species. A rev-minus mutant genome was constructed by insertion of a stop codon at amino acid 54 of the rev coding sequence (RS54M). The rev mutants, 4BSAM and RS54M, were both completely replication defective after transient transfection of CEM cells. Both of these defective mutants were complemented by a rev expression vector cotransfected into U937 cells. However, when the 4BSAM or the RS54M mutant were cotransfected with WT pNL43 into CEM cells, a dose dependent dominant negative effect was observed as measured by p24 production. Cotransfection of a 10:1 excess of mutant to WT DNA inhibited tenfold p24 production by the WT virus. This dominant negative effect was not seen in similar cotransfection studies of pNL43 and a tat-minus mutant genome containing a nonsense mutation at amino acid 8 of the tat coding sequence (TS8M). Rather, the TS8M was complemented by pNL43 with a dose dependent augmentation of p24 production. Two potential mechanisms are proposed to explain the dominant defective nature of the rev proviral mutants. 4BSAM and RS54M may function as proviral sources of an RRE decoy in cotransfection studies with pNL43. Alternatively, or in conjunction with the RRE decoy hypothesis, these rev mutants may over-express nef in the absence of a functional Rev protein to direct the accumulation of singly spliced or unspliced mRNA transcripts. Studies are in progress to better define the roles of RRE decoy and nef over-expression in the negative transdominant phenotype of these mutants.

J 250 HUMAN T CELL RESPONSES TO HUMAN PAPILLOMAVIRUS TYPE 16 IN PATIENTS WITH CERVICAL DYSPLASIA AND HPV-16 INFECTION

B.H. McGovern[1], E. Androphy[2], A.K. Goodman[3] and J. Lieberman[1], Tufts New England Medical Center, [1]Division of Hematology-Oncology and [2]Division of Dermatology, Boston, MA and [3]Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA.

Human papillomaviruses are a heterogeneous group of DNA viruses associated with proliferative lesions of cutaneous and genital epithelium. Epidemiologic evidence implies a role for cell-mediated immunity in the prevention of HPV-related disease. High risk groups, including female renal transplant recipients on immunosuppressive therapy and HIV seropositive females with CD4 counts <200, have an increased incidence of cervical dysplasia and cancer.

We are currently investigating lymphoproliferative and cytotoxic T-cell responses to HPV-16 antigens in immunocompetent patients with cervical dysplasia. The presence of HPV-16 DNA is verified from cervical swabs of patients with cervical dysplasia using the polymerase chain reaction method. Peripheral blood mononuclear cells from HPV-16 infected patients are stimulated in vitro with recombinant vaccinia virus vectors encoding each of the open reading frames of the HPV-16 genome in the presence of IL-2 and/or IL-12. These HPV-specific T-cell lines are assayed for lymphoproliferation and cytotoxicity against HPV-expressing targets. To date, we have identified in one patient with severe cervical dysplasia, a proliferative T cell response to the E6 gene product, which has been implicated in HPV-induced transformation and is expressed in HPV-16 infected cervical carcinoma cell lines.

J 251 MONOCLONAL INTEGRATION OF HIV IN HUMAN NON-B-CELL MALIGNANCIES: EVIDENCE THAT HIV MAY CAUSE TRANSFORMATION THROUGH INSERTIONAL MUTAGENESIS. M. McGrath*, B. Herndier*, and B. Shiramizu*. University of California, San Francisco General Hospital, Departments of Laboratory Medicine, Pathology and Pediatrics, AIDS Program, San Francisco, CA.

Six cases of non-B-cell malignancies from HIV-infected individuals have been characterized by immunocytochemistry. One case has been previously reported from a multisite CD4-positive T-cell lymphoma (Herndier et al, Blood 79:1768-1774;1992). The tumor consisted of large pleomorphic cells expressing HIV p24 antigen and IL-2 receptor (CD25) and was negative for B-cell, monocyte, and myeloid markers. The tumor contained a single clonally integrated HIV-1 and was negative for HTLV-I, EBV, and HHV-6. HIV was found clonally integrated in the other five non B-cell malignancies by Southern blot analysis.

In the first case analyzed, HIV p24 antigen was clearly present in the transformed T cells. In all of the other cases HIV was found almost exclusively within the macrophages which were prominent in these tumors. Two of the cases represent mixed immunotype polyclonal lymphomas as defined by gene rearrangement studies (Shiramizu et al, JCO 10:383-389;1992). The other processes included a CD4 positive T cell lymphoma, an AILD-like B cell lymphoproliferation and a Hodgkin's disease.

In order to test whether HIV was present in a clonal form within these tumors inverse PCR (IPCR) was used to map the HIV integration sites. Five of five of the non-B-cell lymphomas showed amplification of two bands with LTR primers while the Hodgkin's tumor had amplification of one band. Further analysis of all five cases of the non-B-cell lymphomas showed that one of the LTR-amplified bands also contained a segment of the *fur* gene, which is located 5' to the *fes/fps* proto-oncogene on chromosome 15. Molecular studies found that the *fes/fps* gene was expressed within tumor but not in control tissue. These data suggest that HIV may cause T cell and potentially macrophage transformation through an insertional mutagenesis mechanism. Further studies using *in situ* PCR will be required to definitively identify the cells that contain the integrated HIV.

J 252 ANTIVIRAL EFFECTS OF IL-10 AND TGF-β1 IN HIV INFECTED MYELOMONOBLASTIC CELLS

Vanessa McKiel[1,2], Mark Wainberg [2,3], and John Hiscott [1-3].
1. Terry Fox Molecular Oncology Group, Abe Stern Cancer Research Laboratory, Lady Davis Institute for Medical Research, Jewish General Hospital. 2. Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada. 3. McGill AIDS Center.

Proliferation and activation of macrophages and T-cells are dependent on a highly regulated network of inhibitory and stimulatory cytokines. HIV replication in these cells is dependent on cellular activation and HIV infection can alter the kinetics of cytokine transcription. Cytokines which deactivate macrophages and T-cells may therefore be important modulators of an antiviral effect. We have demonstrated that treatment of HIV IIIB infected U937 cells with exogenous rIFNα2 decreased the intracellular accumulation of HIV RNA 5 to 20 fold. Further, treatment with both rIFNα2 and AZT was more effective at limiting the rate and spread of HIV than treatment with either antiviral agent alone. IL-10 and TGF-β1 are cytokines that have been shown to possess macrophage deactivating properties; IL-10 has also been shown to inhibit T-cell proliferation and cytokine production. Thus IL-10 and TGF-β1 alone or in combination with AZT are being analyzed for their antiviral potential. Reverse transcriptase assay and p24 immunofluorescence results of acute HIV IIIB infection of myelomonoblastic PLB 985 cells suggest that treatment with IL-10 alone enhances the spread of HIV; this is reduced by co-treatment with AZT. In contrast to this and previous reports, TGF-β1 had little effect on the spread of HIV in PLB 985 cells; TGF-β1 and AZT treatment resulted in a potent synergy, as a reduction in the spread of virus more dramatic than that observed with AZT alone occurs early in infection. The effects of IL-10, TGF-β1 and AZT are being examined in the T-cell line Jurkat. Elucidation of cytokines that control the degree of HIV replication may have an impact on both clinical treatments and understanding the progression to AIDS.

J 253 ROLE OF THE NEF GENE IN THE REPLICATION OF HIV IN PRIMARY HUMAN MACROPHAGES. Pascal R.A. Meylan, Roland Sahli, and Irène Zbinden. Institute of Microbiology, University of Lausanne Hospital Centre, Lausanne, Switzerland.

Nef is conserved among HIV-1, HIV-2, and SIV, suggesting an important functional role for this gene. Such a role is supported by the observation that in a simian system, *nef* is necessary for optimal in vivo replication and pathogenesis. How *nef* affects replication in vivo is unknown. Nef has been shown to reduce the surface expression of the CD4 antigen in various cell types. This effect might increase the effectiveness of viral propagation, for instance by preventing budding virions from being trapped on producing cells by their own receptor. If this were true, then *nef* should have a reduced effect on HIV replication in host cell types where CD4 has a minor role as viral receptor, as we recently demonstrated in monocytes-macrophages (Virology (1993); 93:256-267). To test the effect of *nef* on HIV multiplication in macrophages, the SalI-BamHI *env* fragment of pNL43 and pNL43ΔNef were replaced by the corresponding fragment from the macrophage-tropic envelope clone pBal (pNL43BE and pNL43BEΔNef). Stocks of corresponding viruses were produced in COS cells and shown to be able to infect primary macrophages. The *nef* deletion in pNL43ΔNef has been shown to confer a major replication defect of HIV in primary lymphocytes and various CD4+ cell lines (C.A. Spina et al, and M. Chowers, Abstracts Q 362 and Q 307, Keystone Symposium 1992, Frontiers in AIDS pathogenesis, Albuquerque, March 29-April 4). Macrophages were infected with stocks of NL43BE and NL43BEΔNef produced either in COS cells or in primary macrophages. In contrast to lymphoid cells, the *nef* deletion conferred only a minor (if any) replication defect in macrophages. Our observation is consistent with the hypothesis that *nef* affects HIV replication through CD4 down-regulation.

J 254 HIV-1 GROWN IN CD4-EXPRESSING CELLS MAY BE COATED WITH CD4. John Mills, Carol Hartley, Mark Gilbert*, Tarek Elbeik*, Jay A. Levy* and Suzanne Crowe. Macfarlane Burnet Centre for Medical Research, Yarra Bend Rd, Fairfield, VIC, Australia 3078 and * University of California San Francisco, San Francisco, CA 94143.

Using a CD4-capture immunoassay (Gilbert *et al.*, 1991, J. Clin. Micro. 29: 142.), several strains of HIV-1 grown in CD4-expressing T-lymphoblastoid cells were found to contain little CD4-reactive gp120 (300pg to 4000pg/ml) relative to virus titre ($10^{3.2}$-$10^{5.0}$ $TCID_{50}$/ml) and p24 concentration ($10^{4.9}$-$10^{6.0}$ pg/ml). HIV-1(SF33) replicates to high titre in CD4-negative neuroblastoma cells (Nb) as well as in the CD4-bearing lymphoid cells (VB cells). HIV(SF33) grown in CD4-negative cells (SF33-Nb) contained a much higher concentration of CD4-reactive gp120 than p24 antigen, in contrast to the same virus strain grown in VB cells (SF33-VB). SF33-VB was found to bind minimally to Sepharose-immobilised rs-CD4 (10% of infectious virus titre and gp120 bound), in contrast to SF33-Nb where 99.9% of the infectious virus and 95% of viral gp120 bound. It was postulated that CD4 derived from host cells might be coating virions, concealing binding domains of gp120. CD4-coating of virions was demonstrated directly by i) the presence of CD4 in sucrose gradient purified virus suspensions by immunoblot assay, and ii) the co-sedimentation of ^{125}I-labelled OKT4 with SF33-VB but not SF33-Nb. Coating of HIV particles with CD4 decreases interactions of virions with CD4, may limit the rate of infection via the CD4 receptor and may contribute to lack of efficacy of soluble CD4 *in vivo*.

J 255 INTERLEUKIN-13 : A NOVEL REGULATOR OF HIV-1 EXPRESSION . A. Minty [1], L. Montaner [2], A. Doyle [2], S. Gordon [2], H. Koziel [3], A. Ezekowitz [3], D. Caput [1] and P. Ferrara [1]. *[1] Sanofi Elf Bio Recherches, 31676, Labège, France; [2] Sir William Dunn School of Pathology, Univ. of Oxford, OX1 3RE, UK ; [3] Harvard Medical School, Boston, MA 02115, USA.*

IL-13 is a 112 amino-acid glycoprotein secreted by activated T4 and T8 lymphocytes (Minty et al, 1993, Nature 362, 248; McKenzie et al, 1993, Proc. Natl. Acad. Sci. USA 90, 3735) . It has been shown to have multiple effects on B lymphocytes and on monocytes. In particular, it inhibits HIV-1 production in primary blood derived human macrophages in vitro (Montaner et al, 1993, J. Exp. Med. 178,743). This inhibition does not appear to be mediated by altered viral entry, reverse transcription or viral release. IL-13 also inhibits IL-6 and TNF-α synthesis by monocytes activated by LPS (Minty et al, op. cit.) and other agents. Since these two cytokines increase HIV-1 expression , their inhibition by IL-13 may contribute to the maintenance of post-integration HIV latency. IL-13 also increases the level of mannose receptors, both on monocytes and on alveolar macrophages, which may aid in combating the Pneumocystis carinii infections frequently found in AIDS patients. These various properties are also shown by IL-4, which shares a common receptor with IL-13 in many cell types. However, IL-4 and IL-13 show different activities on the synthesis of IFN-γ by large granular lymphocytes and by T lymphocytes. IL-13 should thus not prevent a "Th1-type" cellular immune response , as is the case for IL-4. We would suggest IL-13 as a candidate cytokine for reducing systemic virus load in AIDS.

J 256 TH1/TH2 CYTOKINE EFFECTS ON HIV-1 ENTRY/REPLICATION IN PRIMARY HUMAN MACROPHAGES & U-937 CELLS

Luis J. Montaner, Georges Herbein, Philip Griffin, Adrian Minty*, Siamon Gordon. *Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE, UK; *Sanofi Elf Bio Recherches, 31320, Labege, France*

Cytokines have been shown to have regulatory properties on HIV-1 replication *in vitro*, and are presently understood to be involved in the pathogenesis of disease. Since the macrophage is central to primary infection, tropism determination, and pathogenesis (CNS, etc), we are interested in studying these cells and the effects of cytokines on fusion, reverse transcription and replication. We will present studies on the effects of Th-1 (TNF-α, IFN-γ) and Th-2 (IL-4 & IL-13) cytokines on primary human macrophages and U-937 cell line to try to elucidate the potential effects on viral replication that these cytokines may be regulating *in vivo*.. To assess entry we carried out a quantitative 72 hour time-course entry study on primary macrophages with P32 labelled PCR primers to the LTR. Results show that TNF-α decreased total viral load per cell, in contrast with IL-4 and IL-13 where there is an increase of LTR signal. The mechanism for TNF-α viral down-regulation of entry during the first cycle of infection is mediated by a CD4 down-regulation in primary macrophages, while IL-4 and IL-13's increase are probably due to the cytokine-induced aggregation effect, as caused by these cytokines in absence of virus. LPS and AZT controls will be presented. Viral replication was examined using HIV-1 ADA on primary macrophages and HIV-1 IIIB on U-937 cells. The results indicated that Th-2 cytokines and IFN-γ inhibit HIV-1, while TNF-α up-regulates viral production in both primary and U-937 cell line after initial inhibition of entry. These results indicate that: (1) Both Th-1 and Th-2 cytokines may be involved in macrophage viral latency and (2)TNF-α may have multiple regulatory effects on HIV-1.

J 257 LOCAL PATTERNS OF CD4 T CELL DEPLETION IN LYMPH NODES OF HUMAN PBL-SCID MICE INFECTED WITH MACROPHAGE-TROPIC HIV STRAINS, Donald E. Mosier, Richard J. Gulizia, Rosemary Rochford, Klara Tenner-Racz, and Paul Racz, The Scripps Research Institute, La Jolla, CA 92037, and Bernhard Nocht Institut für Tropenmedizin, 2000 Hamburg 36,

Introduction of human peripheral blood leukocytes into SCID mice results in persistent lymphocyte engraftment, including infiltration of empty SCID mouse lymph nodes by human T and B cells. HIV-1 infection of hu-PBL-SCID mice leads to depletion of human CD4 T cells, which is detected both in repopulated lymph nodes and human cells recovered by peritoneal lavage. As previously reported, macrophage-tropic, non-cytopathic HIV isolates cause more rapid and extensive CD4 T cell depletion than T cell-tropic, cytopathic isolates. Using a combination of in situ hybridization and immunohistology, we have analyzed patterns of HIV infection and CD4 T cell depletion in the repopulated lymph nodes of hu-PBL-SCID mice. Macrophage-tropic isolates (e.g., SF162) infect small numbers of human cells, and these infected cells appear to trigger apoptosis in nearby uninfected CD4 T cells, resulting in local concentrations of dying cells. By contrast, infection with T cell-tropic strains of HIV-1 (e.g., SF33) leads to a random distribution of both infected and dying single cells, with no evidence for triggering of apoptosis in nearby cells. Overall viral burden is similar with both strains of virus. The explanation for these different patterns of in vivo CD4 T cell depletion triggered by different HIV-1 isolates is not yet clear, but possibilities include local cytokine/chemokine secretion, aberrant T cell signaling via a locally secreted viral or cellular product, or attraction of non-infected CD4 T cells to infected cells. (Supported by NIH grants AI29182 and AI 30238).

J 258 ICD P24 ANTIGENEMIA IN THE DIAGNOSIS OF HIV INFECTION IN VERTICALLY INFECTED BRAZILIAN CHILDREN, Karin Nielsen, Eneida Santos, Norma Rubini, Waldemir Sales, Fernando Sion, C. Alberto Sa, Lian S. Wei, John Fahey, and Yvonne J. Bryson, Department of Pediatrics, UCLA, Los Angeles CA 90024 and Department of Medicine and Immunology, Hospital Gaffree-Guinle, University of Rio de Janeiro, Brazil.

We conducted a blinded retrospective study of serial plasma specimens from 40 children born to HIV infected women and followed in an AIDS referral hospital clinic in Rio de Janeiro for the presence of immune complex dissociated (ICD) p24 antigen (Ag). A total of 125 plasma samples were pre-treated with glycine and examined through quantitative EIA for p24Ag (pg/ml) (Abbott). All charts were reviewed for verification of HIV status and HIV-related clinical findings with HIV serologies from a 2-year follow-up recorded. ICD p24Ag results were compared to p24Ag values obtained from the same samples. 21/40 children were subsequently shown to be HIV infected. 15/21 (71%) had at least 1 positive ICD p24Ag result with 11/21 (52%) also having a positive p24Ag.. The specificity of ICD p24Ag and p24Ag in diagnosing HIV infection in serial plasma of young children was 95%. The sensitivity of ICD p24Ag was 77.8% and that of p24Ag 67.7%. ICD p24 antigenemia predominated in symptomatic children with failure to thrive, developmental delay, recurrent bacterial infections, hepatosplenomegaly, and parotitis. ICD p24 antigenemia preceded the onset of clinical symptoms in 12 children with an age range of 5 months to 62 months (median age:15 mos) and occurred concurrently with clinical findings in the other 3 children. In developing countries, where PCR and HIV cultures are not available, ICD p24Ag assay is useful for confirmation of HIV diagnosis under 2 years of age before the onset of symptoms. A rising ICD and/or p24Ag may also be useful parameters for initiation of antiretroviral therapy under these circumstances.

J 259 MOLECULAR DETERMINANTS OF SIVsmmPBj PATHOGENESIS, F.J. Novembre, M.M. Saucier, D.C. Anderson, S.A. Klumpp, and H.M. McClure. Division of Pathobiology and Immunobiology, Yerkes Regional Primate Research Center and Emory University, Atlanta, GA 30322

The SIV/macaque system is an excellent model for investigating the pathogenesis of infection and disease of AIDS viruses. A variant SIV from sooty mangabeys (SIVsmm), termed SIVsmmPBj, uniformly induces an acutely lethal disease when inoculated into pig-tailed macaques. Utilizing a molecularly cloned virus, PBj6.6, which reproduces the disease, we have started to map important pathogenic determinants of this atypical virus. Chimeric molecular clones generated between PBj6.6 and other non-acutely pathogenic clones have facilitated a dissection of important viral genetic elements associated with acute disease. The major genetic changes, a duplicated NF-κB site in the LTR and a 5 amino acid insertion in env, may not play a role in the increased virulence. However, multiple determinants, including elements in gp40, gag, and the central regulatory genes, appear to be involved in the acute pathogenesis of PBj-induced disease. One of the unusual characteristics of this virus is the ability to induce proliferation of macaque PBMC *in vitro*. Analysis of our chimeric clones reveals that this feature is directly associated with the ability of a virus to induce acute disease *in vivo*. Key to the development of disease may be a dual effect on lymphocytes that the virus exhibits *in vivo*. While inducing a severe lymphopenia in the peripheral blood, SIVsmmPBj also induces a lymphoid hyperplasia seen at regional lymph nodes and along the intestinal tract where the disease is manifested. The induction of lymphopenia appears to be linked to viral determinants in the central regulatory gene region. Studies using additional chimeric molecular clones will enable an understanding of the molecular mechanisms involved in the acutely lethal disease induced by SIVsmmPBj.

J 260 QUANTITATION OF HIV-1 RNA IN PLASMA USING A BRANCHED DNA (bDNA) SIGNAL AMPLIFICATION ASSAY. Carol Pachl, Sue-Jane Fong, Diana Besemer, Bradley Hoo, Tory Yeghiazarian, Steve Zanki, Robert Kokka, Paul Neuwald, Mickey Urdea, and John Todd. Chiron Corporation, Emeryville, CA 94608

HIV-1 RNA levels in plasma can be quantitated using a solid phase nucleic acid hybridization assay (Quantiplex™ HIV-RNA) based on branched DNA (bDNA) signal amplification technology. RNA quantitation is precise and rapid. The interassay coefficient of variation is $\leq 20\%$, and one operator can quantitate 42 specimens (in duplicate) in 24 hours. HIV-1 seropositive specimens have been analyzed with the bDNA assay, using an assay cutoff of 10^4 HIV-RNA Equivalents/ml, and a detection rate of 87% was seen for specimens from patients with $CD4^+$ T-cell counts less than 500 (n=251) and 40% for specimens from patients with $CD4^+$ T-cell counts greater than 500 (n=97). HIV-1 RNA levels have been measured in clinically stable patients not on therapy and, over a 5 to 6 week period, RNA levels remained constant (n=3) or varied up to 3.5-fold (n=1). In contrast, 3- to 13-fold changes in HIV-1 RNA levels were observed monitoring patients during the course of antiviral therapy. These differences in RNA levels are significant based on reproducibility studies (36 assay runs by eight operators at six sites) which indicate that interassay variation of quantitation is 1.8- to 2.9-fold. A comparison of paired plasma and serum specimens (n=22) revealed that plasma, collected using EDTA as the anticoagulant, is the preferred specimen type for the bDNA assay, with 1.6- to 4.5-fold higher HIV-1 RNA levels measured in plasma as compared to serum. No significant difference in background signal was seen comparing seronegative plasma versus serum (n=10).

J 261 COMPLEX REGULATION OF HIV REPLICATION BY IL-4 AND IL-10. Guido Poli*^, Drew Weissman*, Audrey L. Kinter*, Elisa Vicenzi^, Delia Goletti*, and Anthony S. Fauci*, *Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892 USA; ^AIDS Immunopathogenesis Unit, Istituto Scientifico "S. Raffaele", Milano, Italy.

The effects of IL-4 and IL-10 on acute and chronic in vitro HIV-1 infection of peripheral blood mononuclear cells (PBMC) stimulated with interleukin-2 (IL-2) as well as on different systems of monocytic cell infection were investigated. IL-4 has been previously described as an HIV inductive cytokine in human thymocytes and monocyte-derived macrophages (MDM) infected in vitro, although suppressive effects have also been reported. We have observed that IL-4 suppressed HIV replication in IL-2-stimulated PBMC, a system in which virus production is sustained by secretion of endogenous pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), IL-1, and, frequently, interferon-γ (IFN-γ). In contrast, IL-4 synergized in the induction of HIV expression with pro-inflammatory cytokines exogenously added to the chronically infected promonocytic cell line U1, that is characterized by a state of relative microbiological latency. The role of IL-10 in HIV replication was studied in primary MDM infected in vitro with the macrophage tropic BAL strain of HIV-1. Pretreatment of MDM with IL-10 prior to infection downregulated virus production in a concentration-dependent manner. The same concentrations of IL-10 blocked production of TNF-α and IL-6 from the infected MDM. Furthermore, addition of recombinant TNF-α and IL-6 to the MDM cultures abrogated the suppressive effect of IL-10 on HIV replication, suggesting that IL-10 acted by blocking virus production driven by endogenous TNF-α and IL-6. However, an acceleration of the kinetics of HIV replication was observed when IL-10 was added to the MDM cultures together with exogenous TNF-α and IL-6. These data suggest that in addition to blocking endogenous cytokine production, IL-10 independently synergizes with TNF-α and/or IL-6 once these cytokines are already present. In support of this interpretation, IL-10, similar to IL-4, potently synergized with exogenous TNF-α and IL-1 in inducing virus expression from U1 cells. Thus, both IL-4 and IL-10 exert complex regulatory effects on HIV replication as a function of their ability to modulate either the production or the effect of pro-inflammatory cytokines such as TNF-α and IL-1.

J 262 Recombinant HIV-1 Vpr Protein Induces Cellular Differentiation In Vitro: Y. REFAELI, D.N. LEVY, and D.B. WEINER*; University of Pennsylvania, Philadelphia, Pennsylvania, 19104.

The vpr regulatory gene appears to play a central role in HIV-1 infection of specific cell types in vitro, and may be important for development of high virus load and disease in vivo. We have reported that vpr can contribute to viral infectivity and pahthogenesis through the induction of changes in the genetic program of cells. In order to study the biochemistry and function of vpr, we have constructed recombinant baculoviruses containing the vpr open reading frame from HIV-1 NL-43, and used these constructs to express vpr protein in SF-9 insect cells. Baculovirus produced vpr is found in the supernatants of SF-9 cells and is principally a 15 kd protein identical to viron borne vpr as determined in western blot and silver stain PAGE. In agreement with reports of bacterial and synthetic vpr a fraction of the protein is observed to migrate on SDS-PAGE as an apparent homodimer. We have purified recombinant vpr using affinity chromatography and examined its biochemistry and function in vitro. Baculovirus vpr induces growth inhibition and cell differentiation identical to that previously reported by us to result from transfection of cells with a vpr gene expression vector. We analyzed reactivity of vpr with HIV patient sera samples. Nearly 100% of late-stage HIV+ patients reacted with this baculovirus produced vpr. Using antigen capture ELISA we observed vpr protein in the serum of infected individuals. Thus, the posibility that extracellular vpr may play a role in HIV pathogenesis should be considered. The implications of these data will be discussed.

J 263 EXPRESSION OF HIV-1 NEF PROTEIN IN HUMAN T CELLS INDUCES RAPID INTERNALIZATION AND DEGRADATION OF SURFACE CD4, Sung S. Rhee, Jon W. Marsh, Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892

HIV-1 Nef is a myristylated protein with a relative molecular mass of 27,000, is localized to the cytoplasmic surface of cellular membranes, and has been reported to down-modulate CD4 in human T cells. To understand the mechanism of HIV-1 Nef-mediated down-modulation of cell surface CD4, we expressed the Nef protein in human T cell line VB. Expression of HIV-1 Nef protein down-modulated surface CD4 molecules. In pulse-chase experiments, the CD4 molecules in the Nef-expressing cells were synthesized at normal levels. However, the bulk of the newly synthesized CD4 protein was degraded with a half-life of approximately 4 to 5 hours, compared to the 20 to 24 hour half-life in control cells. This Nef-induced acceleration of CD4 turnover was inhibited by addition of lysomotropic agents NH_4Cl or chloroquine to the media. Surface CD4 binding experiments showed that CD4 molecules in Nef-expressing T cells are transported to the plasma membrane with normal kinetics, but are then rapidly internalized. These results suggest that HIV-1 Nef-induced down-modulation of CD4 is due to rapid internalization of surface CD4 and subsequent degradation in the lysosome.

Upon T cell activation, surface CD4 molecules are phosphorylated by protein kinase C and dissociated from $p56^{lck}$, followed by internalization of CD4. Since Nef-mediated CD4 downregulation does not require phosphorylation of CD4, we determined whether HIV-1 Nef protein triggers $p56^{lck}$ dissociation from CD4. Preliminary studies suggested that a reduced amount of $p56^{lck}$ associated with surface CD4 in the presence of the Nef ptrotein. Studies are currently underway to better understand the mechanism by which CD4 is dissociated from $p56^{lck}$ in HIV-1 Nef-expressing T cells and the effect of free *lck* kinase on the T cell activation pathway.

J 264 FUNCTIONAL CHARACTERIZATION OF CD4 AND CD8 T CELL SUBSETS MISSING IN HIV-INFECTED CHILDREN AND ADULTS. Mario Roederer, Ron Rabin, Eric Wunderlich, Paul Raju, Leonore A. Herzenberg, and Leonard A. Herzenberg. Department of Genetics, Stanford University, Stanford, CA 94305.

We have used four-color immunofluorescence flow cytometry to identify specific subsets of both CD4 and CD8 T cells that are missing in HIV-infected individuals. In infected pediatric patients, there is a greater than 50% loss of naive T cells. The naive phenotype must be defined by at least three simultaneous antigens, as CD4+ (or CD8+) and CD45RA+ L-selectin+, or alternatively, CD4+ (or CD8+) and CD45RA+ CD11a-dim. The loss of this population is relatively uniform among infected children, independent of their age or status. The loss is probably brought about by either of two mechanisms: reduced T cell lymphopoiesis, or a faster transit through the naive compartment. The latter mechanism may involve inappropriate stimulation by the inflamed status of the patients, resulting in generation of improperly matured and dysfunctional "memory" cells.

Four color flow cytometry distinguishes several "memory" subpopulations of both CD4 and CD8 T cells. We are determining the functional capacities of these subpopulations by FACS sorting them at extremely high purity and assaying proliferative capacities and cytokine production in response to various stimuli. The different subsets have easily distinguishable Calcium-fluxes after anti-CD3 stimulation; likewise, their proliferative capacities vary in predictable ways. The different subsets also have distinct cytokine profiles.

The functional analyses of the subsets are critical from the standpoint of the alteration of subset representation in HIV-infected individuals. If the memory cells in infected individuals have been inappropriately matured, then the functional capacity of these cells may be significantly altered. Such mechanisms could contribute to the anergic, immunodeficient capacity of the T cell compartment in infected individuals.

J 265 ASSOCIATION BETWEEN IMMUNOLOGICAL DECLINE AND THE LEVELS AND CELLULAR DISTRIBUTION OF VIRAL DNA AND RNA IN LYMPHOID ORGANS OF SIV-INFECTED MACAQUES. Yvonne J. Rosenberg, M.G. Lewis, E.C. Leon, G. A. Eddy and Jack Greenhouse, Henry M.Jackson Foundation Research Laboratory, Rockville, MD. WRAIR#, Rockville, MD.

Decline in the CD4+ T cells and HIV viral burden in the blood have been used as closely related correlates of disease progression. However since lymphocytes in the blood provide only a small window on the total lymphocyte pool, it is important to understand how viral load in various organs in the lymphoid compartment are associated with their immunological degeneration and disease state. To achieve this, total (pol) viral DNA and unintegrated 2-LTR circular DNA levels as well as viral RNA expression were assessed in blood, spleen, lymph nodes (LN),bone marrow, peyers patches and thymus from SIV-infected monkeys using PCR. Monkeys chronically infected with the SIV-251, SIV-E11S, and SIV-PBj-14 isolates were compared to determine how biologically different isolates affect the clinical course of disease. The results indicate that CD4% decline of LN occurs over a wide range of total viral DNA levels and does not always correlate with high RNA levels. A comparison of DNA levels following infection with different isolates indicates that SIV-251 infection results in >10-fold higher levels of viral DNA than E11S or PBj-14 and may account for the more rapid disease progression. These DNA levels however do not predict progression to AIDS and do not directly correlate with the CD4% decline in nodes. Overall, the best correlate of LN collapse, both architectural and CD4/CD8 ratio decline, seen with advanced disease and/or death, is a significant increase in unintergrated viral DNA levels. This increase in pol and circular DNA is striking in the thymus in late infections. Cell sorting studies of LN cells from macaques with mid- and late stage disease indicate that viral DNA and unintegrated circular DNA is predominantly in non CD4+ lymphocytes, suggesting a transition in viral synthesis from a primarily T-cell source to a predominantly macrophage/dendritic cell source. The association between total viral DNA, unintergrated viral DNA and viral replication in the lymphoid organs and the loss of immune function and lymphoid organ degeneration will be discussed.

J 266 THYMOCYTES AND MACROPHAGES ARE TARGETS OF HIV-1 INFECTION IN THE NEONATAL THYMUS, Michael Rosenzweig, Ronald Collman, Douglas P. Clark, Elizabeth M. Bunting and Glen N. Gaulton, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Pediatric AIDS is a devastating disease that is increasing exponentially worldwide. The importance of *in-vivo* thymic infection with HIV-1 in pediatric AIDS is exacerbated by the fact that the thymus is the site of T lymphocyte ontogeny during fetal and neonatal development. Analysis of thymic infection by HIV-1 was conducted on both fresh thymic tissue isolated from infected neonates, and human thymic organ culture infected *ex-vivo*. The sites of viral replication were first examined via *in-situ* hybridization for the presence and distribution of viral RNA. Viral RNA was detected in focal cortical caps of the thymus in both thymocytes and macrophages. Infected tissue was also subjected to fluorescent antibody staining for HIV-1 viral proteins, which were again found to be present in thymocytes and cortical macrophages. Neonatal thymic organ culture was next used as a model for thymic infection, to further characterize the thymotropism of various HIV-1 isolates. Infection of thymic organ culture explants reflected the macrophage tropism of the particular HIV-1 isolate. Strains of HIV-1 that are lymphocyte tropic replicated poorly, or were unable to cause infection of the thymic explants. In contrast, macrophage tropic strains showed a high level of HIV replication and induced profound thymocyte mortality. Thymic macrophages were isolated from normal neonatal thymus and shown to be capable of supporting *ex-vivo* HIV-1 infection with minimal cytopathology. These studies identify thymic macrophages and immature thymocytes as primary targets of HIV-1 infection. The role of HIV-1 infection of thymocytes and thymic macrophages in the pathogenesis of pediatric AIDS may be linked to disruption of the normal developmental sequence of thymocyte maturation.

J 267 CONSEQUENCES OF HIV-MEDIATED SUPERANTIGEN STIMULATION OF HUMAN T LYMPHOCYTES, Jeffrey L. Rossio, Julian Bess, Jr., Louis E. Henderson and Larry O. Arthur, AIDS Vaccine Program, PRI/DynCorp, NCI-Frederick Cancer Research & Development Center, Frederick, MD 21702

HIV-1 virus, when grown in human cells, bears on its surface structures encoded by MHC Class I and Class II genes (Science 258:1935, 1992). This human cellular HLA antigen on HIV-1 is present in a biologically active form, since it will cooperate with superantigen such as staphylococcal enterotoxin A (SEA) to stimulate resting T lymphocytes from humans or other species. If HIV-1 was grown in mutant cells (174xCEM.T2) lacking HLA antigens, no T cell activation was observed in association with SEA. The Class II-bearing HIV-1 activated T cell cultures to proliferate strongly, as measured by uptake of tritiated thymidine after 72 hours of culture (resting cells + SEA, 482 cpm; SEA alone, 659 cpm; SEA + HIV-1, 4325 cpm). Virus grown in the .T2 cells, lacking HLA-DR, was not stimulatory (434 cpm). A number of purified HIV-1 proteins were examined for superantigen activity in cultures of resting human T cells and antigen-presenting cells (blood monocytes). No superantigen activity was observed in gp120, gp41, p24, p17, p7 or p6 preparations. Human T cells stimulated by HIV-1 and SEA also produced significant amounts of Interleukin-2 (>4800 pg/ml in 72 hours). Proliferation and cytokine production were not dependent upon productive virus infection, since the reaction with SEA proceeded equally well in the presence of AZT. The result of extended culture of resting human T cells with HIV-1 bearing HLA-DR and SEA was apoptotic death, as evidenced by morphological criteria, as well as by the fragmentation of cellular DNA. This programmed cell death was not seen in cultures exposed to SEA with the .T2 virus, which lacks HLA-DR. The presence of functional HLA antigen on the surface of HIV-1 may be significant in viral pathogenesis via induction of anergy and/or apoptosis in human T cells.

J 268 THE SIV_{MAC239} NEF PROTEIN PROMOTES THE RAPID DEGRADATION OF CD4 IN HUMAN T LYMPHOCYTES, Annika Sanfridson and Carolyn Doyle, Department of Immunology, Duke University Medical Center, Durham, NC 27710

The human and simian immunodeficiency virus early gene product Nef has previously been shown to induce the specific down regulation of CD4 from the cell surface in both lymphoid and non-lymphoid cells. We have examined the mechanism by which Nef mediates this effect, using the human CD4+ T cell line CEMss stably transfected with the SIV_{mac239} *nef* gene. The presence of Nef in these cells promotes the rapid degradation of CD4. The biosynthesis and oligosaccharide processing of CD4 in Nef-transfected cells is normal through the ER and Golgi compartments. The degradation of CD4 is a late event in the biosynthetic pathway and is sensitive to treatment with lysosomotropic agents. Both chloroquine and primaquine enhance the stability of CD4 in Nef-transfected cells, suggesting that degradation of CD4 takes place in an acidic compartment. Inhibition of degradation by lysosomotropic agents does not restore the CD4 cell surface levels, as measured by FACS. Quantification of CD4 in Nef-transfected cells reveals that the steady state level of CD4 is dramatically reduced as compared to control tranfectants. The degradation of CD4 in an acidic compartment in Nef-expressing cells correlates with the reduced levels of cell surface CD4.

J 269 HIV-1_{SF2} NEF ASSOCIATES WITH A CELLULAR SERINE KINASE, Earl T. Sawai, Andreas Baur, B. Matija Peterlin, Jay A. Levy, and Cecilia Cheng-Mayer. Cancer Research Institute and Howard Hughes Medical Institute. University of California-San Francisco, CA. 94143-0128.

The Nef gene product of HIV is a myristylated phoshoprotein of approximately 27 kDa. While several studies indicate that Nef suppresses virus replication in T cell lines and transcription from the viral LTR, others report that Nef enhances virus replication in peripheral blood lymphocytes. Furthermore, in SIV-infected rhesus monkeys, expression of Nef is required for maintenance of high viral loads and progression to AIDS. These results suggest that Nef can exhibit pleiotropic effects on virus replication. Since the exact mechanism of Nef's action is not clearly understood, the identification and characterization of its potential intracellular target(s) is necessary. Using T cell lines that constitutively express $HIV\text{-}1_{SF2}$ Nef in the form of a hybrid CD8-Nef fusion protein, a cellular serine kinase was found that specifically associates with Nef. Proteins of 62 kDa and 72 kDa, which co-immunoprecipitated with Nef were phosphorylated in *in vitro* kinase assays. The 62 kDa and 72 kDa phosphorylated proteins also associated with Nef in T-cell lines chronically infected with HIV-1. Nef, however, was not phosphorylated in the *in vitro* kinase assay. This Nef-associated serine kinase activity was not blocked by inhibitors of protein kinase C or protein kinase A, and was lost when Nef was truncated at amino acids 96 or 100. In an attempt to identify the kinase that associates with Nef, immunoblot analyses using antibodies to known serine kinases and other signal transducing molecules have been performed; antibodies against Raf, S6 kinase, GTP-associated protein (GAP), Lck, and MAP kinase do not react with any of the proteins associated with Nef. Kinase activity was also observed when SF2 Nef was expressed in NIH 3T3 or COS-7 cell lines. However, unlike the case of T cells where both of the 62 kDa and 72 kDa proteins are phosphorylated, a 62 kDa protein was the only phosphorylated substrate in *in vitro* kinase assays. We are currently characterizing various Nef mutations and alleles for their ability to associate with this kinase activity. Presently, it is not known whether the 62 kDa protein is the serine kinase or if another protein in the immunoprecipitates is responsible for this activity.

J 270 DEVELOPMENT OF NEW SCID MOUSE MODELS FOR AIDS RESEARCH, Leonard D. Shultz[a], Peter A. Schweitzer[a], T.V. Rajan[b], and Dale L. Greiner[c]; The Jackson Laboratory, Bar Harbor, ME 04609[a]; University of Connecticut Health Center, Farmington, CT 06032[b]; University of Massachusetts Medical Center, Worcester, MA 01605[c]

The objectives of this study were to develop new genetic stocks of immunodeficient mice that will support the growth of human hematolymphoid cells, thus facilitating infection with human immunodeficiency virus (HIV). The development of small animal models for AIDS research has been advanced by the findings that C.B17 mice homozygous for the severe combined immunodeficiency *(scid)* mutation support the growth of human fetal and adult hematolymphoid tissue. Although the use of *scid/scid* mice as hosts for HIV-infected human hematolymphoid cells offers great promise as a small animal model for AIDS research, the engraftment of immunodeficient mice with such cells is limited by the activity of host natural killer (NK) cells and by the extreme radiosensitivity of these mice. The emphasis of our genetic manipulations has been to construct stocks of *scid/scid* mice that have decreased levels of NK cell activity. Depletion of such NK cells has been accomplished by backcrossing the *scid* mutation onto inbred strains of mice with low NK cell activity and onto a strain of mice (C57BL/6J) that enables depletion of NK cells with monoclonal anti-NK 1.1 antibody. Additional series of crosses has produced mice simultaneously homozygous for the beige *(bg)* and the *scid* mutations on different inbred backgrounds. Homozygosity for the *bg* mutation causes depression of NK cell activity. Finally a stock of hematopoietically stem cell defective *scid/scid* W^{41}/W^{41} mice has been constructed to facilitate repopulation with human hematopoietic stem cells without the need for X-irradiation. Construction of this stem cell defective stock utilizes a mutation (W^{41}) at the dominant white spotting *(W)* locus. These NK cell-depleted and hematopoietically stem cell defective mice have been analyzed for histopathological, hematological, and immunological characteristics. The results of these studies and assessments of the ability of these mice to support growth of human hematolymphoid cells will be presented.

J 271 DECREASED EXPRESSION OF COMPLEMENT RECEPTOR 1 (CD35) ON B LYMPHOCYTES IN HIV-INFECTED PERSONS, Gregory T. Spear, Linda Munson, Mark E. Scott Alan L. Landay, Dept of Immunology/Microbiology, Rush Medical School, Chicago, IL

A number of previous studies have shown a decrease in complement receptor 1 (CR1) expression on red blood cells from HIV-infected persons. Studies in our laboratory have found that complement receptor 2 (CR2) is also decreased on both B lymphocytes (Scott et al., 1993, AIDS 7:37) and CD4+ lymphocytes (June et al., 1992, Immunology 75:59) during HIV infection. These results prompted us to assess CR1 expression on CD20+ B lymphocytes, monocytes and neutrophils in blood from HIV-infected persons using flow cytometry.

While 97%±2% of CD20+ B lymphocytes from uninfected control donors (n=9) expressed CR1, only 81%±10% of B lymphocytes from HIV-infected donors (n=9) expressed CR1 (P=0.0004, t test). This reduction is similar in magnitude to, but less dramatic than the reduction in CR2 expression on B cells from HIV-infected persons found in the previous study. In contrast, CR1 on blood monocytes and neutrophils was not reduced in HIV infected persons compared to control donors.

Further studies using three color flow cytometry showed that B cells that lacked CR2 or CR1 from HIV-infected persons also did not express CD10 or B7 indicating that they are not immature or activated. The findings of this study and previous studies showing reduced CR1 and CR2 expression on B lymphocytes in HIV-infected persons could have important implications for the pathogenesis of HIV disease since these molecules have been shown to play a role in activation of B cells.

J 272 SUSCEPTIBILITY OF PBMC FROM DIFFERENT DONORS TO HIV-1 INFECTION. Alexander I. Spira and David D. Ho, The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY.

Selection of HIV-1 populations is known to occur during in vitro cocultivation, as well as during horizontal or vertical transmission of the virus in vivo. Previous reports have indicated that PBMC from different donors variably support the replication of a number of strains of HIV-1. We sought to further elucidate this process using virus primary viruses grown and titrated in normal PBMC, to study the kinetics of their replication in PBMC obtained from a set of normal donors.

For these experiments, viral stocks consisting of both non syncytium inducing (NSI) and SI isolates prepared in the PBMC of a single normal donor. Subsequently, 250 $TCID_{50}$ were inoculated in duplicate into 2 x 10^6 PBMC from different individuals, including those in which the virus was originally grown. Supernatant p24 antigen levels were followed over a period of 14 days. All cultures were infected and HIV-1 grew to p24 levels of >100,000 pg/ml. Thus far, all viruses grew with similar kinetics in each of the donor cells. No PBMC was resistant to infection Viruses grown in the same cells as the original stocks showed no significant difference in susceptibility when compared to growth in different cells. In contrast to previous reports, this study demonstrates that, when using viruses grown and titered in PBMC, any differences seen in growth rates or tropic properties are subtle and not of the magnitude previously thought.

J 273 Follicular dendritic cells (FDC) are permissive for simian immunodeficiency virus (SIV-) infection *in vivo*

L. Stahmer[1], M. Ekman[1], C. Ordonez[1], R. de Mesquita[1], P. Putkonen[2], G. Biberfeld[2] and P. Biberfeld[1]. [1]Immunopathology Lab., Karolinska Hospital/Institute, Stockholm, Sweden,[2]Swedish Institute for Infectious Disease Control, Stockholm, Sweden

AIMS: To study the possible *in vivo* susceptibility of spleen follicular dendritic cells (FDC) as compared to macrophages and, lymphocytes for SIV-infection in monkey lymphoid tissue.

MATERIAL AND METHODS: Lymphnode- and spleen specimens from SIV-infected monkeys (*Macaca fascicularis*) were processed for routine histopathology, immunohistochemistry and transmission electron microscopy (TEM). In addition, cellsuspensions were prepared by enzymatic desintegration of the tissue and macrophages, lymphocytes and FDC were enriched by an immunoaffinity method (MACS). Virus infection was assayed by the above mentioned techniques, PCR and *in situ* hybridization.

The frequency of latent (DNA) and productive infected (mRNA) cells within the different cell populations was assayed by use of nested PCR using limiting dilutions.

OBSERVATIONS AND CONCLUSION: As evaluated by the different methods a prominent accumulation of viral antigen and viral particles in association with germinal center cells of SIV-infected cynomolgus monkeys was observed. he different cell populations were found to contain proviral DNA as evaluated by nested PCR. By in situ hybridization for virus mRNA, rare replication was observed in the lymphocyte fractions and no mRNA was detected in the MF-enrichd fractions whereas the FDC-enriched cell fraction was found to be positive for both, viral mRNA as well as proviral DNA, indicating an viral replication. This was confirmed by EM-studies, which demonstrated budding profiles on FDC-dendrites. Prelimnary results obtained by nested RT-PCR indicate, that the frequency of SIV-infected FDC is higher than that observed for spleen lymphocytes. These findings further support our previous findings, that FDC are acting as a target and a reservoir during HIV/SIV infection in man and monkey respectively.

J 274 THE REGULATION OF THE PROMOTER GENE ENCODING THE p105/p50 SUBUNIT OF NF-kB IS DEPENDENT UPON SPECIFIC kB MOTIF, Rosa M. Ten, William S. MacMorran, Véronique Rolli, Alain Israël*, and Carlos V. Paya, Division of Experimental Pathology, Mayo Clinic, Rochester, MN 55905 and *Institute Pasteur, Paris, France

Our previous cloning and sequencing of the promoter of the gene encoding the p50 subunit of NF-kB indicated that it is upregulated by NF-kB. Furthermore, we have shown that in persistently HIV-infected monocytic cells, which contain increased NF-kB activity, the transcriptional regulation of this promoter is increased. We have further characterized the different regulatory sequences of the p105/p50 promoter that could potentially participate in its autoregulation. One AP-1, four NF-kB like and one HIP-1 motifs are present in this promoter. Gel shift analysis using nuclear extracts from monocytic cells unstimulated or treated with LPS demonstrated specific binding proteins to the AP-1 site, to the three of the four kB sites, but not to the HIP-1 motif. One of the three kB sites (kB4) bound NF-kB complexes with higher affinity than the rest. Deletion and mutation constructs of the p105 promoter cloned upstream a luciferase reporter gene followed by transfection into monocytic cells was performed. The AP-1, kB1, kB2, kB3, and HIP-1 regions were found to be dispensable for the basal and LPS-inducible transcriptional activity of the promoter in uninfected and HIV-infected monocytes. Only the kB4 site was identified as essential in the regulation of the p105 promoter.

These results suggest that the p105/p50 promoter is solely regulated by NF-kB complexes binding with high affinity to only one of the kB motifs in monocytic cells.

J 275 CELLS EXPRESSING MUTATED CDC2 KINASE UNDERGO PROGRAMMED CELL DEATH WITH STRIKING SIMILARITIES TO HIV-DIRECTED CYTOPATHICITY, Huan Tian, Eileen T. Donoghue, Fang Fang*, John W. Newport* and David I. Cohen. Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892; *Department of Biology 0322, University of California, San Diego, CA 92093

Our previous studies have established that HIV can induce a form of programmed cell death with the biochemical signature of mitotic catastrophe (G2/M phase cell cycle death). In general, mitotic catastrophe has been shown to be inducible by single or pairwise disregulation of key mitotic regulatory proteins, such as cdc2. The specific form of HIV-mediated T cell killing is characterized by accumulation of both dramatically hyperphosphorylated p34cdc2, the principal mitotic cyclin dependent kinase, and of the mitotic cyclin, cyclin B. To further investigate the consequences of the apparent disregulation of cdc2 kinase observed during HIV-initiated cell death, we introduced mutant forms of Xenopus cdc2 protein lacking either the regulatory phosphorylation sites T14, Y15, or T161 into the human CD4+ cell line Jurkat. The mutants remained susceptible to HIV infection and to cytopathicity induced by HIV infection. Interestingly, some of the T14 and Y15 transfectants, but not the T161 transfectants, acquired a dominant phenotype with striking morphological and biochemical similarities to HIV-initiated cytopathicity, In particular, transfectants spontaneously formed syncytia, accumulated cyclin B protein, and fragmented their DNA showing they were undergoing programmed cell death. These studies suggest that the partial release from regulatory cdc2 kinase hyperphosphorylation is insufficient to overcome HIV-induced mitotic disregulation and cell death. Importantly, these data show that the direct induction of mitotic disregulation through expression of mutant mitotic kinase cdc2 initiates mitotic catastrophes seemingly indistinguishable from HIV-initiated cell killing, strongly supporting the model that one form of HIV-induced programmed cell death involves mitotic catastrophe.

J 276 THE PRESENCE OF HUMAN p56lck CAN MODULATE THE PATHOGENESIS OF HIV, Michel Tremblay, Réjean Cantin, Richard Bernier, Guylaine Briand and Pierre Bérubé, Infectiologie, Centre de Recherche du CHUL, Département de Microbiologie, Université Laval, Québec, Canada, G1V 4G2.

The CD4 glycoprotein is noncovalently associated with the cytoplasmic *src* family protein tyrosine kinase $p56^{lck}$. This association is thought to be responsible for signalling functions of surface CD4. The role played by $p56^{lck}$ in the process of HIV replication has never been clearly investigated. Thus, to evaluate the putative role of $p56^{lck}$ in the virus replicative cycle, several human CD4+ T lymphoblastoid cell lines that have been previously reported to have no messages of *lck* were transfected with human $p56^{lck}$ using an amphotropic retrovirus vector. The quantification of intracellular human $p56^{lck}$ was achieved by flow cytometry following permeabilization of cell membranes with saponin. Various clones of each cell line, which have demonstrated levels of CD4 expression similar to the parental cell line, were infected with different isolates of HIV-1. Virus replication was evaluated by measuring syncytium formation and reverse transcriptase acitvity in culture supernatants. Results from these studies have indicated that syncytium formation is positively modulated by the presence of $p56^{lck}$. HIV-induced syncytium formation was much more rapid and extensive in cells transfected with $p56^{lck}$. Such effect could not be associated with a more efficient virus entry or with changes in the rate of cellular proliferation. These data suggest that $p56^{lck}$ can play a dominant role in the pathogenesis of HIV.

J 277 NEF INDUCES CD4 ENDOCYTOSIS:REQUIREMENT FOR A CRITICAL MOTIF IN THE MEMBRANE-PROXIMAL CD4 CYTOPLASMIC DOMAIN

Didier Trono and Christopher Aiken, The Salk Institute, 10010 North Torrey Pines road, La Jolla, CA 92037-1099.

CD4 is crucial for antigen-driven helper T cell signalling, and is used as receptor by the human imunodeficiency virus (HIV). The HIV early protein Nef causes a loss of CD4 from cell surfaces through a previously undefined post-transcriptional mechanism. Our experiments demonstrate that Nef acts by inducing CD4 endocytosis, leading to degradation of the receptor in lysosomes. CD4 downregulation is strongly enhanced by the association of Nef with cell membranes, through myristoylation. The study of chimeric molecules reveals that the membrane-proximal portion of the CD4 cytoplasmic domain is sufficient to confer Nef-sensitivity. Within this region, a critical motif functions as a Nef-responsive endocytosis and lysosomal targeting signal. The mechanism of this phenomenon and its consequences on T cell activation pathways will be discussed.

J 278 ASYMPTOMATIC HIV-1 INFECTED SERO-CONVERTERS PROGRESSING TO AIDS HAVE EITHER PERSISTENTLY HIGH OR RISING HIV-1 RNA COPYNUMBER DEPENDING ON CHANGES IN P24 ANTIGENEMIA AND/OR SWITCH IN BIOLOGICAL PHENOTYPE. B. van Gemen[1], S. Jurriaans[2], R. Coutinho[3], D. van Strijp[1], H. Schuitemaker[4], M. Koot[4] and J. Goudsmit[2]. [1] Organon Teknika, Boxtel, The Netherlands, [2] Human Retrovirus Lab., AMC, The Netherlands, [3] Municipal Health Service, Amsterdam, The Netherlands, [4] CLB, Amsterdam, The Netherlands.

HIV-1 viral RNA in serum was quantitated using the NASBA nucleic acid amplification technology. Quantification of RNA was achieved by co-amplification of an internal Standard RNA dilution series, differing only by a 20 nt randomized sequence from the wild-type RNA, thus ensuring equal efficiency of amplification. HIV-1 RNA isolated from an in vitro cultured viral stock solution was used as "Golden standard" to monitor consistency of the quantification method over the time the experiments were performed. Variation in quantification of the Golden Standard ranged from 1.8 till $5x10^{11}$ RNA molecules per ml with a mean value of $3.0(\pm 1.1)x10^{11}$ in 7 duplo NASBA quantifications. The HIV-1 RNA load in serum was determined in longitudinal samples spanning the complete asymptomatic period of 20 seroconverters progressing to AIDS after 445-1994 days. Twelve individuals had stable NSI viruses and had stable and high levels of serum HIV-1 RNA (mean value $6.5x10^5$/ml) during the whole asymptomatic period. Eight individuals showed a NSI to SI virus phenotype switch during the asymptomatic phase. In this group p24 antigen negative sera have a significantly lower average HIV-1 RNA copy number ($3.2x10^4$/ml) than p24 antigen positive sera ($2.3x10^5$/ml), indicating a relation between conversion to p24 antigen positivity and increasing HIV-1 RNA copy numbers. Five of the 8 seroconverters showed a rise in HIV-1 copy numbers accompanying the NSI (mean value $4.6x10^4$/ml) to SI (mean value $4.9x10^5$/ml) switch. The 3 other seroconverters had stable HIV-1 RNA copy numbers with no significant difference between NSI and SI virus phenotypes. Taken together these results indicate that AIDS develops in individuals with high HIV-1 RNA copy numbers from seroconversion till the moment of AIDS diagnosis.

J 279 **HIV-1 INFECTION SELECTIVELY ESTABLISHED BY MONOCYTOTROPIC NON-SYNCYTIUM INDUCING VARIANTS,** Angélique B. van 't Wout, Neeltje A. Kootstra, Greetje A. Mulder-Kampinga, Nel Albrechts, Roel A. Coutinho, Frank Miedema and Hanneke Schuitemaker, Department of Clinical Viro-Immunlogy, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Department of Virology, Academic Medical Centre and Municipal Health Centre, Amsterdam, The Netherlands.

In previous studies we showed that T cell line tropic syncytium inducing (SI) variants of HIV-1 emerge during the later stages of infection and are associated with progression. In contrast, monocytotropic non-syncytium inducing (NSI) variants predominate during the early asymptomatic phase of HIV-1 infected persons. We therefore propose that the monocytotropic variants are responsible for establishing infection in an individual exposed to the mixture of HIV-1 variants. We studied 5 monogamous homosexual couples participating in the Dutch cohort studies of homosexual men and 2 mother-child pairs participating in the Dutch prospective study of HIV-seropositive women and their children. PBMC collected from donor and recipient around the probable time of transmission were used for analysis. We isolated biological virus clones from patient PBMC and determined phenotype by cocultivation with MT2 cells. DNA isolated from the biological clones was amplified by nested PCR for sequencing of the envelop V3 loop. Two of the 5 homosexual donors had a mixture of SI and NSI clones, the other 3 had only NSI clones. In all recipients only NSI clones were detected and in 4 out of 5 couples (including the 2 couples with SI viruses in the donors) a minor variant had established infection. The 2 mothers both had virusses with the NSI phenotype and transmitted a major variant to their child. Our data indicate that either the major or the minor variant can establish infection in the recipient, but only NSI variants are detected in newly infected individuals irrespective of the presence of SI variants in the donor. The finding that monocytotropic NSI variants establish infection has important implications for vaccine development and the understanding of AIDS pathogenesis.

J 280 LACK OF CORRELATION OF VIRUS PHENOTYPE (SYNCTIA INDUCING) WITH ONSET AND PROGRESSION OF DISEASE IN VERTICALLY HIV-1 INFECTED INFANTS, Lorenz Von Seidlein, Eileen Garratty, Ruth Dickover and Yvonne J. Bryson., Department of Pediatrics, UCLA School of Medicine, CA 90024

Vertically transmitted HIV-1 infection may have a bimodal disease presentation with rapid progression to AIDS in some infants before 6 months of age. Factors affecting disease progression include the timing of transmission (*in utero* [IU] vs. intrapartum [IP]), virus load, HIV phenotype, neutralizing HIV antibody, and the infant's immune response. We evaluated the role of HIV phenotype (syncytia inducing [SI] vs. non-synctia inducing [non-SI]), cellular tropism, and the rate of replication *in vitro* (slow low vs. rapid high), on the onset and rate of disease progression in vertically infected infants followed prospectively from birth. Infants were not breastfed and had sequential samples for quantitative co-culture, PCR, and ICD p24 antigen as measures of virus load. Serial HIV isolates from 13 infants were obtained by PBL co-culture from birth up until 45 months of age of age (median:17 mos). 8/13 infants were defined as infected "IU" based on positive cultures/PCR <48 hours of birth, and 5/13 as IP with negative cultures/PCR at birth with subsequent positives. The IU group had a significantly higher virus load and more rapid onset of symptoms and disease progression compared to the IP group. Serial HIV infant isolates (n=33) were titrated in PHA stimulated PBL and MT2 cells for evidence of replication and synctia (ACTG consensus assay) and for tropism in H9, HUT178, MOLT-4, U937 and CEM-ss cell lines. Only 1/33 pediatric isolates was SI in MT2 cells in an *in utero* infected infant whose mother also had SI virus. All others had non-SI isolates transmitted from their mothers which remained non-SI despite the rapid disease progression to AIDS seen in some. Isolates defined as rapid high on PBL were more common in rapid progressors(4/5) vs. slow progressors (0/4). These findings are in contrast to the observed association of SI isolates with disease progression and AIDS in adults. These data suggest that the timing of transmission as *in utero* rather that the transmission or development of SI phenotype may account for the early onset of symptoms in some vertically infected infants.

J 281 TWO POPULATIONS OF CELLS WITH A DENDRITIC MORPHOLOGY EXIST IN PERIPHERAL BLOOD, ONE OF WHICH IS INFECTABLE WITH HIV.

Drew Weissman*,Y. Li*, R. Cherukuri*, J. Ananworanich*, L-J Zhou*, T. F. Tedder*, J. Orenstein* and A. S. Fauci*, * LIR, NIAID, NIH, Bethesda, MD, * Division of Tumor Immunology, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA, and * Department of Pathology, George Washington University Medical Center, Washington, DC.

Dendritic cells (DC) are potent antigen presenting cells derived from the bone marrow. There have been conflicting reports concerning the role of DC in HIV infection regarding their infection, depletion, or dysfunction *in vivo* and whether DC from normal donors are infectable *in vitro*. Standard methods of DC purification include overnight culture, density gradient centrifugation, and depletion of T-cells, B-cells, NK-cells, and monocytes to achieve a highly purified population of DC as defined by function, morphology, and surface antigen expression.

Two populations of cells were identified following standard purification of DC from peripheral blood using a monoclonal antibody (HB15) shown to bind peripheral blood dendritic cells. The $HB15^+$ population did not express T-cell, monocyte, B-cell, and NK-cell markers, had a dendritic morphology by electron microscopy, and had very high levels of HLA-DR. The $HB15^-$ population also had a dendritic morphology but expressed CR1, FcγRI, and FcγRII. The $HB15^+$ cells were much more potent in an autologous MLR than were the HB15/FcγR(I and II)$^+$ population, monocytes, or B-cells in stimulating $CD4^+$ T-cells in the presence or absence of superantigen. The HB15/FcγR$^+$ population was productively infected with HIV-IIIB and HIV-BAL, while the $HB15^+$ cells were not infected with either strain; however, the $HB15^-$ cells when pulsed with virus were able to induce infection in unstimulated $CD4^+$ T-cells. The demonstration of 2 populations of cells with dendritic morphology in peripheral blood, one of which is infectable with HIV, may help to explain the divergent results often observed with DC with regard to HIV infectability *in vitro*, as well as infection, depletion, and dysfunction *in vivo*.

J 282 HIV-1 AND HIV-2 INTERFERENCE WITH T CELL RECEPTOR (TCR)/CD3 FUNCTION AND EXPRESSION, Karen E. Willard-Gallo, International Institute of Cellular and Molecular Pathology, B1200 Brussels BELGIUM

HIV-infection of WE17/10, an IL-2-dependent $CD4^+$ human T cell line, abrogates T cell receptor/CD3 (TCR/CD3) function and expression. We have demonstrated that both HIV-1 and HIV-2 cause a defect in receptor expression due to the specific loss of CD3-γ gene transcripts. We have further shown that this defect does not result from *in vitro* selection of a CD3-γ chain mutant subpopulation nor from changes in the virus itself during *in vitro* infection in a manner that could account for abnormal CD3-γ gene expression.

Examination of receptor density on the surface of WE17/10 cells reveals that TCR/CD3 complexes (and CD3-γ gene transcripts) are quantitatively reduced early after HIV infection (i.e. the cells progress first from **TCR/CD3^{hi+}** to **TCR/CD3^{lo+}** then to **TCR/CD3$^-$**). The passage from TCR/$CD3^{hi+}$ to TCR/$CD3^{lo+}$ is characterized by a progressive decrease in receptor density from 100% to 50% of control values. On the other hand, the change from TCR/$CD3^{lo+}$ to TCR/$CD3^-$ cells occurs by individual cells converting from low postitive to negative.

Receptor function during the transition from TCR/$CD3^{hi+}$ to TCR/$CD3^{lo+}$ cells was examined after synchronizing WE17/10 cells by IL-2 deprivation. Although. stimulation with anti-CD3 + IL-2, leads uninfected cells to downregulate TCR/CD3 expression, increase IL-2 receptor expression, and exhibit enhanced proliferation over untreated controls, the same responses are progressively impaired in TCR/$CD3^+$ HIV-infected cells. Thus, cells expressing 75% of their normal receptor density have limited responses to anti-CD3 + IL-2 stimulation, while those with a 50% decrease in receptor density have completely lost their ability to respond. This data implies that even mimimal interference with TCR/CD3 expression may be sufficient to affect T cell responses via their antigen receptors. Preliminary evidence suggests that interference with TCR/CD3 receptor function and expression in HIV-infected WE17/10 cells results from changes in the expression of a specific viral gene product.

J 283 HIV-1 FROM A LONG-TERM SURVIVOR: MOLECULAR CLONING AND CHARACTERIZATION, Lin Qi Zhang, Yunzhen Cao and David D. Ho, The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY.

Recently, considerable attention has been focused on a unique group of patients who have remained healthy with an intact immune system despite more than a decade of HIV-1 infection. Multiple factors may contribute to the prolonged asymptomatic infection. However, evidence also exists that viral factors, such as lower replication kinetics and cytopathicity, may play an important role in the long-term clinical stability.

In order to investigate possible virus-associated factors involved in the long-term survivors, molecular cloning and biological characterization of HIV-1 have been carried out. Patient PBMC were co-cultured with PHA-stimulated donor PBMC for only a limited period to obtain sufficient proviral copy while minimizing culture selection. The 5' and 3' halves of the HIV genome were amplified by a newly developed PCR method as 4.9 and 4.0 Kb fragments, respectively, and molecularly cloned into a phagemid vector. The intact provirus, containing the entire viral transcription unit plus upstream viral regulatory and cellular recognition sequences, has been reconstructed and tested for phenotypic characteristics *in vitro*. Data generated from these experiments will be presented and discussed.

J 284 MOLECULAR CHARACTERIZATION OF HIV-1 SEXUAL TRANSMISSION. Tuofu Zhu, Ning Wang, Alexander Spira, Yunzhen Cao, Robert Moor-Jankowski, Andrew Carr* and David D. Ho. Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, N.Y., *Center for Immunology, St. Vincent's Hospital, Sydney, Australia.

Several studies to date on the genotypic characterization of HIV-1 in acute seroconvertors have suggested that transmission is selective. However, little is known about the factors involved in the selection. We have therefore characterized the genotype and phenotype of HIV-1 in the peripheral blood mononuclear cells (PBMC) and plasma from a cohort of seroconvertors, as well as in the PBMC, plasma, seminal cells and seminal plasma of the corresponding sexual partners (transmitters). In addition to comparison of DNA and RNA sequences, we have employed the heteroduplex gel shift assay as a complement of large scale sequencing to compare the overall composition of the quasispecies in blood and semen. The envelope gp120 sequences in semen differed to varying degrees from that in the PBMC and plasma in the transmitters. The transmitted virus, similar in seroconvertor's PBMC and plasma, is genetically closer to the viruses in the semen than in the PBMC and plasma of the transmitter. That the transmitted virus is also a minor variant in the semen of the transmitter supports the hypothesis that transmission is governed by selection factors in the new host. We have also found differences in gp120 sequence diversity in seroconvertors compared with viruses in the seminal cells and seminal plasma in the transmitters. The determinants for selective transmission are likely to be complex and widely distributed throughout gp120.

HIV Immune Responses (Humoral and Cellular)

J 300 LOW HIV-1 PATHOGENICITY CORRELATES WITH QUALITY OF IMMUNE RESPONSE AND STRUCTURE OF ENVELOPE PROTEIN, Jan Albert, Gabriella Scarlatti, Thomas Leitner, Mathias Uhlén and Eva Maria Fenyö, Depts. of Virology, Swedish Institute for Infectious Disease Control and Karolinska Institute, S-105 21 Stockholm, Dept. of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

A low proportion of individuals infected by the human immunodeficiency virus type 1 (HIV-1) remain immunologically intact for many years. To identify what distinguishes these long-term survivors is of utmost importance for understanding the pathogenesis of AIDS. We have analyzed HIV-1 V3 loop sequences and presence of autologous neutralizing antibodies in 21 Italian mothers and 12 Swedish homosexual men. The mothers had either transmitted (n=10) or not transmitted (n=11) the infected to their children and the men were selected from a larger cohort of 53 men who have been prospectively followed for many years. The men were divided into non-progressors (n=3), slow progressors (n=3) and rapid progressors (n=6) on the basis of slowly increasing, slowly decreasing and rapidly decreasing CD4+ lymphocyte counts, respectively. Patients, whose PBMC contained a specific mutation in the V3 loop (Arg318 to serine, lysine or leucine) significantly more often had neutralizing antibodies to autologous virus isolates containing arginine at this position. These mutations were also linked a low risk of mother-to-child transmission and long-time survival indicating that Arg318 mutants have a reduced pathogenic potential. We propose that mutations at position 318 presents a means for the virus to evade immune recognition in patients who are able to produce neutralizing antibodies. Thus, we present a model for how the immune system may directly interact with HIV-1 and select for mutants which slow down the pathogenic process.

J 301 DICISTRONIC POLIOVIRUSES WHICH EXPRESS FOREIGN GENES AND NEUTRALIZING ANTIGENIC DETERMINANTS OF HIV-1, Louis Alexander, Hui Hua Lu and Eckard Wimmer, Department of Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, N.Y. 11794

A special property common to all picornavirus genomes is the presence of 'internal ribosomal entry sites'(IRES) which are located downstream from the uncapped 5' termini and confer both cap- and 5' end-independent translation to the viral mRNAs. We have made use of these genetic elements to construct a viral RNA (pPNENPO) that contained two nonhomologous IRES elements (type 1 of poliovirus [PV] and type 2 of encephalomyocarditis virus [EMCV]) and produced a poliovirus (W1-PNENPO) with the following genetic order: PV 5'NTR-EMCV IRES-PV ORF-PV 3'NTR. The insertion of foreign genes into the W1-PNENPO genome between the two IRES elements yielded viable poliovirus with a small plaque phenotype. The foreign genes we have employed in this manner included the coding region for chloramphenicol acetyltransferase (CAT) as well as segments of the HIV-1 envelope glycoprotein gp120. W1-PV-V3-3, a dicistronic PV which contained sequences that included the V3 domain of gp 120 of HIV-1 was used to infect transgenic mice which were engineered to express the PV receptor (PVR+). The genetic stability of these viruses as well as the HIV-specific immune responce in the PVR+ mice infected with these novel agents will be discussed.

J 302 **IMMUNE ACTIVATION IS A MAJOR CO-FACTOR IN THE PATHOGENESIS OF AFRICAN AIDS,** Z. Bentwich, Ruth Ben Ari Institute of Clinical Immunology, Kaplan Hospital, Hebrew University Medical School, Rehovot, Israel.

Hypothesis: Increased activation of the immune system commonly found in the general African population, is a major factor in the pathogenesis of the African pattern of AIDS.

Study: During 1991 a whole community of Ethiopian Jews immigrated to Israel from Ethiopia. We have studied a cohort of these immigrants-300 HIV infected and 100 HIV non infected controls,from the time of their arrival in Israel for both clinical and immune response parameters. The pattern of HIV infection in this cohort is clearly of the African type, while that commonly present in Israel is of the Western type.

Results: On arrival in Israel, infectious and parasitic diseases were highly prevalent. Wide immune activation was present among the HIV non infected as well as the infected immigrants: Blood levels of IgG, IgE, Eosinophiles, IL4, IL6,IL6 receptors, TNF alpha ,soluble TNF receptors, and Isoferitine were all highly elevated. Spontaneous secretion of IL4 and IL6 from PBMC was also significantly higher than in the non Ethiopian controls. Among non Ethiopians, similar findings have been observed only in individuals with HIV infection or among persons belonging to the known risk groups. With the improved living conditions and the sharp decrease in parasitic diseases following their immigration, a gradual decrease in the immune activation has been observed in all immigrants, with a parallel trend for change in the pattern of HIV infection.

Conclusions: These results indicate that: a) The immune system is highly activated in Ethiopians and most probably in other Africans **prior** to HIV infection. b) This activation has some characteristics of TH2 type activation. c)The presence of such activation affects the course of HIV infection and disease and may probably account also for the increased susceptibility to infection seen in Africa. d) A change in environmental factors and particularly the decrease of parasitic diseases may alter this pattern of HIV infection.

J 303 HIV-1 ENVELOPE RESTRICTION; ANTIGEN NOT IMMUNOGEN.

Deborah L. Birx. Nelson Michael, Robert Redfield, Marvin Reitz, Donald Burke, William Blattner*.Department of Retroviral Research, Walter Reed Army Institute of Research, 13 Taft Ct., Rockville, MD 20850 and *NCI, NIH, Bethesda, MD.

We have demonstrated consistent restriction in the cellular recognition of HIV-1 envelopes. This restriction was documented in greater than 75% of HIV-1 infected volunteers despite intact recall responses to other microbial antigens. The restriction was evident utilizing rgp160(BRU), rgp120(IIIB), rgp120(MN), and rgp120(SF2) as recall proliferation antigens. The question remained, was this restriction related to the limitation in envelope antigens available for the assays or a true diminished in vitro cellular recognition of the HIV-1 envelope? In collaboration with the NCI the HIV-1(IIIB)-infected laboratory workers were evaluated. Three of the three IIIB infected individuals failed to recognize any of the HIV-1 envelope antigens in culture despite significant homology between the infecting agent (HIV-1,IIIB) and the rgp160(BRU) and rgp120(IIIB) antigens utilized. Two of the three volunteers had normal recall antigen responses to tetanus, diphtheria, and candida. The cellular restriction of the envelope responses was reversible in 2/3 of the individuals with active immunization with rgp160(BRU), MGS. Although the clinical consequences of this manipulation remains unknown; this cohort represents a unique potential to evaluate the "native" vs. "manipulated" cellular reponses to homologous envelope antigens.

J 304 DEMONSTRATION OF A HIDDEN LINEAR EPITOPE OF HIV-1 gp120 EXPOSED BY EITHER ELIMINATION OF PERIPHERAL MONOSACCHARIDES OF N-LINKED GLYCANS OR BY DENATURING OF THE NATIVE GLYCOPROTEIN.

Anders Bolmstedt[1], Sigvard Olofsson[1], Stig Jeansson[1], John-Erik S. Hansen[2] and John P. Moore[3]. [1]Dept. of Clin. Virology, Univ. of Göteborg, Guldhedsgatan 10 B, S-413 46 Göteborg,Sweden; [2]Dept. of Inf. Diseases 144, Hvidovre Hospital, Denmark; [3]Aaro Diamond AIDS Res. Centre, New York Univ. School of Medicine, 455 First Avenue, New York, New York 10016 U.S.A.

The surface glycoprotein gp120 of HIV-1 is a major target for the host neutralizing immune respons. gp120 is extremely high glycosylated and the three dimensional conformation is very complex containing several neutralizing conformation dependent epitopes.

In the present study we have identified a cryptic linear epitope of HIV-1 gp120 hidden in the native conformation of gp120 but inducible by denaturing of the protein or elimination of peripheral carbohydrates of N-linked glycans.

Purified soluble gp120 was denatured by boiling in presence of SDS and DTT or mock treated prior to capture onto a solid phase. Binding of monoclonal antibodies to gp120 defining specific epitopes was then quantified in an enzyme-linked system. In parallell experiments purified gp120 was linked to a solid phase and subsequently subjected to sequential elimination of peripheral monosaccharide units. Binding of monoclonal antibodies was quantified as above.

We found that this hidden linear epitope, delimited by aminoacids 210 and 220, on gp120 could be uncouvered not only by denaturing of the protein, but also by elimination of peripheral galactose- or fucose units of N-linked glycans.

Our data demonstrate that peripheral carbohydrate structures of N-linked glycans are engaged in modulating the conformation of HIV-1 gp120. These results may be of importance in the strategies to provoke a broadly neutralizing immune respons.

J 305 IMMUNOGLOBULIN A-MEDIATED NEUTRALIZATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE I.

Paul R. Burnett, Victoria Polonis, Thomas C. VanCott, Deborah L. Birx, and Robert R. Redfield, Department of Retroviral Research, Walter Reed Army Institute of Research, 13 Taft Ct., Rockville, MD 20850

The ability of viral-specific human serum IgA to neutralize HIV-1 was studied after screening the sera of 25 HIV-1-infected individuals and identifying 5 with high titer serum IgA specific for both recombinant $gp120_{MN}$ and a synthetic $V3_{MN}$ loop peptide. Protein G immobilized on sepharose was used to deplete these 5 sera of IgG to a level undetectable by nephelometry or viral-specific enzyme immunoassay. Jacalin (a lectin which specifically binds human IgA1) immobilized on agarose was then used to affinity-purify IgA1 from the IgG-depleted sera. The IgG-depleted sera, dual IgG/IgA1-depleted sera, and affinity-purified IgA1 specimens were assayed for neutralization of HIV-1_{MN} *in vitro*, and the results were compared with serum titers of $rgp120_{MN}$-specific IgA and $V3_{MN}$-specific IgA measured by enzyme immunoassay. Both IgG-depleted sera and affinity-purified IgA1 inhibited the infection of CEM-ss cells by HIV-1_{MN} *in vitro*. Specimens showing greater IgA reactivity with HIV-1_{MN} *env* antigens were found to have higher 90% neutralization titers. These data demonstrate that human serum IgA1 can neutralize HIV-1_{MN} *in vitro*.

J 306 COMPARATIVE ANALYSIS OF HIV-SPECIFIC CTL ACTIVITY IN LYMPHOID TISSUE AND PERIPHERAL BLOOD. Butini L., Regnery C., Pantaleo G.*§, Graziosi C.§, Fauci A.S.§, Montroni M. Servizio Immunologia Clinica, Ancona, Italy; *Istituto Scientifico Tumori, Genova, Italy; §LIR, NIAID, NIH, Bethesda, USA

Background: a significant dichotomy in levels of viral burden between peripheral blood (PB) and lymphoid tissue (LT) was observed in patients with HIV disease. High levels of viral replication were observed in LT from asymptomatic subjects with low/absent viremia ("clinical latency" stage), showing that a true latency of HIV does not exist and that lymphoid organs are a major reservoir for HIV. CTL activity plays a major role in immune response against viral infections. HIV-specific CTL have been demonstrated in PB mainly in asymptomatic HIV-infected subjects, but their role in the pathogenesis of the disease has not be fully elucidated.

Objective: to perform a longitudinal comparative analysis of HIV-specific CTL activity mediated by LT and PB-derived MC of the same patients.

Methods: PBMC and LTMC-mediated HIV-specific CTL activity is assayed by 51Cr release using as targets autologous LGL infected with recombinant vaccinia viruses expressing HIV proteins. Quantitative (percentage of specific lysis) and qualitative (viral epitope recognition) differences are evaluated and the results compared with hystopathological patterns.

Results: seven patients have been studied to date. High HIV-specific CTL activity by both PBMC and LTMC was detected in a patient with rapidly progressive disease and high degree FDC network degeneration. In contrast, LTMC-mediated CTL activity was absent, and PMBC-mediated activity was low, in a patient showing no progression of immunodeficiency (long survivor). Intermediate results were obtained in a patient with slowly progressive disease. A longitudinal analysis is conducted and serial lymph node biopsies are performed in selected patients in order to verify the hypothesis of a CTL-mediated immunopathological damage of LT (i.e. disruption of FDC network).

J 307 MECHANISM OF HIV SPECIFIC CYTOTOXIC AND PROLIFERATIVE RESPONSES ELICITED BY PEPTIDE-LIPID COMPLEXES, Mario Canki, Susan Gould-Fogerite and Raphael J. Mannino, UMDNJ-Graduate School of Biomedical Sciences, Department of Laboratory Medicine and Pathology, Newark, NJ 07103.

The Human Immunodeficiency Virus envelope proteins are essential for viral entry, infectivity and pathogenesis. The envelope contains several conserved and variable regions that are targeted by the immune system.

Peptides from the regions of the V_3 loop of HIV III B have been formulated into either plain lipid based complexes or lipid complexes containing Sendai virus surface glycoproteins (F and HN).

These immunogens elicit strong cell mediated responses that are highly specific and predictable in the context of the structure-function relationship of the immunizing complex.

The results of these studies demonstrate strong antigen specific cytotoxic T lymphocyte responses which are MHC restricted and are Ly 2 positive. Induction of helper T lymphocytes was also demonstrated as indicated by proliferative responses.

Peptide-lipid complexes that were less immunogenic due to the properties of the peptide were enhanced to optimal levels by addition of Sendai envelope proteins.

All cytotoxic T lymphocyte responses were specific to peptide-pulsed targets, as well as 3T3 cells transfected with a plasmid expressing gp 160.

These studies demonstrate a specific and predictable way to elicit cell mediated responses in vivo to the V_3 loop of HIV. These observations have implications in design of simple, safe and highly effective subunit vaccines.

J 308 HUMAN FAB TO CONFORMATIONAL AND DISCONTINUOUS EPITOPES OF HIV-1 GP41, Chin-Ho Chen, John E. Monks, Thomas J. Matthews, and Michael L. Greenberg, Department of Surgery, Duke University Medical Center, Durham, NC 27710

HIV-1 infection provokes a vigorous humoral immune response. To better understand the interactions between anti-HIV antibodies and viral antigens we attempted to clone the antibody gene repertoire from peripheral blood mononuclear cells from an HIV-1 infected individual. A cDNA library comprised of more than 5×10^7 members was constructed from kappa light chain and gamma heavy chain Fab regions. The cloned Fab's were subsequently displayed on the surface of a bacteriophage to select for HIV-1 antigen binding reactivities. Fab's were selected with either HIV-1 gp160 or peptides corresponding to the V3 loop in gp120 or the putative leucine zipper region in gp41. Selected and enriched Fab-displaying phage were further engineered to produce soluble Fab secreted into the culture medium. The antigenic specificity of the Fab's was confirmed by ELISA and western blot analysis. Two classes of Fab's were further characterized. The epitope of one class of Fab selected with HIV-1 gp160 was mapped to gp41. This Fab failed to react with a panel of overlapping peptides that spanned the entire extracellular domain of gp41, and appeared to recognize a discontinuous epitope contained within the extracellular domain of gp41. The affinity of this Fab for the extracellular domain of gp41 is approximately 10^{-7}M. Preliminary studies indicate this Fab binds to the surface of HIV-1 infected cells, and studies designed to determine its biological activity will be presented. The second class of Fab was selected with a peptide containing the putative leucine zipper region of gp41. The affinity of this Fab for gp41 was significantly lower that the Fab described above. We will discuss studies designed to determine the nature of the epitope recognized by this Fab as well as its biological activity.

J 309 ANTIGENIC IMPLICATIONS OF HIV-1 ENVELOPE GLYCOPROTEIN QUATERNARY STRUCTURE. Robert W. Doms[1], Patricia L. Earl[2], Deborah Long[1] Bernard Moss[2], and Christopher C. Broder[2] . [1]University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and [2]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892.

Monoclonal antibodies (MAbs) raised against native monomeric or oligomeric HIV-1 envelope (env) glycoprotein revealed significant antigenic differences between env monomers and oligomers, particularly in the gp41 ectodomain. Most MAbs directed against gp41 preferentially reacted with oligomeric env. A subset of these MAbs were oligomer specific - they reacted only with env oligomers. Some of these novel antibodies were neutralizing. In contrast, few MAbs directed against epitopes in gp120 reacted more strongly with env oligomers, and none were oligomer specific. Moreover, approximately 50% of anti-gp120 MAbs preferentially recognized monomeric env, suggesting that epitopes in gp120 can be partially masked or altered by intersubunit contacts in the native env oligomer. Thus, a subunit vaccine that contains the gp41 ectodomain and retains native HIV-1 env quaternary structure may elicit neutralizing antibodies to conserved oligomer sensitive and specific epitopes, and minimize the production of antibodies that preferentially react with monomeric env protein.

J 310 **NATIVE OLIGOMERIC FORMS OF HIV-1 ENVELOPE GLYCOPROTEIN ELICIT A DIVERSE ARRAY OF MONOCLONAL ANTIBODY REACTIVITIES.** Patricia L. Earl[1], Christopher C. Broder[1], Deborah Long[2], Bernard Moss[1], and Robert W. Doms[2]. [1]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, and [2]University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Mice were immunized with native monomeric, dimeric, or tetrameric human immunodeficiency virus type 1 (HIV-1) envelope (env) protein in order to assess the effects of env quaternary structure on the humoral response. 146 monoclonal antibodies (MAbs) were generated and cloned. 55 MAbs recognized epitopes in gp41, 82% of which were conformation dependent. The influence of conformation on gp120 antigenicity was less pronounced, with 38% of the anti-gp120 MAbs binding to conformational epitopes. Most of these blocked CD4 binding. Only 15 of the 88 MAbs to gp120 recognized the V3 loop. This includes 47% of the Mabs derived from animals immunized with monomeric env and less than 5% of the MAbs derived from animals immunized with oligomeric env, suggesting that this region may not be as immunogenic in the context of oligomeric env protein. A set of C terminally truncated env molecules including gp160, 2 sequential truncations in gp41 (635 & 574 residues), gp120, and 3 truncated forms of gp120 (393, 287, & 204 residues) was used to map the region within which each Mab reacts. Interestingly, MAbs to linear epitopes in the C-terminal region of gp120 were not obtained even though it contains a previously identified immunodominant epitope, suggesting that this region of the protein may be masked in the oligomeric molecule. Thus, immunization with oligomeric env may generate a greater proportion of antibodies to conserved, conformational epitopes in both gp120 and gp41 rather than to linear regions of the protein which may or may not be exposed in the native oligomer.

J 311 IMMUNOGENICITY OF HIV-1/GP120 IS DEPENDENT ON PROPERTIES OF THE GLYCOCONJUGATE, Sigrid Eriksson, L. Åkerblom, A. Sjölander, B. Morein and Lena Hammar, Dept of Vet. Vir., BMC, Uppsala, Sweden

Objective. The external envelope glycoprotein of HIV-1, gp120, is under evaluation as antigen in subunit vaccines. This protein i heavily glycosylated. However, the importance of the structure and size of the glycoconjugate for eliciting a protective immune response is uncertain. We present data on the immunogenicity in mice of two envelope glycoproteins, which have been modified by treatment with glycosidases.

Methods. Gp120, purified from HIV-1IIIB infected H9 cell culture medium, and gp160, expressed in Vero cells from the HIV-1IIIB env gene in a vaccinia virus vector, were incorporated into iscoms and treated with 1) buffer (non-modified control), 2) neuraminidase, 3) endoglycosidase-H or 4) glycopeptidase-F. The antigen preparations were analyzed by EM, SDS-PAGE and immunoblot. Groups of 10 mice were immunized two times, four weeks apart, with the 8 antigen preparations. Individual serum antibody titers were measured by ELISA using gp160 and several related peptides as coating antigens.

Results. The serological immune response to the non-modified and modified glycoproteins differed with respect to relative antibody-titre, timing and type of ELISA. As a general the modified proteins gave rise to a lower total titer than the non-modified glycoproteins. This rule was not without exceptions: The Endo-H treated gp120 more efficiently induced antibodies to V3-loop epitopes than the other antigens used. The analyses also indicated a different antibody affinity to gp160 in sera from mice immunized with the deglycosylated antigens. Studies are in progress to iso-type the IgG responses in these groups.

Conclusion. The carbohydrate moieties are important for defining and/or shielding antigenic epitopes.

J 312 PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF $CD8^+$ T CELLS ASSOCIATED WITH LONG-TERM SURVIVAL. John Ferbas, Mary Ann Hausner, Lance E. Hultin, Roger Detels, Janis V. Giorgi, and the Multicenter AIDS Cohort Study. UCLA School of Medicine, Dept. of Medicine/Clinical Immunology and Allergy, Los Angeles, CA, 90024.

In the current study, we characterized HIV-1 infected men from the Multicenter AIDS Cohort Study (MACS) as rapid progressors, intermediate progressors and long-term survivors, and evaluated their cryopreserved PBMC specimens for the levels of the activation antigens HLA-DR (DR) and CD38 on their $CD8^+$ T cells. Our data show that rapid progressors had markedly elevated levels of activated $CD8^+$ T cells that expressed CD38 either alone or with DR and a decreased proportion of DR^- $CD38^-$ (i.e., resting) $CD8^+$ T cells. In contrast, there was less activation of $CD8^+$ T cells among long-term survivors including fewer $CD8^+$ T cells which co-expressed CD38 compared to rapid progressors. Although the DR^+ $CD38^-$ $CD8^+$ cell subset was elevated in long-term survivors as compared to controls, a significant proportion of the $CD8^+$ T cells remained DR^- and $CD38^-$. We hypothesize that the functional role(s) of $CD8^+$ T cell subsets defined by CD38 and DR may differ with respect to their contribution to protective cell-mediated immunity during HIV-1 disease. In this regard, $CD8^+$ cells from the long-term survivors efficiently suppressed HIV-1 replication in vitro in that the removal of $CD8^+$ cells from culture resulted in enhanced levels of HIV-1 p24 in supernatants from autologous $CD4^+$ cells. We are currently designing experiments to define the potential contribution of such $CD8^+$ T cell subsets with regard to suppression of HIV-1 replication in infected individuals.

J 313 REACTIVITY OF SERUM ANTIBODIES FROM SJÖGREN'S SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITH HIV CAPSID PEPTIDES.
William R. Gallaher[1], Hong Luo[2], and Robert F. Garry[2], [1]Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, LA 70112, and [2]Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.
Reactivities to HIV proteins detected by western blotting occur in sera from only 2-4% of normal healthy honors, but in 25-70% of patients with systemic autoimmune diseases such as Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). Since autoimmune individuals constitute a significant fraction of biological false positives in HIV testing, it would be useful to rapidly and specifically distinguish such reactivity in screens for HIV infections that may lead to AIDS. We have done a preliminary linear epitope analysis comparing the reactivities of SS and SLE patients with those found with HIV. Enzyme-linked immunoassays (EIA) were performed using short peptides derived from the sequence of the capsid protein (CA or p24), the major gag protein from HIV. AIDS patients responded to a variety of HIV gag peptides with most reactivity centered about a putative helical region (Hx) and the major homology region of gag (MHR). In SS and SLE patients seroreactivities were limited to only a few peptides. Systemic autoimmune patients failed to react to HIV sequences similar to those of known autoantigens, Sm and La. Some SLE patients responded to the Hx and MHR regions, but nearly all SS patients did not. Most SS and SLE patients recognized one peptide only rarely recognized by AIDS patients. While these distinct epitopes are not contiguous in the linear sequence of HIV p24, they are vicinal on a model that assumes an eight-stranded β barrel structure. The serum reactivities of autoimmune vs. AIDS patients to p24 were thus readily distinguishable by peptide specificity. Indeed, reactivity to the peptide uniquely recognized by SS and SLE patients may serve as a clinically useful marker in EIA to distinguish between autoimmune biological false positives and HIV-specific sera. The source of the reactivity of systemic autoimmune patient serum antibodies to HIV gag proteins is unknown, but may be due to (a) cross-reactivity with as yet undescribed cellular antigens, (b) cross-reactive gag proteins of a human intracisternal A-type retroviral particle associated with SS, to which a large fraction of SS patients specifically react, or (c) other novel human retroviruses.

J 314 ANTIBODY REACTIVITY TO GP120 FROM PROTOTYPE VIRUS STRAINS AND FROM AUTOLOGOUS VIRUS IN SEROPOSITIVE MOTHERS DELIVERING INFECTED AND NON INFECTED INFANTS. Rebeca Geffin, Cecelia Hutto, Maria Fitterman, Manny Master, and Gwendolyn Scott. Department of Pediatrics, Division of Immunology and Infectious Diseases, University of Miami Medical School, Miami, FL 33136.
The correlation between levels of antibody to gp120 and perinatal transmission of HIV-1 was examined in 69 infected mothers born in Haiti, who delivered 69 babies at Jackson Memorial Hospital in Miami, FL. Of the 69 infants, 23 (33%) were infected. Of the 23 mothers that delivered infected infants, 16 (70%) had positive antibody reactivity in western blots to recombinant gp120 from the IIIB isolate, and 7 (30%) did not. Of the 46 mothers who delivered non-infected children, 28 (60%) were seropositive for gp120 and 18 (40%) were not. These results indicate that in this population of mothers, there is no correlation between antibody reactivity to gp120-IIIB and perinatal transmission of HIV-1. In order to elucidate if a negative seroreactivity to the gp120 molecule from the IIIB isolate in some of the mothers was due to differences in the aminoacid sequences between the gp120 from the virus they harbored and the gp120 from the IIIB isolate, reactivity to the autologous isolate was tested. Peripheral blood lymphocytes from 11 of the anti- gp120 negative mothers were cultured, and appearance of virus in the supernatants was monitored using a p24 antigen ELISA. Antibody reactivity to the autologous virus was evaluated using lysed infected cells in western blots. Of the 11 mothers, 9 had a positive antibody reactivity against the autologous virus, one was negative, and one was indeterminate. In addition to the autologous isolate, these sera were tested for their reactivity to recombinant gp120 from the MN isolate using both radioimmunoassay (RIA) and western blots. All of the 11 mothers' sera recognized the gp120 molecule from MN, some with very high reactivity, indicating that the virus harbored in this group of mothers resembles more closely the MN than the IIIB strain. A serum from one mother who was reactive to the gp120 from both MN and IIIB, was used to investigate differences in epitope reactivity to the two strains. In a RIA using either ^{125}I-gp120 from MN or from IIIB, cold gp120 from both strains were used to compete for the binding of the radioiodinated protein to antibodies in increasing dilutions of serum. It was found that while gp120-MN was able to compete completely for the binding of the antibody to gp120-IIIB, gp120-IIIB could only displace about 50% of the counts bound to gp120-MN, indicating that about 50% of the antibody reactivity in this serum was directed against common or conserved epitopes, and 50% was directed against variable epitopes. These results indicate that the determination of antibody reactivity to gp120 or regions of gp120 may be confounded by the virus used as antigen, and that a negative antibody reactivity could indicate differences between autologous virus and the virus used in the assay.

J 315 COMPARATIVE ANALYSIS OF CYTOKINE EXPRESSION IN PERIPHERAL BLOOD AND LYMPH NODES OF PATIENTS WITH HIV INFECTION: LACK OF EVIDENCE FOR TH-1 VERSUS TH-2 IMBALANCE. Cecilia Graziosi, Giuseppe Pantaleo, Kira R. Gantt, James F. Demarest, Oren J. Cohen, Mauro Vaccarezza, and Anthony S. Fauci, Laboratory of Immunoregulation, NIAID, Bethesda, MD 20892.
In this study, we have analysed the constitutive expression of a panel of cytokines in unfractionated mononuclear cells (MC) and sorted CD4+ and CD8+ T cell subsets isolated from peripheral blood (PB) and lymph node (LN) of 11 HIV-seropositive individuals during different stages of HIV infection by semi-quantitative PCR. In all patients analysed, expression of IFN-γ, IL-6, IL-2 and IL-4 was generally higher in the LN compared to PB. High levels of expression of IFN-γ and IL-6 were present in all patients. In contrast, IL-2 was expressed at very low levels in most patients, even in those with only modest decreases in CD4+ T cell counts. IL-4 was consistently negative in PB and barely detectable or absent in LN of 10 patients. One patient showed exceptionally high levels of IL-4 in both PB and LN. However, this patient also showed extremely high levels of expression of all cytokines tested. Levels of expression of IL-10 and TNF-α were high in both PB and LN and did not seem to relate to the stage of disease. Analysis of sorted T cell subsets demonstrated that CD8+ T cells are responsible for the expression of IFN-γ and to a lesser extent of IL-10. In contrast, CD4+ T cells were found to produce cytokine mRNA at very low levels. CD8-/CD4- cell subsets expressed IL-6, IL-10 and TNF-α.
The higher levels of cytokine expression in LN compared to PB indicate a general state of activation of the lymphoid tissue during HIV disease. The finding that CD4+ T cells are very poor producers of cytokines likely reflects the general state of immune dysfunction of CD4+ T cells in HIV infection. Finally, IL-4 and IL-10, the two cytokines recently proposed to be associated with disease progression, are minimally if at all expressed by CD4+ T cells and are either randomly and poorly expressed (IL-4) or constantly expressed (IL-10) by CD8+ T cells and/or non-T cells at all stages of disease.

J 316 HUMORAL AND CELL MEDIATED IMMUNE RESPONSES TO RP400C, A PEPTIDE CORRESPONDING TO THE V3 REGION OF HIV-1_{MN}, Kate A. Hanham, Albert T. Profy, Raman Khiroya, Angela L. Mellon, Paul A. Salinas, Joseph E. Murphy, Alison B. Goldberg, Sonja S. Ramstrom, Rebecca Hunt and Timothy J. Paradis, Repligen Corporation, Cambridge, MA 02139.

Vaccines most likely to be effective against HIV-1 are those which can elicit both neutralizing antibodies as well as cytotoxic T lymphocyte activity. We tested both humoral and cytotoxic responses to RP400c, a cyclic 26 amino acid peptide which contains 23 amino acids corresponding to the V3 region of HIV-1_{MN} envelope protein. BALB/c mice, guinea-pigs, rabbits and cynomolgus monkeys immunized with RP400c formulated in Incomplete Freund's adjuvant (IFA) developed high titer, neutralizing antibodies detectable by ELISA, syncytium inhibition and infectivity reduction assays. Cytotoxic T-cell activity against MN peptide-pulsed syngeneic target cells was also measurable in splenocytes of BALB/c mice, following immunization with RP400c. Specific lysis reached levels up to 40% after mice had received only 2 biweekly immunizations (100μg each). Cross-specificity of both humoral and cytotoxic responses against peptides with sequences corresponding to the V3 regions of non-MN viral isolates were measured, and the results of these studies will be presented. These studies demonstrate that RP400c is immunogenic and capable of eliciting neutralizing antibodies and cytotoxic responses. Our results are in agreement with those of other groups which have demonstrated that the V3 loop contains both B and T cell epitopes. In addition, we have shown that a 26 amino acid peptide corresponding to the V3 region effectively presented these epitopes, without requiring conjugation to a carrier protein. RP400c would appear to be a promising vaccine candidate.

J 317 ANTIBODY CHARACTERIZATION AND VIRUS LOAD IN LONG-TERM ASYMPTOMATIC INDIVIDUALS,

Els Hogervorst[1], S. Jurriaans[1], F. de Wolf[1], A. van Wijk[1], A. Wiersma[1], B. van Gemen[2], F. Miedema[3], J. Goudsmit[1]. [1]Human Retrovirus Laboratory, Univ. of Amsterdam, [2]Organon Teknica, Boxtel, [3]Depart. of Clinical Viro-Immunology, Central Lab. of the Blood Transfusion Service, Amsterdam, The Netherlands.

To obtain information about the immuno-pathogenesis of HIV-1 infection, we studied a group of seropositive individuals, so-called long-term asymptomatics (LTA), who did not progress to clinical manifestations of AIDS and showed no immunological abnormalities within 8 years of follow-up. Of 514 seropositive individuals of the Amsterdam cohort of homosexual men, a group of 10 individual demonstrated persistently normal CD4 counts ($>500/mm^3$) and function (α-CD3 response >1000 cpm), absence of p24 antigen and presence of p24 antibodies. In addition, we studied 2 control groups, one group consisting of individuals who were comparable to the LTA group for 4 years but thereupon showed decreasing amounts of CD4 cells, declining α-CD3 response and finally, clinical symptoms of AIDS (slow progressors). The other control group progressed to AIDS in less than 4 years (rapid progressors). Sera obtained 1 and 5 year after entry of the cohort or seroconversion were tested for neutralization of HIV-1 isolates MN, HXB3 and RF, for the presence of p24 antibodies and for peptide reactivity towards an V3 peptide panel. HIV-1 RNA copy numbers were determined using a quantitative NASBA technique. All individuals showed high neutralization titers to MN. Significant distinction in p24 antibody titers and HIV-1 RNA copy numbers were determined. Differences in RNA copy numbers among the 3 groups, was independent of the virus phenotype present in these individuals. **In conclusion**, we report that neutralizing antibodies cross-reactive to laboratory strains do not appear to be generally involved in disease protection in long-term asymptomatic HIV-1 infected individuals. Significant lower virus load was determined in these individuals, however, virus load was not associated with the syncytium-inducing (SI) or non-SI phenotype of the isolated viruses.

J 318 **HIV-1 SEQUENCE VARIATION AND RECOGNITION BY CYTOTOXIC T LYMPHOCYTES,**

R. Paul Johnson, Spyros Kalams, Alicja Trocha, Mark Dynan, Steve Ngo, and Bruce D. Walker, Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02129.

We have examined the relationship between sequence variation occurring in HIV-1 cytotoxic T lymphocyte (CTL) epitopes in vivo and recognition by HIV-1 specific CTL. In two instances (HLA-B14-restricted CTL epitopes p24/305-313 and gp41/584-592), we have observed the appearance of variant sequences in vivo that were not recognized by CTL clones obtained at several different time points. Lack of recognition of the variant sequences was verified by precursor frequency analysis using bulk PBMC. For both HLA-B14-restricted epitopes studied, the variant *sequences not recognized* by CTL represented the dominant genotype in DNA obtained from unstimulated PBMC at later time points, supporting the hypothesis that these sequences reflected escape variants. Additional evidence in favor of this hypothesis was obtained using an in vitro system in which HIV-1-specific CTL clones were incubated with naturally infected autologous CD4+ lymphocytes, and cultures analyzed for the relative frequency of sequence variation occurring in CTL epitopes. We observed selective replication of viruses not recognized by CTL clones in these cultures, suggesting that the presence of mutations in CTL epitopes confers a selective advantage on the mutated virus.

J 319 T CELL RECEPTOR USAGE BY HIV-1 SPECIFIC CYTOTOXIC T LYMPHOCYTES,

Spyros A. Kalams, R.P. Johnson, A. K. Trocha, M.J. Dynan, H. Steve Ngo, J.T. Kurnick*, B.D. Walker, Infectious Disease Unit and the * Dept. of Pathology Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

Recognition of virus infected cells by cytotoxic T lymphocytes (CTL) occurs through the interaction of the T cell receptor (TCR) with processed viral antigen. We wished to assess the spectrum of TCR usage by HIV-1 specific CTL isolated from infected individuals.

CTL clones were isolated from seropositive individuals and CTL epitopes were defined using autologous EBV lymphoblasts incubated with synthetic HIV-1 peptides. After cDNA preparation from approximately $5x10^6$ cloned T cells, PCR was performed with family specific variable region alpha and beta primers. PCR products were then sequenced directly using the dideoxy chain termination technique.

Ten CTL clones specific for an HLA-B14 restricted gp41 epitope (gp41/584-592) have been isolated from an HIV-1 infected individual over a 31 month span. All ten clones utilize Vα14 and Vβ4 genes. Sequence analysis of the TCR revealed the first nine clones isolated to also be identical at the nucleotide level. The TCR alpha sequence of the tenth clone is likewise identical to the other nine. While this clone shares the same Vβ gene usage as the other envelope specific CTL, it utilizes different Dβ and Jβ segments. The use of these genes is not a feature of HLA-B14-restricted clones in general; an HLA-B14-restricted CTL clone from this subject that is specific for an RT epitope utilizes the Vα21 and Vβ14 genes. A CTL clone from a second subject that shares gp41/584-592 specificity and HLA-B14 restriction utilizes Vβ4, but a CTL clone from a third subject with the same specificity and HLA restriction utilizes Vβ1. Data showing the ability of these envelope-specific CTL clones to recognize peptides representing CTL epitopes of autologous isolates as well as of known HIV-1 variants will be presented.

This study provides evidence that the observed high degree of HIV-1 specific CTL activity in a particular patient may be due to monoclonal or oligoclonal expansion of specific effector cells and that the progeny of a particular CTL clone may persist for prolonged periods in vivo in the presence of a chronic productive viral infection. These data also indicate that structural constraints imposed by the HLA-B14 molecule or the HLA-B14-peptide complex do not limit recognition to CTL expressing Vα14 and Vβ4 genes.

J 320 SIV INFECTION OF MACAQUES: THE ROLE OF VIRAL AND CELLULAR ANTIGENS IN PROTECTION. Kent K, Almond N, Chan WL, Jones W, Kitchin P, Mackett M, Mills K, Page M, Rud E, Trowsdale J, and Stott EJ. National Institute for Biological Standards and Control, Potters Bar, Herts, UK.

Inactivated vaccines composed of virus infected cells or partially purified virus have been shown to induce protection against SIVmac251 grown in human C8166 cells but not when the challenge virus was grown in simian cells. Subsequent experiments in which macaques were vaccinated with uninfected C8166 cells suggested that the observed protection was due, at least in part, to host cell proteins.

To define further the protective role of cellular antigens, macaques have been vaccinated with mouse L cells transfected with MHC Class II (HLA DR4) or with a combination of P815 mouse cells transfected with MHC Class I (A1 and B27) and purified MHC Class I. In both experiments 2 out of 4 vaccinated macaques were protected against challenge with 10 MID_{50} of SIVmac251 (11/88) grown in C8166 cells whereas all control animals (4/4) became infected.

In studies to investigate the role of viral antigens in protection, vaccination with recombinant envelope proteins has failed to confer protection. Passive transfer of immune serum from animals infected with SIVmac251 has also failed to protect against challenge with the homologous simian grown virus. However, infection of macaques with either a full length molecular clone (J5) of SIVmac251 or a variant (C8) which has a 12 base pair deletion in the nef gene prevents superinfection with the heterologous virus. The results of a second attempt to superinfect these animals with a chimeric virus (SHIV-4) composed of HIV-1 HXBc2 env, tat and rev in an SIVmac239 background (Li et.al., 1992) will be presented.

J 321 COMPARISON OF GENETIC SUBTYPE TO NEUTRALIZATION SEROTYPE FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE-1, J.R. Mascola, O.S. Weislow, S.W. Snyder, F.E. McCutchan, J.G. McNeil and D.S. Burke, Division of Retrovirology, Walter Reed Army Institute of Research and Naval Medical Research Institute, Rockville, MD 20850

The human immunodeficiency virus type 1 (HIV-1) can be grouped into at least six separate genetic subtypes (genotypes A-F) based on DNA sequence analysis of the env gene. The possibility that these genotypes correspond to immunologically distinct serotypes may have important implications for vaccine design. Using cross neutralization analysis with virus and plasma from two genotypes (B and E), we have previously shown that plasma neutralization titers were approximately tenfold higher against the homotypic vs heterotypic genotype. Thus, genotypes B and E could be distinguished in a neutralizing antibody assay. Studies are in progress to extend the cross neutralization matrix to multiple genotypes. Preliminary data with a Zambian virus (genotype C) reveals that a panel of eight plasma samples (4 genotype B, 4 genotype E), reacted weakly to the Zambian virus, whereas two genotype C plasma reacted significantly more strongly. These data suggest that HIV-1 genotypes may represent serologically distinct neutralization serotypes. If multiple serotypes of HIV-1 exist, an efficacious vaccine may have to include components of each serotype.

J 322 HIV-1 TAT EXPRESSION IN TARGET CELLS INHIBITS KILLING BY CYTOTOXIC T-CELL LYMPHOCYTES, Masanori Matsui, Robert J. Warburton and Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7290

In progression of disease after human immunodeficiency virus-1 (HIV-1) infection, the functional loss of various cytotoxic T lymphocytes (CTLs) including HIV-specific, influenza virus-specific, allo-specific CTLs is seen. Although several hypotheses have been proposed, the mechanism responsible for CTL dysfunction is still unclear. Tat protein of HIV-1 is essential for replication of HIV-1. Tat has also been reported to suppress antigen-induced lymphocyte proliferation *in vitro*, suggesting that Tat may play a direct role in the immune dysfunction in AIDS. Recently, it has been reported that two-exon Tat (86 amino acids in length) specifically decreased activity of an MHC class I gene promoter (T. K. Howcroft, K. Strevel, M. A. Martin, and D. S. Singer. 1993. Science 260:1320). This data suggests that Tat may downregulate expression of class I molecules on the surface of infected cells, and therefore, may lead to loss of CTL mediated clearance of HIV infected cells. In this study, we have generated a human cell line, T0, expressing Tat (T0-Tat), and have studied the effect of Tat on CTL recognition of human influenza A virus-specific, and allo-specific CTL lines using T0-Tat as target cells. Expression of Tat in target cells inhibits lysis by CTL lines. Surprisingly, surface expression of HLA class I and β2-microglobulin on T0-Tat is not significantly repressed. Pulse-chase analysis followed by Endoglycosidase-H treatment indicates that the rate of maturation of HLA class I molecule in T0-Tat cells is equal to that in normal T0 cells. Because Tat can be secreted from HIV-infected cells and is easily taken up by cells, it was possible that Tat protein secreted from T0-Tat might directly suppress CTL activity. However, addition of either culture supernatant from T0-Tat or T0-Tat cells as cold targets in CTL assays did not inhibit CTL-mediated lysis of T0 cells. The inhibition mechanism of CTL recognition by Tat is currently under investigation.

J 323 AUGMENTATION OF HIV-1 GAG- AND ENVELOPE-SPECIFIC CYTOTOXIC T-LYMPHOCYTE ACTIVITY BY *IN VITRO* STIMULATION OF PBMC FROM HIV-INFECTED CHILDREN. Elizabeth J. McFarland, Harding P, Luckey D, Conway B, Salaman JL, Schooley RT, Kuritzkes DR. Departments of Pediatrics and Internal Medicine, The Children's Hospital and University of Colorado Health Sciences Center, Denver CO 80262 and Department of Microbiology and Immunology, University of Ottawa, Ottawa, CANADA

Adults infected with human immunodeficiency virus type-1 (HIV-1) develop a vigorous HIV-specific cytotoxic T-lymphocyte (CTL) response readily demonstrated in the peripheral blood mononuclear cells (PBMC) without *in vitrro* stimulation. By contrast, HIV-specific CTL activity in unstimulated PBMC from children with vertically-acquired HIV-infection is deficient. We assessed HIV-specific CTL activity in PBMC from eleven vertically-infected children after culture with IL-2, CD3-specific monoclonal antibody, and allogeneic, irradiated normal donor PBMC. Autologous EBV-transformed B-lymphoblastoid cell lines infected with vaccinia recombinants that expressed HIV-1 envelope (Env) and Gag served as targets in a ^{51}chromium-release assay. We demonstrated Gag- and Env-specific cytotoxicity in 91% (10/11) and 78% (7/9) of the infected children respectively. Six uninfected infants born to HIV-infected mothers were similarly examined. Absence of HIV-infection was verified by repeatedly negative HIV-1 culture and PCR. These infants had no Gag- or Env-specific CTL activity in stimulated PBMC. Frequencies of the Gag- and Env-specific precursor CTL (pCTL) were determined by seeding PBMC at limiting dilution prior to culture. Among ten HIV-infected children over age 12 months, HIV Gag- and Env-specific pCTL were identified at frequencies ranging from 0.5-6.3/10,000 PBMC and 0.73-13/10,000 PBMC respectively. Frequencies of HIV-specific pCTL in four uninfected children were below the limits of detection in this assay. Analyses of pCTL using cell populations depleted of either NK cells or CD8+ or CD4+ lymphocytes prior to culture or in assays against allogeneic targets demonstrated that the effector cell phenotype generated was CD8+ and MHC restricted. Further, autologous CD4+ lymphocytes were not required in culture for generation of Gag- and Env-specific CTL activity. Our results suggest that despite the deficiency of circulating activated HIV-specific CTL in PBMC from vertically-infected children, HIV-specific pCTL at high frequencies can be detected after *in vitro* stimulation in the majority of HIV-infected children over age 12 months.

J 324 STUDIES ON THE ANTIGENIC STRUCTURE OF HIV-1 gp120, John Moore, ADARC, New York, NY 10016; Quentin Sattentau, Centre d'Immunologie, Marseille; George Lewis, University of Maryland Medical School, Baltimore; Joseph Sodroski, Dana-Farber Cancer Institute, Boston.

We have been probing the antigenic structure of LAI gp120 with MAbs. Most of the properly folded, monomeric molecule is poorly accessible to antibodies; exposed regions are limited to short segments of C1, V1, V2, V3, C4 and C5. Even less of oligomeric gp120 is exposed on the surface; only stretches of V2, V3, C4 and perhaps V1 are available for MAb binding. The most immunogenic regions of monomeric gp120 lie within C1 from residues 100-120, in C2 from 250-270, and in C3 from 275-285, judged by the high prevalence of antibodies raised in animals against these regions. However, these immunodominant regions are not well exposed on the native molecule. Precise definition of the immunodominant epitopes is in progress. In naturally infected humans, most antibodies are raised against discontinuous epitopes that usually comprise small exposed stretches of the conserved regions. Details of some of these epitopes will be presented, as will data on the rate of induction of such antibodies during primary seroconversion. We are also exploring whether anti-gp120 antibodies against a C1 epitope including the pentamer SLWDQ are able to cross-react with domain 1 of CD4, which also includes the pentamer SLWDQ. This auto-immune reaction has been proposed to have pathogenic consequences. Preliminary results indicate that cross-reactivity is limited to Western blots using denatured CD4 and does not occur under physiologically relevant conditions.

J 325 OLIGOCLONAL SECRETION OF ANTIBODIES TO HIV-1 BY CHIMPANZEE PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO. Stephen M. Nigida, Jr., Carole H. Smith, Michelle R. Shoemaker, and Larry O. Arthur. Viral Diseases and Immunity Section, AIDS Vaccine Development Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

The human immunodeficiency virus (HIV-1) infects cells of the immune system and effects immune functions. Early after infection, a polyclonal hypergammaglobulinemia may be noted in some individuals. Although HIV-1 can infect chimpanzees, no pathological consequences are observed. Peripheral blood mononuclear cells (PBMCs) from chimpanzees responded in vitro to stimulation by the mitogens phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM). PBMCs from HIV-1-infected chimpanzees cultured in vitro in the presence or absence of these mitogens secreted antibodies, detectable by immunoblot, reactive with HIV-1 polypeptides. The secreted antibodies mainly recognized HIV peptides gp160, gp120, p66, p51, and p31. In contrast, plasma antibodies recognized **gag**-coded polypeptides p55, p24, and p17 in addition to the above indicated polypeptides. No antibodies reactive with HIV-1 were noted in PBMC cultures from HIV-1-negative chimpanzees. The amount of antibody detected in vitro increased through six days' incubation. Depletion of T-lymphocytes from these cultures had little effect on the levels of detectable antibody, whereas depletion of B-lymphocytes greatly decreased the amount of detectable antibody. This secretion, in vitro, of antibodies reactive with HIV-1 **pol**- and **env**-coded but not **gag**-coded polypeptides may indicate an antigen-specific, oligoclonal, B-lymphocyte activation in HIV-1-infected chimpanzees. The cause and consequences of this activation remain to be determined.

J 326 IDENTIFICATION OF THREE N-LINKED OLIGO-SACCHARIDES OF HIV-1 GP120 ENHANCING VIRAL SUSCEPTIBILITY TO NEUTRALIZATION BY ANTIBODIES TO THE V3 REGION

Sigvard Olofsson[1], Anna Hemming[1], Gregers J. Gram[2], John-Erik S. Hansen[2], and Anders Bolmstedt[1], Department of Clinical Virology, University of Göteborg, Sweden[1], Department of Infectious Diseases 144, Hvidovre Hospital, Denmark[2]

We have constructed a mutated infectious HIV variant lacking the signals for addition of three N-linked glycans situated in the V4, C4 and V5 regions of HIV gp120. When comparing mutated virus with wild-type virus we found essentially no differences in the phenotypic characteristics of the two viruses except for the expected mobility shift of radioimmunoprecipitated mutated gp120, resulting from the missing N-glycans. Thus, the infectivity titer and the capacity to induce syncytia were similar for the two viruses. We found, however, that mutated virus was significantly more resistant than wild-type virus to neutralization by monoclonal antibodies to the V3-loop, indicating that the three N-glycans are engaged in modulating the three-dimensional conformation of the V3-loop in the infectious virion. In contrast, the sensitivity of neutralization by sCD4 and by the human IgG 2G12, which is a conformational carbohydrate-dependent antibody, was in the same order of magnitude for the two viruses, indicating no major alteration in the three-dimensional conformation of the CD4-binding region of the mutated virus.

The finding that gp120 maintains the three conserved glycans despite the hard evolutionary pressure, would imply that the induction of increased susceptibility to neutralizing α-V3 antibodies per se constitutes a survival advantage. One possibility could be that the increased exposure of the V3 region constitutes a decoy to stimulate the immune response to elicit neutralizing antibodies to the highly variable V3 region rather than to other functional regions of gp120, being more sensitive to changes in the primary structure.

J 327 EVIDENCE OF CD8-MEDIATED SUPPRESSION OF AUTOLOGOUS HIV-1 REPLICATION IN A 5 WEEK OLD HIV-1 INFECTED INFANT. Henry Pollack, Ming-Xia Zhan, Vana Papaevangelou, Sung He Chen, Peter Tao, Keith Krasinski, William Borkowsky, Department of Pediatrics, New York University Medical Center, New York, NY 10016.

Classical methods of CTL assays have failed to demonstrate cell-mediated killing of HIV-1 in infants less than 6 months of age. Using an **in vitro** EBV-stimulated lymphocyte culture technique we have been able to detect evidence of CD8-mediated control of HIV-1 viral replication in a 5-week old HIV-1 infected infant. **METHODS:** PBLs were isolated by density-gradient centrifugation. CD8+ lymphocytes were removed by immunomagnetic beads and purity verified by flow cytometry. Parallel cultures of PBLs containing equivalent numbers of CD4+ lymphocytes and B cells with and without CD8+ cells were set-up. EBV was added and culture supernantants were collected weekly for one month and assayed for p24 antigen and HIV antibody by ELISAs and compared. **RESULTS:** In the lymphocyte cultures depleted of CD8+ cells, a greater than 10-fold increase in p24 antigen production was detected by day 7 compared to the culture containing CD8+ cells. The difference in the level of viral replication persisted throughout the period of culture. No HIV antibody was detected in the supernatants of either culture. **CONCLUSIONS:** Cell-mediated immunity to autologous HIV-1 can be detected at a very early age in perinatally HIV-1 infected infants. It occurs before the infant produces antibodies to HIV and may play an important role in the control of viral replication in very young infants.

J 328THE EFFECT OF VIRAL VARIATION ON CD4 T-CELL RECOGNITION OF HIV-1. Ann D. M. Rees, Maria H. Fernandez and Jonathan Weber. Department of Genito-Urinary Medicine and Communicable Diseases, St. Mary's Hospital Medical School, Praed St., London W2 1NY U.K.

As CD4 T-cell play a central role in the generation of an effective immune response their specificity also deserves consideration in vaccine design. In these studies we have addressed this question by evaluating the impact, on HLA-DR1 restricted CD4 T-cell recognition, of amino acid changes in the V3 loop of laboratory isolates of HIV-1. Using different V3 loop peptides a number of HLA-DR1 restricted CD4 T-cell clones were generated and from studies of their specificity four main conclusions were reached.

1. Multiple distinct, but overlapping, HLA-DR1 restricted CD4 T-cell epitopes were identified within the V3 loop, indicating a high degree of plasticity in the T-cell repertoire recognising this region, suggesting that limited sequence changes in the challenge virus are unlikely to affect vaccine efficacy.
2. Nevertheless, our studies show that even relatively conservative changes in one residue can profoundly alter the recognition of CD4 T-cell clones with distinct epitopes.
3. The sensitivity to these substitutions is not due to an effect on HLA-DR1 binding but may be a result of interference with T-cell receptor interaction.
4. Diversity at the residues identified in these studies has also been observed in patients suggesting our findings are biologically plausible.

Viral variation may, therefore, contribute to pathogenesis if it results in the activation of a functionally distinct, and possibly less effective, CD4 T-cell repertoire. For similar reasons it may also be important to consider the effect of sequence variation on CD4 recognition in the design of sub-unit vaccines.

J 329 ISOLATION AND CHARACTERIZATION OF THREE HLA-B7-RESTRICTED CYTOTOXIC T LYMPHOCYTE CLONES SPECIFIC FOR THE THIRD VARAIBLE REGION OF HIV-1 GP120. Jeffrey T. Safrit, Charla Andrews, Alexander Lee, and Richard A. Koup, The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

We have isolated and characterized three cytotoxic T lymphocyte (CTL) clones from the peripheral blood of two acute seroconversion patients and one patient in the first trimester of pregnancy. These clones were CD8+ and class I HLA-restricted by the B7 molecule. All three clones recognized IIIB and RF but not MN strains of HIV-1. Using vaccinia vectors expressing truncated versions of the HIV-1_{IIIB} envelope, the clones were found to recognize an epitope within amino acids 287-364, but not including 312-328 of gp120. Further mapping of the epitope with synthetic 20-mer peptides overlapping by 10, or 25-mers overlapping by 8, was unsuccessful. The sequence of the region of gp120 recognized by these clones was compared to the predicted HLA-B7 peptide binding motif and a possible matching region was found. Using shorter peptides corresponding to this potential epitope recognition site, the minimum epitope recognized by the clones was determined to be the 10 aa sequence RPNNNTRKSI spanning amino acids 298-307 (BRU sequence). With the exception of the MN strain, this region of the V3 loop is well conserved among clade B isolates of HIV-1. Using peptides based upon virus sequences present within each patient, it was determined the clones are capable of recognizing autologous virus. A serine to arginine change at P9 of the epitope (associated with a switch from NSI to SI phenotype viruses) abrogated clone recognition. An SI virus with this sequence could therefore escape recognition by these CTL. The epitope identified here is not contained in the P18 peptide described as a possible human CTL epitope by Clerici et al. This study demonstrates that human CTL can be generated against sequences within the third variable loop of HIV-1 gp120.

J 330 SULFATED POLYSACCHARIDES MAY MEDIATE S-PROTEIN (VITRONECTIN) DEPOSITION ON rgp120 AND EFFECT COMPLEMENT FUNCTION. Helene R. Su and Robert J. Boackle, Division of Oral Biology, Department of Stomatology, Medical University of South Carolina, Charleston, SC 29403

S-protein (vitronectin) is involved in regulating the rate of complement MAC deposition. Evidence is presented that gp120 coated surfaces have the potential to bind S-protein if gp120 is pretreated with heparin. Thus it is conceivable that S-protein may influence the rate of MAC deposition on HIV and HIV infected cells when heparin is present. Using normal human serum (NHS) as the source of S-protein in an ELISA assay, heparin pre-treatment of immobilized rgp120 caused S-protein deposition in a dose dependent manner, reaching a plateau after incubation with human serum for 20 min. Purified S-protein controls also bound to heparin pre-treated immobilized rgp120. S-protein did not directly bind to gp120. The heparin-mediated serum S-protein binding to rgp120 was not dependent on divalent metal ions. In the presence of 2.0 mM EDTA, heparin mediated S-protein deposition was not affected. However, when comparing the EDTA-serum to freshly-collected EDTA-plasma as a source of S-protein, EDTA-serum provided approximately fifty times more heparin-mediated S-protein binding to rgp120. Dextran sulfate, pentosan polysulfate and fucoidan also mediated a deposition of S-protein on immobilized rgp120.

Heparin or dextran sulfate (with no serum present) enhanced the binding of soluble rgp120 (ABT Inc., Cambridge, MA) to immobilized CD4 (ABT). However when the heparin treated immobilized CD4 surface was incubated with NHS, S-protein deposition correlated with a reversal of the enhanced rgp120 binding. Sulfated polysaccharides may mediate S-protein deposition on rgp120 and effect complement function. This study was supported by NIDR DE08589 and DE05607.

J 331 A SENSITIVE METHOD UTILIZING RT-PCR TO MEASURE COMPLEMENT MEDIATED VIROLYSIS OF HIV. Brenda L. Sullivan and Gregory T. Spear, Department of Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, 60612

Previous studies have shown that anti-HIV antibody plus human serum as a source of complement can inactivate HIV via viral lysis. In these studies, lysis of HIV was measured by release of reverse transcriptase (RT) from the virion. It is possible that virolysis may also result in degradation of HIV genomic RNA due to RNases present in serum. The goal of this study was to determine whether this degradation occurs. Supernatants from H9 cells infected with HIV_{MN} were treated with serum only or serum plus NP40 and incubated at 37°C for 30 minutes. Viral RNA was then extracted, precipitated, serially diluted, reversed transcribed and PCR was performed using the SK38/SK39 primer pair. The amplified DNA was run on an agarose gel stained with ethidium bromide for visualization under UV light. While treatment with serum only had no effect on levels of HIV RNA, treatment with NP40 plus serum caused a 10,000 fold reduction in detectable RNA. Likewise, treatment of virus with anti-cell antibody plus complement resulted in a 100 fold reduction in HIV RNA as compared to complement treatment only. These results suggest that this technique may be useful for determining the antigenic makeup and complement sensitivity of low levels of virus expressed from cells or in plasma.

J 332 HUMAN MONOCLONAL ANTIBODIES (HuMAbs) AGAINST A LINEAR SEQUENCE IN C5 OF HIV-1 gp120 IDENTIFY A NEW ADCC EPITOPE(S) AND REVEAL INTERACTION BETWEEN C5 AND V3 DOMAINS,
Shermaine A. Tilley, Osama Alsmadi, William J. Honnen, Mary Racho, and Abraham Pinter, Public Health Research Institute, New York, NY 10016. We have isolated two HuMAbs, 42F and 43F, from an asymptomatic, seropositive hemophiliac that recognize peptides including amino acids 491-500 (Los Alamos numbering) of HIV-1 gp120. These HuMAbs are of the IgG1 subclass and mediate significant levels of ADCC against CEM.NKR cells chronically infected with the IIIB, MN, SF-2 and RF strains of HIV-1. The peak specific cytotoxicity (SC) measured in a standard, 4 hr. ^{51}Cr release assay varied similarly for both HuMAbs with the strain of virus infecting the cells: SC was 17% vs. IIIB-infected cells (20 µg/ml HuMAb), 17% vs. MN-infected cells (1.3 µg/ml HuMAb), 22% vs. SF-2-infected cells (1.3 µg/ml HuMAb), and 55% vs. RF-infected cells (1.3 µg/ml HuMAb). The level of cytotoxicity directed by 42F and 43F against cells infected with different HIV-1 strains correlated directly with the intensity of fluorescence observed upon staining the live cells with these HuMAbs by flow cytometry. In other studies, we found that binding of 42F to soluble gp120 of the MN strain enhanced the exposure of the V3 loop epitope recognized by our previously described HuMAb, 4117C. This exposure was also induced by a sheep polyclonal antibody, D7324, raised against the C-terminus (residues 497-511) of gp120. However, 42F and D7324 are against distinct epitopes, since the latter does not compete with 42F for binding to recombinant gp160 in ELISA. Experiments are underway to see whether 42F or 43F, though non-neutralizing mAbs, may enhance neutralization of HIV-1 by anti-V3 mAbs. These findings are particularly interesting in light of previous studies suggesting that the epitope(s) recognized by 42F and 43F in gp120 are sequestered by interaction of gp120 with gp41. Perhaps this sequestering is not complete in all strains of HIV-1 and/or represents the favored state in an equilibrium that may be shifted by mAb binding.

J 333 IDENTIFICATION OF AN HIV-1 NEF PEPTIDE THAT BINDS TO HLA CLASS II MHC ANTIGENS. *B. A. Torres and H. M. Johnson* Department of Microbiology & Cell Science, University of Florida, Gainesville, FL 32611.
Several lines of evidence suggest that human immunodeficiency virus (HIV) may encode for a superantigen which may, in part, be involved in the immunological dysfunction seen in AIDS. However, no superantigen has been identified in HIV. Superantigens, such as the staphylococcal enterotoxins (SEs), interact with MHC class II antigens on antigen-presenting cells and T cell receptor (TCR), on T cells, resulting in the expansion of Vß-specific T cell populations which may ultimately be anergized or deleted. The genome of mouse mammary tumor virus, a type B retrovirus, contains an open reading frame (ORF) that overlaps into the 3' long terminal repeat (LTR). This ORF gene product has been shown to be a superantigen which plays an important role in the pathogenesis of the virus. HIV encodes for a 27 K regulatory protein referred to as Nef (Negative Factor), whose gene is also contained in its 3' LTR and which may be important in the pathogenesis of the virus. Overlapping peptides corresponding to the entire sequence of Nef (HIV_{BRU}) were synthesized and tested for their relative abilities to block binding of SEs to MHC class II antigens. An internal Nef sequence, Nef(123-160), blocked binding of several SEs. It significantly inhibited the binding of two highly homologous SEs, SEA and SEE, while it was less effective against SEB and SEC_1. Nef(123-160) bound directly to MHC class II DR antigens as assessed by specific antibodies, and its binding was significantly inhibited by SEE, and also by SEA to a lesser degree. Thus, besides a regulatory role in HIV-1 replication, Nef may have functions such as those associated with superantigens.

J 334 COMPARISON OF THE ABILITY OF VARIOUS GP120/GP160 VACCINES TO ELICIT ANTIBODIES PREFERENTIALLY REACTIVE WITH NATIVE GP120, Thomas C. VanCott, J. Mascola, V. Kalyanaraman*, F.R. Bethke‡, D.S. Burke, R.R. Redfield and D.L. Birx, Department of Retroviral Research, Walter Reed Army Institute of Research and ‡Henry M. Jackson Foundation, 13 Taft Ct., Suite 200, Rockville, MD. 20850 and *Advanced BioScience Laboratory, Kensington, MD.

Vaccination with some preparations of both gp120 and gp160 elicits antibodies that are qualitatively similar in their preferential reactivity to a reduced, denatured gp120 protein. Only a fraction of these antibodies are capable of cross reactivity with the oligomeric gp120/gp41 complex expressed on the surfaced HIV-1 acutely infected cells. This contrasts with the antibody profile induced during natural infection where preferential binding to native monomeric gp120 protein and strong cross reactivity with the oligomeric gp120/gp41 complex is observed. This qualitative difference in antibody profiles induced during gp120/gp160 vaccination and natural infection may explain the ability of HIV-1 sera, but not these vaccinee sera, to neutralize primary HIV-1 isolates in a PBMC based assay. The presence of these vaccine-induced, denatured specific antibodies may be attributable to several factors including the presence of denatured antigen within the preparation, denaturation of the antigen upon formulation with adjuvant or the processing of antigen in vivo. We have extended these studies to examine the impact product variables such as strain, adjuvant formulation and dose schedule have on the induction of native specific antibodies. The ability of these variables to alter the quality of the antibody response and the correlation these changes may have on neutralization of both HIV-1 lab strains and primary isolates will be discussed.

J 335 REGULATION OF HIV REPLICATION IN Th1 AND Th2 CLONES SPECIFIC FOR HIV-1 GAG p24 BY IL-4 AND IFN GAMMA. **Anna Vyakarnam** and Patricia M Matear. Dept. Immunology, UCL Medical School, 40-50 Tottenham Street, London W1P 9PG, U.K.
We have recently reported (Vyakarnam A et al, Proceedings of the IXth International Conference on AIDS, 7-11 June 1993. Abstract number WS - A 15-2) that HIV replicates less efficiently in HIV-1 gag p24 specific Th1 clones compared to Th2 clones of similar specificity. This phenomenon was associated with the induction of cytokine release by the clones in response to activation with specific antigen/APC. One explanation for differennial HIV replication may therefore be the cytokines produced by the clones in response to antigen/APC stimulation - the Th1 clones produced high levels of IFN gamma and IL-2 and no IL-4 whereas the Th2 clones produced low levels of IFN gamma and IL-2 and high levels of IL-4. The relative importance of IL-2, IFN gamma and IL-4 in HIV replication were explored by infecting the Th1 and Th2 clones with the same infectious dose of HIV-1(multiplicity of infection=5) and then culturing the infected cells at different concentrations ranging from $1x10^5$ /well - $6x10^3$/ well in 96-well plates in the presence and absence of each recombinant cytokine and in the presence and absence antigen/APC. We report that although IL-2 improves the efficiency of HIV replication in both the Th1 and Th2 clones, it did not alter the basic difference in HIV replication in the Th1 and Th2 clones in response to antigen/APC activation. Both IL-4 and IFN gamma on the other hand did have an effect on the differential production of HIV from the clones. IFN gamma had dual effects: it reduced partly the capacity of Th2 clones to support HIV and had no effect on HIV replication in Th1 clones- however IFN gamma did not reduce HIV replication in the Th2 clones to the level of the Th1 clones. Furthermore, the converse experiments with neutralising antibodies to IFN gamma showed that the IFN was important for HIV replication in the Th1 and Th2 clones: the overall level of virus produced by the clones was substantially reduced in the presence of anti-IFN gamma antibody. IL-4 had only a weak enhancing effect on HIV replication in the Th1 clones and did not enhance HIV replication in the Th1 clones to the level observed in the Th2 clones. The exogenous addition of IL-4 to the Th2 clones had no effect on HIV replication. Neutralising antibodies to IL-4 however showed that HIV replication in Th2 clones was dependent on IL-4 as virus production in the Th2 clones was inhibited 30-40% in the presence of anti-IL4 antibody. In summary our data show that differential HIV replication in Th1 and Th2 clones cannot be totally reversed by the cytokines IL-4 and IFN gamma and therefore these cytokines only partly account for the differences observed between the two types of clones.

HIV Vaccines; HIV Vaccine Clinical Trials

J 400 EVALUATION OF HUMAN CELLULAR PROTEINS AS IMMUNOGENS IN PROTECTION FROM SIV CHALLENGE, Larry O. Arthur[1], J.W. Bess[1], L.E. Henderson[1], R. Urban[3], D.L. Mann[2], and R.E. Benveniste[2]. [1]AIDS Vaccine Program, PRI/DynCorp, Frederick, MD 21702, [2]Laboratory of Viral Carcinogenesis, NCI-FCRDC, Frederick, MD 21702, [3]Dept. Biochem. Mol. Biol., Harvard Univ., Cambridge, MA, 02138

The possibility that immune responses to cellular antigens might protect against SIV challenge was raised when it was reported that macaques immunized with uninfected human cells were protected from infection with SIV grown in human cells. We have shown that antisera to MHC class I, β2-microglobulin (β2M) and class II will precipitate intact HIV-1 indicating that these proteins are physically bound to the virus. To determine if an immune response to these cellular antigens will protect from SIV challenge, 12 M. fascicularis were immunized with HLA-DR4, class I, mock virus (sucrose gradient banded culture fluid from uninfected cells), glutaraldehyde-fixed HuT 78 cells, and adjuvant. HLA-DR4 and class I proteins were purified by immunoaffinity chromatography to maintain native configuration. The immunized animals were monitored for humoral responses to class II and β2M and then challenged with 20 animal-infectious doses of SIV(Mne) propagated in HuT 78 cells. Animals immunized with HLA-DR and mock virus had the highest titers to HLA-DR antigen and these are the only animals that have scored negative for virus infection through 12-weeks post challenge. All animals immunized with class I, β2M, HuT 78 cells, and adjuvant have become infected with SIV. This is the first demonstration of protection from immunodeficiency virus challenge using purified cellular proteins as immunogens. It is possible that protection from infection by an anti-HLA-DR response may provide an explanation for the observations that some hemophiliacs did not become infected when administered HIV-1-positive factor VIII. These data may also help to explain why the uninfected partner of discordant HIV-1-positive couples are protected from infection, even though they practice unprotected sex.

J 401 EFFICACY OF A CANARY POX HIV-2 VACCINE IN CYNOMOLGUS MONKEYS, Gunnel Biberfeld, P.Putkonen, S.Andersson, B.Mäkitalo, R.Thorstensson; Karolinska Institute and Swedish Institute for Infectious Disease Control, 105 21 Stockholm, Sweden; G.Franchini Laboratory of Tumor Cell Biology, NCI, Bethesda, MD; J.Tartaglia, E.Paoletti, Virogenetics corporation, Troy, New York.

Four monkeys were immunized intramuscularly at 0,1 and 7 months with 10^7 PFU of a recombinant canary pox (Alvac) HIV-$2_{SBL/ISY}$ vaccine expressing the gag, pol and env HIV-2 genes. Another group of four monkeys was immunized at 0 and 1 months with the same vaccine followed by a boost at 7 months with 60μg of a native HIV-2 gp125 preparation in QS21 adjuvant. The HIV-2 gp125 was prepared from the parental HIV-$2_{SBL-6669}$ strain by affinity chromatography using Galantis Nevalis agglutinin lectin. Viral specific antibody responses and lymphocyte proliferative responses to solubilized HIV-2 virions were higher in the monkeys which had received a boost with gp125. In contrast, viral specific cytotoxic T lymphocyte responses were demonstrated in two of three monkeys tested which had received Alvac HIV-2 only but in none of three monkeys tested which had received gp125 at the last immunization. The immunized monkeys together with eight control monkeys were challenged intravenously with HIV-$2_{SBL-6669}$ (grown in monkey blood mononuclear cell cultures) one month after the third immunization. Two of the four monkeys immunized with Alvac HIV-2 followed by gp125 were protected against infection whereas all four monkeys immunized with Alvac-HIV-2 only and 7 of 8 control monkeys became infected as shown by virus isolations and PCR.

J 402 *SALMONELLA DUBLIN* VACCINE STRAIN EXPRESSING V3 LOOP SEQUENCES IN THE FLAGELLIN GENE, Margherita E. Cattozzo[1], Mauro Tognon[2] and Bruce A.D. Stocker[1], [1]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, [2]Institute of Histology and General Embryology, Ferrara School of Medicine, Ferrara - Italy.

The V3 loop region of the surface glycoprotein gp120 of HIV-1 is an immunodominant section of this protein and is responsible for a considerable proportion of the neutralizing humoral response against HIV-1 infection in both naturally infected humans and experimentally infected animals. Though this region is one of the most variable in sequence, some data indicate that the PND (principal neutralization domain) located in the V3 loop contains conserved sequences and that some of peptides from the PND can elicit neutralizing antibodies recognizing multiple isolates.

We plan the use of an attenuated *aroA Salmonella dublin* mutant as a live vector to deliver two representative sequences of V3 loop. The attenuated bacteria, despite their auxotrophy, are able of limited growth *in vivo*. The recombinant strains, given by mouth, might stimulate significant levels of specific mucosal immunoglobulin A against the carrier strain and the HIV antigen and of serum antibodies also.

We chose the V3-loop sequences of isolates SC and WMJ2, RSIHIGPGRAFYATG and RSLSIGPGRAFRTR; these peptides were found to react with, respectively, 65% and 71% of 86 adult and pediatric HIV-positive sera (La Rosa et al., Science 249; 932, 1990). Oligonucleotides specifying these sequences have been inserted into a cloned flagellin gene at the site of a 48-bp deletion in the hypervariable antigenically determinant region. Expression of the V3 sequences by an *aroA* deletion live vaccine strain of *Salmonella dublin* carrying the recombinant plasmids has been shown by Western blot with a polyclonal serum anti HIV-1-IIIB V3 loop peptide.

When the characterization of the two recombinant plasmids is complete we will immunize BALB/c mice and rabbits to determine the anti-V3-loop humoral and cellular responses after i.p. and oral administrations of the live recombinant *Salmonella* strains.

J 403 MACAQUES VACCINATED WITH HLA CLASS I ANTIGEN ARE PROTECTED FROM SIVmac 251 INFECTION, W. L. Chan[1], A. Rodgers[1], S. Ellis[1], C. Grief[1], B. Flannagan[1], J. Bootman[1], N. Almond[1], J. Stott[1], K. Kent[1], and R. Bomford[2], [1]National Institute for Biological Standards and Control, Hertfordshire, UK; [2]Retrovirus Laboratory, Institute Pasteur, Paris, France.

Our earlier reports demonstrated a correlation between protection and the level of anti-HLA class I antibodies in macaques vaccinated with inactivated SIV, infected C8166 cells or uninfected C8166 cells (Chan et al. J. Exp. Med. 176:1203-07, 1992; Vaccine 93, CSHL press pp77-83, 1993). To address the question of whether HLA class I molecules can protect macaques against SIV infection, 4 monkeys were vaccinated with a mixture of murine P815 cells transfected with HLA class I (B27) and HLA A1 and B8 (haplotype of C8166) purified from a human B lymphoblastoid cell line. The adjuvant used was GMDP formulated with squalane and pluronic-in-water emulsion. Control monkeys were vaccinated with P815 cells and adjuvant. All the vaccinees had high antibody responses to HLA I as shown by radioimmune precipitation of C8166 cell lysate, by radioimmunoassay using purified HLA I or by flow cytometry on both P815-B27 (P815 was used as negative control) and C8166 cells. Control animals had antibody titres of <1/30. Pooled sera from the vaccinees, but not the control animals, were also shown to bind to the SIV virus envelope as demonstrated by immuno-gold labelling using electron microscopy. Sera from the vaccinees also inhibited SIV replication in monkey peripheral blood mononuclear cells in vitro. When the animals were challenged with SIVmac 251 (11/88 pool, grown in C8166 cells), all the control animals and only 2 out of 4 vaccinees were infected as detected by virus isolation and PCR of proviral DNA in the PBMC of the animals.

J 404 PREPARATIONS FOR HIV-1 VACCINE EFFICACY TRIALS IN TRINIDAD. Farley Cleghorn, Noreen Jack, Jeffrey Edwards, Michael Greenberg, Jacquelyn Murphy, Rosemary Paul, Frank White, Courtenay Bartholomew, Sten Vermund and William Blattner. NCI and NIAID, NIH; Research Triangle Institute; Duke University; Min. of Health, CAREC and UWI, Trinidad.
Objectives: To describe the epidemiology of HIV-1 among STD clinic attenders in Trinidad and to assess suitability of the site for HIV-1 Vaccine Efficacy trials.
Methods: Cross-sectional, case-control and cohort studies to examine trends in prevalence, define risk factors and estimate seroincidence; identification of cases of primary infection through HIV-1 p24 antigen screening; an infrastructural needs assessment exercise was also conducted.
Results: HIV-1 seroprevalence among STD clinic attenders in Trinidad has increased from 3.0% in 1987/88 to 13.6% in 1991/92, HTLV-I from 1.8% to 4.5% and HIV-1/HTLV-I coinfection from 0.05% to 1.5%. Risk factors for HIV-1 in this group include recent crack cocaine use; a history of >2 episodes of syphilis; sex with commercial sex workers; multiple sexual partners and current genital ulcers and warts for males, while for females having ever had non-gonococcal urethritis; having ever received money for sex; lower age at first sex, and having had more than 2 episodes of gonorrhea were significant. HIV-1 seroincidence based on exact follow-up is estimated to be 4.5 (95% C.I.= 2.5 - 7.6) per 100 person-years.
In addition, between June 1 and July 25, 1993, 1501 STD clinic attenders were screened for HIV-1 P24 antigen. The refusal rate for all clinic attenders was 5%. 16 samples (1%) were P24 Ag positive and 121 (8.1%) were HIV-1 antibody positive by WB. 10/16 (63%) p24 Ag positive samples were also positive by WB, leaving 6 cases of true primary infection. There were 5 males 9 (ages 22-48) and 1 female (age 14). 3/6 were symptomatic in the prior three weeks and 3/5 males had current genital ulcers. 5/6 are HIV-1 culture positive.
Conclusions: In this highly sexually exposed population HIV-1 is primarily heterosexually transmitted, with an increasing prevalence rate and high incidence estimate. Trinidad/Tobago fulfills many of the criteria for an ideal site to evaluate efficacy of HIV-1 vaccine products.

J 405 EPITOPE MIMICRY BY DISPLAY OF FOREIGN PEPTIDES ON THE SURFACE OF FILAMENTOUS BACTERIOPHAGE, Fulvia di Marzo Veronese, Anne E. Willis, Cynthia Boyer Thompson, Ettore Appella and Richard N. Perham, Laboratory of Tumor Cell Biology and Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; Cambridge Center for Molecular Recognition, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, United Kingdom
Hybrid filamentous bacteriophage particles can be constructed in which the wild-type major coat protein ($gVIII_p$) subunits are interspersed with modified coat protein subunits displaying large foreign peptides in their exposed N-terminal segments. Since there are 2700 major coat protein subunits in a single virus particle, each peptide is thus represented many times in the same virion. Such peptides are physically accessible to any potential receptor and are highly immunogenic. We have now inserted a 12 aa HIV-1_{MN} V3 peptide sequence and shown that this construct, with or without adjuvant, evokes high titered cross-reactive antibodies in mice which specifically immunoprecipitate gp120 from cellular and viral lysates in the absence of immunoblot reactivity. Thus, peptide sequences displayed this way are remarkably effective structural mimics of natural protein epitopes. Moreover, these antibodies neutralize HIV-1_{MN} and cross-neutralize additional heterologous viruses. Antibody production can be further ameliorated by simultaneous inoculation with specific T helper epitopes similarly displayed on filamentous bacteriophage particles, indicating efficient processing *in vivo* of these epitopes for presentation by class II molecules to CD4+ T cells. Bacteriophage display is thus a highly promising approach for the favorable presentation of peptide antigens to the immune system, with potential for exploring the immune response to defined sequence epitope and for designing inexpensive and benign vaccines.

J 406 A UNIQUE SUBUNIT VACCINE THAT INDUCES SYSTEMIC AND MUCOSAL, CELL-MEDIATED AND HUMORAL RESPONSES FOLLOWING ORAL ADMINISTRATION, Yvette Edghill-Smith, Masoumeh Kheiri, Kumud Das, Zheng Wang, Eleonora Feketeova, Raphael J. Mannino, and Susan Gould-Fogerite, UMDNJ-Graduate School of Biomedical Sciences, Department of Laboratory Medicine and Pathology, Newark, NJ 07103.
The formulation of a unique, non-replicating, safe subunit vaccine against HIV-1 has become one of the primary focuses of our laboratory. The vaccine should be convenient to administer, have minimal to no adverse side effects, generate strong immunological responses, and maintain long-lasting memory.
Our laboratory has set out to determine the parameters required in an oral subunit vaccine for mounting strong systemic and mucosal immunological responses. We have formulated a unique, insoluble protein-lipid structure which survives the stomach and reaches the mucosal effector sites of the small intestine.
In mice, these formulations generate strong proliferative responses in the spleen and maintain lasting antibody titers after primary oral immunization. On subsequent intranasal challenge with live influenza virus, the animals showed protection indicated by no viral replication in the lungs and trachea compared to unimmunized control animals. The Parainfluenza model has demonstrated strong proliferative and cytotoxic responses as well as strong circulating antibody titers which were maintained for an extended period. Mucosal antibody titers (sIgA) in the saliva was also established.
Formulations containing HIV-1 antigens have induced humoral and proliferative responses after a single immunization. Further investigations relating to the full extent of the immune responses involving the mucosal immune system is ongoing. These vaccine formulations should prove to be effective tools in immunotherapy and preventive regimens for the future.

J 407 HIV-CHIMPANZEE AND SIVsmmPBj14-MACAQUE MUCOSAL CHALLENGE MODELS FOR TESTING VACCINE EFFICACY, Patricia N. Fultz[1], Marc Girard[2], Rebecca Schwiebert[1], Jackie Stallworth[1], Liya Su[1], Jim Mahoney[3] and Elizabeth Muchmore[3], [1]University of Alabama, Birmingham, AL 35294, [2]Institut Pasteur, Paris, France, [3]LEMSIP, New York University, Tuxedo, NY 10987.
Because the major route of transmission of HIV-1 world-wide is across mucosal surfaces as a result of sexual activity, vaccines must elicit immune responses capable of preventing infection by this mechanism. Thus, two animal models are being developed for evaluating candidate HIV-1 and prototypic SIV vaccines. First, infection of adult female chimpanzees with both cell-free and cell-associated HIV-1 has been achieved by placing the viral inocula in the cervical *os* atraumatically with the aid of a colposcope. Infection appeared to be enhanced by adding 25% human seminal plasma to the cell inoculum, probably due to its buffering capacity. Although some animals became infected readily, with isolation of virus from PBMC at 2 weeks after exposure to HIV-1, others appeared to be infected transiently and did not seroconvert. However, in the latter case, PBMC obtained from chimpanzees several months after exposure continued to proliferate specifically in response to HIV-1. Second, the acutely lethal SIV-PBj14 has an advantage over other SIV isolates because it uniformly induces an acute disease syndrome and death following intravenous inoculation of either cell-free or cell-associated virus. In the event vaccine-mediated protection is not achieved, then SIV-PBj14 provides a rapid assessment for protection against disease. The pathogenicity of this virus following mucosal inoculation, however, is not known. The results of exposing female and male pig-tailed macaques to SIV-PBj14 vaginally or rectally, respectively, will be presented. These studies will establish whether mucosal infection of pig-tailed macaques with SIV-PBj14 provides a suitable model for evaluating vaccine efficacy.

J 408 IMMUNITY TO HIV-1 ANTIGENS DISPLAYED ON THE SURFACE OF INOVIRUSES, Leondios G. Kostrikis and David D. Ho, The Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, N Y.

The objective of this study is to develop a novel vaccination strategy, using "live" Inovirus (filamentous bacterial viruses) vectors that display HIV-1 antigens, against HIV-1 infection acquired through the vaginal or gastrointestinal route. The genetically engineered Inoviruses may be used as long-term "HIV-1 antigen delivery vehicles" to deliver antigens to mucosal sites by infecting resident nonpathogenic F-specific *E. coli* strains of the urogenital and intestinal walls of humans. The vectors display on their surfaces critical sites of HIV-1 envelope proteins which have been shown to be involved in antibody neutralization. We have already constructed a recombinant Inovirus that expresses a V3 antibody neutralization epitope. Work is now in progress to generate vectors that will express V2 and gp41 neutralizing epitopes, as well as "mimotopes" of the CD4-binding domain of gp120. In this system, the foreign antigen is expressed on the surface of the progeny virus at about 3,000 copies per virion, and the virus propagates to a titer of up to 10^{13} PFU/ml. The immune response to the constructed vectors, including mucosal immunity, will be evaluated in mice and rabbits using many vaccination strategies. We hope that our findings will contribute to the understanding of mucosal immunity against HIV-1 and to vaccine development. Data from this study will be presented and discussed.

J 409 T CELL AND ANTIBODY RESPONSES INDUCED BY MUCOSAL OR ASSOCIATED LYMPHOID TISSUE IMMUNIZATION OF MACAQUES WITH SIV VACCINES

Thomas Lehner, R. Brookes; L. Klavinskis; L. A. Bergmeier; L. Tao; C. Panagiotidi; J. Walker; L. Hussain; R. G. Ward; N. Graff[†]; A. Gearing[†]; S. Adams[‡] and I. Jones[‡]. Division of Immunology, United Medical and Dental Schools of Guy's & St. Thomas' Hospitals, London Bridge, London SE1 9RT; [†]British Biotechnology Ltd, Brook House, Watlington Road, Oxford OX4 5LY; [‡]Institute of Virology, Mansfield Road, Oxford OX1 3SR.

The rationale for mucosal immunization against genito-urinary and rectal transmission of HIV infection is the high prevalence of heterosexual and homosexual infection with HIV. The first objective of this investigation was to develop an alternative strategy to vaginal, rectal or urethral immunization which is augmented by oral immunization. This was achieved by targetting the genito-urinary-rectal associated lymphoid tissue (GURALT). The vaccine used was SIV gag p27:Ty-VLP or gp120 and p27 linked to cholera toxin B subunit (CTB) for mucosal immunization, or mixed with aluminium hydroxide for GURALT immunization. Both strategies resulted in mucosal secretory IgA and IgG antibodies, serum antibodies, T cell specific proliferative responses and B cell antibody synthesis to p27. The second objective was to map T cell epitopes for which we generated short term cell lines with the particulate SIV p27:Ty-VLP and assayed them for proliferative T cell activity after stimulation with overlapping synthetic peptides (20^{ers}). This showed a hierarchy of 4 T cell epitopes in circulating, splenic and iliac lymph node lymphocytes, which varied with the route of immunization. The cell lines were also assayed for cytotoxic activity by using B cell lines pulsed with the immunodominant T cell peptide determinants. Specific cytolytic activity was detected in T cells against target cells expressing two immunodominant T cell epitopes. The results suggest that both mucosal and GURALT immunization induce specific secretory IgA and IgG antibodies. The diversity in T cell epitope expression and function at different anatomical sites may prevent SIV/HIV transmission through the mucosa, the development of a reservoir of viral latency in the regional lymph nodes, and dissemination of the virus into the spleen and circulation. The protective capacity of these mucosal and GURALT immunized macaques will now be tested by mucosal challenge with live SIV.

J 410 HUMORAL AND CELLULAR RESPONSES TO A HEC/rBCG COMBINATION VACCINE AGAINST SIV.

Nancy J. Leung[1], David A. Anderson[1], Michael A. Jarvis[1], Rebecca F. Cotterman[1], Heather L. Vermazen[1], Andrew White[1], Anna Aldovini[3], Richard Young[3], Murray B. Gardner[2], José V. Torres[1]. Department of Medical Microbiology and Immunology1, Department of Medical Pathology[2], University of California, Davis, CA 95616, and Whitehead Institute for Biomedical Research[3], Cambridge, MA 02142.

An effective vaccine for protection against retroviral infection or disease development should induce both humoral and cellular immune responses. To achieve this, a combination vaccine approach has been designed which will include novel synthetic constructs to elicit a broadly cross-reactive humoral response, and recombinant BCG (Bacille Calmette-Guerin) expressing SIV and HIV proteins to elicit a strong CTL response.

The synthetic constructs, referred to as HECs (Hypervariable Epitope Constructs), have been designed to represent the *in vivo* variability of individual epitopes in the SIV envelope glycoprotein sequences. This was achieved by analyzing the SIV envelope sequences available from the various databases, and using HEC technology to include each variation of the hypervariable and antigenic regions in the peptide mixture. The HECs have been tested in both mice and rabbits and found to elicit broadly cross-reactive antibodies.

Recombinant BCG expressing SIV Gag, Pol, Env, and Nef proteins were constructed by insertion of the respective genes into an *E. coli*/BCG shuttle vector. Each of the four constructs were verified for expression in both *E. coli* and BCG by Western analysis and titered stocks were generated. Each of these BCG recombinants, in combination and individually have been inoculated into mice and rabbits by several routes, and their CTL activity, T-cell proliferation, and antibody responses assessed.

To elicit strong humoral and CTL responses, a combination of the synthetic HEC constructs and all four recombinant BCG constructs was inoculated into mice and rabbits. CTL activity, T-cell proliferation, and antibody responses were analyzed.

J 411 USE OF THE SEMLIKI FOREST VIRUS EXPRESSION SYSTEM TO PRODUCE RECOMBINANT VACCINES,

Peter Liljeström, Peter Berglund, and Marina Fleeton, Department of Molecular Biology, Karolinska Institute, Novum Research Center, Sweden

In the Semliki Forest virus (SFV) expression system DNA of interest is cloned into SFV plasmid vectors that serve as templates for in vitro synthesis of recombinant RNA. The RNA is transfected into animal tissue culture cells where it in the cytoplasm drives its own replication and leads to massive production of the heterologous protein. The SFV expression technology also involves selective in vivo packaging of recombinant RNAs into infectious SFV particles. The resulting high titer recombinant virus stocks can be used to infect any animal cell type, where only the heterologous gene products are expressed.

We have cloned the gag, gag-pol and env genes of HIV-1 as well as the HA and NP encoding genes of influenza virus into SFV expression vectors and shown that high levels of expression of each of these proteins in animal cell cultures. We will present results to show several advantageous features of the SFV technology in terms of recombinant vaccine design: (i) The antigen encoding, self-replicating RNA molecule can be administered either directly (genetic immunization) or by infection with SFV particles; (ii) Large amounts of antigen is produced, whereas no vector proteins are made; (iii) Antigen is expressed within the cell in the same way and site as the authentic antigen, thus resulting in a "high quality product" for immunologic stimulation; (iv) Both shorter peptides and full-length antigens can be expressed, with a choice of circumventing antigenic drift; (v) Second round, non-replicative virus particles can be produced resulting in a second round stimulation of the immune system.

J 412 A PHASE II HIV VACCINE TRIAL IN SERONEGATIVE VOLUNTEERS: EXPANDED SAFETY AND IMMUNOGENICITY EVALUATION OF TWO RECOMBINANT GP120 VACCINES. MJ McElrath, L Corey, ML Clements, R Belshe, M Keefer, B Graham, P Fast, AM Duliege, D. Francis, and the NIAID AIDS Vaccine Clinical Trials Network. Dept. of Medicine, University of Washington, Seattle, WA, 98195.

The first AVEG phase II HIV vaccine trial was initiated in December 1992 to test two mammalian-derived recombinant HIV-1 envelope (rgp120) immunogens among 6 groups of HIV seronegative volunteers with behaviors associated with either relatively low or high risk for acquiring HIV infection. With the exception of teenagers and young adults who are practicing higher risk heterosexual behavior, recruitment and enrollment of the groups has been successful. A comparison of local and systemic clinical responses revealed a similar incidence of side effects among the groups after 2-3 doses of 50 µg SF-2 rgp120 (Biocine) in MF59 or 600 µg MN rgp120 (Genentech) in alum at 0, 1, and 6 months. Moderate to severe local pain and tenderness occurred in 14%, and moderate systemic symptoms occurred in 8%, with no significant difference in frequency of local or systemic symptoms elicited by the two vaccines. Analyses of HIV-specific antibody responses (binding, neutralizing, CD4-gp120 binding inhibition) and cellular responses (lymphoproliferative, CTL) are in progress. Preliminary data indicate that both vaccines appear safe, and there is no evidence of increased local or systemic toxicity in groups perceived at higher risk of acquiring HIV infection versus those at lower risk. The safety profiles of these vaccines appear acceptable and encouraging for further wide scale testing.

J 413 CHALLENGE OF RHESUS MONKEYS WITH SIV AFTER IMMUNIZATION WITH A COMBINATION REGIMEN

M.-L. Michel,* K. Schlienger* ; M. Mancini* ; Y. Rivière** and P. Tiollais* *Unité de Recombinaison et Expression Génétique and **Unité de Virologie et Immunologie Cellulaire, Institut Pasteur, Paris, France

We recently reported that recombinant Hepatitis B surface antigen (HBsAg) particles presenting the HIV-1_{BRU} envelope principal neutralizing determinant on their surface (V3/HBsAg) allowed us to generate proliferative T-cell responses, cellular cytotoxicity and neutralizing antibodies in immunized rhesus monkeys. To evaluate the protective immunity against homologous challenge that may be elicited by the hybrid particles in the SIV/macaque model, we presented the second variable domain (V2) of SIV_{MAC251} gp140 on HBsAg particles. This domain is involved in generation of cell-free virus neutralizing antibodies. We used a combination immunization regimen that included a live recombinant vaccinia virus for priming and the hybrid V2/HBsAg particle for boosting in order to maximize both the cellular and the humoral responses. Four rhesus monkeys received 2 intradermal injections at a 2 month interval of either recombinant vaccinia virus expressing SIV_{MAC} gp140 or wild type vaccinia virus (2 control animals) followed by 2 intramuscular boosts at months 5 and 8 of V2/HBsAg particles without adjuvant (one control animal received the V3/HBsAg particle and the other one received the native particle). Serum antibody responses were determined by ELISA and neutralization assay. Immunized macaques developed persistant antibodies against gp140 after the first injections but the V2/HBsAg boosts did not enhance this gp140 specific response. Despite having a low titer against V2 peptide, neutralizing antibodies were detected after the second V2/HBsAg boost. In light of these results, a third injection of the V2/HBsAg was given intradermally in the Syntex adjuvant formulation (SAF-1) before an intravenous cell free virus challenge with the molecular clone of the $SIV_{MAC\ 251}$. Results of these protection experiments will be presented and discussed.

J 414 POLIOVIRUS ANTIGEN CHIMAERAS PRESENTING *DEFINED NEUT*RALIZING EPITOPES WITHIN THE V2 DOMAIN OF SIV_{mac251} gp140. C.Nichols, C. Vella, T. Corcoran, P. Minor, D. J. Evans and J. W. Almond, Department of Microbiology, University of Reading, P.O. Box 228, Whiteknights, Reading, RG6 2AJ, UK.

The live attenuated Sabin type 1 vaccine strain of poliovirus is being exploited for the presentation of a B-cell epitope of SIV. The 9 amino acids encoding antigenic site 1 have been replaced by 22 amino acids from the second variable (V2) domain of SIV $_{mac251}$ gp140.

Two chimaeras were constructed, S1/SIV/6 with the V2 sequence in place of poliovirus antigenic site 1, and S1/SIV/4, with the V2 sequence within antigenic site 1; i.e., with flanking amino acids from poliovirus. This is to enhance the probability of recognition and hence, increase immunogenicity.

Epitope presentation was assessed by neutralization assays with SIV MAbs directed against the V2 domain and with sera from a number of SIV infected/vaccinated macaques. S1/SIV/4 and S1/SIV/6 were both neutralized by 5 of the V2 MAbs to a high titre. 3 of these MAbs (KK10, 13, 54) have previously been shown to neutralize SIV in vitro. Sera from infected/vaccinated macaques neutralized S1/SIV/4. This has yet to be carried out with S1/SIV/6. These results suggest that the epitope is being expressed in a conformation on the surface of poliovirus comparable to that on the SIV envelope glycoprotein.

The SIV neutralizing activity of the antisera to S1/SIV/4 and S1/SIV/6 is presently being assessed. Epitope mapping studies using overlapping peptides from the V2 region with sera from S1/SIV/4 immunized rabbits demonstrated that comparable residues were immunogenic in both S1/SIV/4 and rec gp140 immunized animals. Recent results have fine mapped KK10 and KK13. Therefore, a third chimaera has been constructed to present this region in a more immunogenic position.

J 415 ENCAPSIDATION OF CHIMERIC HIV-1-POLIOVIRUS MINIREPLICONS WHICH EXPRESS HIV-1 GAG, POL AND ENV PROTEINS, Donna C. Porter, David C. Ansardi, Zina Moldoveanu, Patricia N. Fultz and Casey D. Morrow, University of Alabama at Birmingham, Birmingham, AL 35294

Naturally occurring defective-interfering particles of poliovirus have been characterized which contain deletions in the P1 capsid region maintaining the translational reading frame and capacity for replication. We substituted regions of the HIV-1 gag, pol or env genes into the poliovirus cDNA to further explore the potential use of this system as a vaccine vector. Following in vitro transcription, RNAs were transfected into tissue culture cells. Expression of the HIV-1 proteins was demonstrated by immunoprecipitation with sera from AIDS patients. In order to encapsidate the chimeric HIV-1-poliovirus genomes (minireplicons), in vitro transcribed RNAs were transfected into cells previously infected with a recombinant vaccinia virus (VV-P1) which expresses the poliovirus capsid precursor protein, P1. Re-infection of cells with the encapsidated chimeric genomes resulted in expression of the HIV-1-P1 fusion proteins indicating that the chimeric genomes were encapsidated by capsid proteins provided in trans from VV-P1. The encapsidated genomes could also be serially passaged when cells were co-infected with the minireplicons and VV-P1. Co-infection of cells with encapsidated minireplicons and either type 1, 2 or 3 attenuated poliovirus strains followed by serial passage resulted in the retention of the minireplicon genomes in the virus stocks. Preliminary immunization trials on mice and a single rhesus macaque demonstrated that the minireplicons expressing Gag were immunogenic. These results establish the use of encapsidated poliovirus minireplicons as a new vaccine vector system.

J 416 HBCAG PARTICLES CARRYING PND AND CD4-BINDING SITE SEQUENCES OF HIV-1/GP120 AS SUBUNIT VACCINE CANDIDATES

Albrecht von Brunn, Christine Reichhuber, Peter Rieber* and Lutz Gürtler, L.
Max-von-Pettenkofer-Institut and Institut für Immunologie*, Ludwig-Maximilians-Universität München, FRG

An effective vaccine against human immunodeficiency virus requires the development of immunogens which induce a type- as well as a group- specific neutralizing immune response. Former goal has been achieved in experimental animals by the use of the principal neutralizing domain (PND) located on the third variable region of the outer envelope glycoprotein gp120 of HIV-1. Latter has been attributed to the domain of gp120 (CD4- binding site) which binds to the CD4 receptor.
We have recently shown that upon expression of the V3 loop of HIV-1 (LAI strain) on hybrid HBcAg particles high antibody titers directed against the inserted V3 sequences can be induced. Monoclonal antibodies derived from such immunizations are able to neutralize homologous but not heterologous (MN ,RF, SF-2) virus *in vitro*.
In extension of this work we have inserted at the DNA level the V3 loops of the MN, RF and SF-2 strains at identical internal and C-terminal positions of HBcAg. Furthermore, we have fused 55 amino acids of the sequences involved in the binding of gp120 to the CD4 receptor to the internal position of HBcAg. The insertion of this rather long peptide sequence does allow the formation of hybrid `HBcAg/CD4 binding site´ (HBc/CD4bs) particles as judged by CsCl density equilibrium and sucrose velocity sedimentation gradient analysis. This is somewhat surprising since HBcAg formes protein particles which could be expected to be structurally rather constrained. Binding of HBc/CD4bs proteins to purified sCD4 molecules can be detected with monoclonal antibodies directed against the domains D2. D3 and D4 of sCD4 in ELISA. Experimental animals were immunized with wild type HBc and hybrid particles carrying the V3 sequences and/or the CD4 binding domain of HIV-1. Results on immunogenicity and antiviral acitivity of the respective sera alone or in combination will be presented.

Therapy of HIV Disease; New Approaches

J 500 DEVELOPMENT OF CD4-IgG2 FOR POST-EXPOSURE PROPHYLAXIS OF HIV-1 INFECTION.

Graham P. Allaway, Gary A. Beaudry, Alexia A. Belperron, Jeffrey M. Carver, Karen L. Davis-Bruno, Elisa B. Garcia, Karl W. Hasel, Andrew M. Ryder, Emily L. Wong and Paul J. Maddon. *Progenics Pharmaceuticals Inc., Old Saw Mill River Road, Tarrytown, NY 10591*

Based on their potential to neutralize all strains of HIV-1, CD4-immunoglobulin fusion proteins may be effective inhibitors of post-exposure infection, including mother-to-infant transmission of the virus. We have designed CD4-fusion proteins incorporating the Fc domain of IgG2, which should minimize their role in antibody-dependent enhancement of infection or transplacental transmission of the virus. These molecules include a CD4-γ2 homodimer and a CD4-IgG2 heterotetramer where the variable regions of IgG2 heavy and light chains are replaced by the V1 and V2 domains of CD4. CD4-γ2 and CD4-IgG2 were produced in Chinese Hamster Ovary cells and purified to homogeneity.

Both molecules have half lives in rabbits of around 30 hours, compared with 17 minutes for sCD4. By flow cytometry, neither fusion protein exhibits measurable binding to Fc receptors on U937 cells. sCD4, CD4-γ2 and CD4-IgG2 bind with high affinity to immobilized gp120 from a laboratory-adapted strain and a primary isolate of HIV-1. Of these three CD4-based molecules, CD4-IgG2 exhibits the highest affinity for gp120 and is also the most effective in inhibiting HIV-1 envelope-mediated cell fusion measured in a virus-free syncytium assay. Similarly, CD4-IgG2 neutralizes HIV-1 more potently than sCD4 or CD4-γ2.

Our results indicate that CD4-IgG2 is the most suitable of these molecules for further development as a post-exposure prophylactic agent for HIV-1 infection.

J 501 TWO AND THREE-DRUG SYNERGISTIC INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 *IN VITRO* BY 6-O-BUTANOYLCASTANOSPERMINE (MDL 28574), IN COMBINATION WITH INHIBITORS OF THE HIV REVERSE TRANSCRIPTASE AND PROTEINASE, Tara M Brennan,[1] Debra L Taylor,[1] C Gordon Bridges,[1] Mohinder S Kang,[2] and A Stanley Tyms[1], Marion Merrell Dow Research Institute Laboratories, MRC Collaborative Centre, Mill Hill, London NW7 1AD[1] and Cincinnati, Ohio, USA[2].

MDL 28574, the 6-O-butanoyl derivative of castanospermine is a potent inhibitor of HIV replication. This compound exerts its antiviral effects as a result of inhibition of cellular α-glucosidase 1 of the glycoprotein processing enzymes. Treatment of HIV-infected cells with MDL 28574 caused the intracellular accumulation of glucosylated oligosaccharides and impaired viral glycoprotein processing, resulting in the production of non-infectious virions and inhibition of virus-induced cell fusion. On the basis of its antiviral profile and novel mechanism of action MDL 28574 is currently being considered for clinical trials. Based on the assumption that the future treatment of HIV-infections will involve multi-drug regimens, the anti-HIV activity of MDL 28574 was assessed in combination with the licensed drugs zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC). In addition, MDL 28574 was investigated in combination with other candidate drugs, including clinically investigated non-nucleoside reverse transcriptase (RT) inhibitors and HIV proteinase inhibitors. The data were generated by creating chequerboards of drug concentrations, and assessing the viability of HIV-1_{RF} infected MT-4 cells after six days, using an MTT cell viability assay. Drug interactions were evaluated by the isobologram technique and by calculating combination indices. Synergistic inhibition of HIV replication was observed when the glycoprotein processing antagonist was combined with any of these compounds having alternative modes of action. Furthermore, three-drug combinations using inhibitors of α-glucosidase 1 (MDL 28574), HIV RT (AZT) and HIV proteinase (Ro-31-8959), indicated that an improved antiviral effect was achieved by the addition of a third drug, compared with that seen in two drug regimens. MDL 28574 is a compound which may have an important role in combination chemotherapy for HIV infections.

J 502 COMPARATIVE ANALYSIS OF DIVERSE NONNUCLEOSIDE RT INHIBITORS, Robert W. Buckheit, Jr., Valerie Fliakas-Boltz, W. Don Decker, Joseph L. Roberson, and Cathi A. Pyle, Virology Research Division, Southern Research Institute-Frederick Research Center, Frederick, MD 21701

The National Cancer Institute (NCI) operates a high capacity screening program in which synthetic and natural products submitted from various sources throughout the world are tested for *in vitro* anti-HIV activity. A structurally diverse group of nonnucleoside inhibitors were identified which exhibited potent anti-HIV-1 activity but were totally devoid of activity against HIV-2. Biological and biochemical analysis of these compounds indicated that many common properties were shared among the group, including their reproducible antiviral activity against a panel of biologically diverse laboratory and clinical strains of HIV-1, their relative lack of toxicity, their activity in a wide variety of cultured and fresh human cells (T cells, B cells and macrophage), and their inhibition of reverse transcriptase when evaluated with heteropolymer templates. Each of these compounds needed to be continuously present in the cultures to exert anti-HIV activity. In addition, each of the nonnucleoside inhibitors when tested in combination with AZT exhibited synergistic inhibition of HIV-1, suggesting that combination antiviral therapy with AZT may be therapeutically beneficial. Further analysis of the nonnucleoside inhibitors, however, revealed striking differences in their inhibitory activities. One of the compounds was found to inhibit the murine retrovirus Rauscher, indicating that the HIV-1 specificity of the compounds may not be complete. In addition, drug resistant variants selected in cell culture exhibited differential patterns of cross-resistance to other members of the group of compounds. These results demonstrate that each of the compounds may interact with the reverse transcriptase at slightly different sites, suggesting the potential utility of a therapeutic strategy involving the use of combinations of nonnucleoside RT inhibitors. This work was supported by contract NO1-CM-37818 (NCI).

J 503 **IDENTIFICATION OF A CLEAVAGE SITE FOR HIV-1 PR IN THE TRANSFRAME REGION (p6*) OF A GAG-PR POLYPROTEIN**
M.D. Calvin\Koons, G. Zybarth and C.A. Carter, Dept. of Microbiology, S.U.N.Y, NY. 11743

The mature HIV-1 proteinase (PR; 11 kDa) can cleave all interdomain junctions in the Gag and Gag-Pol polyprotein precursors. To determine the activity of the enzyme in its precursor form, we blocked release of mature PR from a truncated Gag-Pol polyprotein by introducing mutations into the N-terminal Phe/Pro cleavage site of the PR domain. The mutant precursor autoprocessed efficiently upon expression in *E. coli*. No detectable mature PR was released, however, several PR-related products ranging in size from ≈14-18 kDa accumulated. Products of the same size were generated if mutant precursors were digested with wild-type PR. Thus, PR is able to utilize cleavage sites in the region upstream of the PR domain (p6*), resulting in the formation of extended PR species. The cleavage event resulting in the 14 kDa species has not been previously identified. Characterization of this site using purified PR and a synthetic peptide spanning the P5 to P5' residues indicated that the peptide was cleaved very inefficiently. The peptide inhibited cleavage of a Gag substrate *in vitro*. We propose that this region of the Gag-Pol polyprotein interacts with the precursor form of PR to delay PR activation until the appropriate stage of particle maturation.

J 504 CONSTRUCTION AND CHARACTERIZATION OF REPLICATION-DEFECTIVE HIV-1 PACKAGING CELL LINES, R. Carroll[1], J.T. Lin[1], J. Dacquel[1], D. Burke[2], and D. St. Louis[1]. [1]The Henry M. Jackson Foundation, 1500 E. Gude Dr., Rockville, MD, 20850 [2]Division of Retrovirology, Walter Reed Army Institute of Research and the Military Consortium for Applied Retroviral Research, 13 Taft Ct., Rockville, MD, 20850.

We have generated stable HIV-1 packaging cell lines as a component of an HIV-1-based retroviral vector system. Packaging cell lines were constructed by transfecting cells with an expression vector capable of synthesizing HIV-1 structural and regulatory proteins. The transfected cell lines synthesized high levels of structural proteins. The ability of the packaging cell lines to generate transducing particles was measured by transfecting them with a retroviral vector containing LTRs and the packaging signal from HIV-1, an internal promoter, and a neomycin phospho-transferase gene. Supernatant from transfected packaging cell lines was used to infect SupT1 cells, and transducing particle titers were determined by limiting dilution of culture supernatant. Analysis of transduced SupT1 cells indicated that in most cases, vector integration occurred without gross structural rearrangements. Analysis of RNA obtained from the transduced cells indicated that both the internal promoter and the HIV LTR were transcriptionally active. Transducing particle preparations obtained from these stable packaging cell lines were found to be free of replication competent virus.

J 505 BASELINE ZIDOVUDINE (ZDV) SUSCEPTIBILITY, CODONS 215 AND 41 MUTATION GENOTYPE, VIRAL LOAD AND SYNCYTIUM-INDUCING CHARACTERISTICS OF HIV ISOLATES FROM ACTG PROTOCOL 194 SUBJECTS. Winston. Cavert, R.W. Coombs, J. Grimes, D. Kuritzkes, E. Rojo, V. Johnson, J. Kappes, D. Stein, C. Beatty, A. Erice, M. Winters, D. Katzenstein, B. Staes, M. Mirabile, T. Elbeik, L. Corey, and ACTG Protocol 194 Clinics and Virology Laboratories. Univ. of Washington, Seattle, WA 98195; Univ. of Colorado, Harvard Univ.; Univ. Alabama; NIAID; Univ. of Minnesota; Stanford Univ.; Rush-Presbyterian; New York Univ.; Univ. California San Francisco.

AIDS Clinical Trials Group protocol 194 is a placebo-controlled, randomized, multi-center study to evaluate the short-term virologic effect of continuing ZDV, switching to DDI, or adding DDI in ZDV-treated HIV-1 seropositive subjects with CD4 counts between 100 to 300/uL following at least 1 year of prior ZDV therapy. The trial has accrued 84 subjects thus far. The median pre-study duration of ZDV use was 2.6 yrs (25-75% quartile range, 1.7 - 3.6 yrs). The median absolute baseline CD4 count was 180/uL (quartile range, 136 - 241/uL). To date, of 41 subjects evaluated, 20 (49%) had a sensitive baseline ZDV phenotype (IC50 <0.2 uM) by the ACTG/DOD consensus assay, 10 (24%) were intermediate (0.2 - 1.0 uM), and 11 (27%) had high-level resistance (>1.0 uM). For a subset (n=29) of these patients, the presence of a mutation at *pol* codon 215 was associated with increasing ZDV IC50 (Fisher's exact, p=0.09). The frequency of each *pol* genotype was 9 (31.0%) wild-type 215, 10 (34.5%) mutant 215 codon, and 10 (34.5%) mixed. Similarly, the presence of a mutation at codon 41 was also associated with increasing ZDV IC50 (p=0.001). A syncytium-inducing phenotype occurred in 15/29 (52%) of assayed baseline isolates, but did not appear more often with resistant isolates. ZDV IC50 showed no significant correlation with baseline viral load as measured by either quantitative plasma HIV-l-RNA (n=37, bDNA assay, Chiron) or by serum p24 antigen (n=41). These results from this ongoing clinical trial demonstrate that high-level ZDV resistance was present at study entry for approximately one-third of subjects who had received over 2 years of prior ZDV. For this study cohort, ZDV susceptibility may not correlate with HIV-RNA, p24 antigen, or SI phenotype.

J 506 SOURCES OF VARIATION IN THE KINETIC ASSAY OF IMMUNE COMPLEX DISSOCIATED HIV p24 ANTIGEN. Richard M. Donovan, Susan M. Smereck, Lonni R. Schultz, Michael Somero, Norman P. Markowitz, and Louis D. Saravolatz. Division of Infectious Diseases, Henry Ford Hospital, Detroit, MI 48202.

Interpretation of quantitative results from immune complex dissociated (ICD) HIV p24 antigen assays is increasingly important for evaluating new therapeutic approaches, and in the clinical management of HIV infection. This study determined the variation in the ICD HIV p24 antigen assay contributed by: day of assay, individual assay plate, and subject as well as the interaction among these variables. Blood (35 ml) was collected in EDTA from each of 10 antigenemic patients (ICD p24 antigen range = 20 - 325 pg/ml), centrifuged, and the plasma aliquoted among 5 tubes and stored at -20°C. For each of 5 days, one tube of frozen plasma from each subject was thawed and assayed. Three different assay plates were used for each patient each day. Each assay on each plate was performed in replicates of eight using a commercial assay (Coulter Corp., HIV-1 p24 Antigen Assay, HIV-1 p24 kinetic standard, and ICD-Prep Kit) according to the manufacturer's instructions. Results were obtained as colorimetric O.D. changes per unit time and analyzed using SOFTmax software (Molecular Devices Corporation). The amount of p24 antigen was determined from a 4-parameter curve of known amounts of purified p24 antigen prepared in normal human serum and diluted with 200 µl of neutral buffer. The threshold was the mean of the negative control (treated with glycine) plus 0.09 mO.D./min units. A three way analysis of variance was done to assess the variability in the results of the p24 assays, with the assay plate effect nested within the day effect. Both the interaction between day and subject, and between assay plate and subject were highly statistically significant (p=0.0001), with variance estimates of 186 and 220, respectively, compared with a random error of 273. Eight of 50 (16%) subject-days were discordant, and 41 of 150 (27%) subject-assays were discordant. Thus, ICD HIV p24 antigen values that are to be directly compared must be performed on the same day and on the same assay plate.

J 507 PHOTOAFFINITY ANALOGS OF ANTI-HIV NUCLEOSIDE DRUGS: SYNTHESIS, ANTI-VIRAL PROPERTIES AND BIOCHEMICAL USES. Richard R. Drake[1], Feng Mao[1], Alejandro Cantu[2], Sheri Anderson[2], Tammy M. Lutz[1], Robyn Jones[1], Richard Turner[2], David Morrison[2], and Mary Pat Moyer[2]. Department of Biochemistry and Molecular Biology[1], University of Arkansas for Medical Sciences, Little Rock, AR 72205 and Department of Surgery[2], UTHSC at San Antonio, San Antonio, TX 78284.

A series of 5-azido-uridine compounds have been synthesized and tested for their anti-HIV-1 activities. Introduction of the azido group in the nucleoside base (at C5) makes these compounds sensitive to UV photoactivation. Thus, besides being potential new HIV drugs, each compound is a photoaffinity analog that can be used in the study of cellular and viral proteins involved in nucleoside drug metabolism. The following uridine-based compounds have been synthesized: 5-azido-uridine ($5N_3U$), 5-azido-deoxyuridine ($5N_3dU$), 5-azido-dideoxyuridine ($5N_3ddU$), and 3'-azido-5-azido-dideoxyuridine ($5N_3AZU$). These compounds were synthesized by a combination of nitration, reduction, diazotation, and azide exchange reactions. Each of these compounds has shown activity against the replication of HIV strain IIIB in H9 cells as assayed by HIV reverse transcriptase (RT) activity or p24 antigen plates, with little cellular cytotoxicity. When compared with AZT at 1 μM concentrations, $5N_3U$ was 20-fold, $5N_3ddU$ 10-fold, and $5N_3AZU$ 50-fold less effective, though all three compounds showed inhibition of HIV *replication at nanomolar* concentrations. Additionally, $5N_3AZU$, which is the photoaffinity analog of both AZT and AZU, was ineffective at inhibiting replication of an AZT-resistant HIV strain in H9 cells. In separate experiments, 5-azido-UTP and 5-azido-dUTP inhibited the photolabeling of HIV RT by [^{32}P]8-azido-ATP. Collectively, this data suggests that these 5-azido-derivatives can be used as biochemical and pharmacological mimics of AZT for future photoaffinity studies of wild-type and drug-resistant HIV RT. A similar series of 5-azido-cytidine derivatives, including 5-azido-dideoxcytidine ($5N_3ddC$), and 8-azido-purine derivatives are currently being prepared and tested. In summary, these photoaffinity analogs will provide new biochemical tools for analyzing the metabolism of anti-HIV nucleoside drugs and the mechanisms of drug resistance.

J 508 IDENTIFICATION OF A NEW CLASS OF ANTI-HIV AGENTS THAT INHIBIT INTEGRASE, Christine C. Dykstra[1], Terri W. Smith[2], Susan K. Jones[1], Wendy Osheroff[2], Richard R. Tidwell[1], Paula A. Sherman[3], and Ronald I. Swanstrom[2]; [1]Department of Pathology and [2]Department of Biochemistry and Lineberger Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, and [3]Burroughs Welcome Co., Research Triangle Park, NC 27709

In order to improve the therapeutic intervention practices for HIV, there is a need to attack a wider range of targets. Integration of the double stranded DNA copy of the viral RNA genome into host DNA is required for productive infection and the viral integrase protein (IN) is required for this process. We have found that a series of novel DNA minor groove binding agents inhibit the IN DNA cleaving and joining activity. These agents also inhibited the early stages of HIV infection as measured by an assay that assesses the virus life cycle up through the expression of *tat*. There was no effect on reverse transcriptase activity. A greater than 10-fold higher drug concentration was required to observe cell toxicity. Finally, activity was also observed against murine leukemia virus, suggesting general anti-viral utility for these agents.

J 509 **HIV-1 MUTANT ENCODING MHR-DELETED GAG PRECURSORS INTERFERES WITH ASSEMBLY OF WILD-TYPE PARTICLES**
D. Ebbets-Reed, G. Zybarth, L.S. Ehrlich, and C.A. Carter, Dept. of Microbiology, S.U.N.Y, Stony Brook.

Retroviral particle morphogenesis involves assembly of the Gag and Gag-Pol polyproteins at the plasma membrane, budding of an immature particle, and proteolytic cleavage of polyprotein precursors by the viral proteinase to form infectious particles. The CA protein of all retroviruses includes a stretch of 20 conserved amino acids (the Major Homology Region, MHR). Mutations in the MHR have been reported to interfere with viral capsid assembly and with early replication events. We altered this region with point or deletion mutations and examined the effect on particle assembly in transfected mammalian cells. In the cytoplasm of mammalian cells, the MHR deletion mutant was processed as efficiently as the wild-type. However, mutant polyproteins lacking the MHR failed to localize efficiently to the plasma membrane. Fewer particles budded from the plasma membrane and those released contained predominantly unprocessed precursor or aberrantly processed products. Co-expression of the MHR deletion mutant with the wild-type resulted in a decline in wild-type protein localization to the plasma membrane and a concomitant reduction in the release of mature wild-type particles. Recombinant CA proteins isolated from E.coli cells expressing MHR deleted Gag formed some but not all of the oligomeric structures produced by the WT CA. Control of MHR structure during viral assembly could provide a mechanism for temporal or spatial control of polyprotein processing and CA product oligomerization.

J 510 TARGETING THE HOST CELL PROTEASE RESPONSIBLE FOR HIV-1 GP160 PROCESSING IN AIDS, Alex Franzusoff, Alison Volpe, Hilary Chouinard and Joseph Wolf, Department of Cellular and Structural Biology, B-111, U. of Colorado Health Sciences Center, Denver, CO 80262

The HIV envelope (*env*) glycoproteins, synthesized as gp160 precursors, are inefficiently cleaved at a dibasic amino acid site to the mature, membrane-bound gp41 and soluble gp120 forms during transit through the secretory pathway. The gp160 precursor is cleaved by an unknown host cell protease, and this glycoprotein processing is essential for viral infectivity and T-cell syncytium formation. We have successfully reproduced the biosynthesis, processing and cell surface expression of the HIV-1 *env* gene products in the yeast *S. cerevisiae*, in order to identify and characterize the host cell protease by both genetic and biochemical approaches. The most likely yeast protease that recognizes dibasic amino acid-containing substrates is the Kex2 protein, a Golgi-associated enzyme. Genes encoding analogous proteases that function in pro-hormone and secretory precursor protein processing in mammalian cells have been cloned by their similarity with the yeast Kex2 protease. We show that HIV-gp160 processing is abrogated in yeast lacking the Kex2 protease. Notably, cells deficient in this proteolytic activity exhibit normal growth. Furthermore, partially purified Kex2 protease cleaves HIV-gp160 *in vitro*. This work demonstrates the role of Kex2-like proteases in gp160 processing both *in vivo* and *in vitro*, and the feasibility of using the kex2-deficient yeast to identify and characterize the analogous human T-cell protease.

J 511 REQUIREMENT OF HIV-1 *nef* FOR *IN VIVO* REPLICATION AND PATHOGENICITY, B. D. Jamieson, G. M. Aldrovandi, V. Planelles, J. Jowett, L. Gao, L.M. Bloch, I.S.Y. Chen, and J.A. Zack. Dept. of Medicine, Div. of Hematology & Oncology., UCLA School of Medicine, Los Angeles, CA 90024

One approach toward vaccine development is to engineer live genetically attenuated virus. Using simian immunodeficiency virus (SIV), Kestler et al. (Cell 65: 651-662 (1991) demonstrated that *nef* is required for simian immunodeficiency virus (SIV) pathogenicity in vivo, and Daniel et al. (Science 258:1938-1941, 1992) showed that rhesus macaques infected with *nef*-defective virus were protected against a subsequent challenge with wild-type SIV. This is the most successful anti-retroviral vaccine to date. However, SIV and HIV-1 are not genetically identical; therefore, to propose live attenuated vaccines against HIV-1, it is critical to determine whether HIV-1 regulatory gene mutants demonstrate attenuated growth properties in vivo. We investigated the requirement for *nef* for in vivo replication and pathogenesis of two isolates of HIV-1 in human fetal thymus/liver implants in severe combined immunodeficient (SCID) mice. The *nef* mutants of both isolates demonstrated attenuated growth properties. At three weeks post-infection thymocytes infected *in vivo* with HIV-1_{JR-CSF} demonstrated approximately 50 fold greater copy number of HIV DNA than thymocytes infected with the *nef* frameshift mutant HIV-$1_{JR-CSF-X}$. Wild type HIV-1_{NL4-3} grew to 4 orders of magnitude higher copy number than it's *nef* deletion mutant HIV-$1_{NL-\Delta nef}$ at three weeks post-infection. In addition,HIV-1_{NL4-3} induced severe depletion of CD4 bearing thymocytes in all infected thymuses by six weeks post-infection, whereas no CD4 depletion was seen in thymuses infected with HIV-$1_{NL-\Delta nef}$. Our results demonstrate that *HIV-1 nef* is required for efficient in vivo replication and pathogenicity. These results suggest that *nef* may be suitable for further study as a candidate gene for the development of attenuated vaccines. In addition, our results demonstrate that the SCID-hu mouse may be a valuable animal model to assess pathogenic properties of attenuated HIV strains.

J 512 INHIBITION OF METHYLATION IN HIV REPLICATION: POTENTIAL USE AS NOVEL ANTI HIV DRUGS.

Bharat Joshi*, Peter K.Chiang**, Douglas L. Mayers**, Sherwin Lee*, Jay S. Epstein* and Indira K. Hewlett*, * DTTD, CBER, FDA, Bethesda and ** Walter Reed Army Institute of Research, Washington, D.C.

We have studied the effect of inhibitors of methylation on the replication of HIV-1 in H9 cells. The compounds used were 3-deazaaristeromycin (3-deaza-Ari; carbocyclic 3-deaza- adenosine) 3- deaza adenosine (3-deaza-Ado) and 3- deazaneplanocin all of which potent inhibitors of S- adenosylhomocystein (AdoHcy) hydrolase which is necessary for methylation. H9 cells were infected in the logarithmic phase with HIV-1 MN strain at 100ng of viral p24 antigen per 100^6 cells. Cells were then incubated in the presence of the drug and at days 4 and 8 cell free supernatant was collected for analysis of p24 antigen and RT. Cultures were also examined for syncytia formation and cell viability. Greater than 80% inhibtion of syncytia formation and p24 antigen levels were observed with all 3 drugs at nM concentrations without affecting cell viability. When tested against AZT -resistant isolates, these 3- deaza nucleosides were found to be equally effective. Currently, the effect of these compounds on viral load in cells is being evaluated by quantitative DNA and RNA PCR using H9 and normal peripheral blood mono nuclear cells (PBMCs). Experiments are in progress to understand the mechanism of inhibition viz. methylation of specific cellular and viral proteins or genes.

J 513 THE HIV VPX AND VPR GENES MEDIATE VIRION INCORPORATION OF NUCLEASE FUSION PROTEINS, [1]Kappes, J.C., [1]Wu, X., [1]Liu, H-M., [2]Boeke, J.D., [2]Natsoulis, G., [1]Hahn, B.H., [1]University of Alabama at Birmingham and [2]John Hopkins University School of Medicine.

The application of gene therapy for delivery of molecules that possess the ability to selectively interfere with replication of the human immunodeficiency virus (HIV) is a novel strategy for therapeutic HIV disease intervention. "Virion-targeted viral inactivation" represents a new approach to interfere with viral replication. In this approach, a deleterious protein is fused to a viral structural protein so that the fusion is encapsidated where it may inactivate the virion. HIV accessory genes, *vpx* and *vpr*, are packaged into virions in amounts similar to *gag* proteins and are dispensable for viral replication and as such are attractive candidates for application to such a strategy. In order to facilitate efficient incorporation of candidate fusion proteins into virions, the *vpx* and *vpr* packaging signal-sequences were identified. These sequences were then fused in-frame with the staphylococcal nuclease (SN) gene and expressed *in trans* with HIV proviral DNAs. Our results demonstrate stable expression and efficient packaging of *vpr*-SN fusion proteins with HIV-1 *vpr*-deficient and wild-type virions. Similarly, *vpx*-SN fusion proteins packaged efficiently with HIV-2 virions. The use of *vpx* and *vpr* to mediate incorporation of biologically active SN protein into virions, its destruction of the RNA genome and subsequent effect on virus infectivity and replication are currently under study. The potential utilization of HIV accessory genes as mediators of capsid-targeted virion inactivation will be discussed.

J 514 MUTATIONAL ANALYSIS OF RESIDUE 190 OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REVERSE TRANSCRIPTASE

J.-P. Kleim[1], R. Bender[1], C. Meichsner[1], A. Paessens[2], and G. Riess[1]

Hoechst AG, SBU Antiinfectives Research, D-65926 Frankfurt [1], and Institute of Virology, Pharma Research Center, D-42096 Wuppertal [2], Germany

S-2720 and other derivatives of the potent quinoline quinoxaline class of HIV-1- specific nonnucleosidic reverse transcriptase inhibitors (NNRTIs) select for a Gly 190 Glu substitution wihtin the RT in infected cell cultures. This exchange, which has not been shown to occur upon selection for resistant virus with any other RT inhibitor, also confers resistance to compounds which belong to other classes of NNRTIs. Notably, the glutamate in position 190 of the RT causes a decrease in RNA dependent DNA polymerase (RDDP) activity of the enzyme. We replaced the naturally occurring glycine in position 190 of the RT by a number of different amino acids and compared the activities of the recombinant RTs as well as the inhibitor potencies of different NNRTIs against the mutant enzymes. We thereby show that the type of the residue 190 side chain is critical for both reduced susceptibility against NNRTIs and for in vitro RDDP activity of the HIV-1 RT.

J 515 MODIFIED ANTHRAX TOXIN IS CLEAVED AND ACTIVATED BY HIV-1 PROTEASE

Kurt R. Klimpel and Stephen H. Leppla, National Institute of Dental Research, Laboratory of Microbial Ecology, National Institutes of Health, Bethesda, MD 20892

Protective antigen (PA), a protein component from anthrax toxin, must be cleaved in order to intoxicate cells. Following binding to its receptor, a cell associated protease recognizes and cleaves PA after the amino acid sequence RKKR. Cleavage of PA allows binding and internalization of a second toxic protein. We used cassette mutagenesis to replace residues 164167 (RKKR) of PA with five different sequences expected to be recognized by HIV-1 protease. The resulting recombinant proteins were expressed in *Bacillus anthracis* and purified from the culture supernatant.

MUTANT	JUNCTION SEQUENCE SOURCE	AMINO ACID SEQUENCE
PAHIV# 1	Hypothetical	GGSAFNFPIVMGG
PAHIV# 2	Membrane protein-Capsid	QVSQNYPIVQNI
PAHIV# 3	Capsid-Nucleocapsid	NTATIMMQRGNF
PAHIV# 4	Protease-protein 6	TVSFNFPQITLW
PAHIV# 5	Hypothetical	SQNYPVVQ

Recombinant HIV-1 protease was used to digest each PA mutant *in vitro*. PAHIV #'s 1, 2, 3 and 4 were all cleaved by the protease while PAHIV #5 was not. Cleavage was apparent in as little as 5 min. PAHIV#3 was cleaved the most efficiently followed by PAHIV#1 and PAHIV#2. When PA or PAHIV proteins were incubated with the HIV-1 protease for more than 60 min an unexpected second cleavage occurred. This may represent cleavage of PA and PAHIV proteins at residues ^{256}VAAYPIVHV^{264}. In addition to potential anti-viral applications, PAHIV proteins may be valuable tools for studying the action of the HIV-1 protease and its inhibitors in the context of a whole protein.

J 516 IMMUNOTHERAPY WITH AUTOLOGOUS EXPANDED, HIV-SPECIFIC CYTOTOXIC T CELLS IN INFECTED PATIENTS WITH CD4 COUNTS BETWEEN 100-400/MM^3

Judy Lieberman, Jessica A. Fabry, Paul R. Skolnik, George R. Parkerson, Donna M. Fong, Jonathan Kagan, Michael B. Atkins, Daniel Stein, Harold Standiford, Edward Lee, Paul Flyer, Mary Banach, Marcia Scott, Bernard Landry, Divisions of Hematology-Oncology and Infectious Diseases and Geographic Medicine, Department of Medicine, New England Medical Center, Boston MA 02111 and the Division of AIDS Treatment Research Initiative, NIAID, Bethesda MD

HIV-specific cytotoxic T lymphocytes (CTL) are unusually abundant in the blood of asymptomatic HIV-1 infected individuals but become difficult to detect at the time of onset of opportunistic infection. Because the development of opportunistic infections may represent a shift in balance between the host's cellular immunity and viral replication, we are investigating the feasibility, safety and tolerance of treating patients at risk for developing AIDS by infusion of HIV-specific CTL in an attempt to bolster host immunity to control the virus.

The CTL response to HIV-1 is dominated by the recognition of a small number of peptides encoded by HIV-1 structural and regulatory genes. We are able to expand selectively HIV-specific CTL ex vivo by culture with autologous antigen-presenting cells preincubated with immunodominant HIV-1 peptides. In this pilot dose-escalation trial, groups of 5-8 patients with CD4 counts between 100-400/mm^3 are treated by a single infusion of 1, 5 or 25 billion CTL, selectively expanded to recognize and lyse HIV-expressing targets. In the first 6 patients treated with 1 billion HIV-specific T cells and followed for up to 6 months, no toxicity was associated with the infusion. Preliminary data reveal a rise in HIV-specific peripheral blood CTL in most patients. Following treatment some patients show in vitro CTL activity to previously unrecognized HIV proteins. Data will be presented about changes in viral titers in the peripheral blood as well as in surrogate markers of disease including CD4 counts.

J 517 LOW LEVELS OF dNTP INDUCED BY HYDROXYUREA INHIBIT HIV-1 REPLICATION

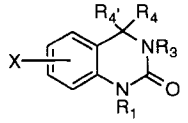
FRANCO LORI[1], WEN-YI GAO[2], ANDREA CARA[1], and ROBERT C. GALLO[1].

[1]Laboratory of Tumor Cell Biology, [2]Laboratory of Medicinal Chemistry, NCI, NIH, Bethesda, MD, 20892.

Incomplete viral DNA originating from reverse transcription before or during virus budding is encapsidated into mature HIV-1 virions. Here we show that the virion associated DNA contributed to the formation of an early pool of incomplete latent viral DNA in infected primary lymphocytes, suggesting that carrying preformed DNA could be advantageous for HIV-1 latency during infection of quiescent cells. This DNA is completed extremely slowly and inefficiently in quiescent PBL compared to that in stimulated PBL. We demonstrate here that this phenomenon is caused by the existence of lower levels of deoxynucleotides (dNTP) in quiescent compared to activated PBL impairing the HIV-1 reverse transcriptase activity. Hydroxyurea treatment of stimulated PBL decreases the levels of dNTP and reduces DNA synthesis rate as well as DNA elongation to levels comparable to quiescent PBL. At concentrations commonly used in human therapy, hydroxyurea inhibits HIV-1 replication in primary human PBL and macrophages and acts synergistically in combination with the nucleoside analogs AZT and ddI. Our data therefore indicate that low levels of dNTP may explain why HIV-1 DNA is synthesized slowly and inefficiently in quiescent PBL and suggest that pharmacologic induction of low dNTP levels represents a novel therapeutic approach for inhibition of HIV-1 replication.

J 518 STRUCTURE - ACTIVITY STUDIES OF NOVEL INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE. Terry A. Lyle, Steven D. Young, Paul S. Anderson, Suresh K. Balani, Susan F. Britcher, Steven S. Carroll, Chris Culberson, Emilio A. Emini, Mark E. Goldman, Carl F. Homnick, Joel R. Huff, William C. Lumma, Julie A. O'Brien, David B. Olsen, Linda S. Payne, Doug J. Pettibone, Julio C. Quintero, William M. Sanders, Phil E. J. Sanderson, William A. Schleif, Steven J. Smith, Mark Stahlhut, Anthony M. Theoharides†, Craig M. Thomas, Lee O. Tran, Thomas J. Tucker, and Catherine M. Wiscount, Merck Research Laboratories, West Point, PA 19486

Reverse transcriptase (RT) has been determined to be a clinically relevant target for the antiviral chemotherapy of HIV infection. All of the drugs currently approved for the treatment of HIV infection are nucleoside analogs and share a common mechanism of action, as well as liabilities due to toxicity. During the past two years, a number of laboratories have identified several classes of non-nucleoside RT inhibitors that apparently share a common allosteric binding site on HIV-1 RT. This presentation will describe structure-activity relationships for a novel series of potent dihydroquinazolinone RT inhibitors as shown below. Pharmacokinetic data and resistance profiles on selected compounds will also be presented.



J 519 DETERMINATION OF INHIBITION CONSTANTS OF VERY HIGH-AFFINITY COMPETITIVE INHIBITORS OF HIV-1 AND HIV-2 PROTEASES, Janet C. Lynn, Paul K. Tomich, Suvit Thaisrivongs, Roger A. Poorman, Ferenc J. Kézdy, The Upjohn Company, Kalamazoo, MI 49001

HIV-1 protease is a well-recognized potential therapeutic target for disrupting the life cycle of the HIV virus, the etiologic agent of AIDS (acquired immunodeficiency syndrome). We have already described a rapid, sensitive fluorometric assay for detecting potential inhibitors of the HIV protease. When the assay is performed under conditions where the substrate concentration is much lower than K_m and conversion to product is greater than 99% complete, then inhibitors produce a measurable retardation of the reaction only when their concentration is much larger than their K_i. This kinetic design obviates the experimental problems associated with low inhibitor and enzyme concentrations. The inhibitor constants measured by this method are more accurate and experimentally more straightforward than those determined by more conventional methods, albeit fully consistent with them. The experimental method and data analysis will be illustrated with U-75875, a high-affinity peptide inhibitor of HIV-1 and HIV-2 proteases.

J 520 TARGETED CLEAVAGE OF PKR mRNA IN INTACT CELLS INDUCED BY 2-5A-ANTISENSE CHIMERAS

R.K. Maitra[1], A. Maran[1], B. Dong[1], K. Lesiak[2], W. Xiao[3], P.F. Torrence[3] & R.H. Silverman[1]

[1]Department of Cancer Biology, Cleveland Clinic Foundation, [2]Laboratory of Biophysics, Center for Biologics Evaluation and Research, F.D.A., Bethesda, MD, and [3]Section on Biomedical Chemistry, N.I.D.D.K., National Institutes of Health, Bethesda, MD.

"Antisense" refers to the concept that nucleic acids which are complementary in sequence may anneal in cells and inhibit gene expression. We have developed a novel antisense strategy in which 2-5A-dependent RNase is directed to specific RNA targets. Chimeric molecules were synthesized in which the 2-5A species, pA(2'p5'A)3, was covalently linked to antisense oligodeoxyribonucleotides. The antisense cassette of the chimera binds to its complementary sequence in RNA while the accompanying 2-5A component attracts and activates 2-5A-dependent RNase which then cleaves the target RNA. Previously we demonstrated that in a cell free system chimeric molecule 2-5A-oligo(dT)18 direct 2-5A-dependent RNase to cleave a modified HIV-Vif mRNA containing an internal stretch of 25 adenylyl residues (Torrence et al., *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1300-1304, 1993). To further develop the 2-5A antisense method, we targeted for cleavage of a naturally occurring RNA sequence in mRNA for PKR, the dsRNA-dependent protein kinase. Accordingly, purified recombinant 2-5A-dependent RNase preferentially degraded PKR mRNA from a mixed population mRNA molecules to PKR and HIV-1 Vif protein. In addition, in the presence of 50 nM of 2-5A-antiPKR, PKR mRNA was cleaved by recombinant 2-5A-dependent RNase whereas no PKR mRNA degradation was observed with 150nM of 2-5A linked to an unrelated oligonucleotide. Furthermore, after incubating HeLa cells with 2-5A-antiPKR for 4 hrs, there was a total ablation of intact PKR mRNA as determined using reverse transcription-coupled PCR or RNase protection assay. 2-5A linked to irrelevant oligonucleotide sequences and antiPKR alone had no effect on PKR mRNA levels and 2-5A-antiPKR did not reduce levels of actin mRNA; thus demonstrating selectivity of action. In addition, 2-5A-antisense did not reduce cell growth rates and therefore it is not cytotoxic. Because 2-5A-dependent RNase is present in most mammalian cells, this new approach to controlling gene expression includes possible therapies for cancer and viral infections including AIDS.

J 521 SELECTION AND ANALYSIS OF HIV-1 VARIANTS WITH REDUCED SUSCEPTIBILITY TO AN INHIBITOR OF HIV-1 PROTEASE. Martin Markowitz, Hongmei Mo, Dale Kempf, Dan Norbeck, David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, New York.

A-84538 is a potent inhibitor of HIV-1 protease in vitro, and is a promising candidate for clinical testing. Using this potent inhibitor in increasing concentrations, we serially passaged HIV-1 NL4-3 in MT4 cells. After nineteen passages a viral isolate with reduced susceptibility to A-84538 was obtained. The ID_{90} of the parental NL4-3 was .07 μM, whereas the ID_{90} of the passage 19 virus was 2.0 μM. This represents approximately a twenty-fold reduction in viral sensitivity to A-84538. PCR was used to amplify the protease coding sequence from cells infected with the resistant HIV-1 variant. The amplified products were cloned into M13, and nucleotide sequencing is now in progress. If specific mutations are found, site directed mutagenesis will be performed to confirm the importance of the changes in conferring the A-84538 resistance phenotype.

J 522 CHARACTERIZATION OF HIV-1 VARIANTS THAT SHOW INCREASED RESISTANCE TO TWO PROTEASE INHIBITORS, Hongmei Mo, Martin Markowitz, John Erickson*, David D. Ho. Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY; *Frederick Cancer Research Center, Frederick, MD.

HIV-1 protease inhibitors show potent antiviral effects on HIV-1 replication in vitro, and several are now in clinical trials. We have studied HIV-1 resistance to protease inhibitors, including two recently developed compounds, MP-134 and MP-167. HIV-1 NL4-3 was serially passaged in the presence of increasing concentrations of MP-134 or MP-167. After 14 passages, the MP-134 ID_{50} of the selected variant increased 6-fold to 30 μM. Similarly, the HIV-1 variant grown in the presence of MP-167 showed a 20-fold increase in ID_{50} (30 μM) for that drug. Both of these viral variants show cross-resistance to the other drug and to C2-symmetric protease inhibitors developed by Abbott (A77003, A80987, and A84538). The nucleotide sequences of the protease coding region of these HIV-1 variants was determined by PCR amplification followed by molecular cloning and sequencing. For the variant selected by MP-134, an isoleucine to valine substitution at position 84 was found in 7 of 10 clones. For the variant selected by MP-167, a glycine to valine substitution at position 48 was found in 8 of 10 clones, while a leucine to phenylalanine substitution was found at position 10 in 5 of 10 clones. All clones from the two sets of variants contained a G57R substitution. The mechanistic significance of these protease mutations is under investigation.

J 523 QC-PCR MONITORING OF PLASMA VIRAL LOAD IN THE EVALUATION OF EXPERIMENTAL TREATMENT FOR HIV INFECTION. M. Piatak, Jr.*, M.S. Saag**, L.C. Yang*, J.C. Kappes**, E. A. Emini***, G.M. Shaw**, and J. D. Lifson*, *HIV and Exploratory Research, Genelabs Technologies, Inc., Redwood City, CA 94063; **Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, 35294; ***Merck Research Laboratories, West Point, PA., 19486*

We have developed a method, QC-PCR, for the accurate and sensitive quantification of HIV-1 nucleic acid species, and have applied this approach to the measurement of virion-associated HIV-1 RNA in plasma of patients undergoing treatment with antiretroviral agents. In QC-PCR, a synthetic internal control template is used to circumvent inherent features of standard PCR methods that can result in inconsistent relationships between input target template copy number and the absolute amount of post-amplification product obtained. QC-PCR employs essentially a titration approach, in which different known copy numbers of the intenal control template, matched to but differentiable from the target template to be amplified, are spiked into replicate aliquots of the test sample, containing an unknown copy number of target sequence. For quantification of HIV-1 RNA, the synthetic template is used in RNA form, providing stringent internal control for both the reverse transcription step and the PCR amplification of the resulting cDNA. After amplification, the amount of target template present prior to reverse transcription and amplification is determined by comparison of the *relative* amounts of products derived from template in the orignal specimen and different known copy numbers of co-amplified synthetic template.

QC-PCR was used to quantify plasma virion-associated HIV-1 RNA in a controlled clinical study in which the pyridinone non-nucleoside reverse transcriptase inhibitor L697,661 was compared with AZT (n=45). Patients discontinued previous therapy for one week prior to initiation of L697,661 or AZT, and were treated for six weeks prior to temporary discontinuation of treatment. Levels of circulating virus measured by QC-PCR correlated well with p24 levels, where measurable. AZT resulted in prompt (within one week), approximately 10-fold decreases in levels of circulating virion-associated HIV-1 RNA, that persisted for the duration of treatment, with a similarly prompt rebound of circulating virus to pretreatment levels upon discontinuation of treatment. Treatment with L697,661 produced similar initial decreases in QC-PCR-measured viral load in plasma, but in contrast to AZT treatment, rebound of circulating virus to pretreatment levels was observed in the face of continuing treatment. The on-treatment rebound in viral load appeared to be related to the emergence of genotypically and phenotypically demonstrable drug-resistant virus in the patients. QC-PCR appears to be promising for monitoring the in vivo activity of antiretroviral treatment, especially in patients with early stage disease, in whom other virologic markers are negative.

J 524 AN ENZYMATIC ASSAY FOR THE MEASUREMENT OF ZIDOVUDINE TRIPHOSPHATE (ZDV-TP) IN PATIENT-DERIVED PERIPHERAL BLOOD MONONUCLEAR CELLS, Brian L. Robbins, Connie McDonald, Patricia M. Flynn, Ranga V. Srinivas, and Arnold Fridland, Department of Infectious Diseases, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105

The *in vivo* concentration of ZDV-TP, the active antiviral metabolite of Zidovudine (ZDV) is a valuable parameter because it may give insight into the efficacy of varied ZDV dosage and the eventual loss of antiviral activity with prolonged ZDV treatment. We describe a new method to measure intracellular ZDV-TP in the peripheral blood mononuclear cells (PBMCs) of patients treated with ZDV utilizing inhibition of HIV-1 reverse transcriptase by ZDV-TP. Intracellular ZDV-TP was determined using the enzymatic assay in PBMCs isolated from healthy individuals incubated with different concentrations of labeled ZDV, and validated by HPLC separation and liquid scintillation counting of the resulting radioactive ZDV-TP. The results from these measurements were virtually identical over a range of ZDV-TP from 150 to 900 fmols with a 90% recovery of ZDV-TP and a limit of sensitivity of 20 to 50 fmol with the enzymatic assay. The intracellular ZDV-TP and plasma ZDV levels were determined in 12 HIV-infected volunteers administered a single 500 or 100 mg oral dose of ZDV. Median intracellular ZDV-TP levels ranged from 5-57 fmol/10^8 cells and 42-92 fmol/10^8 cells in subjects administered 100 and 500 mg of ZDV, respectively. The median ratio of intracellular ZDV-TP was ~2 which was similar to the plasma ZDV area under the curve dose ratio of 2.5. Intracellular ZDV-TP levels appeared to correlate with ZDV levels but not in a predictable fashion over the time frame studied, supporting the direct measurement of ZDV-TP. The enzymatic method of measuring ZDV-TP should prove to be useful in the further study of ZDV metabolism in patient derived PBMCs at the doses of ZDV currently administered.

J 525 SELECTION OF ENHANCED GP120-BINDING VARIANTS OF HUMAN CD4 DISPLAYED ON BACTERIOPHAGE M13, Bruce L. Roberts, Robert Burghoff, Rachel B. Kent, Vicki L. Barniak, William Markland and Robert C. Ladner, Protein Engineering Corporation, Cambridge, MA 02138

While soluble CD4 (sCD4) effectively neutralizes tissue culture adapted strains of HIV1 in vitro, it is less potent when faced with clinical isolates of HIV1. To improve the efficacy of sCD4 we have employed bacteriophage display technology to engineer variants with enhanced affinity for gp120. The D1 and D1D2 domains of human CD4 have been displayed on the surface of bacteriophage M13 as gene III and gene VIII fusion proteins. Anti-CD4 MAb probing studies indicated that correctly-folded CD4 fusion proteins are displayed and are capable of binding immobilized gp120 with high affinity. Substitution of residues 40-43 of human CD4 with the corresponding residues of mouse CD4 greatly diminishes the ability of CD4 fusion phage to bind to immobilized gp120. The region comprising the beta turn of the C'C'' loop of human CD4 (aa 39-45) was variegated and libraries of phage displaying CD4 variants were "panned" to select variants exhibiting the highest affinity for gp120. A variety of isolated variants were expressed in E. coli as malE gene fusions and soluble fusion proteins were purified. Data will be presented which indicates that selected maltose binding protein:CD4 variant fusion proteins show enhanced gp120 binding properties relative to wild type sCD4 in an ELISA based competition assay. These variants will provide a valuable structural basis for further rounds of variegation and selection for engineered CD4 variants with improved gp120 binding capabilities.

J 526 ANALYSIS OF VIRAL MUTANTS RESISTANT TO A77003, A C2-SYMMETRIC INHIBITOR OF HIV-1 PROTEASE. M. K. Singh, T. Toyoshima, H-M. Mo, D. J. Kempf, D. W. Norbeck, J. Erickson, D. D. Ho The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY; Abbott Laboratories, Abbott Park, IL; NCI-Frederick Cancer Research Center, Frederick, MD.

HIV-1 protease inhibitors represent an important and promising class of antivirals for the treatment of AIDS. We have used the prototype C2-symmetric inhibitor A77003 to study the development of viral resistance in vitro. HIV-1-NL4-3 was serially passaged in the presence of increasing concentrations of A77003. After 19 passages, HIV-1 variants emerged showing a 20-fold increase in the ID_{90}. The viral protease genes from various passages were PCR amplified and nucleotide sequences were determined. The predominant changes were identified in residues 8 (R8Q or R8K), and 46 (M46I). Site directed mutagenesis studies with the infectious virus showed that each of the R8Q and R8K mutations contributes to A77003 resistance, while the M46I mutation confers a growth advantage without directly affecting drug sensitivity. Kinetic studies with individually expressed mutant enzymes show that the mutations at residue 8 were associated with an appropriate increase in Ki. The analysis and interpretation of these mutations based on the available three-dimension structure of protease will be discussed.

J 527 INHIBITION OF HIV REPLICATION BY CYCLOSPORIN DERIVATIVES: LACK OF CORRELATION WITH IMMUNOSUPPRESSSION. A.Steinkasserer, A.Billich, P.Peichl, R.Harrison, F.Hammerschmid, W.Schnitzel and B.Rosenwirth, Sandoz Forschungsinstitut, A-1235 Vienna, Austria

It has been argued that immunosuppression may have a beneficial effect in AIDS. It would block T4-cell activation which is necessary for HIV-replication, and would thus have indirect antiviral activity; furthermore, the autoimmune processes which presumably lead to killing of T4-cells would be inhibited. We tested Cyclosporin A, a large number of derivatives, FK506 and several derivatives for inhibition of HIV-1 induced cytopathic effect in the T4-cell line MT4. Most of the CsA derivatives were inhibitory at a concentration below the cytotoxic concentration. On the other hand, FK506 and derivatives were not active in this assay. The most potent CsA derivatives inhibited HIV-1 replication in MT4 cells at concentrations of 0.03 to 0.1µg/ml, but impaired cell proliferation only at concentrations of 3 to 10 µg/ml. Anti-HIV activity, surprisingly, did not correlate with the immunosuppressive capacity of the derivatives but with Cyclophilin binding. FK506 - as expected for an immunosuppressive drug - was able to block HIV replication in primary T4 lymphocytes when added simultaneously with PHA, i.e. before activation, but not when given after stimulation. Immunosuppressive as well as non-immunosuppressive Cyclosporin derivatives, on the other hand, were able to inhibit HIV when given before activation, but also proved active when given 24 hours after PHA stimulation. The antiviral activity of selected immunosuppressive as well as non-immunosuppressive Cyclosporin derivatives was demonstrated in further T4 cell lines, in the promonocytic cell line U937, in HeLa CD4 cells, and in primary T4 lymphocytes and monocytes. The compounds proved equally active against laboratory strains of HIV-1 and against a set of clinical isolates from different geographic locations. Evidence was obtained that there are two steps of virus replication which are inhibited by Cyclosporin derivatives, namely an early step after penetration, but before or at integration of the provirus, and a late step leading to formation of non-infectious particles.

J 528 QUANTITATIVE PCR FOR HIV-1 DNA, Pádraig Strappe, Vincent C Emery & Paul D Griffiths, Division of Communicable Diseases, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.

A quantitative polymerase chain reaction assay has been developed to measure HIV-1 proviral load in PBMC and post-mortem body tissue. HIV-1 DNA is measured by a co-amplification of a 233-bp artificial HIV-1 DNA construct mutated to contain a *Sma-1* enzyme restriction site between 2 conserved *gag* primers. A known amount of construct DNA (1,000 copies) is added to a constant amount of patient DNA. After amplification with radiolabelled primers and enzyme digestion with *Sma-1* enzyme, densitometry can then measure the signal relating to the patient DNA compared to the known amount of construct DNA. RNA transcripts can also be generated from the construct containing the enzyme site to be used in a quantitative RNA PCR. Using this assay, the proviral load was measured in a group of patients with late stage HIV disease who switched anti-retroviral therapy from AZT to ddI. The results show no relative decrease in proviral load expressed per ml of blood in the patients before and after the change in therapy. These results are presented with other parameters of disease progression eg P24 antigen (acid dissociated) and CD4 cell count. Levels of proviral DNA in the post-mortem tissue of one of the patients are also presented expressed per microgram of total cellular DNA. The highest levels of HIV DNA are shown to be in the lymph node and brain (10,000 and 4,000 copies respectively), with levels in the lung, spleen and pancreas approximately 2,000 copies. A study into the variability of such a quantitative DNA PCR is also presented. Using a semi-quantitative point mutation assay the levels of mutations in the HIV-1 reverse transcriptase gene conferring resistance to AZT or ddI are also presented for PBMC and post-mortem tissue DNA.

J 529 RETROVIRAL VECTOR VACCINES FOR AIDS IMMUNO-THERAPY, John F. Warner, Mike Irwin, Lisa Laube, Virginia Lee, Carol-Gay Anderson, Sunil Chada, Gloria Peters, Ulrike Ziegner, Bruce Merchant, Doug Jolly, Steve Mento, and Jeff Galpin*, Department of Immunobiology, Viagene, Inc., 11075 Roselle Street, San Diego, California 92121; *Shared Medical Research Foundation, Tarzana, California 91356.

The cytotoxic T lymphocyte (CTL) response plays an important role in controlling the severity and duration of viral infections. Because effective T cell activation requires the intracellular production and processing of foreign protein antigens, novel methods of introducing genes encoding these antigens into cells could be useful. Gene-based vaccines, using retroviral vectors, represent an efficient means of introducing and expressing genes in mammalian cells and, hence can be employed to provide foreign proteins to the appropriate antigen processing and presentation pathways for CTL activation. We have developed retroviral vectors encoding HIV-1 proteins and examined these vectors for their ability to stimulate immune responses, particularly CTL activity, in several animal models including mice, Rhesus monkeys, and baboons. The retroviral vectors consistently activate $CD8^+$, Class I MHC-restricted anti-HIV-1 CTL responses in these systems. Moreover, the activated CTL show broad crossreactivity against target cells infected with many laboratory and clinical HIV-1 isolates. CTL responses can be induced using *ex-vivo* vector-treated autologous primary cells or by direct administration of the vector. These animal studies demonstrated the ability of retroviral vectors encoding HIV-1 proteins to consistently stimulate the cellular immune response and suggest that these vectors may provide an effective means of inducing or augmenting CTL responses in HIV-infected individuals. Based on these studies, HIV-infected patients have been injected with vector-transduced autologous primary fibroblasts expressing the HIV-1 IIIB envelope protein in a Phase I clinical trial. The results of animal studies and the human trial will be presented.

J 530 TARGETING ACTIVATED HIV-INFECTED T CELLS AND MONOCYTES WITH THE DIPHTHERIA TOXIN-RELATED IL-2 FUSION PROTEINS $DAB_{486}IL\text{-}2$ AND $DAB_{389}IL\text{-}2$: A NEW THERAPEUTIC STRATEGY, Cory A. Waters[a], Lin Zhang[b], Jean C. Nichols[a], Thasia G. Woodworth[a], Donald Craven[c], Charles Flexner[d] and Clyde Crumpacker[b]. Seragen, Inc.[a], Hopkinton, MA 01748, Beth Israel Hospital and Harvard Medical School[b], Boston, MA 02215, Boston City Hospital[c], Boston, MA 02118 and The Johns Hopkins Hospital[d], Baltimore, MD 21287

Monocytes and $CD4^+$ T cells are both targets for HIV infection and represent major reservoirs for the dissemination of infectious virus. Most therapeutic strategies have focused on intervening in the infectious process by interrupting the sequence of events associated with HIV binding, entry, reverse transcription and integration into the host genome. The ability of the virus to generate variants resistant to these therapeutics, however, has represented a major barrier to success. An alternative strategy based on knowledge that productive HIV infection appears to require activation of the infected cell, focuses on targeting the infected cell rather than the virus itself. $DAB_{486}IL\text{-}2$ and $DAB_{389}IL\text{-}2$ are fusion toxins which are selectively cytotoxic for activated IL-2 receptor (IL-2R) expressing cells. Both fusion proteins were constructed by genetically fusing portions of the diphtheria toxin gene (lacking its intrinsic receptor binding domain) to the gene for human IL-2. *In vitro* studies have shown that a range of concentrations of $DAB_{486}IL\text{-}2$ inhibits HIV-1 replication in activated human lymphocytes and monocytes infected by laboratory strains of HIV-1 as determined by the presence of p24 antigen and by H9 cocultivation experiments. In a sensitive RNA:RNA hybridization assay, $DAB_{389}IL\text{-}2$ also inhibits HIV-1 RNA production in PBMC infected with fresh clinical isolates or an AZT-resistant laboratory strain. The results of these experiments, and the safety and evidence of IL-2R targeting without generalized immunosuppression in $DAB_{486}IL\text{-}2$ and $DAB_{389}IL\text{-}2$-treated lymphoma and rheumatoid arthritis patients, prompted us to initiate a Phase I/II double-blind, dose ranging study to evaluate the safety, pharmacokinetics and efficacy of administering $DAB_{389}IL\text{-}2$ to HIV-infected patients with p24 antigenemia and CD4 counts $\geq 300/mm^3$. Eligible patients are randomized to one of three dose levels and receive 5 daily IV bolus doses for 3 courses 2 weeks apart. p24 levels and quantitative viral cultures are evaluated before and after each course and CD3, CD4, and CD8 lymphocytes every 2 weeks. Preliminary safety data will be presented; no safety issues have been reported to date.

J 531 **Protective Anti-HIV Immune responses Induced through in vivo genetic Inoculation:** Bin Wang, Jean Boyer, Kenneth E. Ugen, Michael Adajanian, *Leslie Coney, *Richard Carrano, William V. Williams and David B. Weiner: The University of Pennsylvania School of Medicine, IBAMM, 3600 Spruce Street, Philadelphia, PA 19104., *Apollon Inc. , Malvern PA.

The introduction of non replicating genetic sequences from pathogens into a living animal has great promise for vaccine/immune therapeutic development. Genetic immunization mimics aspects of attenuated vaccines in that synthesis of specific foreign proteins are accomplished in the host cell and become the subject of immune surveillance. It also contains safety aspects of subunit vaccines in that a subset of pathogen genes can be constructed to serve as specific immungens. Genetic immunization is dependent upon injection of a nucleic acid sequence directly into a host target tissue. We have used this technology to immunize small animals (rabbits and mice) with human immunodeficiency virus type 1 (HIV-1) constructs. Through this technology we have achieved relevant immune responses. Specifically, antisera from genetically inoculated animals demonstrate anti-HIV envelope glycoprotein immune responses. The antiserum neutralizes HIV-1 infection and inhibits cell to cell infection in vitro. Antibodies to conformational epitopes were similarly produced. This protocol induced both T cell proliferation and isotype switching consistent with the production of specific T helper immune responses. Furthermore relevant lysis of env expressing target cells was induced by this technique. Through the development of an in vivo murine model we observed that mice can reject lethal cell challenge through specific immune responses directed at HIV proteins. We have applied this approach to immunize non human primates against HIV proteins. Using a similar approach to the small amimal studies we induced anti-HIV immune responses including anti-env T cell responses as well as seroconversion and virus neutralizing antibody responses. This technology has applications for the development of a safe and efficacious immunization strategy against HIV as it provides for relevant antigen production in vivo without the use of infectious agents.

J 532 INTRACELLULAR IMMUNIZATION OF HUMAN HEMATOPOIETIC PROGENITOR CELLS WITH A RIBOZYME AGAINST HIV. Mang Yu, Osamu Yamada, Mark Leavitt, Midori Maruyama, Dennis Young, Anthony Ho and Flossie Wong-Staal, Department of Medicine, University of California, San Diego, CA 92093-0665.

We have previously reported that a hairpin ribozyme designed to cleave HIV-1 RNA in the 5' leader sequence suppresses virus expression in HeLa cells co-transfected with proviral DNAs. More recently, we showed that human T-cell lines and primary T cells transduced with retroviral vectors containing this ribozyme are resistant to challenge with diverse strains of HIV, including several uncloned clinical isolates. A phase I clinical protocol to test the safety and function of the ribozyme in transduced human PBL in vivo has been developed and approved by the NIH RAC. However, we recognize that the ultimate solution for treating AIDS patients may require immunization of the hematopoietic progenitor cells. We have used retroviruses carrying the ribozyme driven by two different pol III promoters to transduce hematopoietic stem cells from different sources, including adult bone marrow, fetal cord blood and mobilized peripheral blood. High transduction efficiency was obtained with immunoaffinity enriched CD34 cells pre-stimulated with a variety of cytokines and growth factors. Ribozyme expression in the CFU as detected by RT-PCR assays was also highly efficient. Moreover, the ribozyme was persistently expressed over fifty days (experiment period). Transduction and ribozyme expression had no apparent deleterious effect on cell phenotype or proliferation, as determined by clonogenic assays and growth curve assessments. The cultured stem cells differentiated into macrophages/monocytes that could be infected by several HIV strains. Experiments to determine whether ribozyme transduced stem cells would yield progeny cells that withstand HIV infection in vitro are in progress.

Late Abstracts

IMMUNOCHEMICAL EVIDENCE FOR CONFORMATIONAL INTERACTIONS BETWEEN THE SECOND CONSERVED DOMAIN AND THIRD VARIABLE DOMAIN OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 GP120, George K. Lewis, Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, MD 21201

Epitope exposure was used as a conformational probe for individual domains of full-length gp120 or fragments of gp120 produced by proteolysis of the V3 domain. A panel of monoclonal and polyclonal antibodies specific for continuous epitopes mapping to the C1, C2, V3, C3, V4, and C-terminal domains of gp120 were used to map the proteolytic fragments by western blotting. A proteolytic cleavage in the tip of the V3 region was defined that produces two fragments of approximately 85kDa and 50 kDa. The 85 kDa fragment spans the C1 region and the N-terminal half of the V3 loop whereas the 50kDa fragment spans the C-terminal half of the V3 region through the C-terminus of gp120. Epitope exposure studies using an antigen-capture assay showed that epitopes of the C2, C3, C3, and V4 regions are poorly exposed on the surface of full-length gp120 and become exposed after denaturation. Similarly, epitopes of the C3 and V4 regions are poorly exposed on the surface of cleaved gp120, also requiring denaturation for their exposure. By contrast, epitopes of the C2 region were exposed on the surface of gp120 that had been cleaved in the V3 region, suggesting that cleavage at this site affects the conformation of the C2 region. Taken together, these results are consistent with the existence of conformational interactions between the C2 and V3 regions of HIV-1 gp120.

IMMUNE AND IMMUNOGENETIC PREDICTORS FOR CMV RETINITIS IN HIV INFECTED INDIVIDUALS

Rachel D. Schrier, William R. Freeman, Clayton A. Wiley J. Allen McCutchan, and The HIV Neurobehavioral Research Center. Departments of Pathology and Medicine, University of California San Diego, La Jolla, CA, 92093 and the University of Pittsburgh, Department of Pathology, Pittsburgh PA 15261

Retinitis caused by Cytomegalovirus develops in approximately 1/3 of all HIV infected individuals, often causing blindness and leading to encephalitis. Retinitis is seen almost exclusively in patients with fewer than 100 CD4 T-cells (per cc). T-cell proliferative response to HIV and CMV are being evaluated in 160 HIV (and CMV) infected, HLA typed, subjects. In the patient group who have developed retinitis, CMV specific proliferative responses, previous to onset of retinitis, were examined. Individuals with CMV retinitis were found to have a history of low CMV specific responses, before CD4 levels dropped, compared to those without retinitis and at similar CD4 levels. In contrast, responses to HIV were not different in those who did and did not develop CMV retinitis, indicating a virus specific immune deficit.

Analysis of the HLA types of 15 individuals with CMV retinitis indicated significant increased frequency of DR7 (observed 57% Vs expected 20%, p=.02) and A2B44 (obs. 40% Vs exp. 20%, p=.03) alleles. HLA B35 and 51 were also over-represented in the sample of 15, but not significantly so when 2 stage analysis was used to compensate for polymorphism. The A2B44, B35, B51 or DR7 alleles could be found in 39% of our HIV study population of 160, but were present in 100% of the 15 retinitis patients, and 6 of these 15 had 2 or more of the alleles. Currently, 8/15 of those with the indicated alleles and with fewer than 100 CD4 T-cells (actively at risk) have retinitis and none of those without identified alleles. All 4 subjects with these alleles who have been autopsied, had retinitis. Individuals with these alleles who have not developed retinitis, have, on average, low T-cell responses to CMV in vitro. These finding suggest that individuals with A2B44, DR7 and possibly B35 and B51, have relatively low immune responses to CMV and an increased risk for retinitis as HIV immunodeficiency progresses.