HIV PATHOGENESIS

Organizers: Margaret Johnston and Warner Greene

April 17-23, 1995; Keystone, Colorado

SPONSORED BY THE UPJOHN COMPANY

Plenary Sessions *Page*

Poster Sessions

HIV Pathogenesis

Molecular Events I

D4-001 OLIGOMERIZATION OF CD4 IS REQUIRED FOR STABLE BINDING TO CLASS II MHC PROTEINS BUT NOT FOR INTERACTION WITH HIV-1 GP120. Toshiko Sakihama, Alex Smolyar and Ellis L. Reinherz. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115.

CD4 is the T cell co-receptor specific for class II major histocompatibility complex (MHC) proteins (1-8). It is also the receptor for the human immunodeficiency virus (HIV) envelope glycoprotein (gp120)(9-12). The extracellular segment of CD4 consists of four immunoglobulin-like domains (D1-D4) joined in a rod-like structure (13-15). Recent studies using site-directed mutagenesis have identified the C'C" ridge within the membrane-distal CD4 D1 as essential for both MHC class II and gp120 binding (16-19). However, in contrast to gp120, MHC class II binding is also affected by amino acid residues that map to the lateral surfaces of D1 and the upper parts of D2 as well as the D1/D2 interdomain groove (20,21). Whereas soluble CD4 inhibits the interaction between CD4 and gp120 with up to a nanomolar affinity, it fails to inhibit class II specific responses of T cell clones (22-25). To explain this paradox and test the possibility that oligomerization of CD4 on the cell surface may be required to form a stable class II binding site, we transfected the F43I CD4 mutant (26), which is incapable of binding to MHC class II (17), into COS7 cells together with wild-type CD4 (wtCD4) (17). Expression of F43I results in a dominant negative effect: no class II binding is observed. In contrast, F43I does not affect the expression of wtCD4 or the binding of gp120 to wtCD4. By production and characterization of chimeric CD4 molecules, we show that domains 3 and/or 4 appear to be involved in the oligomerization process. Several models of the CD4-MHC class II interaction are offered, including the possibility that one or two CD4 molecules initially interact with MHC class II dimers and further associate to create larger complexes important for facilitating TCR crosslinking.

1) Reinherz EL, et al. Proc. Natl. Acad. Sci. USA 76:4061, 1979; 2) Reinherz EL, et al. Eur. J. Immunol. 10:570, 1980; 3) Engelman EG, et al. J. Immunol. 127:2124, 1981; 4) Meuer SC, et al. Proc. Natl. Acad. Sci. USA 79:4395, 1982; 5) Biddison WE, et al. J. Exp. Med. 156:1065, 1982; 6) Krensky AM et al. Proc. Natl. Acad. Sci. USA 79:2365, 1982; 7) Meuer SC, et al. Science 218:271, 1982; 8) Doyle C & Strominger JL. Nature 330:256, 1987; 9) Dalgleish AG, et al. Nature 312:763, 1984; 10) Klatzmann D, et al. Nature 312:767, 1984; 11) McDougal JS, et al. J. Immunol. 135:3151, 1985; 12) Maddon PJ, et al. Cell 47:333, 1986; 13) Wang J, et al. Nature 348:411, 1990; 14) Ryu SE, et al. Nature 348:419, 1990; 15) Kwong PD, et al. Proc. Natl. Acad. Sci. USA 87, 6423:1990; 16) Clayton LK, et al. Nature 339:548 (1989); 17) Moebius U, et al. Proc. Natl. Acad. Sci. USA 89:12008, 1992; 18) Peterson A, Seed B. Cell 54, 65, 1988; 19) Landau NR, et al. Nature 334:159, 1988; 20) Moebius U, et al. Proc. Natl. Acad. Sci. USA 90:8259, 1993; 21) Houlgatte R, et al. J. Immunol. 152:4475, 1994; 22) Hussey RE, et al. Nature 331:78 (1988); 23) Fisher RA, et al. Nature 331:76, 1988; 24) Deen KC, et al. Nature 331:82, 1988; 25) Traunecker A, et al. Nature 331:84, 1988; 26) Moebius U, et al. J. Exp. Med. 176:507, 1992.

Molecular Events II

D4-002 HIV-1 INFECTION OF NON-DIVIDING CELLS: C-TERMINAL TYROSINE PHOSPHORYLATION OF THE VIRAL MATRIX PROTEIN IS A KEY REGULATOR, Philippe Gallay, Simon Swingler, Lauren Strachan, Christopher Aiken and Didier Trono, Infectious Disease Laboratory, The Salk Institute, La Jolla, CA 92037.

Compared to oncoretroviruses, HIV has the ability to infect non-dividing cells. This property is shared with other lentiviruses, and reflects the existence of determinants which govern the active transport of the viral preintegration complex through the nucleopore. It likely plays a crucial role in AIDS pathogenesis because it allows the spread of HIV-1 in such critical targets as terminally differentiated macrophages. Two viral proteins, matrix (MA) and Vpr, participate in this process through distinguishable yet convergent pathways. MA contains two subcellular localization signals with opposing effects. A myristoylated N-terminus governs particle assembly at the plasma membrane, and a nucleophilic motif facilitates import of the viral preintegration complex into the nucleus of non-dividing cells. We find that myristoylation acts as the MA dominant targeting signal in HIV-1 producer cells. During virus assembly, a subset of MA is phosphorylated on the C-terminal tyrosine by a virion-associated cellular protein kinase. Tyrosine-phosphorylated MA is then preferentially transported to the nucleus of target cells. An MA-tyrosine-mutant virus grows normally in dividing cells, but cannot replicate in terminally differentiated macrophages, due to a block in nuclear import. MA tyrosine phosphorylation thus reveals the karyophilic properties of this protein within the HIV-1 preintegration complex, thereby playing a critical role for infection of non-dividing cells.

Viral Load and Immunopathogenesis

D4-003 HOST FACTORS IN THE IMMUNOPATHOGENESIS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) DISEASE, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, Building 31, Room 7A03, 31 Center DR MSC 2520, National Institutes of Health, Bethesda, MD 20892-2520.

A complex array of immunopathogenic mechanisms that are multiphasic and multifactorial are involved in the establishment and progression of HIV disease. Following primary infection, an acute viremia occurs with wide dissemination of HIV. During this early viremic phase, virus enters the lymphoid tissue, trapped within the processes of follicular dendritic cells. In addition, during this phase of primary infection certain patients show major expansions of certain Vβ subsets of CD8+ T cells, which are manifestations of responses to HIV and may be associated with both protective and harmful effects. Thus, events that occur soon after initial infection with HIV are critical to the subsequent course of HIV disease and may be extremely important in the control of the progression of HIV infection. In addition, inappropriate immune activation and elevated secretion of certain immunoregulatory cytokines occur during HIV infection. These cytokines play a role in the regulation of HIV expression in the tissues and, ultimately, influence the course of disease. In studies of HIV-infected individuals who are long term non-progressors despite up to 11 years of infection, it was found that these patients have preserved lymph node architecture, low viral burden and viral expression compared to individuals who are infected for comparable periods of time, but who progress in their HIV disease. The implications of host factors in the immunopathogenesis of HIV disease will be discussed.

D4-004 VIRAL LOAD IN HIV-1-INFECTED CHIMPANZEES: INFLUENCE OF STRAIN AND ROUTE OF INOCULATION, *Patricia N. Fultz*[1], Marc Girard[2], Kalle Saksela[3], David Baltimore[3], Liz Muchmore[4], Sajal Ghosh[1], Sophie Jiang[1], Pam May[1], and Qing Wei[1], [1]University of Alabama, Birmingham, AL 35294, [2]Institut Pasteur, Paris, [3]Rockefeller University, New York, and [4]LEMSIP, New York University, New York.

In HIV-infected humans the amount of detectable virus, or viral load, especially viremia, correlates with disease progression. Because some chimpanzees have been infected with HIV-1 for as long as 10 years, but none have developed AIDS, it is possible that their apparent resistance to disease results from efficient immune responses that maintain a low viral load. Alternatively, there may be a host-specific factor(s), such as an inability to sustain high level virus replication, that renders this species inherently resistant to the pathogenic sequelae of HIV-1. Evaluation of chimpanzees infected by different routes and by different strains showed that viral load is a function of both variables, and after infection is established, of the activation status of the immune system. In chimpanzees, antigenemia and viremia are limited primarily to the acute phase of infection. Sporadic reoccurrences, however, have been documented, and in one chimpanzee that lost detectable antibodies to p24 Gag after 3 years of infection, p24 antigen was routinely detected. In addition, cell-free virus has been isolated from at least 3 animals between 1 and 4 years after infection. Virus isolation from PBMC by cocultivation and/or titration on human PBMC has been successful on >85% of attempts, even with animals infected for 5-10 years. Titration of PBMC or cell-free virus in plasma indicated that acute infection is characterized by high viral loads that decrease with development of immune responses, and that direct isolation of HIV-1 from PBMC is enhanced if $CD8^+$ cells are depleted. Virus also can be isolated frequently from lymph nodes (~90%) and bone marrow (60%), but less frequently from vaginal washes (7%) of HIV-1-infected adult female chimpanzees. In addition, vaginal exposure to HIV-1 sometimes resulted in apparent transient infection without seroconversion. Comparisons of viral load in lymph nodes and PBMC by RT-PCR indicated comparable numbers of infected $CD4^+$ cells were present; however, there appeared to be more cells expressing HIV-1 mRNA in lymph nodes than in the periphery. The above discussion applies to chimpanzees infected with the $HIV\text{-}1_{LAi}$-derived strains LAV-1b and IIIB. Some strains of HIV-1, however, replicate poorly in chimpanzee lymphocytes and establish extremely low levels of infection. One such strain is SF2, which usually can be isolated only during the first 6 weeks after infection; overall SF2 has been isolated from both PBMC and lymph node cells only about 30% of the time, and never after 15 weeks. Furthermore, compared to LAi(IIIB) and LAV-1b, SF2 induced antibodies with delayed kinetics and to lower titers. Viral load can be increased in HIV-1-infected chimpanzees by either specific or nonspecific immune stimulation or by superinfection. The latter was shown by superinfecting SF2-infected chimpanzees with the LAV-1b or LAi(IIIB) strains. It has also been demonstrated that prior immunization or exposure to HIV-1 or HIV-1 antigens can decrease initial viral load and facilitate more rapid clearance of detectable virus from the periphery. The observed variations in levels of infection established by different strains and by the same strain inoculated by different routes indicate that generalizations about the natural history of HIV-1 infection of chimpanzees should not be made until substantial numbers of animals, including those infected with other HIV-1 subtypes, have been studied.

D4-005 SINGLE-CELL ANALYSIS OF HUMAN IMMUNODEFICIENCY VIRUS TRANSCRIPTS, Hairong Peng, Todd A. Reinhart, Ernest F. Retzel, Katherine A. Staskus, Mary Zupancic, and Ashley T. Haase, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455.

Recent analyses of lymphoid tissues of HIV-infected individuals by single-cell techniques provide evidence for three kinds of virus–host cell interactions. $CD4^+$ lymphocytes and macrophages appear to be latently or productively infected whereas follicular dendritic cells are not ostensibly infected but have viral particles on their surface. To further investigate these relationships, and the nature of viral gene expression, we have developed *in situ* hybridization methods with oligonucleotide probes that distinguish multiply spliced from singly or unspliced viral transcripts. We used this method to characterize the changes in transcription that occur in the transition from a latent to a productive infection in a cell culture model of chronically infected T lymphocytes (ACH-2 cells). We will describe results on transcript abundancy gradients that best fit a progression through states of insufficient Tat and Rev in the transition to productive infection. With this technique, and the transcript profile of a productively infected cell defined in this way, we have begun studies of HIV-1 cell interactions and viral gene expression *in vivo*. We will describe the first results of these studies that support the concept that only a small fraction of cells that contain viral DNA also have detectable viral RNA but the cells with viral RNA, mainly T lymphocytes, have transcript profiles consistent with productive infection. As additional evidence of the non-productive interaction of HIV-1 with at least the majority of follicular dendritic cells, we did not detect viral mRNAs for Tat and Rev in this cell type.

D4-006 VIRAL DYNAMICS IN HIV-1 INFECTION, G. Shaw[1*], X. Wei[1], S. Ghosh[1], V. Johnson[1], P. Deutsch[2], E. Emini[2], J. Lifson[3], M. Nowak[4], B. Hahn[1], and M. Saag[1]. [1]University of Alabama at Birmingham, Birmingham, AL, [2]Merck, Inc., West Point, PA, [3]Genelabs, Inc., Redwood City, CA, [4]Oxford University, Oxford, UK.

Although viral replication patterns are certain to contribute importantly to HIV-1 natural history and pathogenesis, remarkably little quantitative information is available regarding HIV-1 dynamics in humans. In this study, we determined the kinetics of viral clearance from plasma in 28 subjects (CD4+ lymphocyte counts of 19-355/mm^3) who initiated treatment with either Nevirapine (NVP), the Merck protease inhibitor L-735,524, or the Abbott protease inhibitor ABT-538. We also determined for NVP-treated subjects the kinetics of virus turnover in *uncultured* plasma and *uncultured* PBMCs using automated population sequencing and by *in situ* expression and drug susceptibility testing of full-length reverse transcriptase (RT) genes. Results: Serial determinations of virion-associated RNA in plasma revealed the half-life ($t_{1/2}$) of circulating virus in the 28 subjects to be 1.8 ± 0.8 days (mean ± 1 SD). There were no differences in viral $t_{1/2}$ among patient groups or in relation to $CD4^+$ cell counts. In NVP-treated subjects, drug resistance conferring mutations were identified at RT codons 181, 188, and 190. Direct sequencing and *in situ* RT functional analyses demonstrated complete viral population turnover from wild-type to mutant in the plasma 14-28 days after initiating antiretroviral therapy. Viral population turnover in PBMCs was delayed and less complete. Conclusions: The short $t_{1/2}$ of virus in plasma, and the rapidity of viral population turnover in plasma indicate that ongoing *de novo* infection of, and virus production by, a relatively short-lived lymphocyte and/or macrophage cell population plays a far greater role in viral pathogenesis than previously recognized. These results, and the methods described here to assess viral population turnover, have broad application in studies of viral pathogenesis, drug resistance, and immune surveillance.

Mechanisms of CD4+ Cell Loss

D4-007 APOPTOSIS OCCURS PREDOMINANTLY IN BYSTANDER CELLS AND NOT IN PRODUCTIVELY INFECTED CELLS OF HIV- AND SIV-INFECTED LYMPH NODES, T. H. Finkel[1], G. Tudor-Williams[2], N. K. Banda[1], M. F. Cotton[1], T. W. Baba[3], R. Ruprecht[3], & A. Kupfer[1], [1]National Jewish Center for Immunology and Respiratory Medicine, Denver, [2] National Cancer Institute, Bethesda, [3]Dana-Farber Cancer Institute, Boston.

We and others have shown a significant increase in the percent of apoptotic cells among the circulating CD4 (and CD8) T cell populations of HIV-seropositive adults and children, and an increase in the frequency of apoptosis with disease progression in infected children. In order to determine relative contributions of direct and indirect killing in HIV infection, we have analyzed lymph node sections of 10 infected children representing different stages of disease and of 2 rhesus macaques neonatally infected with SIV, analyzed early or late in infection. Each section was analyzed for HIV infection by RNA *in situ* hybridization, for apoptosis by nick translation of fragmented DNA, and for surface expression of CD3, CD4 or CD8 by immunohistochemical staining. Although there was a correlation between the amount of apoptosis and productive infection, apoptosis was noted predominantly in bystander cells and not in the productively infected cells themselves. These data suggest that while uninfected cells are killed during HIV infection, productively infected cells are relatively resistant to apoptosis.

What are the mechanisms by which HIV-infected cells engender the apoptosis of bystanders without themselves being affected? Recently, several viral gene products that inhibit cellular apoptosis have been identified. Interestingly, these genes are encoded by viruses known to cause persistent, non-cytolytic infection. We hypothesize that HIV encodes a protein that inhibits apoptosis of its host cell. This would obviously be of selective advantage to the virus. Thus, we suggest that HIV may not kill the cell it infects, but rather uses the infected cell as a factory to produce new virus. In addition, we hypothesize that the predominant mechanisms of cell death in HIV are indirect. Our data suggest that the majority of apoptosis in lymph nodes is found within regions of intense, granular, diffuse HIV RNA signal composed of dense viral particles. These data suggest that, *in vivo*, free virus or virus bound to follicular dendritic cells induces apoptosis in uninfected bystander cells. We are currently expanding our study of apoptosis and HIV infection in order to understand the effect of host and viral factors (stage of disease, treatment, viral strain) on the death of infected and uninfected cells.

Support: AMFAR 770188-11-PF & 02270-16-RG, NIH AI-30575A, AI35513, AI29903A (THF) & AI32330, IP30 28691-01, & an ACS Faculty Research Award (RMR). Acknowledgements:Molecular Histology Laboratory, Inc., Drs. B. Mueller, P. Pizzo, UCHSC ACTU.

Transmission of HIV

D4-008 PROTECTION FROM HIV AND SIV INFECTION, Mario Clerici[1], Ligia A. Pinto[2], Jay A. Berzofsky[2], and Gene M. Shearer[2], [1]Universita degli Studi, Milano, Italy[2], National Cancer Institute, Bethesda.

Identification of the correlate of protective immunity is essential for prophylactic AIDS vaccine design. We recently proposed that strong cellular immunity (CI) and a dominant type 1 cytokine profile will provide protection against disease progression and HIV infection. This immunologic state contrasts with strong humoral immunity (HI) and a dominant type 2 cytokine profile that we consider will not by itself be protective. In theory, prophylactic vaccine strategy might aim for simultaneously maximizing CI and HI. However, it may be difficult to optimize both arms of the immune system, due to the cross-regulatory nature of type 1 and type 2 cytokines. If HIV gains entry to the host as integrated proviral DNA via transferred foreign leukocytes, neutralizing antibodies against HIV should not be protective. Therefore, the most effective defense mechanism will likely involve CI, including HIV-specific cytotoxic T lymphocytes (CTL), and possibly other CI effector mechanisms that control rather than prevent HIV infection. Several laboratories have reported examples of natural immunization of HIV-specific CI without HI that may be controlling limited infection. In collaboration with other laboratories, we found that 35-to-75% of four distinct at-risk or HIV-exposed groups of seronegative individuals respond to HIV *env* peptides by IL-2 production. Detection of HIV proviral DNA in some seronegative CI-responsive individuals and HIV-specific, CD8-mediated, class I-restricted CTL in others each suggest that an infectious event occurred. However, HIV infection may not have occurred in all individuals responsive to HIV antigens by proliferation or IL-2 production. It was also reported that macaques exposed to sub-infectious doses of SIV are protected against subsequent challenge with high infectious doses of SIV. The previously-exposed group remained seronegative, but exhibited an in vitro CI (proliferative) response to SIV *env* peptides and did not develop AIDS-like symptoms. In contrast, the previously-unexposed animals seroconverted upon challenge, did not exhibit CI to SIV, and developed an AIDS-like illness. The above findings strongly suggest that the correlate of protective immunity against HIV development involves potent HIV-specific CI activity. We raise the possibility that the seronegative individuals who were multiply exposed to HIV and exhibited CI had an initial encounter with HIV that resulted in a limited infection that was controlled by a primary HIV-specific CI. This initial encounter activated CI, but not HI, that protected them against subsequent potential exposures to HIV.

Immune Response to HIV

D4-009 HLA-DR IMMUNIZATION PROTECTS MACAQUES FROM CHALLENGE WITH SIV PROPAGATED IN HUMAN CELLS BUT NOT MACAQUE CELLS. Larry O. Arthur[1], Julian W. Bess, Jr.[1], Louis E. Henderson[1], Robert Urban[3], Dean Mann[2] and Raoul 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.

Macaques immunized with uninfected human cells resist challenge with SIV propagated in human cells and MHC-coded proteins are physically associated with virions. To determine if an immune response to these MHC-coded proteins could protect from an *in vivo* virus challenge, macaques were immunized with uninfected human cells, gradient-purified culture fluid from uninfected human cells (mock virus), beta-2 microglobulin (β 2M), immunoaffinity purified HLA class I and class II proteins (HLA-DR) from these human cells and adjuvant and then challenged with SIV propagated in human cells. Macaques immunized with β2M, HLA class I, and uninfected human cells developed high antibody titers to β2M, however, became infected when challenged with the SIV. Macaques immunized with HLA-DR and mock virus developed antibodies to HLA-DR and showed protection from challenge with the same virus stock. The macaques, which resisted challenge, were boosted with the appropriate antigen and challenged with SIV propagated in macaque cells. All animals became infected showing the protection did not extend to monkey class II antigen. Our results indicate that this was sterilizing immunity and that the protection was not due to cross reactive epitopes between class II and the viral envelope glycoproteins. These results are the first demonstration that immunization with a purified cellular protein can protect from virus infection.

HIV Pathogenesis

Models of HIV Pathogenesis

D4-010 PRIMATE MODELS FOR HIV PATHOGENESIS IN INFANTS. M. L. Marthas, K. K. Van Rompay, M. Otsyula, C. J. Miller, A. F. Tarantal, D. Canfield, R. Tarara, N. C. Pedersen, and M. B. McChesney. California Regional Primate Research Center, University of California, Davis, CA 95616-8542. We have developed a model of pediatric AIDS using simian immunodeficiency virus (SIV) infection of newborn rhesus macaques for studies of pathogenesis as well as evaluation of vaccines and anti-viral therapies for infants. The ability of viral variants to determine disease progression in human pediatric AIDS was studied in newborn rhesus macaques inoculated intravenously with one of three SIV isolates previously shown to have distinctly different virulence in juvenile and adult rhesus macaques. All six newborn macaques inoculated with pathogenic, uncloned SIVmac251 developed persistent, high levels of cell-associated and cell-free virus in peripheral blood, had no detectable anti-viral antibodies, and were all euthanized with failure to thrive and other clinical signs of simian AIDS; five animals were killed at 12 weeks after inoculation (pi) and one animal was killed at 26 weeks pi. Two newborns inoculated with a pathogenic molecularly cloned SIV (SIVmac239) also had persistent high virus load in peripheral blood; however, both of these animals had normal weight gain, developed anti-viral antibodies, and survived for more than 30 weeks after inoculation with only mild clinical signs of disease. In contrast, three newborn rhesus inoculated with a virulence-attenuated molecular clone (SIVmac1A11) had transient, low viremia, seroconverted by 10 weeks after inoculation, and remained healthy with normal weight gain for over one year. These results indicate that (1) neonatal rhesus infected with virulent, heterogenous (uncloned) SIV have a more rapid, fulminant disease course than adults inoculated with the same virus, (2) high viral virulence is associated with lack of detectable humoral immune response in SIV-infected infants, (3) neonatal infection with a pathogenic, genetically homogeneous (cloned) viral isolate is associated with a delayed disease course. We also investigated disease pathogenesis in neonatal rhesus inoculated mucosally (oral/conjunctival) with virulent, uncloned SIVmac251. One of three neonates was infected and developed fatal SAIDS 8 weeks after conjunctival inoculation with virus stock A (10^3 TCID/ml). Two neonates inoculated conjunctivally and orally with virus stock B (10^5 TCID/ml) maintained persistent, high levels of virus in plasma and PBMC;both died with simian AIDS by 18 weeks pi. Thus, either mucosal or IV inoculation of newborn rhesus with uncloned, pathogenic SIV results in systemic infection with a similar, rapidly fatal disease course. We are now studying the effects of maternal SIV antibodies on virus load and disease course in SIV-infected newborn rhesus. Three pregnant rhesus macaques were immunized with live-attenuated SIV during the first trimester and boosted early in the third trimester with whole-inactivated SIV to induce maternal SIV antibodies. Infants from the SIV antibody positive dams were inoculated mucosally with virulent, uncloned SIVmac (stock B) within 72 hours after birth; after testing multiple blood samples, virus was recovered from only one of the three infants. Sudies with mucosally inoculated neonatal macaques will allow us to define viral and host factors associated with perinatal SIV transmission and the rate of disease progression after infection occurs.

D4-011 DIFFERENTIAL PATHOGENICITY OF MULTIPLY DELETED SIV IN ADULT AND NEONATAL RHESUS MONKEYS: IMPLICATIONS FOR LIVE-ATTENUATED VIRUS VACCINE STRATEGIES, Ruth M. Ruprecht[1,2], Timothy W. Baba[1,2,3], Yong Seok Jeong[1,2], Rod Bronson[4], Dominique Penninck[4], and Michael F. Greene[2,5]; [1]Dana-Farber Cancer Institute, Boston, MA 02115; [2]Harvard Medical School, Boston; [3]Tufts University School of Medicine; [4]Tufts University School of Veterinary Medicine; [5]Massachusetts General Hospital, Boston, Mass.

Viral infections are often more aggressive in neonates than in adults. Adult macaques did not develop disease following infection with a *nef*-deletion mutant of the simian immunodeficiency virus (SIV) and were protected against challenge with pathogenic virus. This finding led to the proposal to use *nef*-deleted viruses as live attenuated vaccines to prevent human AIDS. We report that four out of four neonatal macaques developed persistently high levels of viremia following oral exposure to an SIV *nef*- and *vpr*-deletion mutant. Severe hemolytic anemia, thrombocytopenia, and profound CD4-cell depletion were observed, indicating that neither *nef* nor *vpr* are determinants for pathogenicity in neonatal macaques. Following *in vivo* transfer of blood from an infected, diseased infant to an adult animal, the virus was attenuated as observed previously (1). In contrast, oral administration of infected blood to a neonate led to persistent infection with high levels of virus. We conclude that *nef*- and *vpr*-deleted viruses are pathogenic in primates and should not be considered as candidate live attenuated virus vaccines against human AIDS.

(1) Wyand MS, Manson AK, Desrosiers RD. Abstract. 12th Ann. Symp. on Nonhuman Primate Models for AIDS, Oct. 12-15, 1994, Boston, MA, p.23.

Supported in part by NIH grants RO1-AI32330 and RO1-AI35533 to RMR who is the recipient of an ACS Faculty Research Award, and by the DFCI CFAR core grant IP30-28691. TWB is supported in part by grant 5T32-AI07386.

Viral Diversity

D4-012 GENETIC DIVERSITY AND RECOMBINATION IN HIV, Beatrice H. Hahn[1], David L. Robertson[2], Joost Louwagie[3], Francine E. McCutchan[4], and Paul M. Sharp[2], [1]University of Alabama at Birmingham, Birmingham, AL 35294, [2]University of Nottingham, Nottingham, UK,[3]Innogenetics, Ghent, Belgium, [4] Henry M. Jackson Foundation Research Laboratory, Rockville, MD.

Retroviruses are highly recombinogenic and this propensity appears to be a consequence of their dimeric RNA genome and a reverse transcriptase enzyme that can switch templates during proviral DNA synthesis. Because recombination occurs during the reverse transcription step, exchanges of genetic material are only possible between genomes that infect the same target cell and are packaged into the same particle. Until recently, HIV recombination events have been thought to involve only the rather closely related members of the quasispecies that evolve over the course of infection, primarily because co-infections with multiply divergent strains have not been observed. However, as more and more isolates are molecularly characterized, evidence is increasing for the existence of mosaic genomes representing recombinants between quite divergent viruses. To revisit the question of HIV superinfection and recombination, we have determined the evolutionary relationships of multiple HIV-1 and HIV-2 strains belonging to different sequence subtypes. Evolutionary trees derived from different genomic regions were generally consistent among the majority of viruses investigated. However, a surprising number of HIV-1 and HIV-2 strains were identified that exhibited significant discordant branching orders depending which regions of their genome were analyzed. For two of these viruses, complete proviral sequences were available ($HIV\text{-}1_{MAL}$ and $HIV\text{-}2_{7312A}$), thus allowing a more comprehensive examination of putative recombination breakpoints. Examination of the linear distribution of phylogenetically informative sites supporting alternative tree topologies revealed complex mosaicism for both viruses, with $HIV\text{-}1_{MAL}$ and $HIV\text{-}2_{7312A}$ representing recombinants between two (and possibly more) sequence subtypes. Mosaic genome structures were also identified in viruses for which only *gag* and/or *env* sequences were available. The majority of HIV-1 recombinants were comprised of subtype A and D sequences, although A/G, A/C, and H/G combinations were also observed. One putative HIV-2 recombinant contained divergent sequences from the same subtype (A). Taken together, these findings indicate that co-infection with genetically divergent viruses *does* occur in both HIV-1 and HIV-2 infected individuals, and that there is the potential for generating hybrid genomes with significantly altered immunogenic or pathogenic properties. Systematic studies are needed to determine how often and under what circumstances individuals acquire such divergent strains. This information will yield important new insights into correlates of protective immunity and is thus essential for ongoing vaccine development efforts.

D4-013 KINETICS OF NSI AND SI VIRAL POPULATIONS IN THE NATURAL COURSE OF INFECTION AND ZIDOVUDINE TREATED INDIVIDUALS, Hanneke Schuitemaker, Angélique B. van 't Wout, Maarten Koot, Ron A.M. Fouchier, Linde Meyaard and Frank Miedema, Department of Clinical Viral Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands.

Biological variability of HIV-1 is an important determinant in the course of infection. In the early asymptomatic phase, NSI dual-tropic HIV-1 variants predominate. In 50% of HIV-1 infected individuals, SI variants emerge in the course of infection preceding accelerated CD4 cell decline and more rapid disease progression. The emergence of SI variants in general precedes an increase in viral burden, due to an expansion of both NSI and SI variants. In some individuals, an increased viral load was observed already prior to SI conversion. The delayed increase in viral load as compared to the onset of increased CD4 cell decline indicates that increased viral burden is not likely to be the only mechanism for enhanced CD4 cell killing.

Upon exposure of PBMC with inocula of comparable $TCID_{50}$, SI variants appeared to infect more T cells as compared to NSI variants. SI variants but not NSI variants could infect all CD4+ Th clones irrespective Th1 or Th2 cytokine characteristics, although kinetics of viral replication were clearly delayed in Th1 type clones. The expanded host range may contribute to the increase in load after SI conversion in vivo.

A virus phenotype dependent efficacy of zidovudine treatment was previously reported. Carriers of NSI variants showed a significant delay of disease progression which was not observed in patients with both NSI and SI variants. All zidovudine treated individuals showed a decreased viral burden within 1-3 months after initiation of treatment. In patients with both NSI and SI clones, a decline in the number of CD4+ T cells harbouring NSI clones was observed, whereas the number of SI infected cells remained constant. Development of zidovudine resistance mutations in the HIV-1 clones with different phenotypes is currently under study although preliminary data show comparable kinetics of resistance development irrespective HIV-1 phenotype. Interestingly, no changes in the composition of virus populations with respect to V3 sequences were observed after development of resistance mutations in the pol gene, pointing to in vivo recombination events.

Role of the Host and Lessons from Cohort Studies

D4-014 **HOST-VIRUS RELATIONSHIPS IN PATHOGENIC AND NONPATHOGENIC HIV AND SIV INFECTIONS** , Mark B. Feinberg, Gladstone Institute of Virology and Immunology, University of California, San Francisco, San Francisco, CA.

The pathogenic consequences of virus infection can potentially be influenced by numerous host, viral and environmental factors. As a means of evaluating the contribution of these various factors to the pathogenesis of HIV and SIV infections, we have applied newly available methods to quantitate and characterize the viral burden and extent of viral genetic sequence diversity present in persons who remain healthy in spite of long-term HIV-1 infection and in non-human primates with asymptomatic SIV infections.

Epidemiologic studies from the San Francisco City Clinic Cohort (detailed in the presentation by Dr. Susan Buchbinder) have identified a group of individuals with documented HIV-1 infections of 11-15 years duration who are clinically well and maintain CD4 counts above 500 cells/ml. Virologic characterization of these individuals has revealed significant diversity within this group with respect to levels of viral burden and cytotoxic T cell (CTL) reactivity. Certain individuals demonstrate exceptionally low levels of plasma HIV RNA (<200 copies/ml) and virus-infected peripheral blood cells(<1 in $5x10^6$ to 10^7 cells), and vigorous, broadly cross-reactive CTL activity. Interestingly, other individuals in this group display persistently elevated levels of viral burden (with plasma HIV RNA copy numbers of 50-100,000/ml), significantly less CTL activity, but stable CD4 counts. These data suggest that long-term, slowly progressing HIV-1 infections may result from a number of distinct types of virus-host relationships.

To investigate the basis for asymptomatic SIV infection in naturally-infected sooty mangabeys, quantitative-competitive PCR (QC-PCR) (to measure viral load) and heteroduplex mobility (HMA) (to measure viral sequence diversity) assays were developed for SIV_{sm} and the closely related virus SIV_{mac} that induces AIDS in rhesus macaques. Interestingly, naturally infected, healthy mangabeys display levels of active SIV replication that equal, and in some cases exceed, those seen in macaques suffering from advanced SIV-induced immunodeficiency. This suggests that greater immunologic control of SIV infection does not explain why infected mangabeys remain free of disease. Further, the fact that SIV replication proceeds at high levels without CD4 depletion indicates that the interaction of virus with mangabey host cells may not be cytopathic. Should this be true, then an active antiviral immune response might not be necessary, and would perhaps be deleterious. The diversity of the plasma viral RNA populations is large in both instances. Examination of the pattern of diversity present should provide clues about the presence or absence of immune selection of viral variants.

Further investigation of these diverse outcomes of virus infection in SIV-infected non-human primates and between HIV-infected humans will provide important clues concerning the pathogenesis of AIDS. The evolution of SIV_{sm} and its sooty mangabey hosts may have selected for a non-cytopathic course of virus infection of host cells, and a limited or absent antiviral immune response. In species that are not natural hosts for T cell-tropic lentiviruses including rhesus macaques and humans, the character of the antiviral immune response may determine the rate of disease progression seen following virus infection, and if incompletely effective, may potentially contribute to immune system compromise.

HIV Replication, Molecular Events, Therapeutics

D4-100 HIV-1 REVERSE TRANSCRIPTASE EXPRESSION AND *trans*-COMPLEMENTATION ANALYSES, M. Ali Ansari-Lari,[1] Lawrence A. Donehower,[2] and Richard A. Gibbs,[1] Department of Molecular and Human Genetics,[1] and Division of Molecular Virology,[2] Baylor College of Medicine, Houston, TX 77030

The maturation of Gag-Pol polyprotein of the human immunodeficiency virus type 1 (HIV-1) by the viral protease occurs during viral budding. The mature core particle contains ribonucleocapsid, reverse transcriptase (RT) and other processed viral proteins. The precise coordination of the viral maturation with the subsequent reverse transcription of the viral RNA is not completely understood. To investigate this process, the transient and constitutive expression of the RT in several human cell lines was analyzed. Cytoplasmic expression of the RT heterodimer was achieved in the absence of protease via the coexpression of its individual subunits. High level constitutive expression of the RT in a Hela cell line that expresses CD4 and has a single integrated copy of a *β-galactosidase* gene under the control of the HIV-1 LTR was established. Two viral constructs containing large in-frame RT deletions in the *pol* gene of PNL4-3 proviral plasmid, and one construct lacking RT activity due to conversion of the conserved YMDD motif to YMAA have been generated. After infection of the indicator cell line with the RT mutant viruses, the RT *trans*-complementation was analyzed by *β-galactosidase* gene expression and by polymerase chain reaction for the detection of 1-LTR and 2-LTR circular forms of viral DNA. The results show that the presence of the RT during the maturation and release of the virus is an indispensable step in its life cycle. We hypothesize the nucleocapsid environment to be inaccessible to RT expression in *trans*. Additionally, the effect of various point mutations and in-frame deletions of the *pol* gene on the Gag-Pol polyprotein packaging and processing will be presented.

D4-101 NEF AND VPR PROTEINS OF HIV-1 ARE POSITIVE REGULATORS OF VIRUS REPLICATION

Chinniah Arunagiri[1], Sonia Sankovich[1], Keith Peden[3], Dale Mc Phee[2], Ian Macreadie[1] and Ahmed Azad[1]
[1]Biomolecular Research Institute, Parkville 3052 Australia, [2]Macfarlane Burnet Centre, Fairfield 3078, Australia, [3]NIH, Bethesda, Maryland 20892, USA.

A molecular clone of HIV, pNL 4.3 was selected to investigate the functions of Nef and Vpr proteins. A *nef* mutant clone was constructed by point mutations of first and second methionine codons and the *vpr* mutant was constructed by point mutation of the first methionine codon, hence, no proteins was translated in the respective mutants. We have also selected another molecular clone of HIV, LAI to construct two frame shift mutants of *nef*. LAI mutants were constructed by frame shift mutation at amino acid 33 and 84 respectively. The mutant clones were assayed for their ability to replicate in primary human CD4 cells at different multiplicities of infection (MOI). We have also assayed for the expression of cell surface molecules by flow cytometry when equivalent MOI were used. The results showed that the effect of Nef on virus replication is MOI dependant and the N-terminus Nef is important for the downmodulation of CD4 and IL-2R receptors. The effect of Vpr on replication of the virus was seen only at low MOI and it did not influence the expression of cell surface molecules. We conclude that the Nef and Vpr proteins are positive regulators of virus replication at low MOI.

D4-102 THE C-TERMINAL DOMAIN OF HIV-1 Vpr CAUSES CELL GROWTH ARREST AND STRUCTURAL DEFECTS

Ahmed Azad*, Chinniah Arunagiri, Dean Hewish & Ian Macreadie
Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052

Objective: To determine the possible role of Vpr in AIDS pathogenesis by studying its effects on host cells.

Methodology: Full length and truncated *vpr* genes were expressed in yeast cells and the effects of expression on cell growth, morphology and viability was studied. The effects of "electroporation" of synthetic C-terminal peptides of Vpr into yeast cells and human $CD4^+$ promonocytic cells was also studied.

Results: The synthesis of Vpr in yeast causes cell growth arrest and gross cell enlargement. Expression of truncated forms of Vpr shows that growth arrest in yeast is caused by the C-terminal third of Vpr and implicates the sequence HFRIGCRHSRIG. Electroporation of yeast cells with C-terminal peptides shows that peptides containing the HS/FRIG motifs cause structural defects that result in osmotic sensitivity. Electroporation of the C-terminal peptides containing the HS/FRIG motifs into human $CD4^+$ promonocytic cells causes enlargement of the cell and the nucleus, and causes increased cell death. Comparison of Vpr sequences from HIV-1, HIV-2 and SIV shows a correlation between the conservation of the HS/FRIG motifs and pathogenicity of primate lentiviruses. The C-terminal peptide containing the HS/FRIG motifs interacts specifically with a cellular protein whose N-terminal sequence has been determined and shows no homology to any known cellular protein.

Conclusion: The function of Vpr may be to bring about cell growth arrest and/or cytoskeletal changes through interaction with cellular proteins to facilitate early events in HIV infection.

D4-103 CELLULAR FACTORS WHICH BIND TO THE NEF PROTEIN OF HIV-1, Andreas S. Baur, Kerstin Mueller, Gabi Sass, Bernd Laffert, Xiaobin Lu, Matija B. Peterlin. Institute of Virology, University of Erlangen/Nuernberg, Schlossgarten 4, 91054 Erlangen, Germany. Howard Hughes Medical Institute, University of California, San Francisco, 3rd and Parnassus Ave. U426, San Francisco, CA, 94143-0724.

Nef of primate lentiviruses is required for viremia and progression to AIDS in monkeys. Recently we have shown that Nef interferes with signal transduction pathways of T cells. This interference leads to induction or inhibition of T cell activation depending on the subcellular localization of Nef (Baur et al., 1994). In order to identify cellular factors which directly bind to Nef and may be responsible for the observed effects, we expressed a chimeric CD8-Nef molecule as well as numerous mutants of the chimera in wild type and mutant Jurkat cells. The resulting cell lines were analyzed for associating proteins by various in vitro assays. Additionally these constructs were transiently expressed in COS cells. We identified two cellular factors which bind to Nef under stringent conditions. First, we found a T cell specific serine kinase which binds to a defined region in the N-terminus of Nef and is clearly distinct from the previously identified serine kinase (Sawai et al., 1994). Second, we found that Lck is involved in the effects of Nef in T cells and binds to Nef under certain conditions. The binding of these proteins to Nef will be shown in detail.

D4-104 HIV-1 NEF INTERACTION WITH β-COP, A COMPONENT OF NON-CLATHRIN COATED VESICLES ESSENTIAL FOR MEMBRANE TRAFFIC. Richard Benarous[1] Serge Benichou[1], Morgane Bomsel[1], Monique Bodéus[1], Hervé Durand[1], Monique Douté[1]and Jacques Camonis[2] [1]INSERM U332, ICGM, 22 Rue Méchain, 75014 Paris, [2]INSERM U248, 10 Avenue de Verdun, 75010 Paris, France.

Nef is a 27 kD myristylated protein conserved in most HIV-1, HIV-2 and SIV isolates. In macaques, the SIV Nef protein plays an essential role in natural infection and is required for high viral load and pathological effects. In cultured cells, the effect of Nef on viral replication is still controversial; however, Nef has been consistently shown to down-regulate the cell surface expression of CD4. This functional interaction may be mediated by proteins required for cell-surface expression of CD4. We used the yeast two-hybrid system to identify cellular proteins that would interact with Nef. A cDNA was isolated which encodes a C-terminal fragment of human β-COP, a major coat component of non-clathrin-coated vesicles. We showed that the Nef-β-COP interaction is specific. Using recombinant proteins, we confirmed that Nef and β-COP interact *in vitro*. Finally, β-COP was co-immunoprecipitated with Nef from lysates of HIV-1 infected cells, by anti-Nef antibodies. These observations indicate that the two proteins are physically associated and suggest that β-COP could be one of the cellular mediators of Nef function in HIV-1 infected cells.

D4-105 EFFECTS OF DELETIONS OF THE AP-1 AND ATF SITES OF THE FELINE IMMUNODEFICIENCY VIRUS (FIV) LONG TERMINAL REPEAT (LTR) ON VIRUS REPLICATION IN PRIMARY FELINE PERIPHERAL BLOOD LYMPHOCYTES (PBL), Luisa Bigornia, Harry Louie, and Ellen E. Sparger, Department of Medicine, School of Veterinary Medicine, University of California at Davis, Davis, CA 95616

AP-1 and ATF sequences within the U3 region of the feline immunodeficiency virus (FIV) LTR are thought to serve as targets of cellular activation pathways mediated by protein kinase C and A, respectively, and may provide sites for regulation of virus replication. The roles of the AP-1 and ATF sites in virus replication were assessed using LTR mutants of the molecular clone, FIV-pPPR. Four to five nucleotide bases were deleted within the AP-1 (TGACTCA) and ATF (TGACGT) sequences using PCR-mediated overlap extension and confirmed by DNA sequencing. Type 1 LTR mutants consisted of AP-1 and/or ATF deletions in the 3' and 5' LTR. The 5' LTR was replaced by a SV40pr/RU5 hybrid promoter of type 2 mutants which contained AP-1 and/or ATF deletions in the 3'LTR. LTR mutant viruses were transfected into Crandell feline kidney cells, which were co-cultivated 24 hours later with primary feline peripheral blood lymphocytes (PBL). Virus production from infected PBL was assessed by a p24 antigen capture assay 2-7 weeks post-infection. Wild-type FIV-pPPR (WT) and the FIV-pPPR construct with the 5' SV40pr/RU5 promoter (pSVWT) exhibited similar rates of virus production. Deletion of the AP-1 site in either Type 1 or Type 2 mutants did not alter virus production in comparison with virus production by PBL infected with WT or pSVWT. However, virus production was reduced in cells infected with mutants containing deletions of the ATF sites. Deletions of both the AP-1 and ATF sites resulted in a significant reduction of virus production. These findings suggest that the ATF site within the FIV LTR plays an important role in virus replication. Experiments are ongoing to assess replication of LTR mutants in PBL and macrophages in varying activation and resting states.

D4-106 **ANTIVIRAL AGENTS REDUCE VIRAL LOAD AND DELAY THE DETECTION OF INFECTED CELLS *IN VIVO* IN THE HIV-INFECTED HuPBMC-SCID MOUSE MODEL.** **Paul L. Black[1], Dennis D. Broud[1], Owen L. Wood[1], Steven C. Kunder[1],Peter J. Dailey[2], Judith C. Wilber[2], Limei Yang[3], Michael Piatak, Jr.[3], Jeffrey D. Lifson[3], and Michael A. Ussery[1]. [1]FDA, Rockville, MD 20857; [2]Chiron Corp., Emeryville, CA 94608; and [3]Genelabs Technologies, Inc., Redwood City, CA 94063.**

In an effort to validate a murine model of HIV infection, we have studied the HuPBMC-SCID mouse model, which involves the I.P. injection of 5-10X10^7 adult human PBMC into 5-7 wk old female SCID mice. Two weeks after reconstitution, mice were infected I.P. with 10^3 $TCID_{50}$ HIV-1 9320 (AZT-sensitive isolate A018, D. Richman). The extent of infection in blood cells, splenocytes, lymph nodes (LN) and peritoneal cells (PC) was assayed by quantitative coculture with human PBMC blasts 1 and 3 wk later. At 1 wk after infection, HIV was recovered from all mice by coculture of cells from all the 4 sites examined. Additionally, HIV could be detected in the plasma of infected mice by coculture and by QC-PCR. In contrast, HIV infection could be detected by coculture in some, but not all animals treated continuously with AZT at 1 mg/ml in the drinking water beginning 1 day before infection. Furthermore, HIV was recovered from fewer sites in the AZT-treated Hu-PBMC-SCID mice, compared with untreated animals, with PC being most frequently positive and LN least. Additionally, viral load was quantitated by the Chiron branched DNA assay, and the results were consistent with those of cocultures. HIV RNA was detected in splenocytes from untreated mice, but not from any AZT-treated mice (or uninfected mice). When HuPBMC-SCID mice were examined 2½-3 wk after infection, the results were quite different. HIV was recovered from all AZT-treated mice, but only about half of untreated mice. The almost complete disappearance of human lymphocytes (especially $CD4^+$ T-cells) from some untreated, infected mice parallels the inability to recover HIV from them. Further experiments are underway to confirm and expand these results, as are studies with an HIV protease inhibitor.

D4-107 ALTERATION OF THE RETROTRANSCRIPTION IN PROLIFERATIVE HUMAN PBL INFECTED WITH VPR NEGATIVE HIV-1, MOHAMAD BOUHAMDAN, JEAN-MARC NAVARRO, ROBERT VIGNE, BRUNO SPIRE AND JOSEPHINE SIRE. INSERM U372, Campus de Luminy, BP178, 13276 Marseille Cedex 9.

Recent studies have demonstrated the detection of the Vpr protein in the viral preintegration complex suggesting a role of Vpr in the early events of the replication. We analysed the different stages of the retrotranscription in proliferative PBL infected with wild-type and *vpr* defective viruses (HIV-1 NDK strain) by quantitative PCR technique. Each step was targeted by an appropriate pair of PCR primers. Viral stocks were propagated on permissive U937 cells and similar amounts of viral particles (quantified by quantitative competitive RT-PCR technique) were used to infect PHA and IL-2 treated PBL. Both viral stocks infect cells in a similar way as determined by measuring the viral RNA entry 2 hours after infection. Both viruses are able to initiate the retrotranscription with the same efficiency as demonstrated by the measure of the strong-stop cDNA with R-U5 primers. The analysis of the intermediate steps of the retrotranscription quantified with Vif primers, indicated that similar levels of DNA were detected 18h post-infection of PBL with the wild-type or *vpr*- viruses. By using U3-Gag primers which amplify products of the reverse transcription following the second jump, we observed a 10 times reduction of viral DNA when comparing *vpr*- virus infection with wild-type virus infection. Lastly, amounts of the 2-LTR circle forms of viral DNA, were reduced for the *vpr*- virus. These results are consistent with a role of Vpr early in HIV-1 infection. In addition, these studies provide a defined function for an accessory gene product of HIV-1, i. e. to ensure the completion of the reverse transcription.

D4-108 THE HIV-1 VPU PROTEIN SPECIFICALLY BINDS TO THE CD4 CYTOPLASMIC DOMAIN: IMPLICATIONS FOR THE MECHANISMS OF CD4 DEGRADATION AND VIRAL PARTICLE RELEASE, Stephan Bour, Ulrich Schubert, and Klaus Strebel, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892-0460.
We have recently demonstrated that co-expression of Vpu and CD4 in HeLa cells results in the degradation of CD4 in the endoplasmic reticulum. The sensitivity of CD4 to Vpu-mediated degradation is conferred by the presence of specific sequences located between amino acids 402 and 420 in the CD4 cytoplasmic domain. To better define the mechanisms involved in CD4 degradation, we investigated the existence and functional relevance of direct interactions between CD4 and Vpu. Co-immunoprecipitation experiments showed that Vpu specifically binds to the cytoplasmic tail of CD4 both in an *in vivo* and *in vitro* system. This phenomenon is relevant to the mechanism of CD4 degradation since amino acid residues 402-420, previously shown to be important for degradation, are necessary and sufficient for Vpu binding. We further demonstrate that a deletion mutant of Vpu as well as a phosphorylation mutant, both biologically inactive with regard to CD4 degradation, retained the capacity to interact with the CD4 cytoplasmic domain. Taken together, these results indicate that Vpu binding is necessary but not sufficient to trigger CD4 degradation. To analyze whether the Vpu-mediated enhancement of virus release similarly involves a direct interaction with HIV-1 Gag proteins, we compared the Vpu-responsive sequence in CD4 with Gag-pol sequences. Interestingly, we found a 5 amino acid sequence identity within a highly conserved region of p55gag which is located between two proteolytic cleavage sites of the Gag precursor protein. The sequence identified is therefore likely to be exposed at the surface of the precursor molecule and could act as a binding site for Vpu. The effect of mutations within this sequence on the enhancement of particle release by Vpu will be presented.

D4-109 DEFINITION OF A MULTI-STEP CASCADE IN SYNCYTIA FORMATION MEDIATED BY HIV-1 ENVELOPE GLYCOPROTEINS Peter J. Bugelski, Yung-Kang Fu and Timothy K Hart, Department of Toxicology, SmithKline Beecham Pharmaceuticals, King of Prussia PA 19406
The mechanism by which cells expressing HIV envelope glycoproteins progress from binding to CD4+ cells to syncytia formation is not entirely understood. The purpose of these investigations was to use physical and biochemical tools (temperature shifts, soluble CD4, serine protease inhibitors and a battery of anti-CD4 monoclonal antibodies) to isolate discrete steps during syncytia formation. Previously (Fu et al, J. Virol 67:3818), we found that preincubation of cells expressing gp160 (TF228.1.16) with CD4+ SupT1 cells at 16°C, which is nonpremissive for syncytia formation, resulted in an increased rate of syncytia formation when the co-cultures were shifted to the syncytia-permissive temperature of 37°C. We have since found that syncytia formation is further enchanced by shifting the co-cultures from 16°C to 4°C prior to incubation at 37°C. These data suggest that two discrete activation states, that we term the first and second activation intermediates (FAI and SAI) are involved in syncytia formation. We have found that acquisition of the FAI (by preincubation at 16°C) is sensitive to serine protease inhibitors (PI) soluble CD4 (sCD4), and anti-CD4 monoclonal antibodies (mAb). Expression of the FAI (by shifting from 16°C to 37°C) remains sensitive to sCD4 and mAb but is insensitive to PI. Similarly, acquisition of the SAI (by shifting from 16°C to 4°C), is sensitive to sCD4, mAb and PI. In contrast, expression of the SAI (by shifting from 16°C to 4°C to 37°C) is senitive only to mAb and cannot be blocked by sCD4 or PI. These data allow us to propose that syncytia formation mediated by HIV-1 envelope glycoproteins proceeds by a multi-step cascade.

D4-110 NOVEL ANTI-HIV-1 ACTIVITIES OF 3-DEAZA-ADENOSINE ANALOGS: INCREASED POTENCY AGAINST AZT-RESISTANT HIV-1 STRAINS, P. K. Chiang[1], J. A. Mikovits[2], R. K. Gordon[1], A. D. Wolfe[1], B. P. Doctor[1], B. Joshi[3], I. K. Hewlett[3], James R. Lane[4], D. S. Burke[1], and D. L. Mayers[1], [1]Walter Reed Army Institute of Research, Washington, DC 20307-5100; [2]Dyncorp, N.C.I.-Frederick Cancer Research and Development Center, Frederick, MD; [3]FDA, Rockville, and [4]SRA Technologies, Rockville, MD
3-Deaza-adenosine (DZA), 3-deaza-(±)aristeromycin (DZAri), and 3-deaza-neplanocin A (DZNep) are powerful modulators of cellular processes. When tested against H9 cells infected acutely with two different strains of HIV-1 and in the chronically infected monocytoid cell lines, U1 and THP-1, the 3-deaza nucleosides caused a marked reduction in p24 antigen production. Similar reductions in p24 antigen were seen in PHA-stimulated peripheral blood mononuclear cells (PBMC) infected with clinical HIV-1 isolates. Strikingly, in comparing the therapeutic indices between the paired pre- and post-AZT treatment HIV-1 isolates, DZNep and neplanocin-A (NepA) showed an increase of 3- to 18-fold in their potency against AZT-resistant HIV-1 isolates. In H9 cells treated with DZNep and DZAri, the formation of triphosphate nucleotides of DZNep and DZAri was observed. The mode of action of DZNep and DZAri appears complex, at least in part at the level of infectivity as shown by decreases in syncytia formation in HIV-1 infected H9 cells, and at the level of transcription as both drugs inhibited the expression of basal or *tat*-induced HIV-1 LTR-CAT activity in stably transfected cell lines. Additionally, DZNep and DZAri demonstrated HIV-1 specificity since they had no effect on the HTLV-1 LTR CAT. Because DZNep induced in H9 cells a rapid expression of nuclear binding factors which recognize the AP-1 transcription site, the anti-HIV-1 activity of the DZA analogs could partly be the induction of critical factors in the host cells. Thus, the 3-deaza nucleoside drugs belong to a novel class of anti-HIV-1 drugs which may have therapeutic potential.

D4-111 HIV-1 VPR AND SIV VPR AND VPX ARE DIRECTLY DEPENDENT ON GAG P6 FOR INCORPORATION INTO VIRIONS
[1]SUNNY CHOE [1]THOMAS LU, AND [1,2,*]NATHANIEL LANDAU, [1]Aaron Diamond AIDS Research Center, 455 First Avenue, New York, N.Y. 10016; [2]New York University Medical School, New York, N.Y. 10016.

VPR is a 96 amino acid protein present at high copy number in HIV and SIV virions. HIV-2 and SIV encode, in addition, a homologous accessory molecule, VPX. We previously showed that the incorporation of VPR into HIV-1 virions during assembly is mediated by the carboxy terminal Gag product, P6. This could have been due either to a direct dependence of VPR on P6 or to an indirect effect in which alteration of P6 caused a conformational change elsewhere in GAG. To discriminate between these possibilities, we constructed mutants of HIV-1 that lack *vpr* and have deletions in *gag* (MA, CA or NC) that do not interfere with particle release. The ability of each mutant GAG protein to incorporate an epitope tagged VPR molecule was assessed in transfected 293 cells. In all three cases VPR was efficiently incorporated into the virus particles. To further study this interaction, we constructed a chimeric provirus based on HIV-1 but containing the P6 region of SIV mac239. The chimeric provirus was then tested in cotransfection experiments for its ability to incorporate epitope tagged HIV VPR, SIV VPX and SIV VPR molecules. The results showed that HIV-1 VPR was not incorporated into the chimeric virions, while SIV VPR and VPX were efficiently incorporated. None of the accessory molecules were incorporated into HIV-1 virions deleted for P6. Taken together, our results suggest that VPR and VPX are both directly dependent on GAG P6 for virion incorporation.

D4-112 THE ROLE OF NF-KB IN MAINTAINING HIV-1 LATENCY IN ASTROCYTES. K.Conant,M.C.G.Monaco, W. Atwood, A. Dayton and E. O. Major. N.I.H., Bethesda, MD
Productive infection with HIV-1 depends on several factors including efficient entry, reverse transcription and integration. Host cell factors play a role in these processes and are essential for efficient transcription once integration has occured. The primary astrocyte is infectable with HIV-1 both in vivo and in vitro but this infection is typically less productive than that of a macrophage. We are examining the possibility that the primary human astrocyte displays a non productive phenotype because, in vitro, it is deficient in necessary host factors. Specifically we are testing whether increases in NF-κB can release these cells from latency. If such is the case, it would imply that in vivo where stimuli that increase this transcription factor may be present, these cells may contribute significantly to disease. We have previously demonstrated that stimuli which are associated with an increase in NF-κB are associated with an increase in HIV-1 expression. In transient transfection assays NF-κB is greater than or equal to Tat or Rev in its ability to increase p24 production from astrocytes cotransfected with pNL4-3 as compared to those transfected with pNL4-3 alone. This could be because NF-κB not only increases Tat expression through its action on the LTR but because this transcription factor may increase the expression of host factors such as those required for efficient Tat or Rev function. We are using EMSA to assess RRE binding proteins in response to stimuli which increase NF-κB and transient transfections to assess possible synergy between Tat or Rev and NF-κB. Also, using stable transfectants we are examining the effect of NF-κB on HIV-1 protein and RNA expression in an astrocyte cell line. Finally, we are examining two other non productively infected cell types, the U1 cell and human tonsillar stromal cells, to determine whether findings in the astrocyte are common to these cell types as well.

D4-113 HIV-1 STRAIN DIFFERENCES IN EXPRESSION IN DIFFERENT CELLS AND IN RESPONSE TO *tat*, TNF-α, J.R. Corboy, R.M. Thompson and P. Garl, Department of Neurology, University of Colorado Health Sciences Center, Denver, CO 80262
Some strains of HIV-1 have a predilection for infection of the central nervous system (CNS), which, in part, is associated with macrophage-tropism. We previously showed that the long terminal repeat (LTR) may also play a role in CNS-specific gene expression, as transgenic mice expressing β-galactosidase (β-gal) under the direction of LTR's derived from the brain of an HIV-1-infected patient (HIV $LTR_{JR\text{-}CSF}$ lac Z, HIV $LTR_{JR\text{-}FL}$ lac Z) express in the brain, whereas transgenic mice constructed with the LTR derived from T-cells(HIV LTR_{IIIB} lac Z) do not. In this study we analyze the different responses of these LTR lac Z constructions to *tat*, the transactivating protein, and tumor necrosis factor alpha (TNF-α), in transient transfections in HeLa cells and astrocytoma-derived U373 cells. The results show that 1) in HeLa cells, transient transfection of HIV LTR_{IIIB} lac Z results in greater baseline expression of β-gal than HIV $LTR_{JR\text{-}FL}$ lac Z, whereas in U373 cells, expression is greater with HIV $LTR_{JR\text{-}FL}$ lac Z; 2) in HeLa cells, cotransfection of a DNA construction expressing *tat* (HIV $LTR_{JR\text{-}FL}$ *tat*) results in a 300- to 500- fold increase in expression with the HIV LTR_{IIIB} lac Z and HIV $LTR_{JR\text{-}FL}$ lac Z constructions, respectively; whereas, in the U373 cells, the expression is increased 125-fold with HIV $LTR_{JR\text{-}FL}$ lac Z, but insignificantly with HIV LTR_{IIIB} lac Z; 3) in HeLa cells, the addition of TNF-α (HIV-$LTR_{JR\text{-}FL}$ TNF-α) in cotransfection increases expression of β-gal 4-5 fold with either HIV LTR lac Z, whereas in U373 cells there is a 2.3 fold increase with the HIV $LTR_{JR\text{-}FL}$ lac Z but none with HIV LTR_{IIIB} lac Z; and 4) in HeLa cells, cotransfection of either lac Z construct with both the *tat* and TNF-α constructs results in a small but reproducible increase in β-gal expression (1.1-1.6 fold) compared to cotransfection with just *tat*; whereas in U373 cells, cotransfection of *tat* and TNF-α with either lac Z construct results in a small but reproducible decrease in β-gal expression (.76 for each) compared to *tat* alone. Thus, we conclude that strain differences in the HIV-1 LTR are very important for deferring differential expression of HIV-1 in neural versus non-neural cells, and that transactivating factors such as *tat* and TNF-α may have different effects depending upon which cells are utilized in vitro, and which strain of HIV-1 is activated upon.

D4-114 **STRUCTURAL AND FUNCTIONAL STUDIES ON HIV-1 VPR SUGGEST THE IMPORTANCE OF A PREDICTED ALPHA-HELICAL AMINO-TERMINAL DOMAIN AND SUGGEST THAT VPR BLOCKS CELL DIVISION IN G2 OF THE CELL CYCLE.**
[1]Paola DIMARZIO, [1]Sunny CHOE, [1]Michael EBRIGHT, [1]Roland KNOBLAUCH, [2]S. ISAKOFF, [1,2,*]N.R. LANDAU. [1]Aaron Diamond AIDS Research Center, 455 First Avenue, New York, N.Y. 10016; [2]New York University Medical School, New York, N.Y. 10016.
vpr is a highly conserved gene of HIV/SIV that is important in AIDS pathogenesis but appears to play no role in infection of transformed cell-lines. Unlike the other HIV-1 regulatory gene products it is abundant in virions. A recent report suggested that Vpr facilitates import of the HIV-1 preintegration complex into the nucleus of non-dividing cells following entry of the virus into the cytoplasm. By studying the incorporation of a panel of mutant Vpr molecules into the HIV-1 virions, we have shown that an N-terminal region of Vpr (amino acids 17-34) which is predicted to form an alpha helix is important for incorporation of Vpr into the virion. In addition, studies with Vpr truncations showed the importance of residues in the C-terminus for virion incorporation. Immunofluorescence studies on the localization of the Vpr mutants show that this region also plays a role in nuclear localization of the protein. Functional studies using novel luciferase and alkaline phosphatase HIV-1 reporter vectors show that Vpr significantly increases the ability of HIV-1 to infect monocytes but does play not a role in resting or activated PBMC. Complementation experiments, in which Vpr was incorporated *in trans* into virions, suggest that Vpr has an additional role subsequent to nuclear import. Interestingly, when expressed in transformed cell lines, Vpr blocks cellular proliferation. Analysis of the DNA content of these cells showed that Vpr blocks the cells in the G2 phase of the cell cycle.

D4-115 HIV-1 TAT BINDS TO A NOVEL PROTEIN OF THE EXTRACELLULAR MATRIX, David A. Elkins and John J. Rossi, Center for Molecular Biology and Gene Therapy and Department of Microbiology, Loma Linda University School of Medicine, Loma Linda CA 92354.
The HIV-1 Tat protein performs an essential function in viral replication as a transcriptional activator. However, it is also shed from infected cells and may then act following uptake into other cells or by a direct effect at the cell surface. A role for extracellular Tat has been suggested in the pathogenesis of Kaposi's Sarcoma (KS); mice transgenic for Tat alone develop liver tumors and a KS-like lesion in the skin, although Tat is not expressed by the tumor cells themselves. Also, several groups have provided evidence that Tat binds to a protein(s) of the cell surface.

We have used the yeast two-hybrid system to isolate proteins from a mouse cDNA library which bind to the single-exon (72 amino acid) Tat protein. One of the strongest interactions is generated by a novel factor whose deduced amino acid sequence reveals homology to a repeated motif common to many proteins of the extracellular matrix and to several growth factors. We are currently defining the specific region of Tat to which it binds, obtaining the complete cDNA sequence and characterizing its expression pattern in mouse tissues. We will discuss the potential role of this protein in KS, suggested by its homologies with proteins involved in angiogenesis.

D4-116 HIV-1 SURFACE ENVELOPE GLYCOPROTEIN INDUCES INTRACELLULAR CALCIUM RELEASE IN CD4-NEGATIVE, GALCER-POSITIVE HUMAN INTESTINAL EPITHELIAL CELLS, Jacques Fantini, Govindan Dayanithi*, Stephen Baghdiguian+, Christian Tourres++, Nouara Yahi, CNRS URA 1455, Faculté de Médecine Nord, and ++Laboratoire de Virologie, CHU de la Timone, Marseille, *CNRS URA 1197, and +INRA-CNRS URA 1184, Université Montpellier 2, France

HIV-1 entry into CD4-negative intestinal cells is mediated by galactosylceramide (GalCer), a glycosphingolipid also expressed in neural cells and previously found to bind HIV-1 surface envelope glycoprotein gp120. In this study, we have investigated the effect of HIV-1 gp120 on the intracellular concentration of Ca^{2+}, a critical second messenger which mediates ion secretory responses (especially chloride secretion) in intestinal epithelial cells. $[Ca^{2+}]_i$ was measured in single human epithelial intestinal HT-29-D4 cells with the Ca^{2+} probe fura-2 and digital imaging microscopy. Treatment of these cells with HIV-1 gp120 induced an important increase of $[Ca^{2+}]_i$. This effect was abolished by preincubation of gp120 with neutralizing antibodies specific for the V3 domain of gp120. These antibodies inhibited the binding of gp120 to GalCer. Moreover, treatment of HT-29-D4 cells with an anti-GalCer mAb induced an increase in $[Ca^{2+}]_i$ and rendered the cells insensitive to HIV-1 gp120 stimulation. The calcium response induced by gp120 resulted from release of Ca^{2+} from caffeine-sensitive intracellular stores. Finally, the viral glycoprotein specifically abrogated the calcium response to the neuropeptide agonist neurotensin, a stimulator of chloride secretion via inositol triphosphate-mediated calcium mobilization. These data suggest that HIV-1 may directly alter ion secretion in the intestine and thus be the causative agent of the watery diarrhea associated with HIV infection.

D4-117 STUDIES ON THE MECHANISM OF ACTION OF N-BUTYL-DEOXYNOJIRIMYCIN AS AN INHIBITOR OF HIV REPLICATION. Fischer, P.B.§; Collin, M¶; Karlsson, G.B.§ James, W.S.¶; Butters, T.D.§; Gordon, S.¶; Dwek, R.A.§; & Platt, F.M.§. §The Glycobiology Institute, Dept. of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom. ¶ The Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom.

The α-glucosidase inhibitor N-Butyldeoxynojirimycin (NB-DNJ) is a potent inhibitor of HIV replication and syncytia formation *in vitro*, and is currently in clinical trials as an HIV therapeutic. However, the exact mechanism of action of NB-DNJ still remains to be established. In this study we examined the effect of NB-DNJ on the output of virus particles and their infectivity, with particular emphasis on viral entry.
Using a 24 hour output assay, NB-DNJ was found to have no significant effect on virus output. The infectivity of the virus particles released, was, however, greatly reduced at concentrations of NB-DNJ higher than 0.5 mM. Using both a PCR-based entry assay analysing the time course of the appearance of reverse transcripts containing a full LTR post entry, and an entry assay based on plaque formation in Hela T4 cells by HIV-Cocal pseudotypes, the reduction in infectivity of virus produced in the presence of NB-DNJ was found to be due to an impairment of viral entry. These results conclusively show that a major mechanism of action of NB-DNJ as an inhibitor of HIV replication is inhibition of viral entry due to an effect on viral envelope components.

D4-118 IDENTIFICATION OF THREE CELLULAR FACTORS THAT ASSOCIATE WITH THE HIV-1 NEF PROTEIN, Romas Geleziunas, Priscilla Hsue, Douglas Brust, Maria Warmerdam, Robert Atchison, Mark A. Goldsmith and Warner C. Greene, The Gladstone Institute of Virology and Immunology, San Francisco General Hospital, UCSF, P.O. Box 419100, San Francisco, CA 94141-9100

The HIV-1 Nef protein is capable of both, CD4 down-regulation and enhancement of viral infectivity in tissue culture. The goal of this study is to identify cellular proteins that physically interact with Nef which may permit the elucidation of the biochemical basis of CD4 down-regulation and enhancement of viral infectivity. We have used the yeast two hybrid assay of protein-protein interaction to identify three distinct cellular proteins that interact with the HIV-1 *nef* gene product. The interactions of these three Nef associating proteins (NAP1, NAP2, NAP3) with Nef led to different levels of transcriptional activation in the two-hybrid system. NAP1 displayed the strongest ability to induce transcription, in conjunction with Nef, whereas NAP3 was the weakest inducer. These NAPs have no identity with any known protein in the Genbank database. NAPs 1, 2 and 3 specifically interacted with Nef since they failed to associate with a variety of viral or cellular proteins including p55gag, the cytoplasmic tail of gp41, Vif, the cytoplasmic tail of CD4, p53 and lamin. Mutagenesis of the Nef gene revealed that the proline rich area $(PXX)_4$ is critical for both interaction with NAP1 as well as for enhancement of viral infectivity, suggesting that NAP1 may lie in the pathway leading to enhancement of infectivity rather than CD4 down-regulation. NAP2 not only associated with Nef but also interacted with the protein tyrosine kinases Lck (a tyrosine kinase that binds to the cytoplasmic tail of CD4) and Fyn but failed to interact with Hck. These interactions have potentially interesting implications for Nef-mediated CD4 down-regulation and alterations in the state of T-cell activation.

D4-119 EVALUATION OF HIV GP120/41-INDUCED CELL FUSION INSIDE HUMAN LYMPHOID TISSUE IN VITRO.

Svetlana Glushakova, Leonid Margolis, Boris Baibakov and Joshua Zimmerberg. LTPB, NICHD, NIH, Bethesda, MD 20892

Although massive fusion between $CD4^+$ cells and those expressing HIV gp120/41 occurs in cell suspensions and monolayers, syncytial cells are only occasionally found in lymphoid tissue of HIV-infected patients. To study gp120/41-induced fusion inside human lymphoid tissue, we monitored gp120/41-expressing cells inside blocks of human tonsils and adenoids kept in long-term histoculture. To express HIV env proteins on the cell surface we (i) infected tissue blocks with recombinant vaccinia virus vPE16, (ii) infected T lymphoblastoid cells with vPE16, labeled them with fluorescent markers and microimplanted them into the tissue block, and (iii) microimplanted prelabeled, stably gp120/41-expressing cells into tissue blocks. In all three types of experiments, massive fusion of native $CD4^+$ cell with gp120/41-expressing cells was revealed, as evaluated with confocal microscopy and cytological analysis of cells retrieved from the tissue block at the end of the experiment. The number of syncytial cells in human lymphoid tissue increased 5-19 fold due to the presence of gp120/41-expressing cells. However, most of the syncytial cells contain 2-4 nuclei. That may indicate the existence of natural restrictions on fusion into larger syncytia inside the real tissue. Small syncytia can be overlooked by regular histological analysis of lymphoid organs of HIV-infected patients due to the densely packing of high nucleus/cytoplasm ratio lymphocytes in these tissues. Massive cell fusion may contribute to $CD4^+$ T cell depletion and other pathological processes in vivo during HIV infection.

D4-120 DEFINING THE IMMUNOGLOBULIN SUPERANTIGEN BINDING SITE OF HIV-1 gp120, Lee Goodglick, Noam Zevit, Mehran Neshat, and Jonathan Braun. UCLA Dept. of Pathology, Los Angeles, CA 90024.

Infection by the human immunodeficiency virus (HIV-1) results in a profound, multifaceted disruption of the immune system. An important manifestation of infection is the depletion of CD4 T cells and the consequent impairment of cellular immunity. In addition, early and dramatic alterations occur to the B cell population resulting in dysfunctional humoral immunity. A recent insight into B cell alterations during the course of HIV infection was the observation of a clonal expansion and subsequent clonal deficit of B cells expressing VH3 immunoglobulin (Ig) heavy chain gene family members. Recently, we demonstrated that the outer envelop glycoprotein of HIV-1, gp120, directly binds to VH3 Ig at a family-specific site; such binding acutely activates VH3-expressing B cells *in vitro* (*Science* 261:1588, 1993). Our results show that VH3 Ig is a natural ligand for HIV gp120, and suggests that the binding to VH3 Ig contributes to B cell dysfunction during the course of HIV infection. In addition, these results classify gp120 as a novel member of the recently described family of B cell superantigens. In this study we have defined the site on gp120 which is responsible for its Ig superantigen (Ig-SAg) binding proerties. To do this we utilized a set of gp120 peptides spanning the complete length of the protein in a competition ELISA system. The majority of gp120 peptides that we examined, including those of the variable region 3 (V3) loop, did not compete for the binding of polyclonal or monoclonal VH3 Ig to native gp120. However, peptides representing homologous 20 amino acid segment of the C2, C3, and C4 domains of gp120 displayed a dose-dependent inhibition of binding of VH3 Ig to gp120. The optimal Ig-SAg motif of gp120 was located in a 10 amino acid stretch of the C2 domain; mutation of residue 1, 8, or 10 of this decamer sequence, abolished binding to VH3 Ig. In conclusion, we have defined the optimal Ig-SAg binding site of gp120. The selective interaction of gp120 and VH3 Ig may therefore represent a novel and important factor in HIV-induced disease. Direct binding of HIV or soluble gp120 may contribute to B cell disorders as well as directly influence viral infection. (NIH CA12800 and AI-28697; UCLA CFAR)

D4-121 **RETROVIRAL NUCLEOCAPSID PROTEINS WITH *CCCC* OR *CCHH* TYPE Zn^{++}-FINGERS FUNCTION IN RNA PACKAGING BUT RENDER VIRUS PARTICLES NON-INFECTIOUS,** Robert J. Gorelick[1], Donald J. Chabot[1], David E. Ott[1], Alan Rein[2], Louis E. Henderson[1], And Larry O. Arthur[1], [1]PRI/DynCorp and [2]ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702.

All retroviral Gag Zn^{++}-fingers utilize three **CYS** and one **HIS** (***CCHC***) to chelate the metal ion. We have introduced amino acid substitutions, exchanging **CYS** and **HIS** in the single Zn^{++}-finger of the MuLV NC protein to create mutants with ***CCCC*** (steroid hormone receptor) type and ***CCHH*** (transcription factor) type Zn^{++}-fingers. Similar mutants are being constructed in the two Zn^{++} fingers of the HIV-1 NC protein. Since both ***CCCC*** and ***CCHH*** Zn^{++}-fingers are found naturally, these mutants should bind Zn^{++} and form Zn^{++}-finger structures. Transfection with the mutant MuLV proviral clones resulted in transiently expressed viruses with properly processed viral proteins. The ***CCCC*** and ***CCHH*** mutants contained ~20% and 100% wild-type levels of full length genomic RNA, respectively. Neither of the mutants were infectious. Based on Hirt analysis, the block in replication appears to be at the level of reverse transcription. Previous studies have shown that mutations abolishing Zn^{++} binding also abolish RNA packaging. Since both the ***CCCC*** and ***CCHH*** mutants contained significant levels of genomic RNA, it seems likely that they formed competent Zn^{++}-finger structures that participated in RNA recognition and packaging at the level of the precursor, $Pr65^{Gag}$. Nevertheless, the mutant viral particles were non-infectious. Retroviral infection processes appear to require unique properties of retroviral ***CCHC*** chelating structures, that ***CCCC*** and ***CCHH*** type chelates do not possess. This is yet another example suggesting that retroviral Zn^{++}-fingers have functions in addition to their role in RNA packaging. These highly conserved structures are exquisitely sensitive to alteration and therefore can serve as excellent targets for antiviral therapies. Research sponsored in part by the NCI under contract NO. N01-C0-74101 with ABL.

D4-122 HIV-1 NEF ASSOCIATES WITH HOST CELL PROTEINS INVOLVED WITH CELLULAR SIGNALLING AND ACTIVATION A.Greenway*[1], A.Azad and D.McPhee[1]. [1]AIDS Cellular Biology Unit, National Centre for HIV Virology Research at Macfarlane Burnet Centre for Medical Research, PO. Box 254, Fairfield, Victoria, Australia, 3078. [2]Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria, Australia, 3052. HIV-1Nef protein causes the loss of cell surface CD4 and IL-2 receptor (IL-2R) from PBMC and CD4+ T-cell lines. As both CD4 and the IL-2 R play critical roles in antigen-driven helper T-cell signalling and T-cell proliferation, respectively, the role of Nef in the viral life cycle may be to perturb signalling pathways emanating from these receptors. However, the intracellular targets for Nef, that result in receptor down-regulation are unknown. Using a recombinant glutathione-S-transferase-full length Nef (GST-Nef 27) fusion protein, produced in *E.coli* by translation from the first start codon of HIV-1 *nef* clone pNL4-3, as an affinity reagent to probe cytoplasmic extracts of MT-2 cells and PBMC, we have shown that Nef interacts with at least seven protein species ranging from 24 to 75 kDa. Immunoblotting identified four of these proteins as $p56^{lck}$, CD4, p53 and $p44^{mapk/erk1}$, all of which are intimately involved in intracellular signalling. To assess the relevance of these interactions and further define the biochemical activity of Nef in signal transduction pathways, highly purified Nef 27 protein was introduced directly into PBMC by electroporation. Nef 27-treated PBMC showed reduced proliferative responsiveness to exogenous recombinant IL-2 The *src*-family kinase $p56^{lck}$ is associated with the beta chain of IL-2 receptor and is intrinsically involved in signalling following IL-2 engagement of its receptor. Normally, stimulation of T-cells by IL-2 or PMA provokes augmentation of $p56^{lck}$ activity and corresponding post-translational modification of $p56^{lck}$. These changes were also inhibited by treatment of PBMC with Nef, suggesting that Nef interferes with activation of $p56^{lck}$, and as a consequence, of signalling via the IL-2 receptor. The effect of HIV-1 Nef on cell activation and proliferation may partly be explained by its interaction with specific cellular proteins. Interaction of Nef with these proteins may modulate their activity such that the cellular response to antigen or lymphokines is severely reduced resulting in the profound immunodeficiency characteristic of HIV infection.

D4-123 MECHANISM OF ENHANCEMENT OF VIRAL INFECTIVITY BY HIV-1 NEF. John Guatelli, Michal Chowers, Mark Pandori, Celsa Spina, Douglas Richman. San Diego Department of Veterans Affairs Medical Center and the University of California, San Diego. 9500 Gilman Dr. La Jolla, CA 92093-0679.

Recent studies have shown a positive effect of the *nef* gene on the infectivity of HIV-1 virions and consequently a positive impact on viral growth rate. To determine if this effect is separable from CD4-downregulation by Nef, relative growth rates were studied using a cell line that expresses a CD4 molecule with a truncated cytoplasmic domain; this truncated CD4 should not respond to Nef. *Nef*-negative virus had an attenuated growth rate in these cells.

Gene expression from viral DNA appears unaffected or negatively affected by Nef. These data suggest that the positive effect of Nef on infectivity must influence early events in viral replication that precede the establishment of viral DNA. To test this hypothesis, the relative accumulation of viral DNA following infection using wild-type and *nef*-negative HIV-1 was compared; cells infected with *nef*-negative virus accumulated less viral DNA.

To determine more specifically the mechanism of enhancement of viral infectivity by Nef, early events in viral replication were analyzed. Wild-type and *nef*-negative viruses bound to cells equally as assessed using flow cytometry. Wild-type and *nef*-negative viruses entered cells equally as assessed using measurement of cell-associated p24. To exclude an effect on RT activity, wild-type and *nef*-negative virions were analyzed for their content of pre-initiated reverse transcripts and their activity in endogenous RT assays; no difference was detected. These data support the conclusion that Nef enhances infectivity by affecting post-entry events that lead to the generation of viral DNA and that these phenomena are independent of CD4 downregulation.

D4-124 **RELATIONSHIPS BETWEEN SI/NSI STATUS, VIRAL LOAD AND env SEQUENCE IN PATIENTS ENTERING A CLINICAL TRIAL.** P.R. Harrigan, I. Kinghorn, A. Kohli, B.A. Larder and the Protocol 34,225-02 Collaborative Group. Wellcome Research Laboratories, Langley Court, Beckenham, Kent, UK BR3 3BS

We examined the functional variation associated with specific HIV envelope sequences in a large number of patients entering a drug therapy trial. Changes in two variable regions of the HIV-1 envelope (the V2 and V3 regions) have been implicated in the switch of HIV biological phenotype from NSI to SI. SI status has been linked to an increased rate of clinical progression of disease. Protocol 34,225-02 was a placebo controlled, double blinded clinical trial comparing AZT monotherapy with combinations of AZT and ddI or AZT and ddC (manuscript in preparation). Inclusion criteria required a CD4 count below 300 cells/mm^3 and less than 4 weeks previous AZT treatment.

The SI status of virus from 163 patients was determined at study entry. Of these, 87 were NSI and 76 were SI. V2 and V3 sequences were determined by direct sequencing of PCR products from the env region of more than 75% of these patients. To date, all but one of the NSI samples examined had a neutral or negatively charged amino acid at position 320, while a large majority of SI samples had a positively charged amino acid residue in the V3 region at positions 306 or 320 (or both). These results are consistent with the results of Fourchier et al. (J. Virol., May 1992) , though a number of apparent exceptions were observed. In a similar manner, V2 sequences were analysed, the results of which will be discussed. Over the following 48 weeks, isolates from 6 patients underwent a switch from NSI to SI. The envelope regions of these samples were sequenced both before and after the phenotypic switch. Finally, the relationship between the envelope sequences, baseline viral load and phenotype was evaluated.

D4-125 HIV-REGULATED TOXIN GENE THERAPY FOR AIDS. Gail S. Harrison, Tyler J. Curiel, Deborah Cook, Elizabeth Shpall, and Julie A. Campain, Division of Medical Oncology, Univsity of Colorado Health Sciences Center, Denver, CO 80262.

We have previously demonstrated that the human T cell line H9, ordinarily permissive for HIV infection, may be rendered resistant to infection with strain IIIB, as well as certain clinical isolates, by intracellular immunization with the gene encoding diphtheria toxin A chain (DT-A) under the control of HIV Tat and Rev. Cloned cells were protected for up to several months. In some cases, protection was complete in that no residual HIV was detected by HIV p24 antigen production, co-culture with parental H9 cells, or by PCR. CD4$^+$ surface expression of DT-A transduced cloned H9 cells was similar to parental H9 in most cases, suggesting that loss of CD4 did not account for the observed protection against HIV. We have recently demonstrated that protection against HIV was also conferred by HIV-regulated DT-A in the monocytic cell line U937 (data to be presented). As these in vitro studies suggest that toxin gene therapy may ultimately be an efficient way to curtail HIV infection, we are interested in examining our system in hematopoietic progenitor cells. Data will be presented showing that high titer retrovirus (including DT-A containing virus) can be produced, and that a gene transfer efficiency of 10-45% can be achieved in CD34$^+$-enriched cells derived from bone marrow, mobilized peripheral blood and cord blood. Additionally, transient assays showed that progenitor cells electroporated with luciferase plasmids expressed the reporter gene at very high levels when driven by either the CMV or RSV promoters. Studies are underway to determine whether long-term expression is achieved using electroporation, as this may be an effective non-viral method of introducing genes into hematopoietic cells in a way which eliminates concerns of the potential presence of helper virus.

D4-126 **CONSTRUCTION AND USE OF AN HIV-1REPORTER VIRUS EXPRESSING HUMANPLACENTAL ALKALINE PHOSPHATASE.**

[1]Jianglin HE, [1]Steven ISAKOFF and [1,2]Nathaniel R. LANDAU, [1]Aaron Diamond AIDS Research Center, 455 1st Ave., 7th Floor, New York, NY10016 and [1,2]New York University Medical School, New York, NY., 10016.

We report here on the use and construction of a novel HIV-1 reporter vector that encodes human placental alkaline phosphatase. HPAP has been inserted into the *nef* position of NL4-3 and contains, in addition, an inactivated *env*, It therefore does not replicate following an initial integration event and allows quantitation of infectivity in a single round of virus replication. Cells infected with the virus derived from this vector stain an intense purple color after incubation with chromogenic alkaline phosphatase substrates NBT/BCIP. This allows for direct visualization and quantitation of the infected cells. Alternatively, infected cells can be quantitated or sorted by FACS using a fluorescent alkaline phosphatase substrate, Vector Red. No problems have been encountered with background staining due to expression of endogenous alkaline phosphatase. Nor is there any staining detected that is due to carry over of input virus. In addition, the assay is rapid, accurate due to clearly discernible purple color that results. The vector can be used in both cell lines and primary cells. Staining is first observed 12 hours after infection and becomes strong at 24-36 hours. This vector may be useful for evaluating inhibitors of HIV infection and for test the function of envelope glycoprotein genes or other HIV regulatory genes. We demonstrate several uses for this vector including those on HIV tropism and studies of HIV integrase function and nuclear import.

D4-127 **A New Class of Anti-viral Drugs Attack Highly Conserved Zinc Fingers in Retroviral Nucleocapsid Proteins**

L.E. Henderson[1]*, R.C. Sowder II[1], D.E. Ott[1], A.R. Rein[2], L.O. Arthur[1] and W.G. Rice[1]. [1]PRI/DynCorp, NCI-FCRDC, Frederick, MD 21702, [2]ABL, NCI-FCRDC, Frederick, MD 21702.

Nucleocapsid (NC) proteins of the Oncovirinae and Lentivirinae subfamilies of Retroviridae contain sequences of 14 amino acids with 4 invariant residues, $Cys(X)_2Cys(X)_4His(X)_4Cys$, that chelate zinc through histidine imidazole and cysteine thiolates forming CCHC zinc fingers. HIV-1 NC contains two zinc fingers separated by only 7 amino acids, and mutational analysis has shown that both are required for packaging genomic RNA and are also essential in the infection process. Retroviral CCHC zinc fingers are ideal targets for rational drug design because of their extreme conservation among Retroviridae and their essential roles in two steps of viral replication. A study of the chemistry of CCHC zinc fingers reveals that the chelated thiolates are reactive with a variety of functional groups including maleimides, nitrosos, disulfoxides, thiocarbamoyl-disulfides, other substituted disulfides and oxidizing agents such as Cu^{+2} ions. These reactions displace zinc, convert the thiolates to disulfides or alkylated derivatives and alter the active conformation of NC zinc fingers. Some are capable of reacting with NC zinc fingers in cell-free virus and in infected cells and are thus promising lead compounds for anti-viral drug development. These reagents block assembly of infectious virus by interfering with the normal functions of the Gag precursor and inactivate cell-free virus by interfering with the role of NC in the infectious process. Lead compounds that are non-toxic *in vitro* have been identified and at least one compound is a FDA approved drug that is non-toxic *in vivo*. The compound also inactivates murine retroviruses (M-MuLV), consistant with the conservation of NC zinc fingers. Preliminary studies indicate that lead compounds can be evaluated for *in vivo* efficacy in murine models. The data suggest moving to *in vivo* SIV testing and small scale human trials. Since these drugs attack highly conserved structures they may circumvent emergence of drug resistant strains.

Research sponsored in part by the NCI under the Contract No. N01-CO-46000 with ABL.

D4-128 A POSTTRANSCRIPTIONAL REGULATORY ELEMENT IN HEPATITIS B VIRUS IS THE FUNCTIONAL EQUIVALENT TO THE REV-RRE SYSTEM OF COMPLEX RETROVIRUSES, Thomas J. Hope, Bruce Atkinson, Ginger R. Lucero, Peggy J. S. Funches, and Arlyne A. Beeche, The Salk Institute for Biological Studies, La Jolla, CA 92037

Human Hepatitis B virus (HBV) is a double-stranded DNA virus with a life-cycle that includes a reverse transcription step. Recently, a new class of regulatory element required for viral gene expression has been identified in HBV. This element has been demonstrated to be required for efficient expression of the HBV Surface protein. The mechanism of action is at the posttranscriptional level where it facilitates the cytoplasmic localization and subsequent translation of unspliced HBV transcripts (1,2). This posttranscriptional regulatory element (PRE) most likely consists of structured RNA and exerts its action through specific interactions with cellular factors which mediate function. To test the possibility that this element was functionally equivalent to the Rev-RRE system of complex retroviruses, we inserted the HBVPRE into the position normally occupied by RRE in our Rev function assay, pDM128. The HBVPRE can transactivate this reporter system to levels seen in the positive control of Rev specific activation. Transactivation is only detected when the HBVPRE is present in the correct orientation. We have characterized this element by deletion and substitution mutagenesis. The function of this element is dependent on a cellular Rev-like protein much like the constitutive transport element recently discovered in Mason-Pfizer monkey virus (3). These results have interesting implications because a regulatory element from a hepadnavirus that does not splice can functionally replace retroviral components involved in the regulated expression of retroviral RNA with a complex splicing pattern. Furthermore, the results argue that the mechanism of the action for Rev-like proteins of complex retroviruses is the facilitation of the nuclear export of viral RNA rather than the inhibition of splicing.

1. Huang J; Liang TJ. A novel hepatitis B virus (HBV) genetic element with Rev response element-like properties that is essential for expression of HBV gene products. Molecular and Cellular Biology, 1993 Dec, 13(12):7476-86.
2. Huang ZM; Yen TS. Hepatitis B virus RNA element that facilitates accumulation of surface gene transcripts in the cytoplasm. Journal of Virology, 1994 May, 68(5):3193-9.
3. Bray M; et.al.. A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev-independent. Proceedings of the National Academy of Sciences of the United States of America, 1994 Feb 15, 91(4):1256-60.

D4-129 THE HIV-1 VPU PROTEIN: A REGULATOR OF PROTEOLYSIS AND PROTEIN TRANSPORT IN THE SECRETORY PATHWAY OF MAMMALIAN CELLS?

M. Abdul Jabbar, M.J. Vincent, and N.U. Raja
Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195

The Vpu protein of HIV-1 is a multi-functional transmembrane phosphoprotein which plays critical roles in both CD4 proteolysis and virus release. Using gp160/CD4 hybrid proteins, we have previously demonstrated that Vpu-dependent degradation occurred in the ER, and that this reaction is sequence specific requiring both the transmembrane and cytoplasmic domains of CD4 [Vincent et al. J. Virology 67: 5538, 1993; Raja et al. Virology 204: 357, 1994]. In the present study, we performed mutational analysis of the minimal Vpu responsive element [VRE (LLSEKK)] in the CD4 cytoplasmic domain and showed that a conformational element rather than the primary amino acid sequence constitutes the VRE that responds to the Vpu protein in the ER. Furthermore, cotransfection studies have revealed that the Vpu protein blocked the shedding/secretion of gp120 in the medium. For example, the chimeric envelope glycoprotein bearing the extracellular-anchor domains of gp160 and the full length cytoplasmic tail was functional in the fusion of HeLa CD4+ cells expressing Vpu. However, biochemical analyses of the chimeric protein revealed that the shedding of gp120 was significantly reduced in the presence of Vpu. Coexpression of soluble gp120 (sgp120) and Vpu had resulted in a block in the secretion of gp120 into the extracellular medium as well. In order to gain insights into Vpu-mediated transport inhibition, we analyzed the expression and maturation of VSV G, a model type 1 glycoprotein in the absence and presence of Vpu. In singly transfected cells, VSV G was transported to the cell surface undergoing complex oligosaccharide modifications in the Golgi complex. However, ER to Golgi transport of VSV G was significantly reduced in cells expressing Vpu in a dose-dependent manner. Thus, these studies have revealed that the Vpu protein of HIV-1 might have modulatory effects in intracellular transport of glycoproteins in mammalian cells. We will discuss the mechanism of ER proteolysis as well as transport inhibition mediated by the HIV-1 Vpu protein in the mammalian secretory pathway.

D4-130 A COEXPRESSION SYSTEM FOR THE HETERODIMERIC REVERSE TRANSCRIPTASE OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 IN THE STUDY OF THE FUNCTIONALITY OF P51, THE SMALL SUBUNIT OF HIV-1 RT. Heidi Jonckheere , Karen De Vreese, Zeger Debyser, Jan Balzarini, Jan Desmyter, Erik De Clercq and Jozef Anné. *From the Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*

The human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) consists of two subunits, which only differ in primary structure at their carboxy-terminus. Although the large subunit (p66) is well characterized, the functionality of the small subunit (p51) is still unclear. To assess the role of the p51 subunit of the HIV-1 RT, we have constructed two compatible plasmids, each carrying the genetic information of one subunit of the heterodimeric HIV-1 RT. The advantage of this coexpression system to many other *E.coli* expression systems is that a mutation can be introduced in only one or both of the two subunits. Three different mutations were investigated for their impact when introduced either solely in p66 or p51 or in both subunits simultaneously. First, we introduced the TIBO-resistance mutation 188Tyr→Leu. Resistance to TIBO was observed when this mutation was introduced in the p66, but not p51, subunit. As second mutation, we introduced the TSAO-resistance mutation 138Glu→Lys. Resistance to TSAO was observed only when this mutation was introduced into the p51, but not p66, subunit. In the helix αL of the RT that is assumed to interact with the primer-template, we mutated Lys395 to Glu. No significant difference in RT activity was observed between wild-type and mutant RT with the 395Lys→Glu mutation in either p66, p51, or both. In conclusion, the unique coexpression system described here provides a useful tool to investigate the role of the p51 subunit of HIV-1 RT in the reverse transcription process.

D4-131 HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 VPU PROTEIN CONTRIBUTES TO THE DOWNREGULATION OF MHC CLASS I MOLECULES ON HIV-1 INFECTED CELLS, Thomas Kerkau+, Ulrich Schubert*, Thomas Hünig+ and Annneliese Schimpl+, +Institute of Virology and Immunobiology, University of Würzburg, Versbacher Straße 7, 97078 Würzburg, Germany, *Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institute of Health, 4/312, Bethesda, USA

One of the known mechanisms by which viruses can escape the immune system is the downregulation of MHC Class I molecules on the surface of infected cells. We and others have previously shown that this phenomenon is also true for transformed cell lines and activated peripheral CD4+ T cells infected with HIV-1. We now show that this effect depends on a functional Vpu protein since we find that virus mutants which are unable to express or to phosphorylate Vpu do not induce a comparable loss of MHC class I cell surface expression. By pulse chase analyses we show that the difference between wild type virus and Vpu mutants is due to an altered stability of MHC Class I complexes. Our results demonstrate that, in addition to its previously described effect on particle release and CD 4 degradation, Vpu plays a crucial role in the downregulation of MHC Class I molecules on HIV-1 infected cells, thereby providing a potential escape route from the attack by Class I restricted cytotoxic T cells

D4-132 INFECTED MACROPHAGES AND DIFFERENTIATED, CHRONICALLY INFECTED THP-1/HIV-1_{IIIB} CELLS ARE MORE SENSITIVE TO CYTOTOXIC EFFECTS OF CATIONIC LIPOSOMES THAN UNINFECTED CELLS, Krystyna Konopka[a], Elizabeth Pretzer[a], Philip Felgner[b] and Nejat Düzgüneş[a], [a]Department of Microbiology, University of the Pacific, School of Dentistry, San Francisco, CA 94115 and [b]Vical, Inc., San Diego, CA 92121

Cationic liposomes may be valuable for the delivery of anti-sense oligonucleotides and ribozymes into HIV infected and uninfected cultured cells. We evaluated the toxicity of three cationic liposomal preparations, Lipofectamine, Lipofectin, and 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide, DMRIE:DOPE, (1:1), to infected and uninfected cells. Monocytes were isolated from HIV seronegative buffy coats by density gradient centrifugation and plastic adherence. Cells were infected with HIV-1_{BaL} on day 8 post-isolation and treated with liposomes, in the presence of 20% FBS, on day 12 post-infection. Uninfected monocytic THP-1 cells and chronically infected THP-1/HIV-1_{IIIB} cells (K. Konopka *et al.*, (1993) *Virology* **72,** 877) were treated with phorbol 12-myristate 13-acetate (PMA) and exposed to liposomes, in the presence of 10% FBS, 6 days post-differentiation. The cells were exposed to Lipofectamine at 3 or 8 μM and to Lipofectin and DMRIE at 15 or 40 μM, for 4 h or 24 h at 37°C. Liposome-related toxicity was evaluated by the Alamar Blue Assay and p24 production. The toxic effect of cationic liposomes was very limited with uninfected cells, although concentrations of liposomes that were not toxic at early time points subsequent to treatment could cause toxicity at later times. A 24 h exposure to 40 μM Lipofectin was toxic to PMA-treated THP-1 cells 9 days post-treatment. In chronically infected THP-1/HIV-1_{IIIB} cells, all three cationic liposomes were toxic at the higher concentration after a 24 h treatment. However, a 4 h treatment of THP-1/HIV-1_{IIIB} cells with the cationic liposomes was not toxic, even up to 14 days post-treatment. In infected macrophages DMRIE, at 15 and 40 μM, and Lipofectin at 40 μM were toxic, while Lipofectamine was not toxic, after a 24 h treatment. Thus HIV infected cells are more susceptible to killing by cationic liposomes than uninfected cells. The molecular basis of this differential effect is unknown.

This work was supported by NIH grants U01 AI-35231 and AI-32399.

D4-133 **DEVELOPMENT OF A QUANTITATIVE IN VITRO ASSAY TO STUDY INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE.**
Paul Krogstad, Osvaldo Rey, and Jude Canon Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024

We have developed a quantitative assay to examine the susceptibility of HIV strains to nucleoside and non-nucleoside inhibitors *in vitro*. Virus is purified by ultracentrifugation or polyethylene glycol precipitation from culture supernatants and placed in endogenous reverse transcription reactions. Viral DNA produced in reaction mixtures in the presence or absence of RT inhibitors is measured using a previously described quantitative polymerase chain reaction technique*. Mutants of HIV_{NL4-3} and other biologically and molecularly cloned strains have been used to examine the ability of this assay to distinguish virus strains with differing levels of susceptibility to RT inhibitors (as determined using the PBMC culture based ACTG/DOD consensus assay). Azidothymidine triphosphate inhibits reverse transcription in this system, but we have been unable to consistently discriminate between AZT resistant and sensitive HIV strains. A biologically cloned HIV variant with decreased susceptibility to nevirapine (due to a T181C mutation) was easily differentiated from highly sensitive strains. This assay may be useful as a rapid method to detect HIV resistance to nevirapine and other reverse transcriptase inhibitors using bulk virus isolates. *Zack et al. Cell 61:213-222

D4-134 STEPWISE ANALYSIS OF HIV REVERSE TRANSCRIPTION: KINETICS AND IMPLICATIONS,
Peng Li, Litsa Karageorgos, and Christopher J Burrell, National Centre for HIV Virology Research, Institute of Medical and Veterinary Science, Adelaide SA 5000, Australia

We have investigated the kinetics of human immunodeficiency virus (HIV) reverse transcription in infected T-cells, using a synchronized one-step cell-to-cell infection model and quantitative PCR assays for the different DNA intermediate structures that are found sequentially during reverse transcription. Different efficiencies arising from using different primers and other PCR conditions were normalized by conversion of each PCR product signal to copy numbers by comparing with standards. After an initial lag period, the minus strand strong-stop viral DNA was detected first, followed by the post-transfer newly extended minus strand viral DNA, then by the plus strand strong-stop DNA and fully extended minus strand DNA. Kinetic data suggested that once the reverse transcription was initiated, the HIV reverse transcriptase synthesized minus strand DNA at a rate of 150-180 bases per minute, and that the first template transfer and the initiation of the plus strand DNA synthesis imposed specific time delays. In contrast, minus strand viral DNA synthesized after the second template transfer appeared at a time point very close to the time of the appearance of the last piece of DNA synthesized just before the second template switch, suggesting that the second switch occurred very rapidly. Taken together, our results define more accurately than previously possible the rates of several of the sequential steps in HIV reverse transcription in infected T-cell lines and implicate different mechanisms for the two distinct template switches during retrovirus reverse transcription.

D4-135 INTERFERING WITH HIV-1 REPLICATION BY AFFECTING CELLULAR FACTORS, Franco Lori,[1] Andrea Cara,[1] Andrei G. Malykh,[1] Daisy Sun,[1] Julianna Lisziewicz,[1] John Weinstein[2] and Robert C. Gallo,[1] [1]Laboratory of Tumor Cell Biology, [2]Labortory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255

HIV-1 DNA synthesis is completed extremely slowly and inefficiently in quiescent PBL compared to that in stimulated PBL. 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 ($\leq$100 μM) 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, indicates 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 an approach for inhibition of HIV-1 replication. Analogs of hydroxyurea show in part the same mechanism of action, though possessing specific individual characteristics. Comparison of efficacy vs. toxicity of these analogs and hydroxyurea will be presented. SIV was inhibited by hydroxyurea similar to HIV-1 in quiescent and stimulated lymphocytes, and was significantly less sensitive to the same drug in macrophages.

D4-136 **CHARACTERISATION OF HIV-1 MUTANT PROTEASES DERIVED FROM INHIBITOR-RESISTANT STRAINS USING MATCHED BACTERIAL EXPRESSION AND PROVIRAL INFECTION VECTORS:** B. Maschera, E. Furfine[1] and E.D. Blair, Wellcome Research Labs, Beckenham BR3 3BS, UK; [1]Burroughs-Wellcome, RTP, NC 27709, USA

Escape mutants of HIV-1 have been observed during passage of virus in the presence of protease inhibitors. To study the effects of individual and multiple mutations on virus and enzyme viability, we cloned PCR mutagenised protease genes into matched viral and bacterial expression vectors.

More than twenty mutant HIV-1 protease enzymes were expressed in *E.coli* and several have been purified. Wild-type and all mutant protease enzyme activities were initially assessed in two ways. Firstly, bacterial viability, which gives a measure of protease toxicity and therefore activity, and, secondly, by SDS-PAGE/anti-protease Western blots, where precursor to product ratios were determined following IPTG-induction. The enzymes purified, initially, contained amino acid substitutions observed during virus selection with compounds Ro31-8959 & Ro XI (Roche) and A-75925 (Abbott), comprising L90M, G48V, L90M/G48V, V32I and the artificial double mutant V32I/L90M. In the Western-blot assay, V32I was as active as wild-type enzyme whereas G48V, V32I/L90M, L90M and G48V/L90M show successively less processing *in vitro*. Effects of mutations on the catalytic parameters K_m, k_{cat} and k_{cat}/K_m will be presented using a fluorogenic substrate (NH_2BzTINle-F(NO_2)ER-NH_2). This allows objective comparison of the inhibition constants (K_i) for each mutated enzyme against Ro31, A-75 and other protease inhibitors, with the wild type enzyme K_i values.

The effects of these alterations in the protease gene on HIV-1 viral replication and drug sensitivity were also assessed. Recombinant viruses bearing the L90M substitution appeared to grow to slightly lower titres in T-cell lines, but nonetheless we established drug sensitivities for all recombinants. Individual mutations G48V and L90M conferred a 2-fold and 5-6 fold increase in virus IC_{50} against Ro31, respectively, whereas G48V/L90M virus was 8-10 times less sensitive to Ro31. V32I was more than 40-fold less sensitive to A-75, and not only was the V32I/L90M virus viable, but it also had dual-resistance, being insensitive to A-75, and 5-7 fold less sensitive to Ro31.

Such studies will allow us to relate enzymatic alterations of drug sensitivity (Ki) to those displayed by virus (IC_{50}), and when combined with crystallography data will permit the atomic definition of resistance.

D4-137 EVOLUTION OF HIV-1 GENOMES UNDER AZT SELECTION. Michael L. Metzker, Xiao-Mei Liu, Susan M. Miller,¶ Dorothy E. Lewis,† and Richard A. Gibbs, Department of Molecular and Human Genetics, †Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX, 77030; ¶Thomas Street Clinic, Houston, TX 77009.

3'-Azido-3'-deoxythymidine (AZT) is effective in inhibiting human immunodeficiency virus type 1 (HIV-1) replication *in vivo*, although resistant strains can emerge in HIV-1 infected individuals after twenty weeks of drug therapy. Different combinations of at least five specific amino acid mutations in HIV-1 reverse transcriptase (RT) have been shown to confer resistance to AZT. Resistant viruses would have a tremendous growth advantage over AZT-sensitive viruses that can lead to an increase in sequence diversification in other HIV-1 genes.

Three asymptomatic HIV-1 infected individuals undergoing AZT therapy are enrolled in a longitudinal study to examine the evolution of multiple HIV-1 genes. Genomic DNA was isolated from peripheral blood mononuclear cells and was amplified by nested PCR. Mutational analyses of each region were performed by *Bst* solid-phase sequencing. Sampling time points were obtained for each individual on a one to two month basis.

Sequencing analyses at the initial time points showed homogeneous signature sequences in both the *pol* and *env* genes from each individual. At eighteen months, each individual has accumulated various mutations in RT known to confer resistance to AZT. Comparative sequencing analyses of the *protease* gene and the V3-V5 domain of the *env* gene are presented, and the possible role of clonal expansion of these genes *via* genetic linkage to resistant RT strains is discussed.

D4-138 THE REPLICATION DEFECT CONFERRED TO AN HIV CLONE BY A *NEF* GENE DELETION DISAPPEARS IN PRIMARY HUMAN MACROPHAGES. Pascal R.A. Meylan, Roland Sahli, and Irène Zbinden. Institute of Microbiology, University of Lausanne Hospital Center, CH-1011 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. The effect of *nef* on HIV replication is debated, but recent evidence using mutants in the pNL43 background suggested that *nef* is necessary for optimal replication and infectivity in T lymphoblastoid cells (Chowers et al, J Virol, 1994;68:2906). Macrophages are an important host cell type. To test the effect of *nef* on HIV replication in these cells, 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). Upon transfection in COS cells, primary stocks were obtained. The wild type stock had a two fold increased infectivity as measured by end-point titration on primary macrophages ($TCID_{50}$/pg p24 antigen ratio) compared to the *nef* deletion mutant. However, when these stocks were used to infect macrophages, in contrast to lymphoblastoid cells, the *nef* deletion mutant did not display any replication defect, although mutiple rounds of infection should result in progressively differing replication curves. After passage in macrophages, a stock of the *nef* deletion mutant no longer displayed any infectivity defect. We are currently testing whether a *nef* allele from a macrophage-tropic isolate (pYU-2) introduced in the same pNL43BE clone and a corresponding *nef* deletion mutant also display the same behavior in macrophages. Our preliminary data suggest that primary macrophages are able to complement the infectivity defect of *nef* mutants observed in other cell types.

D4-139 EXPRESSION OF THE HIV-1 *nef* GENE DURING HIV-1 PRODUCTION INCREASES PROGENY PARTICLE INFECTIVITY INDEPENDENTLY OF gp160 OR VIRAL ENTRY, Michael D. Miller, Maria T. Warmerdam, Kathleen A. Page, Mark B. Feinberg, and Warner C. Greene, The Gladstone Institute of Virology and Immunology, San Francisco, CA 94141

We have previously shown that *nef*+ HIV-1 is more infectious than *nef*- HIV-1 as detected in limiting dilution assays and through a single-cycle infection of an indicator cell line. We now demonstrate that *nef*- HIV-1 can be rescued to wild-type levels of infectivity by co-expressing Nef *in trans* in the cell line producing the virus. Thus, HIV-1 virions produced in the presence of Nef are intrinsically different and *nef* gene expression in the target cell is not required for wild-type infectivity. We show that the major viral structural proteins are quantitatively similar in purified viral preparations of *nef*+ and *nef*- HIV-1. Furthermore, we demonstrate that viral entry is not affected by Nef through an entry assay for HIV-1 and by pseudotyping *env*-defective *nef*+ and *nef*- HIV-1 with an amphotropic envelope. Thus, the increased infectivity of *nef*+ HIV-1 is manifested at a stage after viral entry.

We have also assessed the course of infection immediately following viral entry and observe that reverse transcription proceeds equally well for both *nef*+ and *nef*- HIV-1 infections of H9 cells. It is possible that Nef might exert a function directly within the target cell if it were packaged within the virion. To this end, we observe that Nef protein from *nef*+ HIV-1 co-purifies with HIV particles during sucrose gradient density centrifugation, indicating that Nef is packaged within the virion. Notably, this property of Nef is dependent upon an intact myristylation site, suggesting a viral membrane association for Nef. Taken together, these results suggest a direct role for the myristylated Nef protein in the target cell at a stage between the completion of reverse transcription and subsequent integration.

D4-140 PATTERNS OF SPECIFIC MUTATIONS IN HIV-1 PROTEASE THAT CONFER RESISTANCE TO A PANEL OF PROTEASE INHIBITORS. Hongmei Mo*, Martin Markowitz*, and David D. Ho. Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016.

Using in vitro selection, we have generated HIV-1 variants with resistance to protease inhibitors: L-735,524 from Merck, XM-323 and XR-045 from Dupont-Merck, AG-1343 and AG-1350 from Agouron, and U-103017, U-104904, and U-106893 from Upjohn. Previously, we selected HIV -1 variants resistant to ABT-538 (P22) and A-77003 (P19). P22 was passaged in the presence of increasing concentrations of ABT-538 as well as L-735,524, AG-1343 or AG-135. Passages were also done in parallel in the absence of all protease inhibitors. In addition, P19 was further passaged in the presence of 2.0 μM A-77003 and in the absence of drug. The protease coding region of variant viruses was sequenced and amino acid changes conferring resistance were deduced. We have used site-directed mutagenesis to introduce selected specific mutations into the NL4-3 background. Subsequently, we have tested the activity of the above protease inhibitors against the wild-type virus, HIV-1_{NL4-3}, variants generated from selection experiments as described above, and a panel of HIV-1 mutant viruses including: V32I, L10F, M46I, M46F, G48V, L63P, A71V, V82A, V82F, I84V, R8Q/M46I, R8K/M46I, 10F/G48V, 10F/I84V, V32I/V82A, M46I/I84V. Results of these studies will be described in detail. We conclude at this time that HIV-1 resistance is found for all drugs tested. Residues 8, 48, 82, and 84 appear to be common but not exclusive sites for conferring resistance to protease inhibitors. Finally, there appears to be differing patterns of resistance amongst the various inhibitors, raising the use of combination therapy as a cogent antiviral strategy.

*Contributed equally to these studies.

D4-141 GP120-GP41 STRUCTURE/FUNCTION STUDIES USING MOLECULAR CLONES DERIVED FROM PRIMARY AND T-CELL LINE-ADAPTED ISOLATES OF HIV-1. Daniel S. Nam, Tuofu Zhu, Cecilia Cheng-Mayer, John P. Moore and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016.

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). For example, primary isolates are markedly resistant to CD4 and antibody neutralization. These isolates often exhibit tropism for macrophages. Laboratory strains, on the other hand, are exquisitely sensitive to neutralization and show tropism for T cell lines. In this study, we are examining two HIV-1 isolates, P17 and C17, for which DNA sequence data indicate only four amino acid changes. Three changes occur in the C1 (E67K) and C2 (T249M, A252V) domains of gp120; a single substitution occurs in gp41 (I84F). Also, P17, a primary isolate, is highly resistant to CD4 and antibody neutralization, whereas C17, its T-cell line-adapted counterpart, is highly sensitive.

Based on these observations, we aim to define more precisely the genotypic basis for the phenotypic variation shown by P17 and C17. To this end, we selectively amplified full-length gp160 from P17 and C17 by PCR and initially screened the PCR-amplified gp160 for functionality using a transient expression system. Using these functional clones, we are generating molecular chimeras by means of a reciprocal exchange of these full-length gp160 sequences on the genomic background of the laboratory HIV-1 strain NL4-3. Moreover, of the limited number of amino acid changes, the T249M substitution is notable in that it abolishes a potential glycosylation motif (N-X-T) while the I84F substitution in gp41 may have a role in destabilizing gp120-gp41 associations in C17. To examine whether the amino acid composition of the C1 and C2 domains and gp41 affects CD4 and antibody neutralization, we are generating site-directed mutants of the molecular chimeras. In combination with this approach, these molecular chimeras and their mutants will serve as tools to evaluate how the overall conformation of the envelope glycoproteins influences biologic phenotype.

D4-142 FOLDING, ASSEMBLY AND INTRACELLULAR TRANSPORT OF THE HIV-1 ENVELOPE PROTEIN ANALYZED WITH MONOCLONAL ANTIBODIES Ahlert Otteken, Patricia L. Earl, Christopher C. Broder, and Bernard Moss. National Institute of Allergy and Infectious Diseases, NIH, Bethesda MD 20892-0485.

The HIV-1 envelope (env) precursor gp160 attains an oligomeric form during transport within the secretory pathway of the cell. A unique panel of conformational monoclonal antibodies (MAbs) was used to determine the kinetics of assembly of env protein and the intracellular localization of maturational intermediates. The MAbs were previously characterized with regard to their specificity for gp120 and gp41 subunits, CD4 blocking ability, and selectivity for monomeric and oligomeric forms of gp160. Cells expressing recombinant gp160 were pulse-labeled with [^{35}S]methionine and then chased. MAbs to the V3 loop of gp120 reacted with the env protein at all times consistent with the conformation-independence of the epitope, whereas a 10-min lag period was required for the env protein precursor to fold so as to react with conformational MAbs to gp120 that inhibited CD4-binding. Other MAbs to gp120 were reactive with env protein only during the first 45 min after synthesis, consistent with their preference for monomeric env protein. During the latter time, gp160 molecules acquired the ability to recognize some conformational and oligomer-reactive MAbs to gp41. Remarkably, there was a 2-hour delay before gp160 reacted with stringent oligomer-specific MAbs. Immunofluorescence microscopy indicated that the MAbs with preference for monomers reacted with env protein in the endoplasmic reticulum (ER) whereas the oligomer-specific MAbs stained the Golgi exclusively. Brefeldin A-treatment led to the staining of the ER by oligomer-specific MAbs, consistent with the change in intracellular trafficking induced by this drug. Binding of oligomer-specific MAbs did not occur at 16°C and was prevented by dithiothreitol suggesting a requirement for disulfide bond formation. Thus, the exquisite specificity of MAbs has allowed us to dissect the folding and oligomerization pathway of the HIV-1 envelope protein.

D4-143 **The Fate of the HIV-1 Provirus in Infected Cells: a Novel Role for *vpr*.** Vicente Planelles, Jeremy Jowett, Francoise Bachelerie, Allyson Haislip, Yiming Xie and Irvin .S.Y. Chen. Departments of Medicine and Microbiology and Immunology, University of California, Los Angeles, CA 90024.

We investigated the fate the HIV-1 integrated DNA in infected peripheral blood lymphocytes and immortalized T-cell lines after infection with HIV-1. Post-infection events were studied in the absence of envelope-mediated cytopathicity and viral spread by using a single-step infectious HIV-1. Using this system, we observed that HIV-1 proviruses that lack *envelope* or *envelope* and *nef* were unable to maintain the provirus and establish a chronic infection. We show by mutagenesis analysis that a frameshift in the *vpr* open reading frame enabled HIV-1 to establish a chronic infection and persist in lymphoid cells. It is likely that this is due to growth disadvantage of infected cells.

The *vpr* gene of HIV-1 encodes a 96-amino acid virion-associated protein. Several functions have been ascribed to this protein, including weak transctivation of the HIV-1 LTR, transport of viral nucleocapsids to the nucleus of non-dividing cells and induction of differentiation and growth arrest in rhabdomyosarcoma cells. In an effort to understand how the different reported functions of *vpr* may be interrelated, we are in the process of delineating fuctional domains of this protein by site-directed mutagenesis. Preliminary data suggest that Vpr must be expressed *de novo* in infected cells in order to exert its effect in preventing persistence. This function of Vpr is, thus, independent of its ability to be packaged in the virion.

These observations have important implications concerning the mechanisms by which HIV-1 maintains a persistent infection and suggest a role of *vpr* in modulating viral persistence.

D4-144 Overexpression of SF2/ASF Inhibits Rev/RRE Dependent HIV-1 Gene Expression

Douglas M. Powell,[1] M. Catherine Amaral,[1] Jane Y. Wu,[2] Tom Maniatis,[2] and Warner C. Greene.[1] Gladstone Institute of Virology and Immunology, UCSF, San Francisco General Hospital. P.O. Box 41910, San Francisco, CA 94141-9100 [2] Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge MA 02138

The HIV-1 Rev protein regulates viral gene expression by enhancing the cytoplasmic appearance of unspliced and singly-spliced viral mRNAs encoding the structural and enzymatic proteins of HIV-1. Rev acts by binding directly to a *cis*-acting RNA target sequence, the Rev Response Element (RRE), located in the *env* gene of HIV-1. To identify cellular proteins that interact with the Rev-RRE complex we have screened HeLa nuclear extracts (NE) by RNA electrophoretic mobility shift assay (REMSA). As a first step in the characterization of proteins in the NE, immunoblotting was performed with antibodies specific for known splicing factors. One protein that migrates as a doublet at approximately 33 kD resulted in a mobility shift of the Rev/RRE complex. This protein doublet was identified as SF2/ASF based on immunoreactivity. SF2/ASF is an SR protein that is required for constitutive splicing, and additionally is involved in alternative 5' splice site selection. The SF2/ASF specific antibody resulted in a supershift of the Rev/RRE/SF2/ASF complex. Furthermore, recombinant SF2/ASF produced in baculovirus also resulted in a decreased mobility shift of the Rev/RRE complex. In order to explore the potential effect of the SF2/ASF interaction with Rev on HIV-1 gene expression, SF2/ASF was cotransfected into COS-7 cells together with a genomic HIV-1 Tat expression plasmid, pgTat. This plasmid expresses a two exon, 86 amino acid form of Tat in the absense of Rev and a one exon, 72 amino acid form of Tat in the presence of Rev. Overexpression of SF2/ASF decreases expression of a Rev dependent one exon form of Tat. The effect of SF2/ASF overexpression was also determined in COS-7 cells cotransfected with a replication competent HIV-1 provirus. Viral replication was inhibited in a dose dependent manner by increasing amounts of SF2/ASF. We are currently examining the effect of overexpression of other SR proteins and mutants of SF2/ASF on HIV-1 gene expression.
In summary, the splicing factor SF2/ASF was found to bind to the RRE in the presence of Rev by REMSA. Overexpression of SF2/ASF inhibits HIV-1 gene expression. Characterization of the mechanism of inhibition of HIV-1 gene expression by overexpression of SF2/ASF is currently in progress.

D4-145 **DOWN REGULATION OF CD4 EXPRESSION AND REDUCED VIRUS REPLICATION FOLLOWING EXPOSURE TO LITHIUM GAMMA-LINOLENATE, S.L. Randall, D. Kinchington, M.D. Winther*, D.F. Horrobin*, W.L. Chan, Department of Virology, Medical College of St. Bartholomew's Hospital, London, UK and *Scotia Pharmaceuticals Ltd, Nova Scotia, Canada.**

Studies have shown that HIV-1 infection results in an altered metabolism of fatty acids in the host cell. This altered metabolism presents a possible target for the development of new therapeutic approaches to HIV-1 infection. We report here that the lithium salt of gamma-linolenic acid (LiGLA) at 20μg/ml selectively killed H9 cells chronically infected with HIV-1, strain RF, in vitro. In addition, H9RF cells treated with LiGLA released less infectious virus particles into the culture medium as detected by 'back titration' of culture supernatants on to C8166 lymphoblastoid cells.

When H9 cells were prretreated with 10μg/ml LiGLA before co-culture with H9RF cells, syncytium formation was reduced by approximately 50%. H9 cells treated with LiGLA also showed a marked reduction in cell surface expression of CD4 molecules when analysed by flow cytometry, suggesting that the reduction in syncytium formation could result from a down-regulation of CD4 expression induced by LiGLA pretreatment.

When peripheral blood mononuclear cells (PBMC) from HIV-seronegative individuals were PHA-activated and infected with HIV-IRF in the presence or absence of 20μg/ml LiGLA, virus production, as measured by reverse transcriptase (RT) release into culture supernatants on days 4, 7 and 10 post infection, was significantly reduced in cultures containing LiGLA. There was an 11-, 14- and 9-fold reduction in RT release respectively, compared to untreated, infected PBMCs.

D4-146 AN INTERACTION BETWEEN THE 638-673 MOTIF AND THE 552-595 MOTIF OF THE HIV-1 GP41 PROTEIN OF HIV-1 IS INVOLVED IN MEMBRANE FUSION, Laurence Rimsky, Carl Wild, Barry Kosloff and Thomas Matthews, Department of Surgery, Center for AIDS Research, Duke University Medical Center, Durham, NC 27710.

An anti-viral synthetic peptide, DP178, modeled after residues 643-678 of the HIV-1 *env* protein, completely inhibits virus mediated cell to cell fusion and reduces by 90% the infectious titer of cell free virus at doses as low as one and 100ng/ml respectively (AIDS Res. Hum Retro. 1993, 9: 1051). A virus population resistant to the DP178 was obtained by serially passaging HIV1LAI in the presence of escalating doses of DP178. The region corresponding to the gp41 *env* gene was cloned from this viral population into the pNL4-3 proviral DNA. A resulting virus, NL178, was found to be resistant to doses of DP178 as high as 10 μg/ml. Subsequent analyses of this clone shows a single amino acid change at envelope position 555. The amino acid 555 is located at the N terminal part of a leucine zipper motif which we have previously implicated in the fusogenic process (Proc. Natl. Acad. Aci. USA 89: 10537). The results suggest that the virus target of DP178 is a determinant of the gp41 leucine zipper motif. That interpretation is consistent with other studies in this laboratory which are also suggestive of an association of the DP178 peptide with the leucine zipper motif of gp41. Possible implications of these results for the structure/function of gp41 will also be discussed.

D4-147 **INHIBITION OF A CELL SURFACE REDUCTASE INHIBITS HIV INFECTION OF HUMAN CELLS.**

Hugues J.-P. Ryser, Elinor M. Levy, Richard Mandel, and Gino J. DiSciullo, Departments of Pathology and Microbiology, Boston University School of Medicine, Boston, MA 02118.

The surface of mammalian cells is capable of cleaving disulfide bonds of exogenous proteins that are either receptor-bound, like diphtheria toxin, or otherwise surface bound. This reductive function is markedly decreased (and the cytotoxicity of diphtheria toxin prevented) by inhibitors of protein disulfide-isomerase (PDI), suggesting that PDI catalyzes a thiol-disulfide interchange between its thiols and the disulfides of membrane-bound ligands. We provide evidence that the same reductive process plays a role in the infective HIV-cell interaction by showing that HIV infection of human target cells is markedly inhibited by the membrane-impermeant sulfhydryl blocker DTNB, the PDI inhibitor bacitracin and monoclonal anti-PDI antibodies. This implies that HIV and target cell engage in a PDI-mediated thiol-disulfide interchange and that the reduction of critical disulfide bonds in envelope glycoproteins may cause conformational changes required for virus entry. It is postulated that PDI is situated in the vicinity of the receptor, as in the case of diphtheria toxin, and acts on one of three disulfide bonds situated in proximity of CD4 binding regions of gp120. A highly anionic C-terminal sequence in PDI may provide a point of anchorage to a cationic domain of gp120. The interchange catalyzed by PDI may thus be the molecular event that triggers virus-cell fusion following HIV binding to CD4. These findings suggest new approaches to drug design, namely the development of inhibitors of surface-associated PDI, and of antibodies or rationally designed peptides targeted to epitopes containing disulfide bonds in the conserved CD4 binding regions of gp120.

D4-148 REQUIREMENT FOR THE MEMBRANE-PROXIMAL REGION OF THE HIV-1 TRANSMEMBRANE GLYCOPROTEIN ECTODOMAIN IN FUSION

Karl Salzwedel and Eric Hunter, Department of Microbiology and Center for AIDS Research, University of Alabama at Birmingham, Birmingham, Alabama 35294.

The process of viral entry is a key step in the initiation of HIV infection. We are interested in identifying the domains of the HIV-1 transmembrane glycoprotein, gp41, that are involved in fusing the viral envelope with the plasma membrane of the target cell. The amino-terminus of gp41 has been extensively characterized as the primary fusion domain and presumably interacts directly with the target membrane. In additon, a heptad repeat (coiled-coil) motif has been identified in the ectodomain of gp41 which plays a critical role in the post-CD4-binding events in the fusion process. Moreover, while it has been shown that the cytoplamic tail of gp41 is not required for fusion, we have shown that the fusion event does require a membrane-spanning peptide anchor, since substitution of the membrane-spanning domain of gp41 with a covalently linked lipid anchor did not support fusion.

Here we have set out to determine by site-directed mutagenesis whether a stretch of 17 predominantly hydrophobic amino acids (HXB2 residues 665-683) bordering the membrane-spanning domain on the extracellular side is required for fusion. This domain is unusually rich in tryptophans, containing five tryptophan residues that are highly conserved in all strains of HIV-1. In addition, it overlaps the sequence of a peptide (DP-178) which has been shown to potently inhibit viral-mediated cell-cell fusion in vitro as well as the epitope for a neutralizing human monoclonal antibody (2F5). Both deletion and point mutagenesis approaches have been utilized in these studies. Initial results indicate that deletion of the entire 17 amino acid region abrogates fusion without affecting intracellular transport and processing of the mutant protein. Experiments aimed at defining the sequence requirements of this potential functional domain are currently underway in order to distinguish between a direct role in the fusion event and a purely structural one. The results of these experiments will be discussed in the context of current models for viral-induced membrane fusion.

D4-149 CHARACTERIZATION OF SIAMYCIN I, AN HIV FUSION INHIBITOR, Himadri Samanta, Cliff Bechtold, Carol Deminie, Masud Alam, Keith Riccardi, Burt Rose, Amy Patick, Richard White, Rich Colonno and Pin-Fang Lin, Department of Virology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492

The HIV fusion inhibitor Siamycin I, a 21-mer tricyclic peptide, was isolated from a Streptomyces culture using a cell fusion assay involving cocultivation of Hela-CD4+ cells and monkey kidney cells (BSC-1) infected with a vaccinia vector expressing gp160 (Ref. Tsunakawa, Hoshino, Detlefson, Hill, Furumai, Nishio, Kawano, Yamamoto, Fukagawa, Oki, submitted to J. Antibiotics). Siamycin I is effective against acute HIV-1 and HIV-2 infections in a cell protection assay with ED_{50s} of 0.1-0.6 μM and a CC50 of 150 μM in CEM-SS cells. Inhibition appears to be specific, since Siamycin I will inhibit fusion between HIV chronically-infected CEM-SS and CD4+-C8166 cells (ED_{50} of 0.08 μM), while having no effect on Sendai virus-induced fusion or murine myoblast fusion. Siamycin I seems to bind non-covalently, since inhibition of HIV-induced fusion by this compound is reversible.

Siamycin I does not inhibit gp120-CD4 binding in either gp120 or CD4 capture ELISAs or ^{125}I-gp120-CD4 binding assays. To determine the anti-viral target of this compound, a variant of HIV resistant to Siamycin I was selected by *in vitro* passage of virus in the presence of increasing concentrations of the compound. Drug sensitivity studies of chimeric virus containing the envelope gene from resistant virus suggest that the gp160 domain may be responsible for resistance. Further DNA sequencing of the envelope gene from the resistant and parent viruses revealed a total of 6 amino acid changes in the gp160 domain. Confirming the significance of these substitutions in resistance development using site-directed mutagenesis is in progress.

D4-150 **A Highly Conserved Region And Membrane Targeting Of Nef From Primate Immunodeficiency Viruses Are Required For Association With A Cellular Serine Kinase Activity**

Earl T. Sawai, B. Matija Peterlin*, Jay A. Levy#, Paul Luciw, and Cecilia Cheng-Mayer†, Dept. Medical Pathology, University of California, Davis, CA, 95616. Cancer Research Institute#, Department of Medicine, and Howard Hughes Medical Institute*, University of California, San Francisco, CA, 94143-0128. Aaron Diamond AIDS Research Center†, NYC, NY, 10016.

Among the primate lentiviruses (HIV-1, HIV-2, and SIV), the *nef* gene is highly conserved and encodes a myristylated protein of ~27 kDa (HIV-1) or ~37 kDa (HIV-2, SIV). Previously, we found Nef expressed either as a CD8-Nef fusion protein or as a native protein in virally infected T cell lines associated with a serine kinase activity that phosphorylated two proteins of 62 kDa and 72 kDa in *in vitro* kinase assays. Using transient expression, we have analyzed various alleles and mutants of Nef for association with the cellular kinase activity. We found that the ability of Nef to associate with the kinase activity was highly conserved among other alleles of HIV-1 as well as SIVmac239, and was observed in non-lymphoid cell lines of simian and murine origins. We have provisionally mapped the region of HIV-1_{SF2} Nef that is important for the associated kinase activity to amino acids 45-127. This domain overlaps a central, highly conserved region that is found in all primate lentivirus *nef* genes. Point mutations within this region that abrogate the Nef-associated kinase activity in HIV-1_{SF2} Nef have the same effect when introduced into SIV_{mac239} open Nef. Moreover, myristylation mutants indicate that membrane localization of Nef is important for the associated cellular kinase activity.

To determine the role of the Nef-associated kinase activity *in vivo*, we have infected rhesus macaques with mutant SIV_{mac239} viruses that abrogate this function of Nef. Data from these studies will be presented and discussed.

D4-151 USE OF SIMPLE REV-INDEPENDENT HIV-1 GAG EXPRESSION VECTORS IN GENE THERAPY AND GENE VACCINE APPLICATIONS, Ralf Schneider[1], Seong Song[1], Georgios Nasioulas[2], Andrei Zolotukhin[2], Barbara K. Felber[2], Richard Trauger[3], J. Cox[4], M. Manthorpe[4], George N. Pavlakis[1], Human Retrovirus Section[1] and Human Retrovirus Pathogenesis Group[2] ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702. Immune Response Corp. [3], Carlsbad, CA 92008. Vical Inc.[4], San Diego, CA.

Studies on the regulation of HIV-1 expression by the Rev protein have indicated that the unspliced and intermediate viral mRNAs are defective in the absence of Rev or its binding site on the viral RNA, named RRE. Genetic analysis indicated that multiple inhibitory sequences (INS/CRS) are located throughout the HIV-1 genome in the *gag*, *pol*, and *env* regions. We have demonstrated (1-2) that the multiple INS elements within the *gag* coding region can be eliminated by mutagenesis (changing the nucleotide but not the amino acid composition), leading to constitutive high Gag expression in the absence of Rev. The Gag expression vectors were able to produce viral particles in human and mouse cells in the absence of any other HIV proteins. Additional mutations in the pol region allowed the expression of the protease and the processing of the $p55^{gag}$ precursor. This resulted in the production of processed Gag particles containing lentiviral-like core regions.Mice were injected with the Gag expression vectors. Direct DNA injection of Tat and Rev independent Gag expression vectors in mouse muscle resulted in Gag expression detected by ELISA and in anti-gag antibody response. These results demonstrate the development of simple expression vectors for the efficient production of HIV proteins. We have inserted several Rev- and Tat- independent Gag expression cassettes into retroviral vectors and have constructed cell lines expressing Gag or Gag fragments that are dominant negative inhibitors of HIV-1. These vectors are promising for development of effective gene therapy strategies against HIV-1. INS-free Gag, Pol, and Env expression vectors will allow the efficient production of these proteins in a variety of cells in the absence of HIV-1 regulatory factors. Vectors expressing HIV proteins in a Rev-independent fashion may be useful for other purposes, including the construction of novel gene transfer vectors. One major advantage of such vectors might be the improved gene transfer into quiescent cells. Research sponsored in part by the National Cancer Institute, DHHS, under contract No. NO1-CO-46000 with ABL.

1. S. Schwartz, *et al.*, *J Virol* 66, 7176-7182 (1992).
2. S. Schwartz, B. K. Felber, G. N. Pavlakis, *J Virol* 66, 150-159 (1992).

D4-152 RNA Dependent Cleavage of *Gag*p15 by the HIV-1 Protease

NIJING SHENG AND SUSAN ERICKSON-VIITANEN
The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880

The HIV-1 *gag* polyprotein is processed by the virally encoded protease to yield the structural proteins of the virus. One of these structural proteins, *gag*p15, and its protease cleavage products, p7 and p6 are believed to be responsible for the viral RNA binding which is prerequisite for assembly of infectious virions. In order to better understand the interaction between viral RNA, *gag*p15 protein and HIV protease, we have produced *gag*p15 in an *in vitro* transcription/translation system and as an *E.coli* recombinant protein. *Gag*p15 was properly and specifically cleaved to products of p7 and p6 by the viral protease. In addition, the processing at the p7-p6 junction was RNA dependent, whereas other protease cleavage sites within the *gag* precursor were unaffected by the presence of RNA. Removal of RNA prevented the cleavage of p15 precursor by HIV-1 protease, and rescue of protease susceptibility was accomplished by adding back RNA but not single or double stranded DNA. Gel mobility-shift and nitrocellulose filter-binding experiments indicated that the *gag*p15 specifically bound to its mRNA. The binding was not affected by the presence or absence of Zn^{2+} or Mg^{2+}. Using deletion analysis and synthetic oligonucleotides, we have found that a 24-mer RNA oligo is sufficient to rescue protease susceptibility. Mutation of cysteine residues in the first or second zinc finger did not alter the RNA dependent cleavage; however, mutation of three basic residues located between two zinc fingers blocked HIV protease susceptibility. The results support a new role for the interaction of RNA and nucleocapsid-containing *gag* precursors that may have important consequences for virus assembly.

D4-153 DIVERGENT ANTI-HIV ACTIVITY OF DIDEOXY-NUCLEOSIDES IN RESTING AND ACTIVATED CELLS, Takuma Shirasaka, Sudhichai Chokekijchai, Gilles Gosselin*, Jean-Louis Imbach*, and Hiroaki Mitsuya, National Cancer Institute, Bethesda, MD 20892; *Laboratoire de Chimie Bio-Organique, Université de Montpellier II, Montpellier, France

We have found that anti-HIV-1 dideoxynucleoside analogs (ddNs) can be classified into two groups based on the phosphorylation profiles: (i) cell-activation-dependent ddNs including azidothymidine (AZT) and dideoxythymidinene (d4T) that are preferentially phosphorylated and yield higher ratios of ddNTP/dNTP in activated cells than in resting cells, and (ii) cell-activation-independent ddNs including dideoxy-inosine (ddI), dideoxycytidine (ddC), 2'-β-fluorodideoxy-adenosine (F-ddA), and 9-(2-phosphonylmethoxyethyl)-adenine (PMEA) that produce higher ratios of ddNTP/dNTP in resting cells. We have also determined the anti-HIV-1 activity of ddNs by using phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM) and resting PBM (R-PBM) as target cells. The comparative order of *in vitro* anti-HIV-1 activity based on molarity was: AZT > ddC > d4T > ddI, PMEA > F-ddA in PHA-PBM, and ddC > ddI, PMEA, F-ddA, >> AZT, d4T in R-PBM cells. A compound (bis-S-acetylthioethanol-phospho-diester-ddAMP), which bypasses the intracellular mono-phosphorylation step for intracellular delivery of ddAMP, was highly active both in PHA-PBM and R-PBM, suggesting that the first phosphorylation step of ddI is critical for its ultimate antiviral activity. These data should have practical relevance in the design of anti-HIV chemotherapy, particularly combination chemotherapy with ddNs.

D4-154 REGULATION OF CELL SURFACE LEVELS OF THE HIV-1 ENV PROTEIN BY AN INTERNALIZATION SIGNAL SEQUENCE IN gp41, Robert F. Siliciano and Jennifer R. Rowell, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore MD 21205

Some transmembrane proteins are constitutively internalized from the cell surface by receptor-mediated endocytosis, an efficient process that involves clathrin-coated pits. Tyrosine-containing targeting sequences contained within the cytoplasmic domains of these proteins are essential for clathrin-mediated endocytosis. Very little is known about the internalization of the HIV-1 envelope (env) protein from the surface of infected cells or about how internalization might affect virus assembly, cytopathic potential, virulence, or the ability of the host to mount an effective immune response. The cytoplasmic domain of the HIV-1 env protein contains several tyrosines, two of which are in sequence environments that resemble known internalization signals found in other proteins that undergo rapid constitutive endocytosis. To study the endocytosis of the HIV-1 env protein, we have used a novel flow cytometric assay to measure the internalization of the wild type env protein as well as mutant versions of the env protein containing tyrosine to alanine substitutions or deletions in the cytoplasmic domain. We found that the env protein is internalized at a surprisingly rapid rate, comparable to that of surface receptors that undergo constitutive endocytosis such as the transferrin receptor and the LDL receptor. We have shown that substitution of tyrosine 706 dramatically reduces endocytosis of the env protein while substitution of tyrosine 762 has no effect. In addition, truncation of the cytoplasmic tail beyond tyrosine 706 inhibits endocytosis, indicating that sequences other than the tyrosine motifs described may be involved in endocytosis of the env protein. The role of env protein internalization in virus assembly, cytopathicity, and immunogenicity will be discussed.

D4-155 *TRANS*-COMPLEMENTATION BETWEEN LENTIVIRUS VIF PROTEINS. *J.H.M. Simon, J. Peterson, B.E. Meyer, T. Southerling and M.H. Malim. Howard Hughes Medical Institute and Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6148.*

All known lentiviruses, with the exception of equine infectious anemia virus, possess either an established or a putative virion infectivity factor (*vif*) gene. For HIV-1, the 23 kDal Vif protein is expressed from a partially spliced mRNA in a Rev-dependent manner. Expression of this protein is required for productive HIV-1 infection of primary cells derived from human blood and certain immortalized T lymphoid cell lines.

Using retroviral vectors, we have stably expressed the *vif* genes of human, simian, bovine and feline immunodeficiency viruses and of maedi-visna virus in a number of human T cell lines deemed to be permissive, semi- or non-permissive for productive infection by HIV-1 that lacks a functional *vif* gene (HIV-1/Δ*vif*). The respective capacity of each of these Vif proteins to complement a *vif* defect in HIV-1 and SIV was determined in long term culture experiments.

Our preliminary data suggest that the Vif proteins of HIV-1 (NL4-3 molecular clone) and SIV_{mac239}, but not those of any of the non-primate lentiviruses, are able to restore efficient replication to both HIV-1/Δ*vif* and SIV_{mac239}/Δ*vif* in semi- and non-permissive cells. In addition, none of the non-complementing *vif* genes exerted a dominant negative (suppressive) effect on the replication of wild type HIV-1 or SIV_{mac239} in any of the cell lines tested. The implications of these findings will be discussed.

D4-156 **SIV AND HIV-1 NEF ENHANCE VIRAL REPLICATION AND ARE FUNCTIONALLY INTERCHANGABLE**

Elizabeth Sinclair, Peter Barbosa and Mark B Feinberg
Gladstone Institute of Virology and Immunology

As a strategy for evaluating the *in vivo* contribution of HIV *nef* alleles and LTR regulatory sequences to viral pathogenesis, we have generated SIV/HIV chimeras in which the Nef coding and U3 regulatory regions of SIVmac239 have been replaced by corresponding regions from HIV-1/R73 (SIVR7*nef*+). A *nef*-negative version of this construct, in which the 5' end of the HIV-1 *nef* was deleted was also generated (SIVR7Δ*nef*). Nef has been shown to confer a replication advantage to HIV-1 in primary human T cells and macrophages by enhancing viral infectivity. We therefore used this tissue culture system to compare the phenotype of the chimeric viruses with *nef*+ (SIV*nef*+) and *nef* - (SIVΔ*nef*) variants of SIVmac239. SIV*nef*+ displays a positive growth advantage in comparison with SIVΔ*nef* in this tissue culture system, as has been previously reported for HIV-1. SIVmac239 displays intermediate replication kinetics to the *nef*+ and Δ *nef* variants. SIVR7*nef* +and SIV*nef*+ display similar replication kinetics in primary human PBMC infections. These infections are currently being repeated in Rhesus macaque PBMCs. These data indicate that SIV Nef, like that of HIV-1, does enhance virus replication in primary cells in tissue culture. In addition, HIV-1 Nef can functionally substitute for SIV Nef. Recombinant SIV viruses containing HIV *nef* and/or regulatory elements should permit the experimental analysis of the in vivo contribution of HIV-1 Nef and LTR functions to the pathogenesis of AIDS.

D4-157 **MOLECULAR BASIS FOR CELL-CYCLE DEPENDENT HIV REPLICATION**

M. Stevenson*, A Bukrinskaya*, A. Ghorpade*, N. Heinzinger*, R. Lewis*, L. Ratner#, A. Ragland*, *University of Nebraska Medical Center, Omaha, NE, #Washington University, St. Louis, MO

Virus sequestration within cells of macrophage lineage is a central feature of lentivirus infections. Macrophages are terminally differentiated non-dividing cells. Although onco-retroviruses require host cell mitosis for nuclear localization of viral DNA and provirus establishment, lentiviruses such as HIV have evolved specific mechanisms which allow provirus establishment in non-dividing cells. This property of HIV is governed by the nucleophilic virion proteins *gag* matrix (MA) and Vpr which facilitate nuclear localization of viral DNA in non-dividing cells. HIV variants containing mutations in *gag* MA and Vpr nucleophilic sequences are attenuated for replication in macrophages but are unaffected in ability to replicate in synchronized proliferating host cells such as activated lymphocytes.

Our ongoing studies have now identified that phosphorylation of *gag* MA plays a central role in the functioning of this protein as a nuclear import factor for HIV. Analysis of *gag* MA within virus particle and within preintegration complexes isolated from the nuclear compartment of HIV infected cells has revealed differential phosphorylation of *gag* MA as it undergoes nuclear import. Peptide mapping and mutagenesis studies revealed Tyrosine phosphorylation within the virus particle. Phosphorylation of *gag* MA proceeds in highly purified virus preparations suggesting that Tyrosine kinase is specifically packaged within the virus particle. Upon entry and nuclear targeting of the viral preintegration complex, *gag* MA is almost exclusively phosphorylated on Serine. Use of Tyrosine kinase inhibitors provides evidence that phosphorylation is required in order for *gag* MA to exert its nucleophilic role in transport of viral nucleic acid to the host cell nucleus. These studies reveal novel molecular targets for the intervention of HIV-1 infection at a stage prior to provirus establishment.

D4-158 NEGATIVE REGULATION OF HIV-1 RNA SPLICING: EVIDENCE FOR A CELLULAR FACTOR BINDING TO AN EXON SPLICING SILENCER IN tat EXON 2.

C. Martin Stoltzfus and Brad A. Amendt, Department of Microbiology, Univesity of Iowa, Iowa City, IA 52242

HIV-1 pre-mRNA splicing is regulated in order to maintain pools of unspliced and partially spliced viral RNAs as well as the appropriate levels of multiply-spliced mRNAs during virus infection. We have previously described an element in tat exon 2 that negatively regulates splicing at the upstream tat 3' splice site 3. We have further localized this element, an exon splicing silencer (ESS), to a highly conserved 20 nt sequence spanning the C-terminal vpr and N-terminal tat conding sequences. An additional negative acting region has been found within the second tat/rev coding exon (tat/rev exon 3) that has sequence motifs in common with the ESS element in tat exon 2. This sequence is juxtaposed to a purine-rich exon splicing enhancer element to form a bipartite element which regulates splicing at the upstream tat/rev 3' splice site. To further study the mechanism of regulation by the ESS element in tat exon 2, we used an in vitro splicing system to examine the splicing of HIV-1 RNA substrates containing or lacking the element. No detectable spliceosomes were formed using splicing substrates containing the ESS element. In vitro splicing competition assays indicated that a cellular factor or factors interact with the ESS element to negatively regulate splicing at tat 3' splice site 3. UV-irradiation of the splicing reaction demonstrated specific cross-linking of two cellular proteins (approximately 70 and 90 Kd) to radioactive substrates containing the ESS element. We propose that the ESS binding factor(s) interferes with splicing at an early stage of spliceosome assembly.

D4-159 **CHARACTERIZATION OF A GLIAL CELL-SPECIFIC DNA-PROTEIN COMPLEX FORMED WITH THE HTLV-I ENHANCER;**

Renee Wessner, Maribeth Tillmann-Bogush, and Brian Wigdahl, Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA 17033

Characterization of the cellular transcription factors which interact with the human T cell lymphotropic virus type I (HTLV-I) long terminal repeat (LTR) is essential to dissecting the mechanisms involved in viral transcription that may be pertinent to the oncogenic and neuropathogenic processes associated with HTLV-I infection in both the immune and nervous systems. Electrophoretic mobility shift (EMS) analyses utilizing double-stranded (ds) oligonucleotides homologous to each of the 21 bp repeats and nuclear extracts derived from selected cell lines of lymphocytic, neuronal, and glial origin have demonstrated the differential binding of cellular factors to each of the three 21 bp repeats. Specifically, both a glial cell-specific DNA-protein complex (designated GCS) and 21 bp repeat-specific DNA-protein complexes (designated U1 and U2) were detected. The formation of the GCS DNA-protein complex may involve members of the activating transcription factor (ATF)/cAMP-response element (CRE) binding protein (CREB) family while the formation of the U1 and U2 DNA-protein complexes may involve an Sp1-related factor. In addition, three ATF/CREB-related DNA-protein complexes common to each individual 21 bp repeat (designated C1-C3) were also detected. However, we demonstrated that the abundance of the C1 and C2 DNA-protein complexes detected with the individual 21 bp repeats and glial cell nuclear extract was significantly lower when compared to that obtained with lymphocyte, monocyte, or neuronal nuclear extracts. We also have demonstrated that the ATF/CREB factors participating in formation of the GCS DNA-protein complex are distinct from those participating in formation of the C1-C3 DNA-protein complexes. Based on nucleotide sequence requirements and immunoreactivity, we suggest that the GCS DNA-protein complex may contain a novel glial cell-specific ATF/CREB-related factor(s). Furthermore, we demonstrate that the CRE modulator (CREM) protein in conjunction with CREBP1 interact with each of the three 21 bp repeats to form the C3 DNA-protein complex. However, the abundance of the C3 DNA-protein complex formed utilizing the promoter proximal repeat is dramatically lower compared to either of the other two 21 bp repeat elements. We suggest that the differential binding of cellular factors to each 21 bp repeat element may play a role in LTR-directed transcription in either the immune or nervous system.

D4-160 NUCLEOTIDE SEQUENCE REQUIREMENTS AND THE ROLE OF SP1 IN FORMATION OF AN HTLV-I PROMOTER PROXIMAL REPEAT-SPECIFIC DNA-PROTEIN COMPLEX; Brian Wigdahl, Maribeth Tillmann-Bogush, and Renee Wessner, Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA 17033

Human T cell lymphotropic virus type I (HTLV-I) encodes the trans-activator protein, Tax, which facilitates viral transcription from three 21 bp repeat elements within the U3 region of the long terminal repeat (LTR). Examination of the basal factors interacting with the 21 bp repeat elements through electrophoretic mobility shift (EMS) analyses has demonstrated the formation of DNA-protein complexes common to each of the three 21 bp repeat elements designated as C1-C3, as well as a DNA-protein complex specific to the promoter proximal repeat designated as U1. These studies have indicated that the individual 21 bp repeat elements are not identical with respect to the cellular factors with which they interact. To investigate the nucleotides specific to the promoter proximal 21 bp repeat required for formation of the U1 DNA-protein complex, nucleotide scanning mutational analyses within the promoter proximal 21 bp repeat were performed. EMS analyses utilizing a series of mutated promoter proximal repeat elements have demonstrated that the second and third non-conserved nucleotides between conserved domains B and C are required for formation of both the U1 and the C1-C3 DNA-protein complexes. However, mutations in the first non-conserved nucleotide resulted in the specific formation of the C1-C3 DNA-protein complexes and the complete abrogation of the U1 DNA-protein complex while mutations in the fourth non-conserved nucleotide resulted in the specific formation of the U1 DNA-protein complex and the complete abrogation of the C1-C3 DNA-protein complexes. Competition EMS analyses have indicated that formation of the U1 DNA-protein complex is dependent on a factor capable of recognizing an Sp1 consensus sequence binding site. We also demonstrate utilizing supershift EMS analyses that a member of the Sp1 family of transcription factors is a critical component of the U1 DNA-protein complex. In addition, the interaction of Sp1 with the promoter proximal repeat to form the U1 DNA-protein complex occurs only in the presence of nuclear extract suggesting that this interaction requires the activity of an additional component(s) provided by the nuclear extract. Based on these observations, we suggest that the differential binding of cellular factors to each of the three 21 bp repeat elements may play a role in basal as well as Tax-mediated LTR-directed transcription.

D4-161 NATURAL ENDOGENOUS REVERSE TRANSCRIPTION (NERT) OF HUMAN IMMUNODEFICIENCY VIRUS TYPE I (HIV-1). Hui Zhang[1], Geethanjali Dornadula[1], Masahiro Niikura[1], Omar Bagasra[1], Bernard J. Poiesz[2], Roger J. Pomerantz[1].

[1] Dorrance H. Hamilton Laboratories, Section of Molecular Retrovirology, Division of Infectious Diseases, Jefferson Medical College, Thomas Jefferson University, 329 JAH, 1020 Locust St., Philadelphia, PA, 19107; and [2]Dept. of Medicine and Microbiology, SUNY Health Science Center at Syracuse, NY, 13210.

As detergents have traditionally been used to permeabilize the retroviral envelope, endogenous reverse transcription has long been considered an artificial process which only mimics the natural reverse transcription occuring in the early phase of the retroviral life-cycle. Recently, several groups unexpectedly observed that the endogenous reverse transcription of HIV-1 can take place in situ without treatment with a detergent. Some studies credited this phenomenon to a possible perturbation of the viral envelope during the process of virus purification or freezing and thawing. This phenomenon was carefully examined by our laboratories with different isolation strategies, including isolating the HIV-1 virions after the completion of endogenous reverse transcription. We demonstrated that this phenomenon, for HIV-1, is not caused by an artificial process and therefore, we have termed this process "natural endogenous reverse transcription" (NERT). NERT can also occur in the natural environment for HIV-1 virions; that is human physiological fluids, which have been demonstrated in studies utilizing either quantitative PCR or direct ^{32}P-labelling of de novo synthesized viral DNA. Small amounts of substrates for viral DNA synthesis, deoxyribonucleoside triphosphates (dNTPs), can be detected in the blood plasma and seminal fluid of both HIV-1-infected and -seronegative individuals, which are efficient to support NERT. Further, some physiological substances, such as spermine/spermidine, which are detected in specific extracellular milieus (e.g. seminal fluid), can also potently augment NERT, in a low concentration of dNTPs. Based upon our observations, we conclude that NERT is a very important process for HIV-1, accounting for the intravirion reverse transcripts identified both in vitro and in vivo by several groups, and likely plays an important role for viral intra- or inter-host transmission and pathogenesis.

Mechanisms of Immunopathogenesis, viral Load, Cytokines, SCID/hu Model

D4-200 T CELLS FROM HIV-1 TAT TRANSGENIC MICE SHOW ALTERED CYTOKINE EXPRESSION,

David Abraham, Daniel Pennington, Colin Miles, Sharon Jenkins, Hugh Brady and Elaine Dzierzak, Laboratory of Gene Structure and Expression, National Institute for Medical Research, London NW7 1AA U.K.

Examination of the interaction between HIV regulatory gene products and the host immune system is fundamental in understanding the pathogenesis of HIV and could reveal possible targets for therapeutic intervention in the treatment of the aquired immunodeficiency syndrome (AIDS). The HIV Tat gene is a potential candidate for this type of strategy. Transgenic mice can be used to investigate the in vivo effects of Tat on the developing immune system and on cellular gene expression . We have thus generated transgenic mice harbouring the HIV-1 Tat gene under the control of the human CD2 regulatory elements. This provides copy number dependent, position independent expression of the transgene within the T cell compartment. The CD2-Tat transgenic mice show no signs of aberrant thymic development and have normal levels of T cell subsets in the thymus and peripheral lymphoid organs. However T cells from the transgenic animals exhibited an increase in proliferation in response to antigen. In addition, activated transgenic T cells showed increased levels of TNF-β mRNA and biologically active cytokine. But TNF-α mRNA levels remained unchanged. Although our CD2-Tat transgenic mice are now in excess of one year old we find no evidence of dermal lesions. We are currently examining the effect of this transgene on the level of cytokine production in response to a range of stimuli, which may provide insight into the host immune factors mediating pathogenesis in HIV infection.

D4-201 HIV-1 INFECTED CELLS IN HUMAN LYMPH NODES ARE NOT APOPTOTIC Banda, N. K., Tudor-Williams, G., Kupfer, A., Cotton M. F., and Finkel, T. H. National Jewish Center, Denver, CO 80206

Lymphoid organs play an important role in the pathogenesis of human immunodeficiency virus infection. There is increasing evidence from recent studies that lymph nodes harbor a large viral reservoir during the asymptomatic stage of the infection, although the infectious viral load in the peripheral blood is low. Since lymph nodes are also the organs where T and B cells cluster to respond to foreign invaders, we hypothesized that activation-induced apoptosis might occur in lymph nodes out of proportion to PBMCs in *in vivo* HIV infection. While it has been reported that HIV kills CD4 T cells by direct lysis, our current studies of lymph nodes using nick translation of apoptotic DNA, *in situ* hybridization and confocal microscopy show that productive HIV infection does not induce apoptosis in the vast majority of infected cells. In contrast, bystander T cells that are not infected with HIV undergo apoptosis. These non-infected T cells are present either nearby productively infected cells or in regions of diffuse, granular HIV RNA signal, and there is a correlation between the number of HIV infected cells and the number of apoptotic cells. In sum, our data suggest that HIV infected cells are not apoptotic in the lymph nodes. By analogy with some other viral infections, and based on studies of *tat* and *nef*, it is possible that HIV encodes gene products that protect T cells from apoptosis. Secondly, our data suggest that HIV kills innocent bystander T cells by an indirect mechanism. We are currently investigating the phenotype(s) and mechanism(s) of bystander apoptosis in HIV infected lymphoid organs.

D4-202 **SIV/HIV DESTRUCTION OF ANTIGEN PRESENTING DENDRITIC CELLS OF GERMINAL CENTERS.**

P. Biberfeld[1], I. Stahmer[1], M. Popovic[2], R. Mesquita[1], M. Ekman[1], G. Biberfeld[3] [1]Immunopathology Lab., and, [2]Dept. of Immunology, Karolinska Institute, [3]Dept. of Immunology, Swedish Institute for Infectious Disease Control, Stockholm, Sweden

AIMS: Antigen presenting follicular dendritic cells (FDC), macrophages (MAC) and B/T lymphocytes (Ly) in the spleen of SIVsm infected monkeys were studied *in situ* and *ex-vivo* for latent and productive SIV infection by PCR, RT-PCR, *in situ* hybridization, immunocytochemistry and *in vitro* culture assays for infectious virus.
OBSERVATIONS: PCR showed in limiting dilution analysis comparable or higher levels of proviral DNA in the FDC enriched cell fractions in comparison to the MAC and Ly cell preparations. In contrast, 100-fold higher level of spliced *env* mRNA was expressed by the FDC compared to the Ly and MAC fractions respectively. T cell contamination assayed by RT-PCR of TCR-cβ chain was marginal in the FDC fractions and could not account for the observed differences in *env* transcripts. In situ hybridization for multiple spliced *tat/rev* mRNA transcripts also demonstrated association of SIV messages with FDC rather than contaminating lymphocytes. MAC expressed low levels of *tat/rev* mRNA as also indicated by the RT-PCR results. In co-cultures of FDC with blood donor macrophages, higher expression of viral proteins and RT activity were found in the FDC fraction compared to the lymphocyte and MAC fractions. Studies of monkeys in different stages of disease progression showed association of *env* transcripts with FDC already at early stages of disease, prior to development of immunosuppression and lymphadenopathy. The histological destruction of the FDC network was associated with a decrease in recoverable FDC by fractionation.
CONCLUSIONS: The mechanism(s) resulting in the destruction of the antigen presenting FDC network and germinal centers during SIV/HIV infection are not well understood. The present results indicate that in vivo infection of FDC by SIV can occur already at early stages of infection and therefore may have important direct and/or indirect (CTL?) cytocidal effects.

D4-203 CLEARANCE OF CD3$^-$,CD8$^+$,Bcl-2^{low} LYMPHOCYTES UNDERGOING APOPTOSIS BY ACTIVATED MACROPHAGES IN LYMPH NODES OF HIV-1 PATIENTS, Margarita Bofill, Nicola Borthwick, Wendy Gombert, Arne N.Akbar, George Janossy, Department of Clinical Immunology. Royal Free Hospital School of Medicine. Rowland Hill Street, London NW3 2PF. UK. HIV-1 infection can present with Persistant Generalized Lymphoadenopathy. In this report we have focused on the expanded macrophage and CD8 populations. The principal findings are that: 1) The number and size of the macrophages of the paracortical areas (PC) in HIV-1 infected lymph nodes (LN) (n=22) was increased when compared to healthy controls (n=10). (N° of macrophages per $4.2x10^4$ μm^2 : Controls 29.4 ± 2.13; HIV-1$^+$ 47.8 ± 2.18. Mean macrophage area in μm^2: Controls 135.4 ± 12.64; HIV-1$^+$ 254.2 ± 9.66). 2) While in the controls the macrophages were thin and elongated with the typical appearance of tissue histiocytes, in the HIV-1$^+$ samples the cells were rounded, enlarged and tingible bodies were seen in the cytoplasm of these cells. 3) Apoptotic cells (detected by terminal deoxynucleotidyl transferase (TdT)) were seen in the PC of the HIV-1 LN but were absent in the controls. 4) We further characterized the cells that undergo apoptosis in the paracortical areas of HIV infection by double staining of apoptotic cells by TdT and CD8 and showed that at least a proportion of the apoptotic cells seen in the PC belonged to the CD8 population. As the apoptotic process takes only 4 hours the detection of even small numbers of apoptototic cells in tissues indicates a high cell turnover. Nevertheless, too few apoptotic cells are detected to make an accurate and quantitative assessment. Therefore we looked for other earlier markers of apoptotisis namely presence or absence of Bcl-2 and Fas. 5) We found that a proportion of CD4 cells (in the samples where CD4 was detectable) expressed low levels of Bcl-2 (Controls: 76.6 ± 9.63; HIV-1$^+$ 76.4 ± 3.24) but a much higher proportion of CD8 cells lacked this protein (Controls 82.3 ± 3.11; HIV-1$^+$ 35.2 ± 5.20). That was mirrored by an increase in Fas expression in such cells. Our findings suggest that there is a high turnover of CD8 primed lymphocytes throughout HIV-1 infection.

D4-204 **INCREASE IN HIV-1 REPLICATION FOLLOWING VACCINATION AGAINST OPPORTUNISTIC INFECTIONS**

B. Brichacek*, S. Swindells*, S. Pirruccello*, J. W. O'Brien#, E. Janoff#, R. Kagaruki*, W. Robertson* and M. Stevenson*, *University of Nebraska Medical Center, Omaha, NE, #Department of Veteran Affairs Medical Center, Minneapolis, MN

CD4$^+$ T lymphocytes are one of the primary targets for HIV-1 infection and are important in the maintenance of virus burden. Although HIV-1 can infect both quiescent and activated CD4$^+$ T lymphocytes, integration of viral DNA and productive viral replication requires activation of the T-cell. Since the number of circulating activated CD4$^+$ T lymphocytes is rate limiting *in vivo*, agents, such as foreign antigens, through stimulation of the immune system, could elicit activation of CD4$^+$ lymphocytes and thus influence viral replication and virus burden.

To determine the influence of immunization against opportunistic infections on HIV-1 replication in HIV-1 seropositive individuals, we measured concentrations of extracellular HIV-1 particles in peripheral blood of these patients before, during and after vaccination. Two groups (18 patients) of asymptomatic HIV-1 seropositive individuals were studied. The first group received a subunit vaccine against influenza virus; the second group was immunized with a 23-valent pneumococcal vaccine (12 patients). Virus burden was determined by quantitation of virion associated genomic RNA in plasma.

Our results demonstrate increase in virus burden in majority of individuals after vaccination. Substantial increase in virus burden in plasma was observed by one week post-immunization and correlated with antibody response to the vaccine and with activation of CD4$^+$ cells in peripheral blood. The change in extracellular HIV-1 particles concentration in plasma was also associated with a change in the number of newly infected cells as monitored by detection of nucleus-specific two-long-terminal repeat (2-LTR) circle forms of HIV-1 DNA.

D4-205 VIRAL LOAD AND SURROGATE MARKERS IN A PROSPECTIVE STUDY OF ASYMPTOMATIC HIV-1 INFECTED PERSONS.

S.Bruisten*, J.Frissen#, J.Huisman*, H.Weigel#, P.van Swieten*, E.de Vries#, R.Regez#, R.Harrigan&, M.Tersmette§, P.Reiss§.
*Central Lab of the Netherl Blood Transfusion Service, Amsterdam, The Netherlands #Onze Lieve Vrouwe Gasthuis, Dept Internal Medicin; ßAcademic Medical Centre, Amsterdam, The Netherlands; &Wellcome Research Laboratories, Beckenham, UK

Asymptomatic HIV-1 infected persons visit the clinic every 8 weeks and blood samples are drawn and send to the lab under code. Criteria for intake were: clinically long term stable, >200 CD4+ cells per mm^3, no antiviral therapy in the preceding 6 months. The aim of this study is to find the best parameter for clinical progression and for start of antiviral therapy. Additionally, this study provides baseline data for antiviral therapy studies. The data gathered are: clinical events, viral DNA and RNA load, HIV phenotype, CD4+ cell count, p24 antigen and ß2M. The HIV-1 proviral DNA load is determined using competitive *pol* PCR on nucleic acid isolated from PBMC; the viral RNA is determined by performing a slightly modified version of the quantitative Roche^R PCR on nucleic acid from plasma. The study is still ongoing. Of the 42 study participants, one person died and 7 were lost to follow up (2 years). Only two persons showed progression to AIDS, 3 started antiviral therapy because of a CD4+ count below 350/mm^3, and 29 are stably asymptomatic. The surrogate markers and the viral load markers showed more diversity. The HIV phenotype of 26 of 34 persons was stably non syncytium inducing (NSI). In 5 of 34 a switch from NSI to SI occurred and 3 of 34 persons were stably SI. In most persons the levels of RNA in plasma and DNA in PBMC are positively correlated and stable throughout the observation period. Persons (n=14) with stable low viral loads also show normal ß2M levels (13/14), are p24 antigen negative and have an NSI phenotype (13/14). On the other hand, persons (n=16) with high or increasing viral loads show high or rising ß2M titers (10/16), become p24 antigen positive (7/16), and switch to SI phenotype (7/16). From 4 other persons the observation period is not yet long enough.

D4-206 HIV ON FOLLICULAR DENDRITIC CELLS (FDC) IS HIGHLY INFECTIOUS, Gregory F. Burton, Sonya L. Heath, J. Grant Tew and John G. Tew. Dept. of Microbiology & Immunology, Virginia Commonwealth Univ, Richmond, VA. 23298.

HIV is found in the form of immune complexes on FDC in lymphoid follicles. In this study we sought to test the hypothesis that HIV in the FDC associated immune complexes is infectious and plays an important role in viral pathogenesis. To test this, FDC from uninfected tonsils were pulsed with HIV immune complexes, washed to remove unbound virus and cultured with activated CD4+ T cells. PCR analysis was used to detect infection. HIV immune complexes were formed using HIV and serum from a seropositive patient. These were incubated with sorted FDC, washed and cultured with superantigen activated (SEE) autologous T cells. Infection was present in cultures with HIV-bearing FDC and activated T cells but not in controls. To ensure that infection was transferred on FDC and not by contaminating CD4+ cells and that FDC did not need to be infected to transfer the virus, we used xenogeneic (murine) FDC in place of human FDC. Infection was again observed where HIV immune complexes on FDC were cultured with activated T cells but not with controls of FDC plus virus alone. These results indicate that FDC retained HIV immune complexes can infect activated T cells. Since many HIV seropositive individuals make neutralizing antibody, we sought to determine if FDC could convert neutralized HIV into an infectious form. Several doses (pg to mg) of neutralizing antibody were used to form immune complexes with HIV and these were cultured with susceptible T cells ± FDC. While no infection was seen in cultures of HIV immune complexes and activated T cells, infection was clearly observed when FDC were present. These data indicate that HIV complexes on FDC are infective and that FDC can convert neutralized HIV into infectious virus. This may help explain why HIV infected individuals with high levels of neutralizing antibody still have ongoing infection and supports the hypothesis that FDC may play an important role in HIV pathogenesis. Supported by NIH grant AI32406.

D4-207 HIV-SPECIFIC CTL ACTIVITY IN LYMPHOID ORGANS: RELATIONSHIP WITH DISEASE PROGRESSION.

Butini L., Regnery C.M., Caporaletti T., Maltoni A., Pantaleo G.*, Fauci A.S.*, Montroni M. Servizio di Immunologia Clinica, Ancona, Italy; *LIR, NIAID, NIH, Bethesda, USA

Background: class I MHC-restricted HIV-specific cytotoxic T lymphocytes (CTL) appear in the peripheral blood (PB) very early during infection and are detectable in the majority of the patients in the asymptomatic phase of the disease. CTL activity is supposed to exert a protective effect against viral spreading; however, CTL-mediated mechanisms have been proposed as co-responsible for the damage of some tissues (i.e. lung and CNS) observed in HIV-infected individuals. Lymphoid tissues (LT), the major reservoir for HIV, are the anatomical site where the immune response occurs and harbor the great majority of lymphocytes in the human body; disruption of LT architecture is associated with impairment of immune response and disease progression.
Objective: to evaluate HIV-specific CTL activity in LT and PB from the same patients in association with lymph node histopathological observation and clinical and immunological follow-up.
Methods: a standard 51Cr release assay as well as Limiting Dilution Analysis (LDA) of either activated or precursor CTL have been performed.
Results: eleven patients have been studied. High PBMC and LTMC-mediated CTL activity was detected in two patients with rapidly progressive disease and high-degree FDC network degeneration. In contrast, absence of LTMC-mediated CTL activity and maintenance of lymph node architecture were found in a long-term non-progressor (LTNP). Preliminary LDA experiments show a frequency of activated CTL tenfold higher in the LT of one patient with progressive disease vs. the LTNP. Phenotypic analysis showed higher values of CD8+DR+ cells in LTMC compared to PBMC. A positive correlation was present between the percentage of CD8+DR+ effector cells and the amount of CTL activity.
Conclusions: CTL activity is probably crucial to the containment of HIV spreading during initial HIV disease. However, 0progression of HIV disease is possible even in the presence of high levels of CTL activity, both in the PB and in the LT. Activated CTL may play a role in the disruption of the architecture of lymphoid organs in patients showing progressive disease.

D4-208 **PATHOGENESIS OF SI AND NSI HIV-1 PATIENT ISOLATES, MOLECULAR CLONES AND CHIMERAS IN SCID-hu MICE.** David Camerini*, Beth D. Jamieson, Jerome A. Zack and Irvin S.Y. Chen, Dept. of Microbiology, University of Virginia, Charlottesville, VA 22908* and Depts. of Microbiology & Immunology and Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

We have used 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. Sequential NSI and SI HIV-1 patient isolates and molecular clones of HIV-1 were assayed by injection into conjoint human thymus/liver organs established in SCID mice. Flow cytometric analyses indicated that all 15 thymus/liver implants infected with the SI molecular clone, HIV-1_{NL4-3}, were depleted of CD4-bearing thymocytes by 6 weeks post-infection, while only 4 of 13 implants infected with the NSI molecular clone, HIV-1_{JR-CSF}, had lost these cells. Similarly, 6 of 9 thymus/liver implants infected with SI patient isolates, from three patients who progressed rapidly to AIDS, showed depletion of CD4-bearing thymocytes. In contrast only 1 of 15 implants infected with earlier NSI isolates from the same patients during their asymptomatic stage and one long term survivor, were depleted of CD4+ thymocytes by six weeks post-infection. Nevertheless, the viral load in infected implants, determined by quantitative PCR, was similar for sequential patient isolates whether NSI or SI, but 10 to 100 fold lower for virus isolated from a long term survivor. These data suggest a significant difference in the pathogenic potential of NSI and SI isolates of HIV-1 and support the idea that disease progression in some patients may result from the appearance of more pathogenic HIV-1 species. We have created chimeric HIV-1_{JR-CSF} strains substituted with the V1 to V3 region of the *env* genes of two NSI and two SI patient isolates and have injected them into human thymus/liver grafts in SCID-hu mice. We are currently examining whether these V1 to V3 HIV-1 *env* fragments are sufficient to determine pathogenesis in the SCID-hu mouse.

D4-209 MIP-1α IS INDUCED BY AND IT INHIBITS HIV INFECTION OF BLOOD-DERIVED MACROPHAGES. B. Canque, M. Rosenzwajg, and J.C. Gluckman. CNRS URA 1463, hôpital Pitié-Salpêtrière, 75651 Paris Cedex 13, France.

Cytokines produced by Macrophages (MA) regulate immune responses and hematopoiesis. It is assumed that HIV may affect cytokine (TNF-α, IL-6) production by MA, though conflicting results have also been reported. Conversely, cytokines may enhance (IL-3, IL-4, M-CSF, GM-CSF, TGF-ß1) or inhibit (IL-10, IL13, IFNs) HIV replication by MA. MIP-1α is a chemokine that also influences hematopoiesis. We investigated production of MIP-1α by blood-derived MA infected by HIV-1_{Ba-L} or HIV-1_{Ada}. Viral p24 and cytokines in culture supernatants were measured by ELISA. Compared to controls, levels of MIP-1α, but not of IL-1, IL-6, GM-CSF, G-CSF, TNF-α nor TGF-ß1, increased from 2- to >10-fold at peak p24 production; levels of other chemokines (RANTES, IL-8) did not change. No modification of already high MIP-1α levels was noted with normal blood lymphocytes replicating either HIV-1_{Ba-L} or HIV-1_{LAI}. MIP-1α increased production by HIV-infected MA was blocked with ≥10 µM Zidovudine added 24 h post-infection, which indicates involvement of virus replication in this effect. Alternatively, incubating MA with ≥10 pg/ml of recombinant huMIP-1α before adding the virus, and continuously thereafter, resulted in a ≥90% inhibition of virus replication that was abrogated by anti-MIP-1α IgG. Virus inhibition by MIP-1α was time-dependent, since it was no more noted when MIP-1α was added >1 day post-infection. MIP-1α did not affect HIV infection of normal blood lymphocytes. MIP-1α treatment did not affect MA cell viability nor expression of CD4 or other membrane markers. It did not modify production of cytokines and chemokines, but for TNF-α the protein and mRNA levels of which were then decreased. Thus, HIV infection of MA promotes MIP-1α production, which may then act as a chemo-attractant for HIV-susceptible CD4+ lymphocytes and which may also be involved in some aspects of impaired hematopoiesis due to HIV. Alternatively, MIP-1α antiretroviral activity makes it a candidate molecule for the suppression of HIV infection within monocytes and MA in vivo.

D4-210 **Early thymic epithelial and T cell alterations in SCID-Hu Lung/Liver/Thymus mice after progressive and systemic HIV-1 infection**

J.-Y. Cesbron[1], M. Raphael[2], H. Agut[3], P. Guiet[2], M. Grandadam[3], D. Canddotti[3], P. Grenot[2], F. Puech[4], and B. Autran[2]

[1]INSERM U415-Institut Pasteur Lille (France); [2]CNRS URA 625 Groupe Hospitalier Pitié-Salpétrière Paris (France); [3]CNRS EP 57 GH Pitié-Salpétrière; [4]Service de Gynécologie Obstétrique CHUR Lille.

The SCID-Hu mice model already allowed studies on the ongoing HIV induced CD4+ T cell depletion after direct viral inoculation in the thymic implant. However, it might not be representative of a systemic and progressive infection. The present study was designed both to develop a systemic and multifocal HIV-1 infection in SCID mice and to investigate the consequences of a systemic viral infection for thymic T cell maturation. Thus, we engrafted both human foetal thymus/liver coimplants and intraperitoneal fragments of autologous lung in SCID mice. The recovered lung tissue bearded close resemblance to normal human lung, both in the bronchiolar and in the alveolar compartments. The thymus human immune reconstitution appeared similar to the normal thymus with (i) an appropriate distribution of immature (CD1a,b,c, CD4, CD8) or mature thymocytes (CD4+CD8-,CD4-CD8+,CD3+, and (ii) a normal proliferation capacity (CD3 +CD28, CD3 + IL-2 costimulation).

A persistent productive HIV infection was obtained both in lung macrophages and in thymic T cells after viral intraperitoneal inoculation of 2 cell-free HIV-1 primary isolates, with a dose-dependant efficiency. Using 10,000 to 20,000 TCID50, 100% human lung or thymus recovered issues were found positive for viral infection (600 to 50,000 HIV-1 provirus copies per 10^6 thymic cells). The thymic abnormalities appearing before depletion of mature CD4+CD8- T cells were: (i) a massive CD8+ T cell amplification and (ii) a depletion of the immature CD1+4+8+T cells. The proliferative capacity of infected thymocytes to CD3+IL2 or CD3+CD28 costimulation was decreased in each case, even in the absence of CD4+ depletion, suggesting early functional abnormalities. Histological analyses showed that the macrophage compartment was preserved while the KL1+ thymic epithelial cells were amplified, in the cortex. This thymic epithelial amplification was observed whatever the intensity of the T cell depletion.

This systemic and multifocal HIV infection in SCID-Hu^{LLT} mice allowed to detect early thymic abnormalities involving both epithelial cells and immature thymic T cells. This model should be relevant for the analysis of AIDS physiopathology.

D4-211 RESTING CD4+ T CELLS WITH INTEGRATED PROVIRUS DO NOT ACCUMULATE UNTIL LATE IN HIV-1 INFECTION, Tae-Wook Chun, Diana Finzi, Karen Chadwick, Joseph Margolick and Robert F. Siliciano, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore MD 21205

It is generally presumed that the potential of HIV-1 to establish latent infection at the cellular level contributes in a fundamental way to its capacity to persist for long periods of time in the host and to avoid eradication by immunologic effector mechanisms such as virus-specific CTL. There are two possible forms of latent infection of $CD4^+$ T cells. A labile preintegration form of latency is observed following infection of quiescent G_0 $CD4^+$ T cells. In principle, a second, more stable form of latency may occur in T cells that have undergone integration of proviral DNA. Post-integration latency may be established *in vivo* if productively infected, activated $CD4^+$ T cells return to a resting G_0 state without succumbing to viral cytopathic effects or host cytolytic mechanisms. A clear understanding of AIDS pathogenesis requires an analysis of how the viral load in $CD4^+$ T cells is distributed between three potential states: pre-integration latency in resting $CD4^+$ T cells, productive infection in activated $CD4^+$ T cells with integrated provirus, and post-integration latency in resting memory $CD4^+$ T cells. In this study, we provide direct quantitative *in vivo* evidence for the existence of the third state, latently infected resting $CD4^+$ T cells with integrated provirus. To do, we have developed rigorous cell purification strategies that allow isolation of very pure populations of resting $CD4^+$ T cells. With these purified populations, we have defined the molecular state of the provirus in resting T cells using a novel assay that unambiguously distinguishes unintegrated and integrated HIV-1 DNA. Our results provide direct evidence that latent integrated provirus is present in resting G_0 T cells. Surprisingly, this reservoir is found in individuals later in the course of infection but is not readily detected in healthy asymptomatic individuals early in infection. Thus, resting T cells with integrated provirus do not accumulate early in HIV-1 infection. These results highlight the importance of targeting other reservoirs of HIV-1 infection, including the pre-integration state and persistent non-cytopathic infection of macrophages.

D4-212 IMMUNOLOGIC ANALYSES OF HIV-INFECTED PROGRESSORS AND NON-PROGRESSORS. M. Clerici, L. Meroni$^+$, C. Balotta$^+$, E. Ferrario, C. Riva$^+$, D. Trabattoni, A.L. Ridolfo$^+$, M.L. Villa, G. M. Shearer*, M. Moroni$^+$and M. Galli$^+$. Cattedra di Immunologia and $^+$Clinica Malattie Infettive, Universita' degli Studi di Milano, Italy; *Experimental Immunology Branch, NCI, NIH, Bethesda, MD, 20892, USA

We analyzed cytokine production and surface markers expression of peripheral blood mononuclear cells (PBMC) of 26 human immunodeficiency virus type-1 (HIV) seropositive (HIV^+) individuals who had been infected with HIV for more than 8 years and showed different patterns of disease progression. Thus, 15 of the patients had remained asymptomatic with a CD4 count higher than 500mm^3 and were considered to be long time non progressors (LTNP), whereas 11 mostly symptomatic patients showed a reduction of the CD4 counts (> 500/mm^3 ---> < 400/mmc) in the same period, and were considered as patients with progressive HIV infection (PI). None of the patients had been treated with antiretroviral therapy prior to the study. 14 HIV^- individuals of comparable age and sex were utilized as controls. In vitro production of interferon gamma and interleukin-2 (IL-2)(type 1 cytokines), and of interleukin-4 (IL-4) and interleukin-10 (IL-10)(type 2 cytokines) was evaluated by antigen-or mitogen-stimulated PBMC. We report that: 1) both groups of HIV^+ patients produce lower amounts of IL-2 than the HIV^- controls; 2) type 2 cytokines production is comparable in HIV^- and LTNP patients, but is significantly increased in PI patients; and 3) the elevated production of type 2 cytokines is paralleled by an increase in CD57+ CD4+ CD7- lymphocytes, recently shown to be augmented in the progression of HIV infection, and producers of type-2 cytokines. Thus, whereas a high IL-2/low IL-4, low IL-10 cytokine production pattern is present in HIV^- controls and in LTNP HIV^+, the progression of HIV disease is associated with a low IL-2/ high IL-4 and high IL-10 cytokine profile. These findings provide the first demonstration that an in vivo dominant type 2 cytokine profile with defective IL-2 production is associated with progression to AIDS, further support the type 1/type 2 cytokine model in HIV infection, and suggest the monitoring of cytokine production as a surrogate marker of prognosis.

Supported by grants from Istituto Superiore di Sanita' " VI Progetto AIDS 1993; VII Progetto AIDS 1994; VIII Progetto AIDS 1995"

D4-213 **INTERLEUKIN (IL)-12 CAN FUNCTION ALONE AND IN SYNERGY WITH IL-2 TO SUPPORT HIV-1 REPLICATION IN ACTIVATED HUMAN T CELLS**

K. A. Clouse, K. A. Weih, N. D. Weber, S. Daniel and L. M. Cosentino*; Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892, USA; (*Current address: Biotech Research Laboratories, Rockville, MD)

Interleukin-12 (IL-12) is a heterodimeric cytokine produced by activated monocytes and B lymphocytes. It is essential for the development of NK cell-mediated immunity and for differentiation of the T_H1 lymphocyte subset in cellular immune responses. Since decreased NK cell function and progressive loss of T_H1 cells are common in HIV disease, therapeutic use of IL-12 has been suggested. It was previously reported that IL-12 neither stimulates nor inhibits HIV-1 replication in resting human PBMC. We also found that IL-12 does not support HIV replication in unstimulated PBMC. However, the observations that IL-12 can enhance T cell proliferation, induce T cell production of immunoregulatory cytokines, and synergize with IL-2 to augment this latter response suggest that the effects of IL-12 on replication of HIV may be influenced by cellular activation. Thus, PBMC stimulated with mitogen prior to infection with HIV were evaluated in the presence and absence of IL-12. In cells cultured with IL-12, virus replication was detected at levels above those for HIV control cultures containing medium alone, but lower than levels detected for infected cells maintained in medium containing IL-2. Upon addition of IL-12 at varying concentrations to medium containing a constant amount of IL-2, HIV replication increased in a manner that was dependent on the dose of IL-12. Studies are in progress to determine the basis for this effect. Thus, the ability of IL-12 to influence HIV replication in this system appears to be dependent on the state of lymphocyte activation, which can be influenced by mitogens, antigens and other cytokines.

D4-214 CHARACTERIZATION OF ENV GENE EXPRESSION IN HIV-1 FROM LONG-TERM NON-PROGRESSORS.

Ruth I. Connor, Linqi Zhang, Yunzhen Cao and David D. Ho. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

Most individuals infected with HIV-1 progress to AIDS within 10 years after seroconversion; yet a small number remain clinically healthy, with normal CD4 cell counts, for more than a decade after infection. A concerted effort is now underway to define the virologic and immunologic determinants of nonprogression. Our studies have focused on the env gene of HIV-1, which is functionally involved in determining cell tropism and syncytium-formation in vitro. Changes in determinants which map to env have been associated with altered tropism and increased viral cytopathicity in vitro and are implicated in disease progression. However, the role of env in HIV-1 from long-term non-progressors is undefined. To address this question, we are studying a well characterized cohort of 10 long-term non-progressors. In initial experiments, viral DNA was detected in PBMC from each of these individuals by quantitative PCR; however, infectious virus was only recovered from 4 subjects. These isolates were non-syncytium inducing and replicated poorly in PBMC cultures. In further experiments, the env gene was amplified by PCR directly from patient PBMC and the PCR products cloned into expression vectors. Env expression vectors were co-transfected in the presence or absence of Rev, and expression of gp160/120 in cell lysates was determined by immunoprecipitation using pooled anti-HIV-1 antisera. Additional experiments are now in progress to assess the functional properties using in vitro complementation assays. The results of these experiments will be presented.

D4-215 VIRAL BURDEN, VIRULENCE AND VIRAL THRESHOLD IN HIV-1 DISEASE: IMPLICATIONS FOR CLINICAL TRIAL DESIGN AND STUDIES ON PATHOGENESIS.

Robert W. Coombs* and Patricia S. Reichelderfer, *Departments of Laboratory Medicine and Medicine, University of Washington, Seattle, WA 98195 and Division of AIDS, National Institutes of Health, Bethesda, MD 20892

HIV-1 disease involves a complex interaction between the virus and the host immune response. To date, clinical trial design has focused on drug licensure and group versus individual response, often mixing antiviral activity with antiviral efficacy, and ignoring potential complicating factors. We have analyzed data from two clinical trials (ACTG 143 and 116B/117) and a natural history study, and propose a model of HIV-1 infection that shows the dynamic relationship between CD4-cell response [CD4(T)], CD4-cell proliferation [CD4(P)], change in CD4-cell count due to virus load [CD4(L)] and virulence (V) (equation 1: CD4(T) = CD4(P) - [CD4(L)*(V)]). A series of equations are derived which, when log transformed, describes a four-quadrant plot in which antiviral activity is defined independently of pathogenesis; i.e., the influence of change in log virus load (*x*-axis) is defined relative to the change in log CD4-positive cell count (*y*-axis). Quadrants **A** (*x*-, *y*+) and **D** (*x*+, *y*-) define the effect of a change in virus load on the change in CD4-cell count; quadrants **B** (*x*+, *y*+) and **C** (*x*-, *y*-) respectively define the nullifying effect of either (i) decreased or increased virulence factors or (ii) increased or decreased CD4-proliferation on the change in virus load. The antiretroviral response is best assessed at higher viral loads in the diagonal **AD** while the influence of pathogenetic (e.g., viral phenotype or immunologic) or other factors on this response is assess by the diagonal **BC**.

The model predicts the results of natural history studies, the effect of antiretroviral therapy, the differential effect of therapies, the effect of a virulence factor (such as syncytium-inducing phenotype), and the effect of lower-limit CD4-cell and viral thresholds. As such, this model has a potential application to the design of clinical trials and the individualized patient management of antiretroviral therapy.

D4-216 THE EFFECT OF GM-CSF ON HIV-1 INFECTION OF MONOCYTE MACROPHAGES.

Suzanne Crowe#, Anne Maerz#, Angel Lopez+
#National Centre in HIV Virology Research, Macfarlane Burnet Centre for Medical Research, Yarra Bend Road, Fairfield, 3078 Vic. +The Hanson Centre for Cancer Research, Frome Road, Adelaide SA 5000.

Aim: Published data suggest that GM-CSF increases HIV replication in monocyte-derived cell lines (Ref 1) and primary monocyte-derived macrophages (2). The objective of this study was to further investigate the effects of GM-CSF on monocyte-derived-macrophages infected with HIV-1 *in vitro*.

Methods: Monocytes isolated by density gradient centrifugation and plastic adherence were cultured in suspension for 5 days. The cells were infected with HIV for 2 hours, then washed and cultured adherent for 10 days in the presence or absence recombinant human GM-CSF derived from both yeast (Genzyme) and *E.coli* expression systems. All experiments were performed with rigorous exclusion of LPS contamination. Infection was determined p24 EIA and flow cytometry. HIV cDNA was quantitated by PCR using the primer pair SK38/39, which detects almost full-length transcripts.

Results: GM-CSF, from both sources, inhibited HIV infection of macrophages in a dose dependent response. Preliminary results indicate that the block in viral replication is occurring after the reverse transcription step.

References: (1) Folks et al Science 1987;238:800-802
(2) Koyanagi et al Science 1988;241:1673-1675.

D4-217 QUANTITATION OF SIV RNA IN PLASMA DURING ACUTE INFECTION OF MACAQUES USING A BRANCHED DNA (bDNA) SIGNAL AMPLIFICATION ASSAY.

P. J. Dailey, J. A. Kolberg, M. Zamroud, M. G. Lewis, and M. S. Urdea. Chiron Corp., Emeryville, CA 94608 and Henry M. Jackson Foundation, MMCARR, Rockville, MD 20850.

A simple, rapid, and reproducible method to quantitate SIV RNA in plasma would be useful in the study of the pathogenesis of SIV in primate models as well as evaluation of therapeutic agents and vaccines. HIV-1 RNA levels in plasma can be reliably quantitated using a solid phase nucleic acid hybridization assay based on branched DNA (bDNA) signal amplification. Target probes for HIV-1 were designed in pol and were predicted to cross-react with SIV pol (65% homology). Our objective was to determine if the HIV-1 RNA assay (Quantiplex™ HIV-RNA) could be used to quantitate SIV RNA during acute infection of macaques with SIV. Plasma samples were obtained from three rhesus macaques at 0, 1, 2, 3, 5, and 12 weeks post-challenge with SIV_{mac251}. Relative quantitations were obtained using the bDNA assay based on an HIV-1 RNA standard curve. Plasma viral RNA quantitation results were compared to p27 antigen levels. A peak of SIV RNA was detected in plasma obtained two weeks after challenge in all three animals. This corresponded to the peak of plasma p27 antigen and detection of cultivable virus in PBMCs. In two animals that developed immune responses to SIV (EIA using whole HIV-2 lysate), viral RNA declined to undetectable levels by week three and by week five, respectively. A third animal did not develop an immune response to SIV, and SIV RNA levels continued to increase through week 12. In all cases, there was a strong correlation between SIV RNA levels and p27 antigen ($r=.89$, $p<.001$). In this initial study, the HIV-1 RNA assay was used to detect and determine the relative quantity of SIV RNA in acute infections of rhesus macaques. Since quantitations measured by the bDNA assay are based on an HIV-1 standard, the relative reactivity of SIV needs to be determined. Studies to evaluate the relative quantitation between HIV-1 and SIV are ongoing.

D4-218 QUASISPECIES TURNOVER: A LINK WITH DISEASE PROGRESSION?

Eric L. Delwart[1], Haynes W. Sheppard[2] and James I. Mullins[3] 1. Aaron Diamond AIDS Research Center, New York University, New York, NY 10016 2. Viral and Rickettsial Disease Laboratory, California Dept. of Health Services, Berkeley, CA 90704 3. Dept. of Microbiology and Medicine, University of Washington, Seattle, WA 98915.

HIV-1 quasispecies sequence changes in vivo are being analyzed using DNA heteroduplex analyses. Proviral envelope sequences in un-cultured PBMC samples collected from the time of seroconversion and up to eight years thereafter have been PCR amplified from 20 individuals with very different rated of CD4 decline. The rate of clearance of the earliest variants from subsequent quasispecies the culturable variants in vivo are being measured to correlate quasispecies properties with disease progression.

D4-219 **Th1/Th2 OR Th0 (?) CYTOKINE PATTERNS IN HEALTHY HIV INFECTED INDIVIDUALS.**

Fakoya A., Matear PM., Filley E., Rook GAW., Stanford J., Gilson RJC., Maini M., Weller IVD., Vyakarnam A. UCL Medical School, London W1P 9PG. U.K.

We followed IL-4 and IFN gamma production in PBMC cultures of 16 healthy seropositive individuals (mean CD4 count $619\pm162x10^6$/ml) in three consecutive bleeds over a period of four weeks. Both spontaneous and anti-CD3/PMA induced cytokine production was measured (at 48H and 7 days) by sensitive immunoassay. Cytokine levels in patients cultures were compared with PBMC cultures of 14 seronegative healthy volunteers.

5/16 patients produced high spontaneous IL-4 (>70pg/ml) compared to 0/14 controls. Of these 5 patients, four produced high IL-4 in two out of the three consecutive bleeds. Their PBMC could not be induced to produce higher levels of IL-4 upon stimulation suggesting that the cells were already maximally activated for IL-4 production *in vivo*. However, PBMC from patients who did not produce high spontaneous IL-4 produced significantly higher levels of this cytokine compared to controls in response to anti-CD3/PMA stimulation((20±38.1pg/ml versus 5.1±13.2 pg/ml; p=0.005). There was little spontaneous IFN gamma production in patients or controls. In contrast to IL-4 production, all patients and controls studied produced IFN gamma to anti-CD3/PMA stimulation although the levels produced by patients was significantly higher (905.2±719 IU/ml versus 187±348.6 IU/ml; p<0.005). There was no correlation between the induction of HIV-1 p24 in these cultures with the production of either cytokine. Further studies are in progress to measure IL-2 and IL-10 levels in the same cultures.

This data suggests that HIV infection can lead to a significant increase in the production of IFN gamma and in a proportion of patients in the production of IL-4. Whether this pattern reflects an expansion of a Th0 as opposed to a Th2-like population is under investigation by T-cell cloning. The possibility that an early increase in IL-4 production might predict a more rapid CD4 decline is being studied prospectively.

D4-220 A NOVEL MECHANISM FOR HIV-1 MODULATION OF ANTIGEN PRESENTING CELL FUNCTION.

Sarah Fidler, Lucy Dorrell, Simon Ball, Jonathan Weber, Catherine Hawrylowicz & Ann D.M. Rees. Department of Genito-Urinary Medicine & Communicable Diseases and Department of Immunology, St. Mary's Hospital Medical School, Praed St., London W2 1NY U.K.

Defective antigen presenting cell (APC) function has been proposed to play a role in HIV pathogenesis but the precise mechanism is, as yet, unclear. Using a panel of HLA-DRβ1 *0101-restricted T cell clones (TLC) with diverse patterns of recognition of the gp120 V3 loop we have found that, in contrast to APC from HLA-matched healthy volunteers (HIV-APC), only 2 of the 10 TLC tested consistently responded to peptide presented by HLA DRβ1 *0101 APC from patients with AIDS (HIV+APC). When HIV infected individuals at different stages of diseases were compared, an intermediate group of TLC which responded to APC from clinically asymptomatic patients was identified. Patterns of recognition of substituted peptides did not suggest that responding TLC had a higher overall affinity for the peptide-MHC ligand. HIV- and HIV+APC expressing comparable levels of Class II and co-stimulatory molecules retained their discriminate ability to support prolifertion of some, but not all of the T-cell clones. All of the TLC in this study were of Th0-type and interaction with non-stimulatory APC did not result in a change in cytokine secretion patterns.

When the activation requirements of all the TLC were compared, responding TLC were found to be less CD4 dependent. This suggests that engagement and subsequent down-modulation of CD4 by viral Gp120 on the membrane of HIV-1 infected APC might explain the differentiall responsiveness of the TLC. Heterogeneity in the ability to present peptide to some of the TLC correlated with disease progression, and may, therefore, explain the early loss of responsiveness to recall antigens.

D4-221 HUMAN IMMUNODEFICIENCY VIRUS GLYCOPROTEINS INDUCE INTERFERON-α PRODUCTION BY PERIPHERAL BLOOD MONONUCLEAR CELLS, Patricia Fitzgerald-Bocarsly, Hang-min Zheng, Nilesh Patel and Stephen Feldman, Dept. of Lab. Med. and Pathol., UMDNJ-New Jersey Medical School, Newark, NJ 07103.

H-9 or U937 cells chronically infected with HIV-1 induce interferon-α (IFN-α) production by a population of low-frequency PBMC. The responding cells were found to be similar to the "natural IFN producing cells" or NIPC which respond to herpes simplex virus and distinct from the monocytes which produce IFN in response to Sendai virus in terms of their frequency and sensitivity to the lysosomotropic drug chloroquine. NIPC are believed to represent a subpopulation of peripheral dendritic cells. The ability of U1 cells to induce IFN-α in PBMC was upregulated by pre-treatment of the cells with PMA for 24-48 hr, which increased cell surface gp120 expression and viral production, resulting in both higher IFN production and an increased frequency of the responding cells as measured by IFN bioassay and ELISpot, respectively. In addition to the induction of IFN-α by HIV-infected cells, free HIV-1 ADA was also able to induce IFN-α in the low-frequency NIPC population. To evaluate the role of viral glycoprotein in induction of IFN-α by HIV-1, we have utilized several approaches. First, we have used antibody to gp160 or gp120 and soluble CD4 in the induction assays and found that these antibodies as well as the soluble CD4 could partially block IFN-α production in response to H9-$HTLV_{IIIB}$. Moreover, cell-free, membrane extracts from H9- $HTLV_{IIIB}$ but not uninfected H9 cells were able to induce IFN-α production by PBMC. Finally, we have utilized vaccinia virus constructs expressing the gp120 gene from HIV-1 to infect HELA cells, and used these cells to stimulate PBMC for IFN-α production. HELA cells infected with the gp120 expressing vaccinia but not the parental vaccinia construct induced IFN-α production in the PBMC, although at lower efficiency than the HIV-infected cell lines. We conclude that HIV-1 induces IFN-α production in a low-frequency, NIPC population of PBMC and that viral glycoproteins are involved in this induction.

D4-222 RNA SPLICING PATTERNS IN HIV-1 INFECTIONS :CORRELATION TO DISEASE PROGRESSION Manohar R. Furtado, Department of Pathology, Northwestern University, Chicago IL 60611

An increase in the unspliced RNA/spliced mRNA ratio during the course of an infection is characteristic of acute HIV-1 infections in culture and chronically infected cells induced by cytokines. We observed similar changes in HIV-1 transcripts in sequentially obtained patient PBMC. Six rapid progressors who exhibited a decline in their CD4+ cell counts to below $200/mm^3$ within 24 months after seroconversion and four nonprogressors whose CD4+ cell counts did not drop below $200/mm^3$ for over 36 months after seroconversion were studied.

Further, we specifically amplified and quantitated the various multiply spliced mRNAs that encode *tat*, *rev* and *nef*. Amplifications were within the linear range and internal controls were co-amplified to correct for variations in the efficiency of reverse transcription. The cDNAs generated were resolved by PAGE, transferred to nylon membranes, probed with splice junction specific and exon specific probes and quantified.

Large increases in the relative amounts of *rev* transcripts, moderate increases in the relative levels of *tat* transcripts and decreases in the relative amounts of *nef* transcripts were characteristic of the rapid progressors. Conversely, the relative levels of the *tat*, *rev* and *nef* transcripts exhibited moderate to no change in the nonprogressors. The *rev* transcript levels exhibited a strong inverse correlation to CD4+ lymphocyte decline. Mechanisms that can specifically alter *rev* transcript levels are being evaluated.

D4-223 DIVERGENT EFFECTS OF CHRONIC HIV-1 INFECTION ON HUMAN THYMOCYTE MATURATION IN SCID-HU MICE, Harris Goldstein, Tobias R. Kollmann, Ana Kim, Massimo Pettoello-Mantovani, Arye Rubinstein, and Marsha M. Goldstein, Departments of Pediatrics, and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

We have recently developed a modified SCID-hu mouse model in which the implanted human thymus and liver (hu-thy/liv) and human peripheral T cells become infected with HIV-1 after i. p. inoculation (*J. Exp. Med.* 1994;179:513). Using this model, we evaluated the effect of HIV-1 infection on thymic maturation and observed that different HIV-1 strains had divergent effects of thymic maturation. While minimal effects on continued thymopoisis in the hu-thy/liv implant were observed after chronic infection with two primary patient isolates, $HIV\text{-}1_{28}$ and $HIV\text{-}1_{59}$, and with $HIV\text{-}1_{ADA}$, $HIV\text{-}1_{Ba\text{-}L}$, $HIV\text{-}1_{JR\text{-}CSF}$, $HIV\text{-}1_{JR\text{-}FL}$, and $HIV\text{-}1_{SF162}$, significant thymocyte depletion was detected after infection with $HIV\text{-}1_{IIIB}$, and $HIV\text{-}1_{RF}$. Thus, the effect of HIV-1 infection on thymocyte maturation may depend upon the strain of HIV-1 infecting the thymus and this SCID-hu model can be used to evaluate the *in vivo* biological behavior of different HIV-1 isolates. Although minimal effects on thymopoiesis were observed in the hu-thy/liv implanted in SCID-hu mice 6 months after infection with $HIV\text{-}1_{28}$, significant changes were seen in the human T cell population circulating in their peripheral blood. These changes ranged from a reversal of the CD4/CD8 ratio of peripheral human T cells to less than 1 in some SCID-hu mice to the complete depletion of peripheral human T cells observed in other SCID-hu mice. Since these effects were associated with the detection of HIV-1 infection of the peripheral human T cells, these modified SCID-hu mice should prove to be a valuable model for investigating the effects of chronic HIV-1 infection on the peripheral human T cell population.

D4-224 HIGH LEVEL VIREMIA WITH HIGH DIVERSITY UNDERLIES NONPATHOGENIC SIV INFECTION OF SOOTY MANGABEYS. Robert M Grant[1], Brian Corliss[1], Silvija Staprans[1], Harold McClure[2], Mark Feinberg[1].

1. Gladstone Institute of Virology and Immunology, POB 419100, SF, CA 9414, and 2. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322. Simian immunodeficiency virus from macaques (SIV_{mac}) appears to have arisen from inadvertent spread of virus from sooty mangabeys naturally infected with SIV_{sm}. SIV infection in rhesus macaques causes an AIDS-like disease while SIV infection of sooty mangabeys is not pathogenic. To study the viral-host interactions that preclude a pathogenic outcome in sooty mangabeys, we developed a quantitative competitive PCR (QC-PCR) assay to determine the number of SIV_{sm} plasma viral RNA copies and a heteroduplex mobility assay (HMA) to characterize the diversity of the plasma SIV_{sm} RNA population. The QC-PCR primers amplify a segment of the GAG region while the HMA primers amplify a segment of the envelope gene including the first and second hypervariable regions. Naturally infected sooty mangabeys were found to have high level viremia in plasma ranging from $1.5x10^7$ to $1.0x10^8$ RNA copies/ml. The high numbers of viral RNA copies were confirmed by limiting dilution and non-competitive PCR. Diversity of the plasma viral RNA population was large as indicated by a broad band of heteroduplexes showing retarded mobility during PAGE. The quantity of SIV RNA copies in plasma is similar to what we have found in macaques that develop AIDS following SIV infection. This suggests that greater immunologic control of SIV infection does not explain why sooty mangabey's remain free of disease. Further, replication of SIV appears to proceed at high rates without CD_4 T cell depletion, suggesting that the interaction of the virus with host cells is not cytopathic in this system. The viral diversity found in these animals may be due to a high mutation rate creating defective virions, a multi-compartmental infection, or selective pressure from as yet unidentified host factors.

D4-225 **Increased HIV Plasma Viremia Following Influenza Vaccination of Seropositive Adults**

Brian Hamilton[1], Stephen Follansbee[2,3], Silvija Staprans[4], Tarek Elbeik[5], Mark Feinberg[3,4,5]. 1. Children's Hospital, Oakland, CA, 2. Davies Medical Center, San Francisco, CA, 3. Bay Area Community Consortium, San Francisco, CA, 4. Gladstone Institute, San Francisco, CA, 5. UCSF, San Francisco, CA.

HIV-1 expression and replication are known to be activated by immunologic stimulation of T lymphocytes in tissue culture. To investigate whether a specific antigen exposure also activates virus replication in HIV-1-infected individuals, we studied the virologic and immunologic consequences of influenza vaccination of seropositive adults. Thirty HIV-1-infected individuals (stratified by CD4+ T cell levels) and 10 seronegative individuals were studied. Plasma and PBMC's were isolated at baseline weeks 1, 2, 4 and 13 following vaccination. Immune responses to vaccine antigens (measured by hemagglutination inhibition and T cell proliferation assays) and changes in plasma HIV-1 RNA levels (measured by bDNA and/or QC-PCR methods) were evaluated. A substantial but transient increase in HIV RNA levels was observed in 78% of HIV-1 infected individuals. Activation of HIV-1 replication following vaccination was correlated with an individual's ability to recognize and respond to vaccine antigens. These data support the suggestion that stimulation of virus replication by environmental antigens may contribute to T cell depletion following HIV-1 infection.

D4-226 *IN VIVO* PATHOGENIC PROPERTIES OF HIV-1_{NL4-3} and HIV-1_{JR-CSF} B.D. Jamieson, D. Boldt-Houle, S. Pang, G.M. Aldrovandi, J. Zha and J.A. Zack, Dept. of Medicine, Div. of Hem./Onc., 11-934 Factor, UCLA, Los Angeles, CA, 90024

We have investigated the *in vivo* pathogenic properties of two strains of HIV-1 in human fetal thymus/liver (thy/liv) implants in severe combined immunodeficient mice (SCID) mice. HIV-1_{NL4-3} and HIV-1_{JR-CSF} were studied to determine their *in vivo* replication kinetics and their ability to induce CD4+ thymocyte depletion. HIV-1_{NL4-3} replicated *in vivo* with faster kinetics reaching ~10-fold higher viral titers than HIV-1_{JR-CSF}. HIV-1_{NL4-3} also induced CD4+ thymocyte depletion with faster kinetics than HIV-1_{JR-CSF}. Examination of the TCR Vß repertoire revealed that this depletion of CD4+ thymocytes was not due to a superantigen effect. These results suggest that virus load and cell depletion are linked and imply that genes which regulate viral replication are important in the cytopathic process of HIV-1. In the SCID-hu model, this pathogenesis most likely occurs in the absence of an immune response. Therefore, we investigated whether the absence of immune selection resulted in extensive genetic variation and the emergence of a more pathogenic strain. To this end, the V4 region of the envelope gp120 recovered from biopsy samples at six weeks post-infection was sequenced revealing that little genetic variation had occurred in either HIV-1_{JR-CSF} or HIV-1_{NL4-3} infected implants. The overall mutation rate demonstrated in both of these viruses was more reflective of an acute HIV-1 infection than the chronic phase of disease. These results demonstrate that two strains of HIV-1 exhibit different pathogenic potential and suggest that the SCID-hu mouse model can be used to study the *in vivo* pathogenicity of different HIV-1 isolates.

D4-227 **Immunomagnetic capture linked RT-PCR for detection of HIV-1 RNA in serum and culture fluids.** Bharat H. Joshi, Jay S. Epstein and Indira K.Hewlett. Laboratory of Molecular Virology, Division of Transfusion, Transmitted Diseases, Center for Biologics, Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892.

A sensitive immunomagnetic capture assay coupled with RT-PCR has been developed for detection of HIV-1 in culture and body fluids. Anti-IgG activated magnetic beads were incubated with either anti p24 or anti gp 120 monoclonal antibodies or a combination of both at 4°C for 16 hours. The sensitivity of the assay was directly proportional to the amount of antibody used to coat beads. Virion enrichment was greater with anti gp120 coated beads than anti p24 antibody coated beads while the combination of both yielded maximal enrichment. By this method an equivalent of 10ng of p24 antigen per 100ug of coated beads could be detected maximally. HIV-1 RNA detection was done from serially diluted culture fluid that had an equivalent of 50 fg of p24 antigen input corresponding to 250-350 HIV-1 RNA copies and less than 100 HIV-1 virions. An analysis of a panel of 9 sera from HIV-1 infected seropositive individuals revealed that detection of HIV was achieved using this assay from 10 ul of serum. No signals were seen in sera from uninfected individuals.

D4-228 THE CYTOPATHICITY OF A SIVMne VARIANT IS REGULATED BY BOTH 5' AND 3' GENETIC DETERMINANTS, Jason T. Kimata and Julie Overbaugh, Dept. Microbiology, SC-42, University of Washington, Seattle, WA 98195

We have been using the simian immunodeficiency virus (SIV) macaque model of AIDS to address the hypothesis that rapidly replicating cytopathic lentiviral variants that are present late in infection and associated with CD4+ cell decline are more pathogenic than the slower replicating noncytopathic viruses from the early asymptomatic stage of infection. Previously, we demonstrated that both a molecular and biological clone of SIVMne undergo genetic and phenotypic changes in association with disease progression when they are inoculated into pig-tailed macaques. To determine whether the late viruses are more pathogenic and to identify the viral genetic determinants responsible for the change in phenotype, we cloned a variant of SIVMne from peripheral blood mononuclear cells (PBMCs) of a macaque that was inoculated with the molecular clone (SIVMneCl8) of SIVMne. At the time the PBMCs were isolated, the animal had a depressed CD4+ cell count and AIDS. Restriction map analysis and DNA sequencing demonstrate that the late variant (SIVMne170) is genetically distinct from SIVMneCl8. Furthermore, in T-cell cultures, the variant replicates rapidly, induces syncytia, and is cytopathic compared to SIVMneCl8. To identify determinants of cytopathicity, recombinant viruses have been constructed between the PBMC variant and the parent virus, SIVMneCl8. A phenotypic analysis of these chimeras demonstrates that syncytium-inducing phenotype maps to *env*. Interestingly, a recombinant virus consisting of the 5' half of SIVMne170 and 3' half of SIVMneCl8 replicates efficiently and is highly cytopathic compared to SIVMneCl8, although it does not induce syncytia, suggesting that the cytopathicity of SIV can be regulated by a 5' determinant independent of Env mediated syncytium-associated cytolysis. Studies are being performed to identify the specific determinant. These data demonstrate that in addition to *env* determinants, genetic changes outside of the *env*, LTR, and regulatory gene regions of the SIV genome may influence viral phenotype *in vitro* and pathogenicity *in vivo*. Finally, we have isolated a variant from lymph node cells to determine if a virus from this tissue undergoes similar changes as the PBMC variant virus. Dissection of the different determinants of cytopathicity in the SIV system may allow testing of which viral property contributes to disease progression.

D4-229 **INTERFERENCE OF INTERLEUKIN-10 WITH HIV-1 REPLICATION IN PRIMARY MONOCYTE DERIVED MACROPHAGES,**

Neeltje A. Kootstra, Angélique B. van 't Wout, Han G. Huisman, Frank Miedema, and Hanneke Schuitemaker.

Department of Clinical Viro-Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

Previously we demonstrated an inhibitory effect of IL-4 on establishment of HIV-1 infection in primary macrophages. The reported similarities between the biological effects of IL-4 and IL-10 prompted us to study the effect of IL-10 on HIV-1 replication.

Treatment of primary macrophages with IL-10 resulted in inhibition of HIV-1 infection. This inhibitory effect was specific for macrophages since IL-10 did not interfere with HIV-1 replication in primary T-cells. Semi-quantitative PCR analysis excluded an inhibitory effect of IL-10 on virus entry and reverse transcription. Effects of IL-10 on HIV-1 LTR-driven CAT activity also could not be demonstrated in a transient expression system in primary derived macrophages. In agreement with this, Northern (RNA) blot analysis demonstrated equal amounts of viral RNA species irrespective of IL-10 treatment, also excluding an inhibitory effect on elongation of virus transcription. MDM treated with IL-10 after HIV-1 inoculation showed accumulation of apparently mature p24 protein suggestive of an inhibitory effect at the level of virus assembly. IL-10 treatment of MDM prior to HIV-1 inoculation did not result in accumulation of p24 protein. Immunoblot analysis indeed showed the absence of mature p24 and gp120 but accumulation of the Pr53 *gag*-encoded protein in HIV-1 inoculated IL-10 pretreated MDM suggesting an inhibitory effect at the level of protein processing. A combination of IL-4 and IL-10 resulted in a cumulative inhibitory effect on HIV-1 replication in MDM.

The recent observations that in the course of HIV infection a shift occurs in the production of IL-2/IFN-γ towards enhanced IL-4 and IL-10 production and the reported shift from preferential macrophage-tropic towards preferential T-cell-tropic HIV-1 variants with progression of disease suggest that cytokines have an important role in the in-vivo regulation of HIV-1 tropism.

D4-230 IMMUNOSUPPRESSIVE EFFECTS OF APOPTOTIC DEBRIS FROM HIV-INFECTED T CELLS ON T CELL PROLIFERATION. 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 (LAV-1) undergo apoptotic cell death (J. Clin. Invest. 87:1710, 1991). Supernatants containing subcellular apoptotic debris from these cells were tested in an IL-1 assay using the murine Type 2 helper CD4+ T cell line, D10.G4.1. This cell line proliferates in response to stimulation of the TCR (using ConA or the anti-idiotypic anti-TCR monoclonal antibody, 3D3) plus IL-1. No IL-1 activity was present in the CEM or CEM/LAV-1 supernatants. However, in the presence of a submaximal amount of rHu-IL-1 β, supernatants from the apoptotic HIV-infected CEM cells reproducibly suppressed the proliferative response of the D10.G4.1 cells. This suppression occurred regardless of whether ConA or 3D3 MAb was used to stimulate the TCR. Most surprisingly, the suppressive activity was not a soluble factor since it could be pelleted by centrifugation at 15,000 g for 15 min. Further experiments are currently ongoing to test the ability of HIV-infected apoptotic debris to influence the activities of human non-CD4+ T cells reactive to antigens from *M. tuberculosis.* These findings suggest that apoptotic debris, which has been shown to accumulate during HIV infection, may not be immunologically neutral and instead may have effects on the immune system which continue after the apoptosing cells have died (as reviewed in Immunol. Lett., in press).

D4-231 **ANTI-APOPTOTIC MOLECULES INCLUDING SOLUBLE CD95 (FASΔTM) INHIBIT DEATH OF LYMPHOCYTES FROM HIV-INFECTED PATIENTS *IN VITRO***, John F.Krowka[1], Michael S. Ascher[1], Haynes W. Sheppard[1], Michael C. Kiefer[2], Ian C. Bathurst[2], L. David Tomei[2] and Philip J. Barr[2]. California Dept. of Health Services[1], Viral & Rickettsial Disease Laboratory, Berkeley, CA 94704 and LXR Biotechnology Inc.[2], Richmond, CA. 94804.

The FAS/APO-1 glycoprotein is an apoptosis-associated molecule which has recently been given the designation CD95. It is a member of the molecular family which includes CD40 and the receptors for tumor necrosis factor and nerve growth factor. It occurs in two distinct molecular forms, as a transmembrane protein and as a soluble protein in which a hydrophobic stretch of 17 amino acids has been deleted. Because of its role in apoptosis we wished to test the hypothesis that HIV-induced modulation of CD95 expression plays an important role in increased levels of lymphocyte death that have been observed in HIV-infected (HIV+) patients. Flow cytometric analysis revealed increased levels of CD95 expression on lymphocytes from HIV+ donors. RT-PCR analysis demonstrated increased levels of mRNA encoding the soluble form of CD95 (FASΔTM) in lymphocytes from HIV+ donors. Both FASΔTM and additional anti-apoptotic compounds were able to block *in vitro* DNA fragmentation of lymphocytes from HIV+ donors. Studies of the levels of CD95 in the serum of HIV+ individuals and controls are currently in progress. These data suggest that CD95 plays an important role in lymphocyte death and that FASΔTM as well as other molecules may be useful therapeutically to inhibit lymphocyte death in HIV+ patients.

D4-232 IN VITRO EFFECT OF IL-12 AND ANTI-IL-10 IN RESTORING CELL MEDIATED IMMUNITY IN HIV INFECTED INDIVIDUALS. A Landay[a], M Clerici[b], F Hashemi[a], B Sha[a], H Kessler[a], J Berzofsky[b], GM Shearer[b]. Rush Medical Center[a], Chicago, IL, and National Cancer Institute[b], Bethesda, MD.

Various cytokines have been shown to either upregulate (IL-12) or downregulate (IL-10) cellular immune responses and may play a role in the natural history of HIV infection. We investigated the ability of IL-12 and anti-IL-10 to restore or augment cell mediated immune responses in patients with HIV infection as measured by *in vitro* T helper cell activity. Peripheral blood mononuclear cells from 34 HIV infected individuals (200-500 CD4+ cells/μl, n=22; CD4<200 cells/μl, n=12) were stimulated *in vitro* with influenza (FLU), HIV envelope peptides (ENV), alloantigen (ALLO), and PHA and evaluated by tritiated thymidine uptake. A positive response was considered to be a stimulation index (SI) greater than or equal to 3. Four response patterns were seen in patients with 200-500 CD4+ cells: ENV+, FLU+, ALLO+, PHA+ (1/22); ENV$^-$, FLU+, ALLO+,PHA+(11/22); ENV$^-$, FLU$^-$, ALLO+, PHA+(8/22); and ENV$^-$, FLU$^-$, ALLO$^-$, PHA+ (2/22). Addition of IL-12 or anti-IL-10 increased the number of ENV responses to 7/22 patients from 1/22. The response to FLU was increased in 2 patients from 12/22 to 14/22 in the presence of IL-12 and in 5 patients from 12/22 to 17/22 in the presence of anti-IL-10. Patients with CD4<200/μl demonstrated T helper responses that were depressed further with 7/12 being ENV$^-$, FLU$^-$, ALLO+, PHA+,and 5/12 FLU$^-$, ENV$^-$, ALLO$^-$ and PHA+. Even with this depression of T cell activity, we observed restoration of *in vitro* FLU responsiveness in 2/12 of the individuals with IL-12 and 3/12 with anti-IL-10. The ability to restore *in vitro* proliferative responses by cytokine or cytokine antibody may provide new insights into their role in the natural history of HIV infection and possibly for therapeutic intervention.

D4-233 Systemic HIV Activity Does Not Drive Peripheral CD4 T Cell Decline: a Non-Cytopathic Model for HIV-related CD4 T Cell Depletion. P. Bagnarelli*, D. Mathez**, P. Clementi*, J. Leibowitch**. * Laboratory of Virology, Di Torrette Hospital, Ancona, Italy. ** Laboratory of Immunology, Raymond Poincaré Hospital, Garches, France.

Blood CD4 T cell-borne HIV measured as infectious, QC-PCR DNA, QC-PCR RNA burden, are shown to faithfully represent retroviral activity/burden within peripheral lymphoïd tissues. Plasma HIV RNA measured by sensitive QC-PCR essentially concur with blood CD4 T cells infectious titers. In most semi-long term survivors (> 5 years since contamination), rates of CD4 T cell decline follow a single exponential in the individual patient with no stoïchiometry with systemic retroviral activity in that patient. Rather than depleting CD4 T cells via accelerated cytopathic damage, infection by HIV of non-circulating cells in lymphoïd/lymphopoïetic tissues would locally interfere with the generation/expansion of CD4 T cells, independently of systemic retroviral productive infection.

D4-234 REDUCED PROGRAMMED CELL DEATH IN HEALTHY LONG TERM POSITIVES VS ASYMPTOMATIC PROGRESSORS, Teri J. Liegler, Susan P. Buchbinder and Warner C. Greene, Gladstone Institute of Virology and Immunology, PO Box 419100, San Francisco, CA, 94141-9100, San Francisco Department of Public Health, PO Box 421873, San Francisco, CA, 94142-1873.

Factors influencing the rate of HIV disease progression are complex and poorly understood. Inappropriate CD4 T cell death by apoptosis has been proposed as one mechanism involved in HIV pathogenesis. However, it remains unknown whether lymphocyte apoptosis drives disease progression or is simply a nonspecific consequence of other more direct mechanisms. To address this issue, we quantified *in vitro* apoptosis of peripheral lymphocytes from 4 groups of HIV negative and positive individuals: 1) (HLP) asymptomatic healthy long term HIV positives with CD4 T cell counts >500/mm^3 and 10-15 years infection, 2) (RSC) HIV+ asymptomatics with a relatively recent infection (1-6 years) showing equally high CD4 T cell counts, 3) (PWA) persons with AIDS and 4) (NEG) HIV negatives. PBMC were incubated either in the presence or absence of serum derived growth factors and mitogens. Cell viability was quantified by flow cytometry using acridine orange/ethidium bromide staining or *in situ* DNA end labeling. By comparing the averages and distribution of viability measurements, the following observations were made: 1) All HIV+ subgroups show increased apoptosis when compared to NEGs, regardless of treatment *in vitro*, 2) HLPs show significantly less spontaneous (non-activation induced) apoptosis than RSCs, who show significantly less than PWAs, 3) In serum deprived or mitogen treated cultures, overall increased apoptosis was seen and HLPs could not necessarily be distinguised from other HIV+ groups. Both CD4 and CD8 T lymphocytes underwent apoptosis, determined by simultaneously assaying DNA fragmentation and cell surface phenotype. However, in serum deprived cultures, a complete loss of CD3/4 lymphocytes was observed in approximately 20% of the cultures, some with an input CD4/CD8 ratio of 1.0, suggesting that CD4 T lymphocytes may be the primary target of HIV-induced cytopathicity and that CD8 T cell apoptosis occurs by a secondary mechanism. Our results present evidence of decreased apoptosis in HLPs when compared with other asymptomatics before signs of net CD4 depletion and disease progression, suggesting that the factors promoting apoptosis contribute to and are not a consequence of CD4 T cell depletion. It is clear, however, that other elements contribute to the longevity of HLPs since, once activated or deprived of growth factors *in vitro*, cytopathicity appears similar to that seen in asymptomatic progressors.

D4-235 **SERA FROM HIV-1-INFECTED PIG-TAILED MACAQUES NEUTRALIZE THE ANCESTRAL VIRUS** Yiling Liu, Edward Hunter, Mark G. Lewis, Jing Miao, Karen Corts and Suzanne Gartner. Henry M Jackson Foundation Research laboratory and MMCARR, Rockville, MD

We are attempting to develop an animal model for HIV-1 infection in pig-tailed macaques. Previously we reported persistent virus infection in some animals following inoculation with a chimpanzee-passaged isolate of HIV-1_{IIIB}. (This chimpanzee isolate is designated as HIV-1_{CH69}.) At week 61 we recovered an isolate (HIV-1_{255-61}) from one of the HIV-1_{CH69}-infected macaques which exhibits biological properties suggestive of enhanced adaptation to macaques. Cell-free inoculation of naive pig-tailed macaques with HIV-1_{255-61} led to the establishment of persistent infection in several recipients. We evaluated sera from two of these animals, 2364 and 2369, for their ability to neutralize HIV-1 infection. Macaque 2364 had antibody to HIV-1 p24, gp41 and gp160 at week 7 post inoculation, and to p17 and gp120 at week 11. Macaque 2369 developed antibody to p17, p24, gp120 and gp160 at week 8, and to gp41 at week 13. These responses have persisted at similar or increased levels. Sera recovered from 2364 at week 20 and from 2369 at week 32 were assayed for neutralization using three virus isolates, HIV-1_{255-61}, HIV-1_{CH69} and HIV-1_{IIIB} and three target cells, human T lymphoblasts, pig-tailed macaque T lymphoblasts and H9 cells. A pool of HIV-1 patient serum was used as a positive control. The patient serum was able to neutralize HIV-1_{255-61} and HIV-1_{IIIB} infection of all three cell types, as well as HIV-1_{CH69} infection of macaque T cells and H9 cells, but not HIV-1_{CH69} infection of human T cells. Sera from both macaques were unable to neutralize HIV-1_{255-61} and HIV-1_{CH69} infection of any kind of cells, but were able to effectively neutralize HIV-1_{IIIB} infection of H9 cells. Additional neutralization experiments are in progress using other sera as well as other members of the HIV-1_{IIIB} family of viruses. Also, sequencing analyzes are underway to identify the molecular determinants associated with these biological properties. Data obtained thus far indicate that there are only three amino acid residues within the gp120 that could conceivably account for the divergence in neutralization potential of the macaque sera. Alternatively, determinants outside of gp120 may be responsible. Moreover, the inability of the patient serum to neutralize HIV-1_{CH69} infection of human T cells appears to be a consequence of an amino acid difference at the tip of the V3 loop. Virus neutralization studies using sera from HIV-1-infected macaques, coupled with genetic analyses of the viruses, is providing new insight into HIV-host cell interactions.

D4-236 IL-15 INDUCTION OF LYMPHOKINE ACTIVATED KILLER CELLS AND IFN-GAMMA BUT NOT IL-2 FROM PBMCs OF HIV-POSITIVE PERSONS.DR Lucey, LA Pinto, FR Bethke, J Rusnak, GP Melcher, FN Hashemi, A Landay, H Kessler, RJ Paxton, K Grabstein, GM Shearer. EIB/NCI/NIH, Bethesda, MD. Wilford Hall Med Ctr, San Antonio, TX. Rush-Presbyterian Med Ctr, Chicago, IL. Immunex Corp, Seattle, WA.

IL-15 is a recently reported14-15 kD cytokine which stimulates T-cell proliferation, LAK cell generation and cytotoxic T-cells from healthy donors. IL-15 binds to the IL2 receptor (IL2R) beta and gamma chains, but not the alpha chain (Grabstein et al. Science 1994;264:965). We asked whether IL-15 stimulates PBMCs to produce IL-2 or IFN-gamma in vitro and whether IL-15 induces LAK cells from PBMC's of HIV-positive persons at different stages of disease. PBMC from 10 donors (8 HIV-positive: CD4+ T-cell counts of 30/ul to 860/ul) were cultured for six days with recombinant simian IL-15 (3ng/ml, 30 ng/ml, 100ng/ml) or rh IL-2 (20U/ml). The 2 HIV-negative controls & 7/8 HIV-positive patients demonstrated induction of cytolytic activity against both Daudi lymphoblastoid cell line (LAK) targets and K-562 targets in response to IL-15. This cytolytic activity was blocked by antibody (Mikß1: gift of Dr T.A. Waldmann) against the IL2R beta chain, but not by antibody (anti-tac)against the alpha chain. PBMC from an additional 8 donors (4 HIV-positive: CD4+ T-cell counts of 54/ul to 450/ul) were cultured with 1, 10, 50 or 100ng/ml of IL-15 and supernatants assayed by EIA (Endogen) for IL-2 and IFN-gamma after 1, 2, and 3 days in culture. IL-2 was not found in any of the 8 donors while IFN-gamma was found in 4/8 donors (2 HIV-positive and 2 HIV-negative) in an IL-15 concentration -dependent pattern. Our preliminary studies suggest that IL-15 can induce LAK cells and IFN-gamma production from PBMCs of some HIV-positive as well as HIV-negative donors.

D4-237 LYMPH NODE FINE NEEDLE ASPIRATION PROVIDES A RELIABLE INDEX OF HIV CLINICAL STATUS AND MEASURE OF VIRAL BURDEN, William Lyman, Mark Suhrland, Carol Harris, Ruy Soeiro and Antonio Cajigas, Departments of Pathology and Medicine, Albert Einstein College of Medicine, Bronx, NY 10461

Reliable markers of HIV disease status remain elusive. To date, indices of clinical status have focused on variations in peripheral blood T cell number, the percentage of CD4+ lymphocytes, the ratio of CD4+ to CD8+ cells, and viral burden estimated by a number of different laboratory tests. However, the correlation of each variable with clinical status and their value as predictors of disease progression remain questionable. Recent studies indicated that events in the lymph nodes (LN) of HIV+ people may correlate better with clinical status. However, these investigations were based on excisional biopsies which require surgery and preclude the possibility of analyzing the same LN later in disease. We tested the hypothesis that fine needle aspiration biopsy (FNAB) can overcome these limitations and provide a reliable measure of clinical status. The study participants had persistent generalized lymphadenopathy without clinical evidence of lymphoma or nodal infections due to organisms other than HIV. Seven men and five women ranging in age from 23 to 55 and CDC Stages A2 to C3 enrolled in this study. From each participant, LN and blood samples were submitted for cytologic examination, flow cytometric analysis of lymphocyte subsets, and estimation of viral burden determined by PCR. Flow cytometry measures included total T cells, B cells, CD4+ cells, CD8+ cells, and natural killer (NK) cells. The relative percentages of T, B, and NK cells in these tissues were different and reflected the expected distribution of these cell types. The percentages of CD4+ and CD8+ cells were different between LN and blood but not statistically significant. In contrast, the ratio of CD4+/CD8+ cells in LN and blood was different and statistically significant ($p<0.001$) for patients in CDC categories A2-B2 but not different for categories B3-C3. More importantly, the ratio of CD4+/CD8+ cells in the LN but not in the blood correlated ($r=-0.78$) with clinical status. PCR data indicated that LN tissue harbors more virus than blood but this difference did not correlate with CDC stage. Thus, FNAB in combination with flow cytometry may prove to be an important tool in HIV clinical staging.

D4-238 KINETICS OF VIRUS LOAD, CD4+ CELL DECLINE, AND ANTIBODY TITERS IN A PATHOGENIC HIV-2 MACAQUE MODEL. Jan McClure[1], M. Scheibel[1], A. Watson[1], A. Schmidt[2], J. Steele[2], N.Dorofeeva[2], H. Ochs[2], D. Looney[3], F. Wong-Staal[3], W. Morton[2], S-L. Hu[1]. [1]Bristol-Myers Squibb, 3005 First Avenue, Seattle, WA., [3]Veterans Affairs Medical Center, University of California San Diego, La Jolla, CA., [2]University of Washington RPRC; Seattle, WA.

Objective: To define the kinetics of viral replication, $CD4^+$ cell depletion, and antibody titer in macaques infected with pathogenic HIV-2_{287} and to evaluate the effects of virus dose, mode of transmission, and host vaccination on these parameters.
Methods: HIV-2_{287} was derived from the lymph node of an HIV-2_{EHO} infected macaque (*in vivo* passage 2) with AIDS-like disease. HIV-2_{287} cell-free virus stock was prepared and titered *in vitro* and *in vivo* in Macaca nemestrina. Cell-associated viral load was determined by limiting dilution co-culture and plasma viremia was determined by RNA QC-PCR. Antibody responses were assessed using EIA and radioimmunoprecipitation. Lymphocyte subsets were analysed by FACS.
Results: All naive of macaques inoculated with HIV-2_{287} experienced high virus burden, high levels of viremia, rapid $CD4^+$ cell depletion, and seroconversion. The inoculating virus dose had an effect on the virus load and the time to onset of $CD4^+$ cell decline and progression to disease. HIV-2_{287} is transmitted perinatally and resulted in similar patterns of infection in terms of the viral kinetics and $CD4^+$ cell decline. Prior exposure of macaques to non-pathogenic HIV-2_{KR} significantly altered the response to an HIV-2_{287} challenge.
Conclusions: The predictable, rapid progression to AIDS in 100% of the naive macaques inoculated with HIV-2 $_{287}$ makes this a unique model for the evaluation of therapeutic and prophylactic approaches and investigations into the viral and host factors involved $CD4^+$ cell depletion.

D4-239 EFFECTS OF INTERLEUKIN-12 (IL-12) ON OUTGROWTH OF ENDOGENOUS HIV FROM PBMC ISOLATED FROM HIV-INFECTED ADULTS. EJ McFarland, PA Harding, RT Schooley, DR Kuritzkes, Univ of Colorado Health Sciences Center, Denver, CO 80262

IL-12 is a cytokine which enhances lymphocyte proliferation and natural killer cell activity in PBMC from HIV-infected individuals. Addition of IL-12 to exogenously infected HIV-1 co-cultures of normal PBMC and to cultures of chronically HIV-infected cells lines did not enhance viral growth. We wished to determine whether IL-12 had the potential to increase outgrowth of endogenous virus in PBMC from HIV-infected individuals. PBMC, freshly isolated from HIV-infected adults (CD4 lymphocyte count range 55-557), were enriched for CD4+ cells by positive selection. Cells bearing CD4 were cultured for 25-30 days at 0.72-3.5 x 10^5/ml in 48 well plates in the presence of PHA (5ug/ml) with or without the addition of exogenous IL-12 (8U/ml) or IL-2 (5%). Supernatants were collected every 3-4 days for 25-30 days and assayed for p24 antigen production by ELISA. The kinetics of p24 antigen production between 0-20 days of culture were similar under all three conditions for all six patients tested. The mean log difference in p24 antigen production between cells cultured either with PHA alone as compared to those cultured with IL-12 plus PHA was -0.18 ($\pm$0.19) at day 13-15 and -0.11 ($\pm$ 0.20) at day 19-21. Likewise, p24 antigen production by cells cultured with PHA alone was similar to those cultured with IL-2 plus PHA with a mean log difference of -0.48 ($\pm$ 0.49) at 19-23 days. In cultures from two patients, p24 production by cells cultured with PHA alone reached a plateau at day 19-20, but p24 production in the presence of IL-12 continued to increase; in these cultures, the cells grown with PHA were less viable after day 20. In cultures from the remaining four subjects p24 antigen production in each condition was similar throughout the culture. These data suggest that IL-12 does not promote endogenous viral outgrowth in activated PBMC isolated from HIV-infected individuals.

D4-240 A PROPOSED MECHANISM OF A TH-2 MEDIATED ESCAPE OF IMMUNE CLEARANCE BY HIV: THE MACROPHAGE "TROJAN HORSE" HYPOTHESIS RE-VISITED

Luis J. Montaner and Siamon Gordon. *Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK*

Th2 cytokines are regarded as factors detrimental to HIV immunity; responsible for the preferred phenotype of virus-expressing T cells and the negative regulation of Th1 responses in the host. We believe however, that Th2 cytokines may help both virus and host for different reasons. From the host's perspective, Th2 cytokines not only inhibit effective Th1 responses, but dampen a chronically activated immune system and reduce the potentially enhancing effects of TNF, IL-1 and IL-6 in virus expression and immune dysfunction. Supporting this hypothesis, we show that in either primary HIV-macrophage or HIV-PBMC infected cultures, viral inhibition results from combinations of IL-4 or IL-13 with TNF-apha or IL-1-beta respectively. In addition, macrophage-derived IL-10 has been shown to increase from the point of sero-conversion without decreasing early CTL responses, suggesting a potential role as an immune deactivation counter-response to infection. Hence, Th2 cytokines might actually be a factor in reducing viral load if one accepts the correlation between TNFα-IL1-IL6/NF-Kb/HIV. However, Th2 cytokines may be part of a general mechanism of viral persistence *in vivo* since the preferential viral production by Th2 T-cells downregulates Th1 responses and mediates a population of NEWLY-infected macrophages which would not express virus (data to support this regulation will be shown). An inhibition of newly infected macrophages would potentially protect these cells from immune-mediated clearance (i.e., CTL) directed towards nearby infected Th2 cytokine-producing T-cells. Furthermore, CTL-derived INF-gamma should also act to inhibit macrophage viral output. This mechanism is enhanced by the increase of HIV-1 entry into macrophages mediated by IL-13 and IL-4 resulting in higher viral output once the cytokine virostatic regulation is removed and another cycle of replication in Th2 T-cells begins. In conclusion, Th2 cytokines should be considered as a potential mediator pathway, shared between host and virus as a response to an uncontrolled virus-induced activation and a mechanism for persistence, respectively. We believe the Th2 cytokine pool is not homogeneous and that a therapeutic use of the Th2 anti-inflammatory property without directly affecting Th1 CD4 differentiation may be possible by the use of IL-13. The data to support this abstract/mechanism will be shown.

D4-241 PATHOGENESIS OF CD4 T CELL DEPLETION IN HU-PBL-SCID MICE, Donald E. Mosier[1], Richard Gulizia[1], Jacqueline Glynn[1], Ling Yin[1], and Jay A. Levy[2], [1]The Scripps Research Institute, La Jolla, CA 92037, [2]UCSF, San Francisco, CA 94143

We have previously shown that molecularly cloned HIV-1 isolates differ in their ability to cause depletion of CD4 T cells in hu-PBL-SCID mice. HIV-1_{SF33}, a T-cell tropic, cytopathic, SI isolate caused slow depletion of CD4 T cells, while HIV-1_{SF162}, a MØ-tropic, non-cytopathic, NSI isolate caused rapid loss of CD4 T cells. Several other macrophage-tropic isolates also caused rapid CD4 T cell depletion. We have extended these studies to better define the biologic mechanisms involved in these phenomena. Hu-PBL-SCID mice were infected with 100 tissue culture infectious doses of cell-free HIV or autologous PBL infected 3 days earlier with HIV *in vitro*, and CD4-, CD8-, CD3-, and CD45-positive human cells were enumerated by flow cytometry at 2 and 4 weeks after infection. Viral load was determined by quantitative PCR analysis of proviral copy number. Introduction of HIV-1_{SF33} as infected cells rather than free virus led to a much more rapid and complete loss of human CD4 T cells. The proviral copy number was increased 2.5-fold by cell-associated infection, but was still less than that achieved by infection with cell-free HIV-1_{SF162}. Examination of local lymph nodes repopulated by human cells by in situ hybridization, antibody staining for cell phenotypes, and the TUNEL assay for apoptosis showed differences in the localization of infected and dying cells following infection with different virus isolates and with cell-free versus cell-associated HIV-1. HIV-1_{SF162} infection efficiently established foci of infected cells when introduced either as cell-free or cell-associated virus, but HIV-1_{SF33} only caused infection of single cells when introduced as free virus. Cell death appeared to be triggered both in infected and uninfected cells, and bystander cell death was more prominent with the macrophage-tropic virus. Infection with chimeric SF2/SF162 viruses mapped these biological properties to *env*. Moreover, *nef* deletion mutants showed a reduced capacity to replicate in this model, and caused little if any CD4 T cell loss. We conclude that the ability to rapidly spread from cell-to-cell may be important in establishing foci of infection *in vivo*, and that high local concentrations of virus may contribute to both direct and indirect mechanisms of CD4 T cell death. Both *env* and *nef* gene products may be important for these properties of different virus isolates (Supported by NIH grants AI29182, AI30238, and AI34742).

D4-242 THE MECHANISM OF INHIBITION OF HIV REPLICATION IN MONOCYTES AND MACROPHAGES BY INTERLEUKIN 10

Hassan M. Naif, Joon Chang, Michael Ho-Shon, Shan Li, Anthony L. Cunningham, Department of Virology, ICPMR, CIDM, National Centre for HIV Virology Research, Westmead Hospital and The University of Sydney, Australia.

The mechanism of inhibition of HIV Ba-L replication in primary monocytes and macrophages was investigated using a semi-quantitative PCR for reverse transcribed HIV DNA, northern hybridisation for HIV mRNA expression and p24 antigen ELISA for extracellular HIV production. Interleukin 10 (IL-10) inhibited HIV replication when added prior to infection and but only partial effect was observed when IL-10 was added after HIV inoculation. IL-10 also markedly inhibited transcription of both unspliced and spliced HIV RNA with pre-incubation of IL-10 reaching a nadir at 7 days and recovering to normal levels by 10 days after a single application. The inhibitory effect was time and concentration dependent, with the optimum concentration being 25 ng/ml. Lesser inhibition of HIV RNA expression was also observed with addition of IL-10 after HIV infection. IL-10 also downregulated the expression of cellular genes including the transferrin receptor and 28S rRNA and also more markedly GAPDH. Monocytes were completely recovered from this inhibitory effect at day 10 after a single application of IL-10. However, IL-10 did not inhibit cellular mRNA expression in the macrophage cell line THP-1. All inhibitory effects were reversed by pre-incubation of IL-10 with neutralizing polyclonal antibody. Ethidium bromide/acridine orange staining revealed that cell detachment or loss of viability was minimal during the treatment period. Interaction of IL-10 and IL-4 in combination were antagonistic rather than synergistic, but IL-4 did not abrogate the inhibitory effect of IL-10 on HIV RNA. In addition, IL-10 prevented stimulation of HIV RNA expression by TNF-α but only if pre-incubated with monocytes for 48 hr before the addition of TNF-α. This data suggested that the inhibitory effect of IL-10 on HIV replication was maximal at 48 hr pre-incubation. It is mediated either by inhibition of the activation and differentiation processes of monocytes leading to a downregulation of cellular RNA transcription, or possibly through the effects of cellular gene expression on HIV mRNA. However, IL-10 had no effect on the levels of HIV cDNA or the process of initiation and completion of reverse transcription.

D4-243 SECRETION OF PREFORMED, MEMBRANE-BOUND 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.

Immune system deregulation and dysfunction are hallmarks of infection by the human immunodeficiency virus type 1 (HIV-1) and progression to AIDS. This is observed in both efferent arms of the immune system: cell-mediated and humoral. In HIV-1-infected humans, diminished responses to alloantigens and recall antigens may be noted. Early after infection, a polyclonal hypergammaglobulinemia may also be noted in some individuals. Although HIV-1 experimentally infects chimpanzees, no pathological consequences are observed. The mechanisms of this apparent resistance to HIV-1 pathogenesis are unknown, but may be related to decreased virus production by chimpanzee cells or to control of the infecting virus by the chimpanzee immune system. 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. No antibodies reactive with HIV-1 were noted in PBMC cultures from HIV-1-negative chimpanzees. The secreted antibodies mainly recognized HIV peptides gp160, gp120, p66, p51, and p31. In contrast, in addition to the above indicated polypeptides, plasma antibodies recognized the ***gag***-coded polypeptides p55, p24, and p17. This phenomenon is T-cell independent as 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. The reactive antibody appears to be preformed and on the lymphocyte membrane as treatment with trypsin or washes at 37°C reduced the amount detectable antibody. Similarly, cryopreserved cells no longer secrete these antibodies. 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 and secretion remain to be determined.

D4-244 THE INDUCTION OF TGFβ1 PRODUCTION BY HIV-1 AFFECTS THE DEVELOPMENT OF THYMOCYTES.

Colin O'Conell, Michael Rosenzweig, Bruce Freedman and Glen N. Gaulton. Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104-6142 USA.

The objective of this research is to determine the mechanisms whereby HIV-1 infection alters the development and function of lymphoid progenitor cells in fetal and neonatal thymus. The potential mechanisms for this inhibition include cytopathology as a result of HIV-1 infection of thymocytes, and/or the indirect actions of accessory cells or cytokines. An *ex vivo* system of human thymic organ culture (hu-TOC) was used to assess the regulation of cytokine production by thymic stromal cells and thymocytes in the absence and presence of HIV-1. Macrophage tropic HIV-1 isolates displayed aggressive replication in hu-TOC. Infection was assessed by PRC techniques in isolated thymic subpopulations from infected subjects and hu-TOC. HIV-1 was detected primarily in stromal cells with a macrophage-like $CD14^+$ $CD4^+$ phenotype, and to a lesser degree in all major thymocyte subsets. These results were verified by *in vitro* infection of isolated thymic macrophages. Analysis of cytokine production within infected macrophages showed that HIV-1 stimulated a near 10-fold increase in the production of TGF-β1, and the addition of exogenous TGF-β1 to hu-TOC profoundly affected thymocyte maturation. Gene therapy techniques were used to assess whether TGFβ1 production induced during HIV-1 infection of hu-TOC was responsible for altered thymocyte development. TGFβ1 antisense oligonucleotides were first introduced into either thymic stromal cell or thymocyte progenitors using amphotropic retroviral vectors, and the impact of these agents on thymocyte maturation in hu-TOC were studied. The effects of TGF-β1 antisense within hu-TOC will be presented as a therapy to inhibition of thymocyte progenitor function by HIV-1.

D4-245 QUANTITATION OF HIV-1 RNA IN PLASMA USING BRANCHED DNA (bDNA): EFFECT OF PATIENT BIOLOGIC VARIATION AND SPECIMEN MATRIX. Carol Pachl, Barbara Judson, Torange Yeghiazarian, Pamela Johnson, Diana Besemer, Roseann White, Steve Zanki, David Chernoff and John Todd. Chiron Corporation, Emeryville, CA 94608

HIV-1 plasma RNA levels change during disease progression and in response to antiviral therapy. To accurately measure the level of HIV-1 RNA, it is critical to understand the effect that biologic variability, specimen matrix and commonly used drugs have on RNA measurements. HIV-1 RNA was quantified using the Quantiplex™ HIV RNA assay, which is based on branched DNA (bDNA) signal amplification technology. The bDNA assay has been shown to provide precise quantitation, with an interassay variation in quantitation of ≤3-fold. Accurate quantitation of HIV-1 subtypes A-F has also been demonstrated. Biologic variability in HIV-1 RNA levels was assessed in 4 clinically stable individuals every 7 days over a 5-6 week period, and RNA levels remained constant (n=3) or varied 3.5-fold (n=1). A larger study (n=20 subjects) is in progress to monitor RNA variability over 56 days (samples taken day 0, 1, 2, 7, 14, 21, 28, 56). These samples are being collected into 3 anticoagulants (EDTA, ACD, heparin) in order to determine the effect of anticoagulant on RNA variability. A previous study showed that RNA levels were highest in specimens collected in EDTA. Diurnal variation is also being evaluated by monitoring RNA levels in 14 subjects (samples taken every 4 hrs for day 1 and every 8 hrs for day 2). Specimen matrix effect on RNA quantitation has been evaluated in specimens with elevated levels of lipids, hemoglobin or bilirubin (n=10 each group), and no effect was seen except for a reduction in quantitation using specimens containing ≥2 mg/ml hemoglobin. Finally, the effect of nucleoside analogue drugs, and drugs commonly used for OI prophylaxis, on RNA quantitation is being analyzed by spiking these drugs into HIV-1 positive and negative specimens (n=5). The effect of these variables on quantitation of HIV-1 RNA in plasma will be presented.

D4-246 IL-12 INDUCES HIV-1 REPLICATION IN CD8-DEPLETED PBMC FROM ASYMPTOMATIC BUT NOT SYMPTOMATIC SEROPOSITIVE INDIVIDUALS. Miguel-Angel Perales[1], Paul R. Skolnik[2] and Judy Lieberman[1], Divisions of [1]Hematology-Oncology and [2]Infectious Diseases, New England Medical Center and Tufts University School of Medicine, Boston, MA 02111.

IL-12, a cytokine which promotes T_H1 differentiation and enhances the cytolytic activity of T cells and NK cells, is currently being investigated in HIV-1 infected patients. Because stimuli that activate T cells generally activate HIV expression, we investigated the effect of IL-12 on viral replication in T cells from HIV-1 infected donors. CD8-depleted PBMC from 12 HIV-1 seropositive patients at various disease stages were incubated for 1 week in the presence of no cytokines, IL-2 (20 U/ml), IL-12 (10 U/ml) or the combination of IL-2 and IL-12. HIV-1 replication, measured by p24 Ag in the culture supernatant, was increased by IL-2 in most patients. IL-12, however, induced significant p24 levels only in patients who were either asymptomatic or had generalized lymphadenopathy. The levels of viral expression induced by IL-12 seen in those patients were comparable to those induced by IL-2. Furthermore, whereas in the asymptomatic donors, IL-12 and IL-2 had a synergistic effect on viral expression, IL-12 partially inhibited the IL-2 induced viral expression in symptomatic patients. To further investigate the possible role of patient differences in T_H subset representation on the varied effect of IL-12, the levels of IL-4 and IFNγ in the culture supernatants were also measured. In all infected asymptomatic donors, incubation of CD8-depleted PBMC with IL-2 induced IL-4 secretion, while addition of IL-12 to IL-2 decreased levels of IL-4 and increased IFNγ secretion. IL-4 secretion induced by IL-2 or the combination of IL-2 and IL-12 was significantly higher in cultures from the asymptomatic patients than from 5 seronegative controls. Comparable high levels of IFNγ secretion were induced in the presence of combined IL-2 and IL-12 in seronegative and asymptomatic seropositive individuals. Levels of both IL-4 and IFNγ induced by cytokines were reduced in the symptomatic patients. Synergistic enhancement of IFNγ secretion was induced by IL-2 and IL-12 in patients with ARC symptoms but not in patients with a history of AIDS-defining opportunistic infections. These findings support a progressive loss of cytokine-stimulated CD4 T cell function that correlates with disease stage and suggest that IL-12 immunotherapy may be most effective in patients who have symptoms of HIV infection but have not yet developed opportunistic infections.

D4-247 MUTATIONS IN THE SIVmac239 TM CYTOPLASMIC TAIL INCREASE EXPRESSION OF ENVELOPE GLYCOPROTEINS ON INFECTED CELLS. Monica M. Sauter[1], Beth S. Haggarty[1], Josephine Romano[1], Patricia N. Fultz[2], James A. Hoxie[1]; [1]Univ of Penn, Phila, PA 19104; [2]Dept of Microbiology, Univ of Ala, Birmingham, AL 35294.

We have described the *in vitro* derivation of a SIVmac variant, CP-MAC, from the BK28 molecular clone (La Branche, C., et al, J. Virology, 68: 5509, '94). CP-MAC was shown to exhibit a striking increase in the level of envelope glycoproteins on the surface of infected cells. Of the 11 coding mutations in CP-MAC *env* gene, the determinant for high surface envelope glycoprotein expression has been mapped to a single amino acid change of a Y to C, at position 723 in the TM cytoplasmic tail (LaBranche, C. et al, submitted). To determine if this mutation had the same effect on an SIV that did not have a premature termination codon in TM, we introduced a Y to C mutation at the analogous position (721) in SIVmac239, which has a full length TM cytoplasmic tail. This virus (SIVmac239C) was shown to replicate with RT levels similar to the parental virus in CEMx174 cells, as well as in rhesus and pig-tailed macaque PBL. CEMx174 cells chronically infected by SIVmac239 or SIVmac239C were analyzed for the surface expression of envelope glycoproteins by FACS with monoclonal antibodies to TM and SU. In 3 different experiments, cells with the Y721C mutation exhibited a 3 to 4 fold increase in the level of surface envelope glycoproteins. Sequence analysis of viral DNA from infected cells showed that the Y721C mutation was stable and that no new mutations in TM had occurred during replication. To better characterize the determinant responsible for this effect, we introduced other amino acid substitutions at position 721, including F, A , S and I. FACS analysis of chronically infected CEMx174 cells showed that substitution of F resulted in a minimal (< 2 -fold) increase in surface envelope glycoprotein expression while A, S and I substitutions produced increases that were comparable to that observed for the Y721C mutation. These data suggest that a structural determinant involving an aromatic amino acid at position 721 in the SIVmac239 TM cytoplasmic tail can modulate envelope glycoprotein expression on infected cells. Because SIVmac239C is able to replicate in rhesus macaque PBL, future studies will address the *in vivo* consequences of this mutation on viral pathogenesis and the host immune response.

D4-248 **INDUCTION OF HIV-1 REPLICATION CAN BE DISSOCIATED FROM INDUCTION OF CELL PROLIFERATION IN A PRIMARY CD4 CELL MODEL.** Celsa A. Spina and Douglas D. Richman. University of California and the VA Medical Center, San Diego, CA 92093.

HIV-1 can enter quiescent CD4 lymphocytes, establish a latent nonproductive infection, and subsequently be induced to replicate through T cell activation and proliferation. It is not understood which components of the T cell activation cascade are essential to virus induction or how different steps in cell activation influence the regulatory control of HIV replication. To study these required virus-cell interactions, we have used an *in vitro* cell model of acute infection of quiescent, primary CD4 lymphocytes with the NL4-3 clone of HIV, and induced T cell activation by CD2 and/or CD3 cross-linking compared to phorbol ester (PMA) stimulation. When PHA mitogen or high concentration anti-CD3 antibody was used to activate infected CD4 cells, the level of HIV replication was, in general, directly correlated with the level of induced cell proliferation. However, moderate virus replication (4-6X) could be obtained in the absence of cell division with the use of low dose PHA. Maximal virus replication (100-500X) was induced with suboptimal doses of anti-CD3 in combination with anti-CD28 costimulation. In contrast to the effects of surface receptor cross-linking, PMA + ionomycin activation resulted in only minor induction of HIV replication in the presence of high levels of induced cell proliferation. Addition of PMA to anti-CD3 stimulus caused enhanced CD4 cell proliferation, but significantly inhibited virus replication. These results demonstrate that induction of HIV replication is not linked universally to cell proliferation; rather, specific signal transduction pathways contribute essential factors to virus growth in addition to, or separate from, those required for cell division.

D4-249 **IMMUNE ACTIVATION OF SIV-INFECTED RHESUS MACAQUES INCREASES VIRAL LOAD AND MAY ACCELERATE DISEASE PROGRESSION,** Silvija Staprans[1], Ann Rosenthal,[2] Elizabeth Sinclair[1], Jim Carlson[2], Nick Lerche[2] and Mark Feinberg[1], 1. Gladstone Institute of Virology and Immunology, University of California, San Francisco, San Francisco, CA 94141; 2. University of California, Davis, Davis, CA 95616

Antigenic stimulation during HIV infection leads to transient yet substantial increases in plasma viremia. The antigen-responding population of T cells may therefore be preferentially targeted for infection and the cytopathic effects of viral replication. To investigate whether there is selective depletion of cells responding to the challenge antigen, we utilized the SIV model of AIDS, and inoculated SIV-infected rhesus macaques with conventional antigens (DPT) or superantigens (SEC_3). Increased plasma viremia, monitored by QC-PCR, was observed in all animals following antigenic stimulation. Levels of cellular activation and viral replication in lymph nodes are being investigated by immunohistochemistry and in situ hybridization in superantigen challenged animals. To determine whether repeated antigenic stimulation leads to depletion of the antigen-responding population of T cells, SIV-infected animals have been repeatedly challenged with tetanus. Antigen-specific immune responses, specifically the ability to boost antibody titers and mount T cell proliferative responses, are being monitored in these animals. Preliminary observations suggest that such repeated immunization accelerates disease progression.

D4-250 HIV-1 INDUCED THYMOCYTE DEPLETION IS ASSOCIATED WITH INDIRECT CYTOPATHICITY AND INFECTION OF PROGENITOR CELLS *IN VIVO*. Lishan Su, H. Kaneshima, M. Bonyhadi, S. Salimi, D. Kraft, L. Rabin and J. M. McCune, HIV Group, Systemix, Inc.1501 California Ave., Palo Alto, CA 94304

To understand HIV-1 pathogenesis *in vivo*, the SCID-hu (Thy/Liv) model has been used to study the mechanisms of HIV-1 induced human T cell depletion. HIV-1 infection of SCID-hu Thy/Liv mice leads to massive human T cell depletion, especially of CD4+ T cells. An active cell death program (apoptosis) is induced in these T cells by HIV-1 infection.

Direct and indirect cytopathic mechanisms have been proposed to account for the loss of CD4+ T cells after infection with HIV-1. We report here that HIV-1 infection of the human thymus *in vivo* results in thymocyte depletion by at least two different mechanisms: indirect apoptotic destruction of uninfected cells and direct infection and destruction of intrathymic ($CD3^-CD4^+CD8^-$) progenitor cells. These mechanisms are differentially induced by distinct isolates of HIV-1.

D4-251 HIV-INDUCED T CELL SYNCYTIA TRANSLOCATE AND PHAGOCYTOSE THROUGH THE EXTENSION OF GIANT PSEUDOPODS, Andrew Sylwester[a], Damon Shutt[a], Deborah Wessels[a], Seamus Murphy[a], Jan Stites[a], Jack T. Stapleton[b], Ronald Kennedy[c], and David R. Soll[a], [a]Department of Biological Sciences, [b]Department of Internal Medicine, University of Iowa, Iowa City, IA 52242, and [c]Southwest Biomedical Research Foundation, San Antonio, TX 78284

In contrast to the previous view that HIV-induced T cell syncytia are inert, disorganized fusion products in the throes of death, we have discovered that HIV-induced T cell syncytia are motile, and translocate through extension of giant pseudopodia. Using immunofluorescent staining of nuclei and cytoskeletal elements, we have found that syncytia mimic the nuclear and cytoskeletal organization of single T cells. Through the use of a computer-assisted motion-analysis system, we have also found that syncytia translocate at velocities and with the directionality of single T cells. In addition, syncytia up to 100 times the volume of single cells exhibit the same 1.6 min behavior cycle as single T cells. This has been demonstrated for SupT1 cells and CD4-positive peripheral blood T cells, and suggests that motility cycles, previously described in cell types ranging in complexity from soil amoebae to human polymorphonuclear leukocytes, are size-independent. Using transmission electron microscopy, we have also found that HIV-induced SupT1 syncytia phagocytose single SupT1 cells. Giant syncytial pseudopods wrap around T cells during the process of engulfment. These latter results suggest for the first time that, at least *in vitro*, there are two avenues of syncytium-mediated T cell death, fusion and phagocytosis.

D4-252 **Quantification of HIV-1 RNA in plasma using the NASBA® amplification system compared to an in-house quantitative RNA-PCR and the Amplicor® HIV monitor test.** A-M Vandamme[1], J-C Schmit[1], S Van Dooren[1], K Van Laethem[1], E Gobbers[2], W Kok[2], N Albrecht[3], R Huygen[4], J Desmyter[1], and E De Clercq[1] (1) Rega Institute for Medical Research, Leuven, (2) Organon Teknika, Turnhout (3) Roche Diagnostic Systems, Gent, Belgium (4) Pharmacia Biotech, Roosendaal, The Netherlands.

Quantitative NASBA is a new way of assessing plasma viral load based on co-isolation and isothermal co-amplification of HIV-1 RNA with 3 internal standards followed by electro-chemiluminescent detection of amplification products. Linearity of the assay extended over a 4 log range, and the detection limit was between 10 and 100 $CCID_{50}$/ml of plasma using 0.1 ml as input. The high reproducibility was confirmed by a standard deviation lower than log 0.23 for samples from 100 to 10 000 $CCID_{50}$/ml. The in-house test involved extraction of HIV-1 RNA using RNAzol (Biotecx Laboratories), cDNA synthesis and amplification with fluorescently labeled primers using the Perkin-Elmer RNA-PCR kit, and detection and quantification of the amplified product on the A.L.F. DNA sequencer using an external RNA standard. The Amplicor test was performed according to the manufacturers recommendations.
For five patient samples tested with all three techniques, one was negative in all assays, two had a comparable viral load (within one log) in all tests, one was about 1.5 log higher when using the external standard compared to the other 2 assays, and one was two logs lower in the Amplicor test compared with the other two assays. An additional 13 samples were tested with Amplicor and NASBA. From the 18 samples, 12 viral load values (67 %) were within one log when both methods were compared, 4 (22 %) had a difference of 2 logs, and 2 (11 %) had a difference of 1 log. For most samples, the three methods give comparable viral load values. However, for a significant number of samples the three assays perform differently, with no consistent deviations observed with one particular method. Since the NASBA and the Amplicor test have proven to give reproducible results, other factors such as the type of viral strain are possibly involved. Therefore, to follow the change in viral load in a patient, one method has to be used.

D4-253 **FACTORS GOVERNING THE DIFFERENTIAL REPLICATION OF HIV IN Th1/Th0 CELLS.**

Vyakarnam A.*,Matear PM*., Bruisten S+., Huisman H+. *UCL Medical School, London W1P 9PG. U.K; +Central Blood Transfusion Service., Amsterdam.

Previous work from our laboratory has shown that HIV replicates less efficiently in CD4+ Th1 compared to Th0 clones. We now report on some of the mechanisms which underlie this difference. Differential virus replication in Th1/Th0 cells is:
(i) not due to unequal virus entry as competitive quantitative PCR on the clones immediately after virus infection showed that both the Th1 and the Th0 clones had similar number of integrated HIV DNA copies per cell (ranging from 0.2-8 DNA copies/10e3 cells).
(ii) linked to cell specificity - HIV specific Th1 cells supported HIV replication less efficiently than Th1 cells which were PPD-specific or derived by random activation.
(iii) linked to cytolytic potential - Th1 cells killed autologous antigen coated BCL targets more efficiently (by 3-5 fold) than Th0 cells. Direct cell-cell killing by HIV specific Th1 cells could explain why these cells were less efficient than non-HIV specific Th1 cells in supporting HIV.
(iv) not linked to the activation state of the cells: the differences in virus replication between Th1 and Th0 cells were maintained whether or not the cells were activated with cognate antigen or mitogen.
(v) linked to intrinsic differences in cell survival upon activation: Higher proportion of Th1 compared to Th0 cells underwent cell death upon activation. This could reduce HIV replication in activated Th1 cells.
(vi) linked to differential cytokine production - Th1 but not Th0 cells produced factors which inhibited HIV in secondary cultures. In contrast, cell free supernatants of Th0 cells contained factors which enhanced HIV.

Our results could have several important implications (a) they explain how Th1/Th0 or Th2 cells could play an opposing role in HIV infection; (b) the preferential replication of HIV in Th0 cells could explain why these cells might be lost in sites of active replication and (c) The loss of Th0/Th2 cells could have a dual effect on disease: on the one hand Th0 cells might be important in promoting disease; on the other hand the loss of these cells could lead to the overproduction of cytokines like IFN gamma which would normally be inhibited by IL-4 producing Th0 or Th2 cells.

D4-254 THE CORRELATION BETWEEN VIRAL LOAD AND DISEASE OUTCOME IN SIV INFECTED MACAQUES. Andrew Watson, Jan McClure, Jane Ranchalis, William Sutton, Bruce Travis and Nancy Haigwood. Bristol-Myers Squibb, Seattle, WA. 98121.

A quantitative approach has been used to define more precisely the relationship between virus load and disease outcome in macaques infected with SIV. A procedure for quantitating plasma viremia was developed from a method described by Piatak et al (Science. **259**, 1749,1993) and is based on the inclusion of a fixed copy number of an internal control RNA template into the reverse transcription and amplification reactions. The assay is stable, sensitive and has been used to quantitate viremia in macaques infected by SIVsmE660, SIVmne and HIV-2EHO$_{287}$. Results from the infection of ten *M. mulatta* with SIVsmE660 for over six months show that plasma viremia falls into at least three broad patterns: (i) viremia levels peaking at approx. week 2 post-infection, declining by week 4 and then following individual profiles which can vary by greater than 2 $\log_{10}$ particles/ml between animals, (7/10) (ii) immediate high viremia which fails to decline and increases until early death (2/10) and (iii), consistently very low viremia, with no detectable acute phase. Infection detected at limit of assay sensitivity and confirmed with other procedures (1/10). The plasma viremia patterns observed were also found to be in good general agreement with the infected PBMC frequency data. After 28 weeks of infection, viral load has emerged as a major determinant of clinical outcome with more rapid disease progression associated with high virus levels. Thus, the two animals which maintained the highest viremia levels have died while those with the lowest levels of viremia remain healthy. A full analysis of the relationship between viral load and disease outcome in SIVsmE660 infected *M. mulatta* will be presented.

D4-255 GENETIC CHARACTERIZATION OF THE HIV- 1 LTR REGION OF LONG-TERM SURVIVORS,

Linqi Zhang, James Ip, Yaoxing Huang and David D. Ho, The Aaron Diamond AIDS Research Center, New York University, School of Medicine, 455 First Avenue, New York, NY 10016

Although the great majority of HIV-1 infected individuals develop AIDS within approximately 10 years, there is a small but significant number of individuals who remain clinically healthy without disease progression. While multiple factors may contribute to the prolonged asymptomatic infection, HIV-1-related factors must also play an important role. In this study, we have characterized the LTR regions of 10 long-term survivors. A region of the 5' LTR region was amplified by PCR directly from PBMC from each case and subsequently cloned, sequenced, and analyzed. Of the 10 long-term survivors studied, 9 were PCR positive. Sequence analysis has demonstrated that there is a substantial degree of sequence variation in the U3 region whereas in the R and U5 regions, sequences are relatively conserved. Both deletion and/or duplication have been found in the U3 region, localizing primarily in the upstream NF-kB sites, in three patients (CD, D, BO). In one case (D), NF-kB sites have been completely deleted in multiple samples over 6 years as well as in the 3' LTR. In addition, significant G to A hypermutation has been observed in one patient (RP) which resulted in the loss of NF-kB sites as well as Sp1 binding sites. Functional consequences of these mutations are currently being studied.

Early Events, Immune Responses, Viral Diversity, Host Factors, Vaccines, Primate Models

D4-300 **HIV-2 SEQUENCE VARIATION IN GUINEA-BISSAU: CORRELATION TO CLINICAL OUTCOME**

Jan Albert, Thomas Leitner, Anders Nauclér, Department of Clinical Virology, Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden.

The clinical manifestations of HIV-2 infection in Guinea-Bissau shows an interesting dichotomy in that the highest incidence of infection is seen in comparably old individuals (50-59 years), whereas the mean age of HIV-2 associated AIDS cases is lower (approximately 40 years). One explanation for this could be that the two groups are infected with distinct virus strains which differ in virulence. The purpose of this study was to genetically characterize and compare HIV-2 strains from patients with different clinical manifestations.

Blood samples were obtained from clinically and immunologically well-characterized HIV-2 infected individuals in Guinea-Bissau; ten were asymptomatic and less than 25 years old, 10 asymptomatic and more than 50 years old and 10 had overt HIV-2 associated AIDS. Regions corresponding to the HIV-2 matrix (MA) protein and envelope V3 domain were directly sequenced from uncultured lymphocytes.

All individuals were shown to carry virus strains belonging to subtype A of HIV-2. Phylogenetic tree analyses of MA and V3 sequences did not reveal any specific clustering pattern for sequences from AIDS patients or asymptomatic individuals.

This study shows that all examined HIV-2 infected individuals from Guinea-Bissau carried virus strains which belong to subtype A and which are genetically closely related despite the fact that the clinical manifestations varied widely. Thus, these differences in clinical outcome must be due to as yet unidentified minor genetic differences between different HIV-2 strains and/or host-specific differences.

D4-301 **ADAPTATION OF HIV-1 NEF-SPECIFIC CTL REPERTOIRE TO EPITOPE VARIATIONS WITH DISEASE PROGRESSION**

B. Autran[1], G. Haas[1], U. Plikat[4], Y. Dudoit[1], O. Bonduelle[1], C. Katlama[2], B. Maier[3],A. Meyerhans[4], H.G. Ihlenfeldt[5], G. Jung[5],and P. Debré[1]. [1] URA CNRS 625, Immunlogie cellulaire and [2] Dept. Mal. Infect., Hôp. Pitié-Salpétrière, Paris, France, [3] Max-Planck-Inst. für Immunbiologie, Freiburg, [4] Inst. für Virologie, Freiburg, [5] Universität Tübingen, Germany.

Cytotoxic T cell (CTL) responses specific for HIV proteins play a major role in controlling HIV infection though they appear unable to definitely control virus replication and progression to AIDS. In an effort to investigate the virus mutations as the cause of such CTL final inefficiency, we studied HIV-specific CTL responses in a cohort of 50 patients ($CD4^+$ counts: 150-800/ mm^3) over three years. A part from the HIV-LAI proteins recognition we focused our attention on Nef and simultaneously analyzed autologous virus variations and CTL recognition of 5 epitopes containing the anchor motives for HLA-A2 and B7. Four patients at 2 time points were selected for which CTLs recognized Nef. Their CD4 counts ranged between 150-400/mm^3. Nef-specific CTL were detected in 50 % of the patients, while CTL specific for Env, Gag, Pol were detectable in 80% of patients at all stages of the infection. We characterized 3 new CTL epitopes restricted by HLA-A2: Nef 136-145, Nef 180-189 and Nef 190-198 and two epitopes in association with HLA B7 at Nef 68-77 and Nef 128-137. Three patterns of epitope variation and CTL evolution were obtained: First, we observed constant CTL recognition over time of Nef 180-189 and 190-198 epitopes despite high levels of viral mutations in these regions. epitope-specific CTL detectable in 1992 recognized the major viral variants that disappeared two years later. Second, dominant viral variants occurred in the HLA-B7 restricted epitope Nef 128-137, which were only weakly recognized by CTL in 1992. However, these CTL were amplified in vivo 2 years later in parallel to the relative decrease of the initial dominant variant. These 2 patterns indicated an apparent control of virus replication by CTLs. Third, we found a constant CTL recognition of the HLA-A2-restricted epitope Nef 136-145, which displayed little variation ; the major variant persistance indicating an insufficient control of viral replication in vivo. Interestingly, we did not detect mutations at amino acid positions containing HLA-A2 or -B7 anchor motifs that would abolish CTL recognition. Altogether these data strongly suggest: that 1/ the epitope-specific CTL repertoire is maintained with disease progression, 2/ and is adaptating to viral epitope variation and controlling viral replication in patients with less than 200 $CD4^+/mm^3$, and 3/ viral variations or persistence occur independently of a CTL escape phenomenon. The in-vitro amplification of CTL recognizing and controlling viral variants at advanced stages of the disease appear therefore feasible and might be useful for application in immunotherapy.

D4-302 PRESENCE OF NEUTRALIZING ANTIBODIES IN THE CERVICAL VAGINAL SAMPLES FROM HIV-INFECTED WOMEN. Padma Baskar, Anne Rampalo* , Thomas Quinn * and James E.K. Hildreth. Department of Pharmacology and Molecular Sciences and Division of Immunology and Infectious Diseases *, The Johns Hopkins School Medicine, Baltimore, MD, 21205.

Over the past decade, the acquired immunodeficiency syndrome (AIDS) epidemic has spread worldwide, and currently 14 million people are estimated to be infected with HIV, the virus that causes AIDS. The World Health Organization has estimated that 80% of all HIV transmission is now through heterosexual contact. Male to female transmission appears to be more efficient than female to male, and therefore, there may be biological factors that may affect transmissibility as well as susceptibility to HIV infection among women. Systemic immunity alone may be inadequate to interrupt heterosexual transmission. Therefore, this study is undertaken to investigate the potential role of neutralizing antibodies in vaginal secretions and sera of infected women. Examination of cervical vaginal samples (CVL) and plasma samples from HIV-infected women showed the presence of HIV specific IgG antibody. In addition, CVL samples showed weak reactivity for IgA antibody by Western Blot analysis. To assess the functional activity of these samples, neutralization assays were performed. The majority of the plasma samples had significant but varied neutralization titers against $HIV\text{-}1_{RF}$, comparable to that of pooled positive sera. Neutralization activity in CVL samples was low compared to the plasma. However, concentrated CVL samples showed dose-dependent virus neutralization. The plasma and CVL samples from two HIV-uninfected individuals had no neutralization activity. These results indicate the presence of HIV-specific, neutralizing antibodies in the CVL of HIV infected women.

D4-303 **FUSION INHIBITORY ACTIVITIES OF ANTI-ENV ANTIBODIES ASSAYED BY A VACCINIA-BASED QUANTITATIVE REPORTER GENE ACTIVATION ASSAY**

Edward A. Berger, Paul E. Kennedy, Christopher C. Broder, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, 20892 USA

We have developed a quantitative, sensitive, and highly versatile vaccinia-based assay for cell-cell fusion mediated by the CD4/HIV-1 env glycoprotein interaction, based on the activation of the *LacZ* reporter gene by T7 RNA polymerase selectively in fused cells. The assay has a rapid throuput (1 day) with considerable biosafety advantages compared to methods using live HIV. Moreover it can be readily adapted to analyze fusion mediated by envs from diverse HIV isolates, tested against different CD4+ cell types (e.g., continous CD4+ cell lines, PBMCs, primary macrophages). We have optimized the assay to quantitate the fusion-inhibitory activities of various antibody reagents. Sensitivity for detection of fusion inhibition was improved by experimental manipulations that reduced the expression level of vaccinia-encoded env. MAbs directed against the V3 loop of gp120 showed extremely potent fusion inhibition when tested against the homologous env, consistent with the HIV neutralizing actiivities of these antibodies. Certain antibody reagents capable of blocking CD4/gp120 binding also inhibited cell fusion in this system. We are extending our analyses to other classes of MAbs (e.g., MAbs reactive with gp41 epitopes; MAbs preferentially reactive with different oligomeric forms of env, etc.) with the goal of testing whether inhibition of cell fusion correlates with neutralization of HIV virus. We are also extending these studies to analyze fusion-blocking antibodies in various types of serum (e.g., HIV-infected individuals, vaccine animal models, vaccine trial participants).

D4-304 CLONING AND CHARACTERIZATION OF A LARGE PANEL OF ANTIBODIES TO GP41.

James M. Binley[1], Henrik J. Ditzel[1], P.H.W. Parren[1], R. Michael Hendry[2], Lynette S.W. Sawyer[2], Nancy Dunlop[3], Peter L. Nara[3], Carlos F. Barbas[1], and Dennis R. Burton[1], [1] The Scripps Research Institute, La Jolla, [2] California Institute of Public Health, Berkeley, CA, [3] Laboratory of Tumor Cell Biology, NCI, NIH, Bethseda, MD.

Combinatorial antibody libraries expressed on the surface of filamentous phage have been used to isolate antibodies with specificities to the V3 loop, V2 loop, and CD4 site of gp120. A large panel of human Fab fragments to a recombinant HIV-1 gp41 protein was cloned from 6 phage-displayed antibody libraries prepared from seropositive donors. All 23 Fabs were found to be dependent on structural determinants of gp41. Of epitope involving residues 644-663 ("Mt12 region"). The other 2 Fabs reacted with an epitope involving the immunodominant region (residues 579-613). Sequences of the heavy chain CDR3 of 6 of these were highly homologous. Although 4/6 of these appear to have arisen from the same germline progenitor, two come independent germline rearrangements (70.5-76.4% homology to other members of the group). This implies a scenario where a highly immunogenic structural region of gp41 affinity selects for specific sequence binding motif under the conditions of persistent infection. None of these antibodies exhibited virus neutralization. Cross competition analysis between members of the initial antibody panel revealed a series of overlapping specificities between and within the two immunogenic regions. Interestingly, members of the group of 6 Fabs with heavy chain CDR3 motif more effectively inhibited Fabs to the immunodominant region (579-613) than other Fabs to the "MT12 region". This assigns the 6 "CDR3 motif" antibodies to an epitope touching the "MT12 region" but overlapping the immunodominant region. The cloning of further more rare gp41 specificities is being explored by masking gp41 at the highly immunogenic epitopes above.

D4-305 MICRONUTRIENT NUTRITION IN HIV-1 INFECTED HETEROSEXUAL ADULTS, John D. Bogden, Joan H. Skurnick, Herman Baker, Francis W. Kemp, Alice Sheffet, Gloria Quattrone and Donald B. Louria, Department of Preventive Medicine and Community Health, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2714

There is compelling evidence that some micronutrients can profoundly affect immunity; knowledge of the changes in micronutrient status that occur during HIV infection may provide insight useful for making clinical decisions and for understanding disease progression. We surveyed vitamin supplement use and serum concentrations of 23 nutrients in a cohort of 64 HIV seropositive subjects participating in a study of heterosexual HIV transmission. Only 9 (14%) reported a weight loss of 5 kg or more in the 6 months prior to blood withdrawal; none showed evidence of wasting. Seronegative controls (n=33) were also studied. Two blood samples were collected 1-4 months apart for most subjects. Blood concentrations measured were retinol, ascorbate, alpha-tocopherol, total carotenes, vitamins B6 and B12, folic acid, thiamin, niacin, biotin, riboflavin, pantothenic acid, glutathione, biopterin, inositol, free and total carnitine, free and total choline, copper, zinc, magnesium and selenium. Seronegative controls had significantly ($p < 0.05$) higher plasma concentrations of carotenes, glutathione, total choline, and magnesium than HIV+ subjects. The percent of HIV+ subjects with low concentrations was greatest for vitamin C (20%), total carotenes (26%), and magnesium (51%). Within each of the 3 categories of HIV disease (CDC Stage A, B or C), subjects ingesting vitamin supplements tended to have fewer low antioxidant (ascorbate, alpha-tocopherol, carotenes, retinol) concentrations than those not taking them. Across all HIV status categories, supplemental intake significantly reduced the number of low antioxidant concentrations (stratified exact trend test, $p = 0.0006$). The frequent occurrence of abnormal micronutrient concentrations, as found in these HIV+ subjects, may contribute to disease pathogenesis. (Supported in part by NIH Grant NO1-A1-95013).

D4-306 COMPARISON OF DIRECT AND STIMULATED ASSAYS OF HIV-1-SPECIFIC CTL ACTIVITY IN A PROSPECTIVE STUDY OF INFECTED INDIVIDUALS.

Robert C. Bollinger, Michel N. Lubaki, Stuart C. Ray, Ramesh Paranjape, Robert F. Siliciano. Division of Infectious Diseases, The Johns Hopkins Medical School, Baltimore, MD 21205

In an attempt to determine a more sensitive and efficient method for measurement of CTL activity, we have prospectively compared a direct assay and an antigen-specific stimulation assay for measurement of HIV-1-specific CTL activity. Fresh PBMC isolated from infected individuals were assayed for direct CTL activity specific for HIV-1 *env*, *nef* and *gag* expressed by vaccinia infected autologous B-LCL in a standard ^{51}Cr release assay. In addition, an aliquot of the fresh PBMC were stimulated in vitro, using an antigen-specific stimulation technique with psoralen/UV treated B-LCL infected with vaccinia expressing HIV-1 *env* (vPE16), *gag* (vvgag) or *nef* (vvnef). Responding cells were then assayed after 10 days for HIV-1-specific CTL activity. Our preliminary results suggest that direct HIV-1-specific CTL activity, presumably reflecting an activated CTL population is generally low at all stages of HIV disease and decreases with decline in CD4 count. In a some patients a significant decline in CD4 count late in disease appears to be associated with a transient increase in direct CTL activity. Measurement of viral load from stored plasma from these patients in ongoing to determine if this increase in direct CTL is associated with a corresponding increase in viral replication in vivo. In contrast to the direct assay, the antigen-specific stimulation procedure greatly increases the sensitivity of detection of CTL activity from patients at all stages of disease and may reflect the level of memory CTL activity. In most patients, the CTL activity detected with this stimulation procedure is well preserved until very late in the course of HIV disease. Confirmation of this observation is ongoing using a limited dilution analysis to quantitate CTL precursor frequencies from these patients.

D4-307 IDENTIFICATION OF AN N-LINKED GLYCAN IN THE V1-LOOP OF HIV-1 gp120 INFLUENCING NEUTRALIZIATION BY ANTI-V3 ANTIBODIES AND SOLUBLE CD4

Bolmstedt A.[1], Gram G.[2], Hemming A.[1], Jansson B.[1], Åkerblom L.[3], Nielsen J-O[2], Hansen J-E[2] and Olofsson S.[1] [1]Department of Clinical Virology, University of Göteborg, Sweden; [2]Laboratory of Infectious Diseases 144, Hvidovre Hospital, , Denmark; [3]The National Veterinary Institute , Biomedical Center, Uppsala, Sweden.

Glycosylation is necessary for HIV-1 gp120 to attain a functional conformation and individual N-glycans of gp120 are important, but not essential, for replication of HIV-1 in cell culture. We have constructed a mutant HIV-1 infectious clone lacking a signal for N-linked glycosylation in the V1-loop of HIV-1 gp120. Lack of an N-linked glycan was verified by a mobility enhancement of mutant gp120 in SDS-gel electrophoresis. The mutated virus showed no differences in either gp120 content per infectious unit or infectivity, indicating that the N-linked glycan was neither essential nor affecting viral infectivity in cell culture. We found that the mutated virus lacking an N-linked glycan in the V1-loop of gp120 was more resistant to neutralizing by monoclonal antibodies to the V3-loop and neutralization by soluble recombinant CD4 (sCD4). Both viruses were equally well neutralized by ConA and a conformation dependent human antibody IAM-2G12. This suggests that the N-linked glycan in the V!-loop modulates the three-dimensional conformation of gp120, without changing the overall functional integrity of the molecule.

D4-308 SERUM LEVELS OF SUPERANTIGEN/gp120-BINDING VH3 ANTIBODY IS A POLYMORPHIC PHENOTYPE ASSOCIATED WITH SUSCEPTIBILITY TO HIV TRANSMISSION, Jonathan Braun, Janet Townsley-Fuchs, Larry Kam, and Lee Goodglick. Department of Pathology and Lab Medicine, and the Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Recently, a superantigen for the common VH3 immunoglobulin gene family has been identified in the second constant domain of HIV-1 gp120. Since HIV has apparently evolved an epitope capable of binding a major subset of prevalent antibodies, we have tried to imagine why this would be an advantage to the virus. Our concept is that this superantigen system acts to direct the virus to Fc receptor-bearing cells, thereby enhancing the efficiency of infection. In collaboration with the UCLA Multicenter AIDS Cohort Study, we have sought epidemiologic evidence for this hypothesis. We evaluated two predictions. First, we asked whether SAG-binding Ig levels are a polymorphic trait in the general population. Over 100 low-risk seronegative individuals were evaluated for their level of gp120/SAG-binding serum IgM. We observed that these antibody levels varied dramatically among individuals (40-fold). However, among 16 individuals monitored over 6 months, the level in each individual was constant over time. Thus, the level of SAG-binding IgM is a natural polymorphic trait in the general population. Second, we asked whether individuals highly susceptible to HIV transmission correspond to those with the high-phenotype SAG-binding Ig trait. Initially seronegative MACS subjects were grouped by statistical criteria (level of high risk behavior; duration of time to seroconversion) into those who were highly susceptible or resistant to infection. Sera from individuals >2 years prior to seroconversion were tested for levels of superantigen-binding Ig. Among 16 resistant individuals, the levels had a distribution similar to the general population. In contrast, antibody levels in 16 susceptible individuals were remarkably elevated (mean, 8-fold greater than the resistant population; $p<0.016$). These findings strongly support the prediction that the high antibody phenotype is a host susceptibility factor in HIV transmission.

D4-309 ANTIGENIC TARGET SITES FOR HIV-1 SPECIFIC ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

Kristina Broliden, Agneta von Gegerfelt, Anders Vahlne*; Swedish Institute for Infectious Disease Control, Dept of Virology, 105 21Stockholm, Sweden, * Dept of Clinical Virology, Huddinge Hospital, Stockholm, Sweden.

Hyperimmune antisera derived from monkeys against forty peptides together representing the entire envelope gp120 of HIV-1 (HTLV-IIIB) were used to map antibody-dependent cellular cytotoxicity (ADCC) target regions. Four regions corresponding to amino acids 65-125, 152-230, 248-330, and 445-466 were found to contain epitopes inducing ADCC activity not only against HTLV-IIIB infected cells but also against cells infected with SF2 and fresh clinical isolates of HIV-1. Furthermore, a mouse-human chimaeric monoclonal antibody directed against the V3 region of gp120 could mediate ADCC indicating the importance of this region as a target for ADCC. When comparing seroreactivity to the individual peptides with ADCC titers none of the regions seemed to be dominant. These results describe regions involved in the functional immunity against HIV-1 and will probably be of importance in the development of a vaccine against HIV.

D4-310 ANALYSIS OF CROSS-CLADE NEUTRALIZATION BY HIV-1+ HUMAN SERA

Yunzhen Cao, John Moore, John Leu and David D. Ho, The Aaron Diamond AIDS Research Center and NYU School of Medicine, 455 First Avenue, New York, NY 10016

To determine whether there might be neutralization serotypes of HIV-1 that correspond to the genetic subtypes, we have performed neutralization checkerboard analyses using primary isolates from clades A-E and autologous and heterologous sera from the same clades. For one checkerboard, we used sera that had been screened for high-titer anti-gp 120 antibodies. In general, neutralization of the primary isolates by the HIV-1^{+} sera were sporadic; a few sera showed reasonable neutralizing activity, and a few viruses were moderately neutralization-sensitive. However, we are unable to detect any pattern of neutralization that was clade-specific, and we conclude that there is no simple relationship between the genetic clades of HIV-1 and neutralization serotypes. Rather than a single, common neutralization serotype of HIV-1, our data are more consistent with there being a no serotype of any significance.

D4-311 ANALYSIS OF WIDELY DIVERGENT SIMIAN IMMUNODEFICIENCY VIRUSES IN FERAL AND PET SOOTY MANGABEYS IN WEST AFRICA, Zhiwei Chen, Paul Telfer, Patricia Reed, Linqi Zhang, Agegnhu Gettie, Mary P. Avalle, David D. Ho and Preston A. Marx, AIDS Animal Models Laboratory at LEMSIP, The Aaron Diamond AIDS Research Center and Department of Microbiology, New York University Medical Center, New York, NY 10016

Sooty mangabeys (SM, *Cercocebus torquatus atys*) are presumed to be the natural host of the simian immunodeficiency virus (SIV), which gave rise to HIV-2. Here we describe SIV isolates from feral and pet SMs for the purpose of identifying and characterizing unknown viruses and to provide information as to the origin of HIV-2. In a feral troop, four of ten sooty mangabeys had SIV antibody, and the first SIV from a feral SM, SIVsmSL92a (Sierra Leone 1992), was isolated. Among 91 households with pet SMs, four animals had SIV antibody and another new virus, SIVsmSL92b, was obtained.

For genetic analysis, proviral *gag* and *env* fragments were amplified by nested PCR directly from uncultured host cells derived from four feral SMs of a single troop and three pet SMs. Different phylogenetic approaches showed that these viruses are members of SIVsm/HIV-2 lineage. They are widely diverged and form the most divergent group reported thus far in a single monkey subspecies, suggesting that like SIVagm, SIVsm existed in SMs long before the AIDS epidemic in humans. The topology of the phylogenetic tree, based on SIVsm sequences, indicated that SIVs previously isolated from macaques (SIVstm and SIVmac/mne) originated from only two cross-species transmissions from SIVsm infected sooty mangabeys.

Cross-species transmission to humans was also addressed in West Africans. One of 14 people who had contact with sooty mangabeys harbored SIV-like DNA and antibodies. In fact, the DNA sequences were closely related to a SIVsm found in a household pet sooty mangabey in Liberia. Our data suggest that HIV-2 subtypes may arise from both multiple and continuous entries from SIVsm infected African sooty mangabeys into the human population.

D4-312 CELLULAR IMMUNE RESPONSES FOLLOWING PRIMARY HIV INFECTION, Elizabeth Connick, Donna Hoak, Rick Schlichtemeier, Patricia Uherova, Franklyn N. Judson, and Robert T. Schooley, Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, CO 80262.

We examined natural killer (NK) cell activity, cytotoxic T lymphocyte (CTL) activity, and lymphocyte proliferative responses, as well as isolated virus from peripheral blood mononuclear cells (PBMC) of 10 recent HIV seroconverters. These subjects were prospectively identified from a cohort of 768 high risk HIV seronegative homosexual men tested for HIV antibodies at 6 month intervals.

METHODS: PBMC were separated from whole blood at weeks 0, 6, and 12 following seroconversion. NK activity was assayed using K562 cells in a standard ^{51}Cr release assay. CTL activity was assessed in a ^{51}Cr release assay using EBV transformed B cells infected with vaccinia recombinants expressing the HIV-1 envelope (MN) or gag antigens, or *E. coli* beta-galactosidase as a negative control. Lymphocyte proliferative responses to candida, tetanus, and PHA were assayed by ^{3}H-thymidine incorporation. Virus was isolated from PBMC by co-cultivation with allogeneic PBMC stimulated with PHA and IL-2.

RESULTS: Seven of 10 subjects reported a flu-like illness, which may have represented their primary HIV infection, in the 6 months preceding seroconversion. Natural killer cell activity was variable, but tended to decrease over time. Seven of 9 subjects tested demonstrated antigen-specific lymphocyte proliferative responses at the first time assayed, and all had proliferative responses to PHA. None of 4 subjects assayed thus far has demonstrated HIV-specific CTL activity in fresh PBMC. The 3 asymptomatic seroconverters had some of the highest CD4+ lymphocyte counts and recovery of virus from culture was less frequent from these 3 subjects than from the symptomatic seroconverters.

CONCLUSION: HIV-specific CTL activity in fresh, unstimulated PBMC has not been detected thus far in this small group of seroconverters, but we continue to follow their cellular immune responses. More sensitive techniques to detect CTL may be necessary and are currently under investigation. The difficulty in recovering virus from asymptomatic seroconverters may reflect a lower virus burden and possibly more effective immune responses to the virus. This subset of asymptomatic seroconverters merits further investigation as they may provide important insight into the most critical immune responses to HIV.

D4-313 ADHESION TO EXTRACELLULAR MATRIX PROTEINS BY LYMPHOCYTES ISOLATED FROM HIV-I TRANSGENIC MICE, Sarah Culkin*, Paul Klotman^, Benjamin Weeks*, *Department of Biology, Hamilton College, Clinton, NY 13323, ^Mount Sinai Hospital Medical Center, NY, NY 10029

While many complications of AIDS are secondary to immunocompromise, increasing evidence supports a more direct role for the virus or viral gene products. For example, AIDS-related dementia complex, HIV-associated nephropathy, and Kaposi's sarcoma may result from either replication of virus in a particular cell type or delivery of viral gene products by infected cells. Thus infected cells may act as a viral reservoir to target organs. In that regard, we previously demonstrated that HIV-I-infected peripheral blood lymphocytes demonstrate both enhanced adhesive (J Cell Biol. 114:847-853, 1991) and invasive properties (AIDS Res & Hum Retrovir 9:513-518, 1993) in vitro. The purpose of the current study is to determine whether enhanced properties of adhesion can be seen in vivo using an HIV transgenic mouse model.

We have developed a mouse model of HIV-I nephropathy, AIDS associated cachexia, and pediatric AIDS. The mouse is transgenic for a deletion mutant (d1443) of the infectious proviral clone pNL4-3 which is under the control of the viral LTR. The construct deletes gag/pol but encodes the HIV-I env, the regulatory genes tat, rev, and nef, as well as the accessory genes vpr and vpu. Expression of Tat- and Rev-specific messages are detectable in lymphoid tissue. Singly spliced and unspliced messages are also detectable in lymphoid tissue. Lymphocytes were isolated from the transgenic mice and tested for adherence to extracellular matrix proteins. Peripheral blood lymphocytes and splenocytes showed a 50% increase in adhesion to fibronectin. Isolated thymocytes demonstrated a 300% increase in fibronectin attachment. All assays were compared to identical assays on cells isolated from non-transgenic mice.

Further characterization of this mechanism was done by treating H9 lymphocytes with exogenous HIV proteins. The HIV-2 protein, Vpx, induced homotypic aggregation of lymphocytes in suspension. Additionally H9 lymphocytes treated with Vpx had a forty fold increase in adhesion to fibronectin. Treatment with the HIV-I proteins gp120, rev, and nef had no effect on lymphocyte adherence or aggregation.

D4-314 BINDING OF SOLUBLE CD4 TO gp120 PROMOTES BINDING OF HIV-1 VIRIONS TO CD4-NEGATIVE CELLS, Sandra Demaria and Yuri Bushkin, Laboratory of Molecular Immunology, Public Health Research Institute, New York, NY 10016.

Binding of HIV-1 virions to cells is mediated by the interaction between viral gp120 and its cellular receptor, CD4. CD4-negative human and mouse cell lines can bind HIV-1 virions only when they are modified to express human CD4 (Maddon *et al., Cell* 47:333, 1986). It has been proposed that HIV entry is mediated by a CD4-driven activation of viral fusion which is induced upon binding to cellular CD4 and requires conformational changes in gp120 (Allan, *Science* 252: 1322, 1991; Moore *et al., ibid.*). Recombinant soluble CD4 (sCD4) is thought to mimic cellular CD4 since its binding induces conformational changes in the gp120/gp41 envelope complex of cell-line adapted isolates of HIV-1. At 37°C, these conformational changes lead to dissociation of gp120 from gp41 while at 4°C they occur without dissociation of gp120. We have found that incubation of HIV-1_{IIIB} virions with sCD4 at 4°C enables the binding of virions to CD4-negative human cells. Accumulated data indicate that other cell surface molecules, in addition to CD4, may be involved in the process of virus binding and play a role in the subsequent fusion of viral and cellular membranes. Upon treatment of cells with heparitinase, the sCD4-induced binding of virions is decreased. This suggests that cellular heparan sulfate proteoglycans may mediate, in part, the interaction of virus with cells in the presence of sCD4. Moreover, sCD4 can induce the binding of virions to CD4-negative mouse cells, suggesting that these cells express surface molecules capable of interacting with regions of HIV-1 envelope other than the CD4-binding site. Therefore, it is important to identify the cellular molecules that bind to HIV-1 virions in the presence of sCD4 and their interaction sites on gp120. This will contribute to the understanding of viral entry and may help to explain different susceptibilities of human and mouse cells to HIV-1 infection.

D4-315 AIDS-associated Kaposi's Sarcoma secretes a Neutrophic Growth Factor(s)

Carol L. Derenzo and Benjamin S. Weeks. Biology Department, Hamilton College, 198 College Hill Rd, Clinton, New York 13323

Kaposi's Sarcoma (KS) is an AIDS-associated cancer. KS is found in endothelial and epthelial tissues, but the origin of the cells is unknown. Cells from KS lesions have been isolated and cultured in order to investigate their properties. Cultured KS cells have been shown to secrete several growth factors. Here, we investigated the ability of KS cells to secrete neurotrophic growth factors. We did this by treating neuronal cells with the medium from cultured KS cells. This medium contains factors which the KS cells secrete and is referred to as KS conditioned medium (KSCM). KSCM increased neurite outgrowth in neuronal cells (PC12 cell line) by six fold. Antisera to nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) were tested for their ability to inhibit KSCM-mediated neurite outgrowth and they had no effect. In addition we show that PC12 neuronal cells do not respond to brain derived neurotrophic factor (BDNF) and neurotophic factor 4 (NT-4). This indicates that the KS cells secrete a neurotrophin(s), however it is not NGF, bFGF, BNDF or NT-4. Future experiments will focus on identifying which neurotrophin(s) is secreted by the KS cells and if this neurotrophin(s) plays a role in AIDS-associated neurological disorders.

D4-316 THE HUMORAL RESPONSE TO OLIGOMERIC HIV-1 ENV PROTEIN. R.W. Doms, P.L. Earl, J.R. Mascola, J.A. Hoxie, C.C. Broder, and S.T. Abedon. University of Pennsylvania, Philadelphia PA 19104; National Institutes of Health, Bethesda MD 20892; and Walter Reed Army Institute of Research, Rockville, MD 20850.

The humoral response to HIV-1 infection is generally studied using monomeric forms of env protein or peptides corresponding to env protein sequences. Because HIV-1 envelope protein complexes that maintain quaternary structure have been shown to elicit qualitatively and quantitatively diferent immune responses, we developed an oligomeric HIV-1 env capture ELISA to study the humoral response using a more native, oligomeric antigen. A soluble oligomeric form of HIV-1_{IIIB} env (gp140) containing all of gp120 and the entire gp41 ectodomain was captured to the solid phase by any one of a number of monoclonal antibodies. Once bound, the protein retained its native oligomeric structure: it bound sCD4, was recognized by a large panel of MAbs to conformational epitopes in gp120 and gp41, and was recognized by MAbs that bind to oligomer specific epitopes in gp41. In addition, the captured gp140 bound two or more copies of the same MAb. Thus, captured oligomeric env closely reflects the antigenic characteristics of env protein on virions and the surface of infected cells. We compared the reactivities of serum samples from individuals infected with either clade B or clade E HIV-1 strains, and found that both reacted equally well with captured oligomeric gp140 to dilutions of $\geq$ 1:10,000. We next determined if incubation of captured gp140 with various serum samples could block subsequent binding of MAbs to epitopes in gp120 and gp41. Clade B serum samples consistently prevented binding of oligomer dependent MAbs to gp41 and, to a slightly lesser extent, MAbs to the CD4 binding site in gp120. V3 loop MAbs were never blocked, consistent with findings that few individuals are infected with IIIB-like strains. Clade E serum samples, on the other hand, showed equivalent or greater blocking of oligomer dependent gp41 antibodies and considerably less blocking of CD4 binding site MAbs. Thus, captured oligomeric IIIB env protein retains highly conserved epitopes, particularly in gp41, that are recognized by antibodies raised against different clades.

D4-317 PROTEINASE-RESISTANT FACTORS IN HUMAN ERYTHROCYTE MEMBRANES MEDIATE CD4-DEPENDENT FUSION WITH CELLS EXPRESSING HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ENVELOPE GLYCOPROTEINS. Tatjana Dragic*, Laurent Picard°, MArc Alizon°, [1]Aaron Diamond AIDS Research Center, New York, NY 10016, Institut Cochin de Genetique Moleculaire, 75014 Paris, France

Most non-human CD4+ cells are resistant to HIV-1 entry and to fusion with cells expressing HIv-1 envelope glycoproteins (Env). The role of human-specific factors in Env/Cd4-mediated fusion is shown by the ability of transient cell hybrids, formed between CD4+ murine cells and human HeLa cells, to fuse with Env+ cells. This type of assay can therefore be used to determine if the human factors required for Env/Cd4-mediated fusion are expressed by a given cell line.

Hybrids formed between CD4+ murine cells and human erythrocytes were permissive for fusion with cells expressing HIV-1 envelope glycoproteins. Experiments with erythrocyte ghosts showed that the human factors allowing Env/CD4-dependent fusion were associated with plasma membrane and were fully active after extensive digestion of human erythrocytes by proteinase K or pronase. The extent of proteolysis was determined by the loss of detectable cell-surface biotinylated proteins as well as the loss of detectable, specific membrane-associated antigens. The human factors controlling HIV-1 entry remain to be identified, but novel strategies taking into account the role of non-protein components of the human membrane should be considered.

D4-318 OLIGOMERIC HIV-1 ENVELOPE ELICITS A SPECTRUM OF CONFORMATION-DEPENDENT, CROSS-REACTIVE ANTIBODIES

Patricia L. Earl[1], Christopher C. Broder[1], Stephen T. Abedon[2], Robert W. Doms[2], and Bernard Moss[1] NIAID, NIH, Bethesda, MD 20892[1] and U. of Pennsylvania, Philadelphia, PA 19104[2]

The native HIV-1 envelope (env) protein exists as oligomeric complexes of gp120/gp41 molecules. We expressed a soluble, oligomeric form of env and used it to prepare a panel of 138 monoclonal antibodies (MAbs). The panel was distinguished by the relatively high number of MAbs directed to conformational epitopes and to epitopes in gp41. Of the 52 gp41 specific MAbs, only 9 reacted with denatured env in Western blots. The epitopes recognized by these MAbs were mapped to amino acids 503-574 (1 MAb), 575-635 (5 MAbs), and 636-678 (3 MAbs) of truncated env molecules. Four MAbs were further mapped to amino acids 592-608 and one to amino acids 637-660. Many of the 43 conformation-dependent anti-gp41 MAbs exhibited oligomer-sensitivity, i.e., they reacted preferentially with oligomeric env; 5 of these reacted exclusively with oligomeric env. These 5 oligomer-specific MAbs all bound to the same or overlapping sites on gp41 as determined by blocking assays. The area involved in the binding was localized to the region near the 2 cysteine residues in the ectodomain of gp41. None of the oligomer-specific MAbs blocked binding of other members of the panel of oligomer-sensitive MAbs indicating the existence of at least 2 distinct oligomer-dependent epitopes in gp41. In addition, the anti-gp41 MAbs generated to oligomeric env exhibited broad cross-reactivity with the envs from diverse HIV-1 isolates. Greater than 80% of the MAbs reacted with the envs from 8 laboratory isolates (4 T-cell tropic and 4 macrophage tropic). Also, 24-47% of the MAbs reacted with env from 3 highly divergent primary isolates including 2 from a different clade. The oligomeric structure of the env from these 3 primary isolates was similar to that of the well characterized IIIB env.

D4-319 ANTIGEN-SPECIFIC ACTIVATION/EXPANSION OF HUMAN ANTI-HIV CTL USING RECOMBINANT ALVAC (CANARYPOX)-BASED VECTORS, Guido Ferrari[1], Cynthia Place[1], John Bartlett[2], Dewey Moody[3], James Tartaglia[4], Enzo Paoletti[4], and Kent Weinhold[1], Department of Surgery[1] and Medicine[2], Duke University Medical Center, Durham, NC 27710; Applied Immune Sciences[3], Santa Clara, CA 95054; Virogenetics, Inc.[4], Troy, NY 12180

The development of adoptive immunotherapeutic strategies for AIDS will depend on technologies capable of driving effector cells *ex vivo* in an antigen-specific manner while maintaining the broadest possible T-cell repertoire. In an attempt to fulfill this requirement, two ALVAC (canarypox)-based recombinants containing HIV-1 multigene expression casettes were evaluated for their capacity to induce activation/expansion of HIV-specific CD8+ cytotoxic lymphocyte precursors (CTLp) obtained from HIV-1 infected donors. These two vectors, vCP205 encoding HIV-1 gp120+TM (28 amino acid transmembrane anchor sequence) in addition to Gag/protease and vCP300 encoding gp120+TM + Gag/protease as well as Nef and Pol CTL determinants, are pancytotropic but replication incompetent in mammalian cells. Bulk peripheral blood lymphocytes (PBL) or enriched CD8+ T cells were stimulated for 10 days with autologous ALVAC recombinant-infected PBL (R:S=5:1) in the presence of IL-7 (1000U/ml) and CTL activity was assessed. Parallel *in vitro* stimulations with fully replication competent vaccinia/HIV-1 constructs were performed. Both vaccinia and ALVAC constructs were potent activators of HIV-1 CTL, indicating that vector replication was not a prerequisite for CTLp stimulation. Activation by ALVAC was IL-2 dependent and highly specific as vCP205 elicited only Env, and Gag CTL while vCP300 elicited broader reactivities against Env, Gag, Pol, and Nef determinants. Enriched CD8+ T cells activated by vCP205 expanded nearly 10-fold over a 30 day period at which time overall CTL activities declined. Potent reactivities were restored following vector restimulation. Despite their potency as stimulators, ALVAC infected BLCL were poor CTL targets. These data highlight the capacity of ALVAC vectors to drive CTLp in an antigen-specific manner, illustrating their therapeutic utility.

D4-320 REPLICATION OF MACROPHAGE-TROPIC AND T-CELL-TROPIC STRAINS OF HUMAN IMMUNODEFICIENCY VIRUS IS AUGMENTED BY MACROPHAGE/ENDOTHELIAL CELL CONTACT, Patrick N. Gilles[1], Janet L. Lathey[1] and Stephen A. Spector[1,2]*, Department of Pediatrics[1] and Center for Molecular Genetics[2], University of California, San Diego, La Jolla, CA 92093.

Macrophages perform a central role in the pathogenesis of human immunodeficiency virus type-1 (HIV-1) infection, and have been implicated as the cell type most prominent in the development of central nervous system impairment and possibly maternal-infant transmission. In this study, we evaluated the interaction between macrophages and endothelial cells on HIV-1 replication. Upregulation of HIV-1 replication was consistently observed in monocyte-derived-macrophages (Mϕ) co-cultured with either umbilical vein endothelial cells or brain microvascular endothelial cells (EC). HIV-1 p24 antigen production of laboratory-adapted strains and patient-derived isolates was increased 2- to 1000-fold in macrophage/endothelial (Mϕ/EC) co-cultures, with little or no detectable replication in cultures containing ECs only. The upregulation of HIV-1 in Mϕ/EC co-cultures was observed not only for viruses with the nonsyncytium inducing, macrophage-tropic phenotype, but also for viruses previously characterized as syncytium inducing and T-cell-tropic. In contrast, co-cultures of Mϕs with glioblastoma, cortical neuronal, fibroblast, and placental cells failed to increase HIV-1 replication. Enhancement of HIV-1 replication in Mϕ/EC co-cultures required cell-to-cell contact; conditioned media from EC or Mϕ/EC co-cultures failed to augment HIV-1 replication in Mϕs. Additional evidence for the need for cell-to-cell contact was demonstrated by the ability of antibody to leukocyte function-associated antigen (LFA-1), a Mϕ adhesion molecule, to inhibit HIV-1 replication in Mϕ/EC co-cultures. Thus, these data indicate that Mϕ/EC cell contact enhances HIV-1 replication in Mϕs for both macrophage-tropic and previously characterized T-cell-tropic strains, and that antibody against LFA-1 can block the necessary cell-to-cell interaction required for the observed upregulation. These findings may have important implications for understanding the ability of HIV-1 to replicate efficiently in Mϕ and may play an important role in HIV-1-related CNS impairment and maternal-infant transmission.

D4-321 ACQUISITION OF FUNCTIONAL HYALURONATE RECEPTORS BY HIV, Margaret M. L. Guo and James E. K. Hildreth, Department of Pharmacology and Molecular Sciences, The Johns Hopkins School of Medicine, Baltimore, MD, 21205

We and others have shown that HIV acquires a number of cell surface proteins from host cells including leukocyte adhesion receptors. Whether the adhesion receptors on the virion surface can function similarly to those on the cell surface has yet to be determined. We have used the inducible binding of CD44 to hyaluronate as a model system to determine if the adhesion phenotype of HIV is the same as that of the cells that produced it. CEMx174 cells can be induced by phorbol myristate acetate (PMA) to bind hyaluronic acid by way of CD44 expressed on the cell surface. CEMx174 cells chronically infected with HIV are similarly inducible. Virions produced by the stimulated cells but not by the resting cells bind hyaluronic acid. The binding to hyaluronic acid of both the cells and the virions can be blocked by excess soluble hyaluronate and an anti-CD44 monoclonal antibody. This adhesive phenotype on the virions is stable for at least 24 hr whereas the cells lose the ability to bind hyaluronic acid 6 hr after removal of PMA. Flow cytometry analysis reveals that expression of CD44 on CEMx174 cells does change appreciably after PMA treatment. Therefore, the binding of HIV to hyaluronate reflects the acquisition of modified CD44 molecules or hyaluronate co-receptor. Our data show that the CD44 molecules on the virion surface to be functional in binding hyaluronic acid and therefore we have shown here for the first time that the adhesion phenotype of HIV correlates directly with the phenotype of the cell in which the virus was produced. Functional adhesion receptors on the virus could have profound effects on virus binding, tropism, and infectivity.

D4-322 SIV INFECTION OF CYNOMOLGUS MACAQUES: ANALYSIS OF CTL DURING THE EARLY PHASE OF INFECTION, AND IMMUNIZATION/PROTECTION EXPERIMENT USING RECOMBINANT SIV PROTEINS AS SOLID MATRIX-ANTIBODY-ANTIGEN (SMAA) COMPLEXES. T Hanke[1], RE Randall[2], A Gallimore[1], F Gotch[1], A McMichael[1] and J Stott[3]. [1]Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, U.K.; [2]School of Medical and Biological Sciences, University of St.Andrews, St.Andrews, U.K.; [3]NIBSC, Potters Bar, U.K.

Cynomolgus macaques were infected with SIVmm251(32H), either pathogenic clone pJ5 or clone pC8, which carries an in-frame 4-amino acid deletion and 2 conservative amino acid substitutions in nef, and euthanized at 1, 2 or 3 weeks post-infection. The specificities of cytotoxic T cell (CTL) responses from blood, lymph nodes and spleen were analysed. The results indicated that (i) at all time points, the CTL responses were weak and variable in their antigen specificities, with possibly more frequently observed recognition of nef, (ii) the levels of specific lysis remained similar over the period studied, (iii) there was no overall difference among the CTL responses in spleen, lymph nodes and blood, although there were variations among organs within an individual animal, and (iv) there was no obvious difference in the potency of CTL induction between SIV clones pJ5 and pC8.

An immunization/protection experiment was carried out using recombinant SIV proteins assembled into solid matrix-antibody-antigen (SMAA) complexes. Cynomolgus macaque vaccinated with a mixture of env, p17, p27, reverse transcriptase (RT), vpr and vpx-containing SMAA complexes developed antibody responses against all SIV antigens and transient RT-specific CTL. None of these animals were protected against subsequent challenge with 10 MID_{50} of monkey cell-grown SIVmm251(32H)(pJ5).

D4-323 CHANGES IN CLONAL PATTERNS OF ANTI-p24 AND ANTI-gp41 ANTIBODY REFLECT RATE OF PROGRESSION TO AIDS. D. Henrard, J.F. Phillips, B. Phelps, and J.J. Goedert Abbott Labs, N. Chicago, IL and NCI, Bethesda, MD.

Objective: Determine whether early changes in clonal patterns can predict clinical progression among subjects with similar titers of anti-p24 and anti-gp41 specific antibodies.

Methods: The first 2-3 yearly serum samples collected from 16 seroconverters who developed AIDS within 4-7 years (group I, n=7) or remained AIDS-free for 7-11 years (group II, n=9) were analyzed. Specific antibody clonotypes were separated by isoelectric focusing (pH 6-9), transferred to nitrocellulose coated with p24 or gp41 antigen and detected by goat anti-human antibody conjugated to alkaline phosphatase. Clonal evolution was expressed as median (range) number of new vs disappearing (N/D) bands.

Results: The initial median (range) number of clonotypes was similar in each group: 16 (4-20) vs 15 (9-33) for p24 and 22 (11-30) vs 18 (14-27) for gp41 antibody. Over time, however, median N/D for p24 clonotypes was 3/8 (range 6/8-0/0) in group I vs 15/14 (range 6/4-6/14) in group II. Similarly, median N/D for gp41 clonotypes was 8/11 (range 7/7-0/0) in group I vs 20/12 (range 19/2-10/12) in group II.

Discussion: The initial number of p24 and gp41 antibody clonotypes did not predict progression to AIDS. Over time, however, a high N/D for p24 or gp41 antibody clonotypes, presumably reflecting rapid changes in virus-immune system interactions early after infection, correlated with a slower rate of disease progression.

D4-324 PRODUCTION OF POTENT NEUTRALIZING AND FUSION-INHIBITING MONOCLONAL ANTIBODIES TO SIVmac. James A. Hoxie[1], Ména Ahuja[1], Beth S. Haggarty[1], Thomas J. Palker[2], and David C. Montefiore[2]; [1]Univ of Penn, Phila, PA 19104; [2]Duke Univ Medical Center, Durham, NC 27710.

Neutralization determinants on SIV env glycoproteins are poorly characterized. To date, only a limited number of neutralizing monoclonal antibodies (MAbs) have been produced and in general, these MAbs have been unable to inhibit SIV-induced cell fusion. In order to develop MAbs with fusion-inhibitory activity, we immunized mice with Sup-T1 cells chronically infected with CP-MAC, an *in vitro* derived variant of SIVmac/BK28 that expressed a high level of env glycoproteins on the surface of infected cells (LaBranche, C. et al, J. Virol. 68: 5509, 1994). MAbs were screened for the ability to inhibit CP-MAC induced syncytia formation on Sup-T1 cells. Seven MAbs were derived that inhibited cell fusion (at concentrations ranging from 0.01-8.1 µg/ml) in coculture assays using CP-MAC-, as well as SIVmac251-infected cells . In addition, all MAbs were able to neutralize infection of CEMx174 cells by SIVmac251 (50% inhibition activity at 0.04-0.8 µg/ml). Three of these 7 MAbs were also able to neutralize the genetically heterologous SIVmac isolate, B670, while no activity was found for any of the MAbs against a sooty mangabey isolate, SIVmmE543. All MAbs were shown to be reactive with conformational determinants on SU and to inhibit infection at a step subsequent to CD4 binding. Although epitopes have not been defined, competition studies have determined that these MAbs are at least partially competitive with one another, indicating that their biological activity is restricted to a limited region on the SU molecule. The utilization of cells that express high levels of conformationally-intact surface env glycoproteins may represent an effective approach to elicit humoral immune responses with enhanced anti-viral activity. For CP-MAC as well as SIVmac239, a mutation has been defined in the TM cytoplasmic tail that can increase the expression of env glycoproteins on the cell surface (see accompanying abstract by Sauter, M. et al). Future studies will evaluate the extent to which the level of surface env glycoproteins on infected cells can affect the host immune response and will explore the relevance of these observations for vaccine strategies against SIV and HIV.

D4-325 SENSITIVITY OF HIV-1 TO NEUTRALIZATION BY ANTIBODIES AGAINST O-LINKED CARBOHYDRATE EPITOPES DESPITE DELETION OF O-GLYCOSYLATION SIGNALS IN THE V3 LOOP

Britt Jansson[1], John-Erik Stig Hansen[2], Gregers Gram[2], Henrik Clausen[2], Jens Ole Nielsen[2] and Sigvard Olofsson[1], [1]Dept. of Clin. Virology, Univ. of Göteborg, Guldhedsgatan 10 B, S-413 46 Göteborg, Sweden; [2]Dept. of Inf. Diseases 144, Hvidovre Hospital, 2650 Hvidovre, Denmark.

Indications have previously been found to suggest that threonines in the V3 loop of HIV-1 gp120 are glycosylated with the short-chain O-linked oligosaccharides Tn or sialosyl-Tn that function as epitopes for broadly neutralizing carbohydrate specific antibodies. In this study we examined whether mutation of such threonines could decrease the sensitivity to infectivity inhibition by Tn or sialosyl-Tn specific antibodies. Potentially O-glycosylated threonines in the V3 loop of cloned HIV-1_{BRU} was mutagenized to alanine thus abrogating any O-glycosylation at these sites. Additionally, one of these T-A mutants (T308A) also abrogated the signal for N-glycosylation at N306 inside the V3-loop. The mutant clones were compared with the wild type virus as to sensitivity to neutralization with monoclonal and polyclonal antibodies specific for the tip of the V3 loop of BRU or for the O-linked oligosaccharides Tn or sialosyl-Tn. Deletion of the N-linked oligosaccharide at N306 increased the neutralization sensitivity to antibodies specific for the tip of the loop, which indicates that N-linked glycosylation modulates the acessability to this immunodominant epitope. However, none of the mutants with deletions of O-glycosylation signals in theV3 loop displayed any decrease in sensitivity to anti-Tn or anti-sialosyl-Tn antibody. This indicates that these broadly specific neutralization eptopes are located outside the V3 loop of gp120.

D4-326 CHARACTERIZATION OF SIVsmB7: A BIOLOGICALLY CLONED DELETION MUTANT OF SIVsmH3, Edmundo Kraiselburd, Jim Smith, Nancy Leung, Angel Salaman and José V. Torres, University of Puerto Rico School of Medicine, Rio Piedras, PR 00935 and Medical Microbiology and Immunology, University of California, Davis, CA 95616.

A stable cell line producing the virus-like particle SIVsmB7, has been in culture for almost two years and does not show any cytopathic changes. PCR analysis of genomic DNA from the cell line producing SIVsmB7 show that viral DNA is present and cell-free supernatants contain high concentrations of SIV p27 antigen and relatively low levels of reverse transcriptase activity. Surface expression of viral antigens was observed when the chronically infected cells were incubated with sera from a SIVsmE660-infected rhesus macaque. Furthermore, radiolabeled virus bound CEMX174 cells, indicating that the envelope glycoproteins are appropriately expressed on the virus particle surface and capable of binding the CD4 receptor. Given that the env glycoproteins were intact, it was interesting to note that the SIVsmB7 cell line did not demonstrate any syncytia formation during its continuous propagation. Analysis of the surface CD4 expression on the SIVsmB7 cell line showed that it was phenotypically $CD4^-$, and as expected, cocultivation of the SIVsmB7 cell line with $CD4^+$ CEMX174 cells resulted in syncytia formation. Although cocultivation of CEMX174 cells with the SIVsmB7 cell line resulted in cytopathic effects, a productive infection did not occur. The RT and p27 levels were similar to baseline levels from SIVsmB7 cells alone. Cell-free supernatants as well as pelleted SIVsmB7 virus was unable to infect CEMX174 cells or primary PBMCs and macrophages from both rhesus macaques and humans. No viral DNA could be detected in the CEMX174 cells (PCR and Southern blot analysis were performed on genomic DNA samples obtained 22 hr and 44 hr postinfection). The defect present in the viral genome has been characterized at the molecular level and includes a deletion of 1.6kb (*integrase, vif, vpr, vpx*). We have demonstrated that this VLP lacks infectivity *in vivo*. Rhesus macaques inoculated with high concentrations of this VLP showed no plasma viremia and the PBMC were PCR negative. However, a virus neutralizing SIV-specific immune response was elicited in all the macaques tested. Due to the high constitutive expression of this VLP and the lack of infectivity, we are now testing this preparation as a vaccine. We found that this vaccine is immunogenic in rhesus macaques and elicits SIV-specific antibodies that have neutralizing capabilities. Molecular characterization of this defective virus may also provide insights into the mechanisms of retroviral pathogenesis.

D4-327 PEPTIDE MAPPING OF HUMORAL RESPONSES FROM RABBITS IMMUNIZED WITH AN OLIGOMERIC GP160 ENVELOPE PROTEIN IN SEVERAL ADJUVANTS, Lawrence Loomis, T. VanCott*, M. Krider, M. Mitchell, R. Kaminsky, V. Kalyanaraman#, G. Lowell, D. Burke*, R. Redfield*, D. Birx*; H. M. Jackson Foundation, Rockville, *Division of Retrovirology, WRAIR, Rockville, and #Advanced Bioscience Laboratories, Kensington, MD, USA.

We have synthesized sets of overlapping 12-mer peptides which encompass the entire sequence of the HIV-1 envelope from various clades, including: B clade (lab isolates LAI/LAV, MN, SF2 and primary isolates US3 from North America and BK132 from Thailand), A clade (DJ263), C clade (DJ259), D clade (SE365), E clade (NT235) and F clade (BZ126). These peptides were used to investigate the linear antibody response from rabbits immunized with an affinity purified, oligomeric HIV-1 envelope protein, ogp160, harvested from infected cell cultures. Sera from rabbits immunized with rgp160 (baculovirus, MicroGeneSys) were used for comparison. The ELISA peptide mapping technique (PEPSCAN) was used. A wide variety of adjuvants was tested for immunomodulation of activity with this protein, including alum, Freund's' adjuvant, several adjuvants obtained from RIBI Immunochem Research, Inc., and proteosomes (meningococcal derivatives with and without immunomodulators). Strong immunogen-directed antibody responses were elicited by the antigen in each of the adjuvants tested. Antibodies elicited by the antigen in Freund's adjuvant were broadly reactive and were strongest in regions of the envelope that have previously been characterized as "constant" by genetic typing. Antibodies recognized specific epitopes in all 6 clades investigated, including: 6 in gp120 -- 3 in C1, 1 in C2, 1 in C3, 1 in C5 -- and 2 in gp41. This reactivity was generally similar to the pattern of reactivity elicited by a monomeric rgp160 in Freund's. These results will be compared to those obtained using both products in other adjuvants.

D4-328 PIG-TAILED MACAQUES INFECTED WITH HIV-2_{KR} ARE PROTECTED AGAINST DISEASE AFTER CHALLENGE WITH A DIVERGENT VIRULENT HIV-2 ISOLATE.

D. Looney[1,2*], J. McClure[3], S. Kent[5], M. Robertson, S. Klucking, G. Kraus[2], P. Badel[2], J. Steele[5], N. Dorofeeva[5], A. Schmidt[4], C. Bartz[4], P. Greenberg[5], S.-L. Hu[3], W. Morton[4], F. Wong-Staal[2]. [1]VA Medical Center San Diego, [2]University of California San Diego, [3]Bristol-Myers-Squibb, [4]University of Washington Regional Primate Center, [5]University of Washington, Seattle.

Objective: To investigate protection induced by live virus infection, pig-tailed macaques previously inoculated with an avirulent, infectious, molecular HIV-2 clone (HIV-2_{KR}) were challenged with HIV-2_{287}, an HIV-2 isolate producing rapid decline in CD4 lymphocyte numbers and immunodeficiency.

Methods: HIV-2_{KR} pools were produced by transfection into Molt-4/Clone-8 cells. The HIV-2_{287} challenge pool was grown in *M. nemestrina* PBMC. Viral burden was assessed by quantitative viral culture, plasma antigen EIA, and internal standard DNA & RNA PCR. Antibody response was assessed using EIA and immunoblotting. Neutralization assays were performed on C8166 (*lck*$^+$ clone9, J. Corbeil) cells. CTL activity and CTL precursor frequencies were also determined using published methods.

Results: Pig-tailed macaques inoculated with 10^4-10^1 SFU HIV-2_{KR} intravenously were all infected as determined by nested PCR. Virus was reisolated from 3 macaques, and transient (~50%) decline in CD4$^+$ lymphocyte number was seen in all. No neutralizing antibody, ADCC, or CTL directed against homologous virus was detected in KR infected animals prior to challenge with HIV-2_{287}. Prior inoculation with HIV-2_{KR} failed to protect from infection, however, animals inoculated with 10^4 SFU of HIV-2_{KR} were protected from CD4 decline and disease following challenge with either 10^5 or 10^1 $TCID_{50}$ of HIV-2_{287}. Protected animals displayed lower levels of proviral burden (1-2 logs). Both protected and unprotected animals displayed low levels of plasma antigenemia and RNA. Two animals previously inoculated with ~5000x more viral antigen of an HIV-2_{KR} mutant (KRΔnef) incapable of replication in macaques do not appear to be protected from disease after HIV-2_{287} challenge.

Conclusions: The virological and immunological basis of protection induced by avirulent HIV-2 in this non-human primate model may provide important information about HIV pathogenesis and new goals and strategies for the design and evaluation of HIV vaccines.

D4-329 INCREASED ADHESION AS A MECHANISM OF ANTIBODY DEPENDENT AND ANTIBODY INDEPENDENT COMPLEMENT MEDIATED ENHANCEMENT OF HIV INFECTION.

Lund, O.; Hansen, J.; Sørensen, A.M.; Mosekilde, E.; Nielsen, JO. and Hansen, J-E.S. Laboratory of Infectious Diseases, Hvidovre Hospital, 2650 Hvidovre, Denmark.

Enhancement of HIV infection by complement alone or in conjunction with antibodies was studied experimentally and theoretically. Experimental studies showed that while HIV positive sera neutralize HIV infection, the addition of fresh complement abrogated neutralization and could even cause enhancement. Enhancement was blocked by anti complement receptor 2 (CR2) antibodies, and infection under enhancing conditions could be blocked by soluble CD4 (sCD4). Complement antibody mediated enhancement (C'ADE) was dependent on the alternative complement activation pathway, as factor B-deficient serum could only enhance after addition of factor-B. The observed enhancement was also partially antibody dependent since addition of antibodies increased the level of enhancement. Under C'ADE conditions, infection reached a plateau within 5 minutes and was not caused by activation of cells by factors in the human serum. On the contrary, preincubation of cells with complement decreased the level of enhancement. A theoretical model of HIV infection *in vitro* was developed which exhibited similar enhancement in an antibody and complement concentration dependent way. Model studies indicated that the enhanced infection process could be explained by virions binding more efficiently to cells, due to complement deposition on the viral surface. The model also indicated that the saturation of the enhanced infection process seen after a few minutes could be caused by saturation of the complement receptors. The effect of neutralizing antibodies can thus be overcome by the enhancing effect of complement that facilitates the contact between gp120 and CD4. These studies demonstrate that the main features of the complement dependent enhancement phenomenon can be understood in terms of a simple mathematical model.

D4-330 SECRETORY LEUKOCYTE PROTEASE INHIBITOR: A HUMAN SALIVA PROTEIN EXHIBITING ANTI-HIV ACTIVITY.

Tessie B. McNeely, David J. Dripps, Stephen P. Eisenberg, and Sharon M. Wahl; NIDR, NIH, Bethesda, MD; Synergen, Inc., Boulder, CO. Infection of adherent primary monocytes with HIV-1_{Ba-L} is significantly suppressed in the presence of human saliva. Saliva present for 1 hour during exposure of monocytes to virus inhibited HIV propagation for three weeks postinfection. Human plasma and synovial fluid had no such antiviral activity. Activity was localized to the soluble fraction, and of the many saliva proteins examined, only Secretory Leukocyte Protease Inhibitor (SLPI) had anti-HIV activity at physiological levels. SLPI anti-HIV activity was dose-dependent with > 90% inhibition of RT activity at 1-10 µg/ml. SLPI also partially inhibited HIV-1_{IIIB} infection in proliferating human T-cells. SLPI appears to target a host cell associated molecule, because equivalent anti-HIV activity was observed when SLPI was preincubated with monocytes, then washed away before addition of HIV. In addition, no interaction with viral proteins could be demonstrated. However, SLPI activity was not due to direct interaction with, or down regulation of the CD4 antigen. Partial removal of SLPI in saliva caused a reduction in anti-HIV activity of saliva. These data indicate that SLPI has anti-HIV activity and may contribute to the activity in saliva associated with the infrequent oral transmission of HIV-1.

D4-331 HIV REVERSE TRANSCRIPTPASE MUTATION ENABLES EVASION OF CTL MEDIATED IMMUNITY

UC MEIER, P KLENERMANN, S McADAM, B KOEPPE, S ROWLAND JONES, ANDREW McMICHAEL, B LARDER, R PHILLIPS
Molecular Immunology, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU

HIV-reverse transcriptase (RT) is an important target for cytotoxic T-cells. We studied an HLA-B8-restricted epitope (aa18-26) which is located in the N-terminal finger-domain important in positioning the template DNA or RNA during synthesis; another commonly recognized epitope HLA-A2 (aa309-317) is located in a region involved in the dimerisation of the p66 and p51 subunits. In HIV positive patients who make CTL responses to RT, we observed aminoacid variation within proviral sequences encoding these epitope regions. Variation in both epitopes affected CTL recognition and mutant epitopes were found to antagonise CTL responses, as has been observed in HIV- gag (1). We expressed full length RT containing these epitope variants to test whether these mutations affect enzyme function and evaluated proviral clones which combined CTL antagonist and escape sequences. Generation of CTL escape mutations with antagonist activity which retain enzyme function may confer a survival advantage to a wide diversity of HIV and provide a potent means of immune escape.

(1) Klenerman, P. et al., Nature (1994) 369,403-407

D4-332 CHARACTERIZATION OF T-CELL DEFECTS IN EARLY HIV INFECTION,

Linde Meyaard, Heleen Kuiper, Sigrid A. Otto, Berend Hooibrink, Katja C. Wolthers, René A.W. van Lier and Frank Miedema.

Dept. of Clinical Viro-Immunology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, incorporating the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, The Netherlands.

Early in HIV infection CD4+ and CD8+ T cells are qualitatively affected. Loss of responses to recall antigen precedes impaired responses to allogeneic MHC and mitogens. To get more insight in these T-cell defects we investigated whether specific T-cell subsets and/or T-cell signals are affected in HIV-infected individuals.

First, we studied whether functional loss of T cells affects both naive and memory T cell subsets in the course of HIV infection. The proliferative response of CD4+ T cells from HIV-infected individuals to alloantigens, to which normally both naive and memory T cells respond, was studied by limiting dilution analysis. The decreased proliferative response to alloantigens in HIV-infected individuals was associated with a decreased precursor frequency of alloreactive cells. The frequency was decreased in both the CD45RA+ and the CD45RO+ subset of CD4+ T cells and analysis of four individuals in the course of HIV infection revealed similar kinetics of the decline in function in both subsets.

Furthermore we studied whether in the presence of impaired responses of T cells from HIV-infected individuals to CD3/TCR mediated signalling, costimulation through CD28 and CD27 after interaction with their natural ligands CD80 and CD70 is intact. The impaired T-cell proliferative responses to signal one in combination with CD80 or CD70 as present in a large fraction of asymptomatically HIV-infected individuals was due to impaired responses of signal one but not to impaired responses to costimulation, since CD80 or CD70 did enhance signal one-mediated proliferative responses to a normal extent. Moreover, in individuals with proliferative responses to signal one that were decreased to 50% of normal T-cell responses, costimulation even was increased compared to controls.

We conclude that in HIV infection the function of both CD45RA+ and CD45RO+ cells is affected. In these cells the response to costimulation is relatively preserved compared to responses to the first signal and point to the defect in T cells being primarily in the CD3/TCR-mediated pathway.

D4-333 HIV-1 SPECIFIC CTL DURING THE CLINICAL COURSE OF HIV-1 INFECTION

Frank Miedema[1], Carel A. van Baalen[2], Agnes M. Holwerda[1], Susana R. Kerkhof-Garde[2], Ireneus P.M. Keet[4], Jan K.M. Eeftinck Schattenkerk[3] and Michèl R. Klein [1]

[1]Dept. of Clinical Viro-Immunology of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, incorporating the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam. [2]Dept. Virology, Erasmus University, Rotterdam [3]Academic Medical Centre, Amsterdam. [4]Municipal Health Service, Amsterdam, The Netherlands.

Clinical course of HIV-1 infection may be influenced by an effective host immune response against HIV-1 and/or by viral properties. To investigate the cellular immune response to HIV-1 we analyzed CTL activity against different HIV-1 proteins in long-term asymptomatic HIV-1 infection as well as during rapid progression to AIDS.

Longterm asymptomatic individuals (n=6) with CD4 counts >500 cell/μl after more than 8 years of infection were selected from The Amsterdam Cohort Study on AIDS versus 6 subjects who progressed to AIDS <5 years. CTL activity was measured on ^{51}Cr labelled HLA matched or autologous B-LCL, infected with rVV expressing HIV-1 Ag. Limiting dilution CTL assays were performed longitudinally with PBMC after Ag-specific stimulation. Sequences of CTL epitopes were determined in homologous virus isolates.

In long-term asymptomatics, stable CD4+ counts and preserved CD3 mAb-induced T-cell reactivity coincided with persistent HIV-1 Gag-specific CTL responses and very low numbers of HIV-1 infected CD4+ T-cells for >8 years. These data suggest that attrition of immune functions is delayed and that host cellular immune responses may have contributed to maintenance of the asymptomatic phase. In five out of six rapid progressors Gag-specific CTL were also elicited. However, despite strong and mounting Gag-specific CTL responses early in infection, the number of HIV-1 infected CD4+ T-cells increased in parallel in the course of infection. Apparently CTL failed to keep viral replication in check. During subsequent clinical progression to AIDS loss of Gag-specific CTL coincided with precipitating CD4+ counts and severe deterioration of T-cell function.

These results warrant more detailed studies on the quality and quantity of CTL during HIV-1 infection to understand why CTL failed to contain viral spread in rapid progressors.

D4-334 ANTIBODIES THAT FORM IMMUNE COMPLEXES CAPABLE OF BINDING COMPLEMENT RECEPTOR TYPE 1 (CR1/CD35) AS A CORRELATE OF IMMUNITY IN HIV-1 AND SIV INFECTION AND VACCINATION, David C. Montefiori,[1] Brahmajothi Vasudevan,[1] Louis N. Martin,[2] Giuseppe Pantaleo,[3] Anthony S. Fauci,[3] Jintao Zhou[1] and the NIH AIDS Vaccine Evaluation Network, [1]Duke University Medical Center, Durham, NC 27710, [2]Tulane Regional Primate Research Center, Covington, LA 70433, [3]Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892

CR1 can participate in a number of physiological processes that might possibly influence HIV-1 pathogenesis and immunity. Among those involving immune complexes are: 1) virus clearance through the mononuclear phagocytic system, 2) retention of virus in lymph nodes and 3) infection-enhancement. We developed an *in vitro* assay to quantitate and characterize virus-specific antibodies capable of forming immune complexes with HIV-1 and SIV that bind CR1. The procedure involves incubating virus and antibodies with fresh normal human serum as a source of complement and then capturing immune complexes on the surface of 96-well immuno-plates coated with recombinant soluble CR1. Trapped virus was then quantitated by p24 immunoassay. HIV-1 trapping occurred with sera from infected individuals and from rgp160-vaccinated volunteers but not with sera from HIV-naive individuals. No trapping was observed in heat-inactivated or C3-depleted human serm, confirming the need for complement. Similar results were obtained with SIVdelta/B670 using sera from infected macaques. HIV-1 trapping was possible using laboratory strains of HIV-1 and with PBMC-grown primary isolates, and was consistently and equally observed among sera from progressors and long term non-progressors using H9-grown HIV-1 IIIB. Vaccinee's sera that had little or no neutralizing activity sometimes demonstrated high activity in this assay, suggesting that envelope-specific, non-neutralizing antibodies have potential to facilitate CR1-mediated processes with possible relevance to HIV-1 pathogenesis and immunity. Additional evidence in macaques suggests that *in vitro* activity might correlate with trapping of virus in lymph nodes during the acute stage of SIV infection.

D4-335 SYNTHETIC PEPTIDES ENTRAPPED IN MICROPARTICLES CAN ELICIT HIGH LEVELS OF CTL ACTIVITY, Douglas F. Nixon, Pei-de Chen, Peter J. Kuebler, Catarina Hioe, Julie Richardson, Manmohan Singh, J. Paul McGee and Derek T. O'Hagan, United Biomedical, Inc., Hauppauge, NY 11788.

Objective:

To assess the ability of peptide epitopes encapsulated in microparticles to stimulate CTL.

Methods:

The immunogenicity of three CTL epitopes, influenza NP147-158, plasmodium berghei CS protein 252-260 and HIV gp120 "peptide p18" were studied in BALB/c mice. Peptides were formulated as microparticles prepared with poly (D,L lactide-co-glycolide) using a solvent evaporation technique. The microparticles were administered as a suspension in phosphate buffered saline. 100μg of peptide or controls (the weight equivalent of blanks) were administered to groups of 3 mice intra-peritoneally or sub-cutaneously at 1,10 and 20 days. 7 days following the last immunization splenocytes were cultured in vitro in the presence of appropriate peptide or control with rat con A supernatant as a source of growth factors. CTL activity was measured in a standard 4 hour chromium release assay and results expressed as % specific lysis.

Results:

CTL could be elicited in vivo with peptide entrapped in microparticles. CTL were CD8+ and could recognize processed peptide (infection with virus). Levels of lysis were as high as 98% specific lysis at an E:T ratio of 50:1.

Conclusion:

These results demonstrate that peptides administered in microparticles can induce a systemic CTL response in vivo. Peptide vaccines using such a formulation could be used to stimulate CTL responses as part of a prophylactic vaccines or as immunotherapeutics.

D4-336 **CONSERVATION OF ANTIGENIC SPECIFICITIES IN DIVERGENT V3 LOOP RESIDUES FROM HIV-1 SUBTYPES A THROUGH F.** Gary A. Pestano[1], Karlene Hosford[1], Janice Riley[1], Alexander Spira[2], John Mascola[3], Jerry Guyden[1], David D. Ho[2] and William M. O. Boto[1], Biology and Chemistry Dept., The City College of New York, CUNY, NY 10031, Aaron Diamond AIDS Res. Fdn., 455 First Av, NY 10016, and Walter Reed Army Inst. of Res., 1600 E. Gude Drv., Rockville, MD 20850.

This report describes the possible impact of genetic variation on the seroreactivity of the putative principal neutralizing determinant (PND) encoded in clones from the globally divergent HIV-1 clades. New Ugandan HIV-1 clones from the subtypes A, C, and D, North American clones from the B subtype and reference clones from subtypes E and F were analyzed in this report. A region of the ENV gene encoding the C2 to the V5 domains was PCR-amplified from the lysates of peripheral blood leukocytes or from short-term cultured isolates. Computer-assisted analyses were conducted on the amino acid sequences to predict highly antigenic residues. Despite marked sequence variation, an analogous antigenic site corresponding to the PND was predicted in the V3 loop for all of the clones. Synthetic peptides comprising this epitope were tested in ELISA for antigenic reactivity with sera from asymptomatic Ugandan, New York and Thai donors. Peptides derived from UG06c [A], RT3 [B], UG045 [C], UG044 [D], CM244 [E], and RMA [F] displayed broad patterns of reactivity with the Ugandan, New York and Thai serum samples. The highest degree of cross-reactivity was detected against the peptide from the North American clone, RT3. Analogous peptides derived from clones expressing aberrant V3 loop cap residues, RT1 [B], UG23c [D], and UG042 [D], were significantly less reactive. Secondary structure analysis of the diverse V3 loop residues indicated a good correlation of possible β-turn occurrence with peptide reactivity. ELISA inhibition assay further suggested that UG045, CM244 and RMA share a common antigenic specificity in the V3 loop. Serological analysis of the neutralization epitopes encoded in the globally diverse HIV-1 clades could complement the current search for a broadly reactive candidate vaccine.

D4-337 **IgM ANTIBODY REACTIVITY AGAINST MYELIN GLYCOLIPIDS IN PLASMA SAMPLES FROM HIV-POSITIVE PATIENTS**

Steven Petratos, Victor J. Turnbull, Roula Papadopoulos and Michael F. Gonzales. Neuropathology Research Laboratory, Department of Anatomical Pathology, The Royal Melbourne Hospital, Parkville 3050, Victoria, Australia.

Peripheral neuropathies are now well documented amongst patients with human immunodeficiency virus (HIV) infection. Of the eight clinically defined neuropathies, three have been hypothesised to be immune mediated; viz distal predominantly sensory neuropathy (DPSN), mononeuritis multiplex (MM), and inflammatory demyelinating polyneuropathy (IDPN). Recently there has been increasing interest in the role of glycolipids as neuritogens in demyelinating conditions and conduction block. Gangliosides (especially GM1) have been implicated in multifocal motor neuropathy and motor neuron disease. Antibodies against sulphatide (GalS) have been detected in some patients with sensory neuropathies. We have screened, via a TLC-immunostaining procedure, 21 HIV-positive plasma samples that contained high titre IgM reactivity against peripheral myelin, previously determined by ELISA. Of the 21 samples tested for IgM reactivity, 18 (86%) showed anti-galactocerebroside (GalC) reactivity, 8 (38%) showed anti-ganglioside reactivity and 2 (10%) showed strong anti-sulphatide binding. Of 36 negative controls screened only 2 showed mild anti-ganglioside binding and 1 showed anti-GalC reactivity. No reactivity was detected against GalS. Positive controls comprised of plasma samples from IgM paraproteinaemia patients with biopsy proven neuropathy. Various other neurologic control groups with and without HIV-infection have also been studied. These data suggest that antibodies directed towards glycolipids in peripheral myelin may be a significant finding in the pathogenesis of HIV-associated inflammatory demyelinating neuropathies.

D4-338 CYTOKINE PRODUCTION BY HIV-INFECTED CELLS FROM TERM PLACENTA. M. Plaud Valentin, and L. Meléndez-Guerrero. School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, P.R. 00936

A recent focus of investigation has been on the role of the placenta in maternofetal infection. It has been shown that trophoblasts and placental macrophages (Hofbauer cells) are susceptible to HIV. The interactions between these two cell populations, the regulatory substances secreted and the mechanisms that regulate HIV expression remains to be understood. Since cytokines can regulate HIV expression in T cells and macrophages, we will study the cytokines produced by uninfected and HIV infected trophoblasts and Hofbauer cells. Supernatants from uninfected placenta cell cultures were assayed for the presence of the following cytokines: TNF-α IL-1β and IL-6. The percentage of macrophages and trophoblasts in the first day of culture were approximately 43% and 30% respectively as determined with monoclonals antibodies for trophoblasts (Trop-2) and macrophages (CD11c) by flow cytometry. Unseparated placenta cells produced minimal basal levels of TNF-α IL-1β and IL-6. These cytokines increased dramatically upon LPS stimulation. IL-6 showed the most elevated levels. Following 7 and 14 days post-infection the levels of p24 antigen in mixed cultures increased, showing active HIV production by these cells. Upon HIV infection with the monocytotropic strain HIV-BaL, mixed cultures still produced similar levels of IL-1β and IL-6, but TNF-α showed a significant increase indicating that HIV stimulated TNF-α production in placenta cells. After purification with monoclonal antibodies and immunomagnetic beads the effect observed in placenta cells was increased in the Hofbauer cells population. These results support previous findings that Hofbauer cells are the placental cell population being infected by the virus and that HIV activates TNF-α production in placental cells. Furthermore this study indicates that the cell being infected by HIV and the one that produces increased TNF-α levels is the Hofbuaer cell.

D4-339 CUTANEOUS DENDRITIC CELL-T CELL CONJUGATES ARE ACTIVELY INFECTED WITH HIV-1, Melissa Pope[1], Nancy Gallo[2], Lloyd Hoffman[3], Stuart Gezelter[1], and Ralph M. Steinman[1]. [1]Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021, [2]New York Firefighters Skin Bank, New York Hospital, New York, NY 10021, [3]Division of Plastic Surgery, Cornell University Medical College, New York, NY 10021

Both dendritic cells and memory T cells [$CD4^+$ and $CD8^+$] migrate from organ cultures of split thickness skin that has been removed from donors undergoing reductive plastic surgery. The skin cell emigrants consist of free dendritic cells, free T cells, and dendritic cell-T cell conjugates. The three cell subsets can be separated by cell sorting; conjugates continue to form when sorted dendritic cells and T cells are recultured together. The skin cell emigrants are permissive to infection with several HIV isolates. Multinucleated syncytia are the major site of virus production. Infection with HIV-1_{mn} is dependent on the presence of both dendritic cells and memory T cells, which fuse together to form the syncytia.

As well as furthering our infection studies using the primary patient-derived isolates, we have investigated cadaver skin as a more abundant source of cutaneous dendritic cells. Cadaver skin, taken within 24 hours of death, does provide much greater numbers of cells. Similar numbers [52,000 ± 5324 leukocytes per cm^2 skin] of functionally competent dendritic cells and memory T cells migrate from cadaver skin relative to fresh skin, with some of the dendritic cells and T cells forming stable conjugates.

Like the cells migrating from skin from live donors, cadaver-derived skin cell emigrants are sensitive to infection with HIV-1. The kinetics of infection of the skin cell emigrants with several HIV-1 isolates has been investigated. Dendritic cells and $CD4^+$ T cells together are necessary for infection with either syncytia-inducing or non-syncytia-inducing HIV isolates. AZT prevents infection with all HIV isolates tested. Cutaneous dendritic cells and memory T cells alone do not support productive infection with HIV-1, but together they create a microenvironment that is permissive to productive and cytopathic infection.

D4-340 DIVERSITY AND TISSUE SPECIFICITY OF HIV-1 IN FEMALE EARLY SEROCONVERTORS IN EAST AFRICA

Mary Poss[1], Hal Martin[2], Kishor Mandaliya[3], Bhavna Chohan[3], Joan Kreiss[2], and Julie Overbaugh[1], Department of Microbiology,[1] and Departments of Epidemiology and Medicine[2], University of Washington, Seattle, WA 98195, Coast Province General Hospital, Mombasa, Kenya[3]

HIV-1 infection is now prevelant in most geographical areas of the world including areas in East Africa. This virus exists as a diverse array of quasispecies that can be demonstrated at both the population and individual host level. Diversity is frequently defined by variations within the gene encoding the viral glycoprotein, *env*. A mature protein product of this viral gene, gp120, is a likely target for vaccine development. Effective virus prophalaxis must be approached from a knowledge of both global viral epidemiology and viral pathogenesis in infected individuals. Critical to this evaluation is understanding properties of transmitted viruses and their subsequent evolution within a host.

We addressed these questions by examining *env* variation in genital mucosa and PBMC from individuals shortly after seroconversion and at regular intervals thereafter. The cohort evaluated in this study are female sex workers from Mombasa, Kenya who attended a clinic monthly for evaluation of HIV infection as well as other sexually transmitted diseases. One week following a positive HIV ELISA and every three months thereafter, women returned to the clinic for collection of blood and cervical swabs. These samples were sent to our lab in Seattle, WA for evaluation of *env* variable regions V1-V3 by nested PCR.

We report that virus heterogeneity is present in both cervical mucosa and PBMC early in infection. These two distinct tissue populations of viruses are related but unique from one another. At three months following seroconversion, a genotype emerged in PBMC that reflected a minor variant seen at seroconversion. The population of virus at this later time point was more homogeneous than what was seen at seroconversion and had started to undergo some genetic variation. Our findings differ from analysis of sequence variability in other cohorts and may be unique to HIV-infected women or to this East African cohort, or may represent an important general aspect of pathogenesis not yet observed in early HIV infection. Any of these consideration are significant in addressing the worldwide spread of HIV.

D4-341 STRAIN-SPECIFIC CTL ACTIVITY DIRECTED AGAINST HIV-1 *env*

Stuart C. Ray, Bharati R. Dhruva, Robert F. Siliciano, and Robert C. Bollinger, Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Objective: In assays for the detection of CTL directed against HIV-1, recombinant vaccinia vectors expressing antigens from standard isolates are generally used as targets. Given the extreme diversity of HIV-1 strains, it is unclear whether the true response is accurately measured, either qualitatively or quantitatively, using constructs from these laboratory-derived HIV-1 strains.

Methods: We have generated recombinant vaccinia constructs expressing gp120 cloned from HIV-seropositive patients' PBMC (autologous) and from isolate HXB2 (heterologous), and have used them as targets in direct ^{51}Cr-release assays of CTL responses in these patients, with vaccinia and EBV background lysis reduced through the use of cold target inhibition. We have also used these constructs in an antigen-specific *in vitro* stimulation protocol, in which fresh PBMC were incubated with psoralen/UV inactivated autologous B-LCL expressing either autologous or heterologous gp120.

Results: Preliminary data show a similar low level of CTL activity directed against autologous and heterologous gp120. Both constructs induced a strong antigen-specific proliferative response in patient PBMC. In contrast to the direct assay, ^{51}Cr release assays of the stimulated cultures showed greater lytic activity directed against the autologous than the heterologous antigen. This finding was supported when cold targets expressing autologous but not heterologous gp120 effectively blocked lysis of hot targets expressing the autologous construct.

Conclusion: These studies provide direct evidence for autologous isolate-specific CTL directed against epitopes in gp120, CTL that would not be detected in standard assays.

D4-342 LOW VIRAL LOAD AND HIGH ANTI-HIV-1 CYTOTOXIC T LYMPHOCYTE MEMORY RESPONSES CORRELATE WITH LACK OF HIV-1 DISEASE IN LONG-TERM NONPROGRESSORS, Charles Rinaldo, Xiaoli Huang, Zheng Fan, Ming Ding, Lisa Beltz, Alison Logar, Dennis Panicali, Gail Mazzara, Jim Liebmann, Martin Cottrill and Phalguni Gupta, University of Pittsburgh, Pittsburgh, PA 15261 and Therion Biologics, Cambridge, MA

We have studied long-term nonprogressors in comparison to long-term intermediate and advanced progressors as defined by rate of $CD4^+$ T cell decline for HIV-1 specific cytotoxic T lymphocytes (CTL) and levels of HIV-1 DNA and RNA in blood. Lack of progression of HIV-1 infection strongly correlated with very low copy numbers of HIV-1 DNA and RNA in PBMC and plasma and the presence of high levels of memory $CD8^+$ CTL (CTLm) specific for Gag, Pol and Env. In contrast to the CTLm responses, $CD8^+$ effector CTL (CTLe) capable of directly lysing Gag, Pol and Env expressing targets without prestimulation in vitro were present at similar levels in all three groups of HIV-1 infected subjects. $CD8^+$ CTLm may have a critical role in controlling HIV-1 viral replication and protecting against immunosuppression and disease progression in long-term survivors.

D4-343 **ANERGY INDUCTION TO COMMON ANTIGENS DUE TO PRESENTATION BY MHC CLASS II ON HIV-1**, Jeffrey L. Rossio, Theresa A. Wiltrout, Louis E. Henderson and Larry O. Arthur, AIDS Vaccine Program, NCI-FCRDC, Frederick, MD 21702.

We have shown that HIV-1 grown in human PBMC bears on its surface numerous cellular antigens, including MHC gene products class II and class I. Human peripheral blood T lymphocytes isolated from a tetanus/diphtheria immunized, boosted normal donor were purified and cultured for 1 to 5 days in the presence of HIV-1 and an antigenic peptide (aa 830-844) derived from tetanus toxoid. The HIV-1 used was produced from the same cells used for assay; thus it presented antigen using autologous, histocompatible MHC class II molecules. The T cell cultures were then challenged with the antigenic tetanus peptide and autologous antigen-presenting cells (APC, peripheral blood monocytes). Proliferative responses were assessed after 5 days by ^{3}H-thymidine incorporation during the last 6 hours of culture. T cells previously exposed to antigen presented on HIV-1 showed marked reduction in response to normal antigenic signals presented by bona fide APC. Reduction in responsivity (anergy induction) was directly related to the amount of HIV-1 used, and exposure to HIV-1 alone, without antigen, did not result in anergy. Preliminary experiments indicate that induction of anergy is dependent on class II molecules, since HIV-1 propogated in cells with class II (CEMx174.T1) has surface class II and induces anergy, while virus propogated in a mutant clone lacking surface class II (CEMx174.T2) has no class II and does not induce anergy. The presence of interleukin 2 during the challenge phase significantly reversed the anergy. One implication of these results is that the loss in immune responsivity in HIV-infected individuals may proceed in part through a non-cytolytic, non-infective mechanism, and that the hyporesponsiveness observed may be specific and antigen-driven.

D4-344 CHARACTERIZATION BY COMPETITION ELISAs OF V3 LOOP-mAb 391/95-D BINDING. S. J. Seligman[1], M. K. Gorny[2], and S. Zolla-Pazner[2,3]. [1]SUNY/Health Science Center, Brooklyn, NY 11203; [2]NYU Medical Center, New York, NY 10016 and [3]Veterans Affairs Medical Center, New York, NY 10010.

A series of HIV-1 neutralizing mAb reacts with sequences in the crown of the V3 loop of HIV-1 (Gorny et al, J Immunol, 150, 635-643, 1993). The human mAb 391/95-D, 1 of 2 mAb that was not completely characterized by Pepscan analysis, was studied using competition ELISAs. The solid-phase antigen was biotin-YNKRKRIHIGPGRAFYTTKN attached to streptavidin-coated plastic plates. The fluid-phase competing antigens included series of peptides terminally deleted at either the N or the C terminus. The deletion series defined a core epitope of IGP and an extended epitope of KRIHIGPGRAFY. Affinity estimates for the 12-mer were $>10^6$ fold higher than for the trimer. The assays also permitted evaluation of the contributions to affinity of individual amino acids. For example deletion of an N terminal arginine or of a C terminal tyrosine decreased affinity more than 100 fold. Accordingly, deletion of single amino acids from competing peptides may be useful for estimating the minimal sequence of amino acids necessary to elicit neutralizing antibody and hence for predicting minimal specific sequences in sub-unit vaccines.

D4-345 THREE REGIONS OF GP160 CONTAIN OVERLAPPING CTL EPITOPES RESTRICTED BY MULTIPLE HLA CLASS I ALLELES. Premlata Shankar, Jessica Fabry, Donna Fong and Judy Lieberman, Division of Hematology/Oncology, New England Medical Center, Boston, MA 02111

CTL play an important role in the resolution of initial viremia and in delaying the onset of symptomatic disease in HIV-1 infection. We have identified overlapping CTL epitopes in HIV-1 gp160 which are recognized in the context of multiple MHC Class I alleles. In the N terminus of gp160, a 20mer peptide VPVWKEATTTLFCASDAKAY spanning aa 49-68 was recognized in four subjects. Using shorter 10 aa peptides, three distinct epitopes VPVWKEATTT (aa49-58), VWKEATTTLF (aa51-60) and LFCASDAKAY (aa59-68) could be identified within this sequence. The MHC restriction element for the aa 49-58 and the aa 59-68 epitopes was mapped to HLA-B55 and A24 respectively. A 15mer peptide ERYLKDQQLLGIWGC spanning aa 591-605 in gp41 which was recognized by four subjects was also found to contain three distinct epitopes; a B14 restricted epitope ERYLKDQQL, a B27 restricted epitope RYLKDQQL and an epitope YLKDQQLL, which was recognized in the context of A24 as well as B8. In the C terminal region, a 22mer peptide YRAIRHIPRRIRQGLERILL spanning aa 844-863 was identified in six subjects. The restriction element was B8 in 4 cases and A30 in one case. In a sixth subject, who does not express A30 or B8, the restriction element remains to be identified. Such broadly recognized multideterminant peptides may be useful components of an effective vaccine.

D4-346 EVALUATION OF ANTI-CARBOHYDRATE ANTIBODIES IN SERUM FROM HIV INFECTED PATIENTS. Sørensen, A.M.; Sørensen, T.; Clausen, H.; Nielsen, C.; Nielsen, JO. and Hansen, J-E.S. Laboratory of Infectious Diseases, Hvidovre Hospital, 2650 Hvidovre, Denmark.
Broadly HIV neutralizing carbohydrate Abs (anti-GalNAc) can be found in serum from HIV-infected at low titres.

Background: Abs directed against GalNAc (Tn) have previously been shown to be broadly HIV-neutralizing which is why Tn is proposed as a vaccine candidate.
Purpose: We wanted to evaluate the presence of Tn-Abs in serum from HIV-infected patients and to examine if virus isolated from patients with Tn-Abs could escape from these.
Materials/Methods: The presence of Tn-Abs in serum from 50 HIV infected patiens were tested. Virus was isolated from one patient with a peak in Tn-Abs. Inhibition experiments using Tn-Abs were performed with these isolates. Further, the proliferation response against Tn of PBMC from this patient was evaluated.
Results/Conclusions: Tn-Abs were found in serum form 20% of the HIV infected patients. Using a competitive ELISA, it was shown that the Abs were specific for Tn. In a consecutive series of serum from one patient, we observed a peak in the concentration of Tn-Abs. Virus isolated during the Ab peak was found to be less sensitive to Tn-Abs than virus isolated later. Further, PBMC from this patient proliferated when exposed to the Tn structure.

HIV infected patients can respond with an anti-Tn immune response, and wild-type HIV contains the Tn epitope during the entire course of HIV infection.

D4-347 IDENTIFICATION OF THE INFECTED TARGET CELL IN THE MUCOSA AND KINETICS OF VIRAL SPREAD FOLLOWING INTRAVAGINAL INOCULATION OF $SIV_{MAC}251$ INTO RHESUS MACAQUES. Alexander I. Spira[1], Bruce K. Patterson[2], Agegnehu Gettie[1], Preston A. Marx[1], Richard A. Koup[1], Steven M. Wolinsky[2], and David D. Ho[1]. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY[1], Northwestern University, Chicago, IL[2].

We have inoculated 4 female rhesus macaques intravaginally with $SIV_{mac}251$, a virus previously shown to induce infection by this route. One macaque was sacrificed 2 days (# 4140), 5 days (# 4167), 7 days (# 150G), and 9 days (#103D) after inoculation. At necropsy, blood, cervix, vagina, uterus, spleen, draining (internal iliac, common iliac, and aortic) lymph nodes, and peripheral (axillary and inguinal) lymph nodes were obtained from each animal and stored. To monitor for infectious virus, cells from each sample were cultured with CEMx174 cells and assayed for SIV gp120 production and cytopathic effects (syncytium formation). Only PBMC and internal iliac lymph nodes from macaque # 4167 were positive by culture for SIV. DNA was extracted from paraffin embedded samples and analyzed for the presence of SIV gag sequences by nested PCR. All 4 macaques were PCR positive in the vagina, cervix, draining and peripheral lymph nodes, and spleen, suggesting the rapid dissemination of virus in the first few days of infection. We have also begun in situ PCR studies to localize virus in the mucosa and submucosa of the cervix and vagina. Vaginal tissue from #4167 demonstrated the presence of SIV in non-lymphocytic cells immediately underlying the stratified squamous epithelium of the vagina. Studies are ongoing to clarify the identity of this infected cell in the mucosa.

D4-348 THE EFFECT OF SALIVARY ACIDIC PROLINE RICH PROTEIN - ELASTASE DIGESTS ON DIPEPTIDYL PEPTIDASE IV ACTIVITY, Helene R. Su and Robert J. Boackle, Division of Oral Biology, Department of Stomatology, Med. Un. of S.C., Charleston, SC 29425

HIV and HIV infected cells enter the oral cavity via gingival crevicular exudates in individuals with AIDS and periodontal disease. However, infectious HIV is infrequently isolated from whole saliva in individuals with AIDS. PMN leukocyte elastase present in gingival crevicular exudates has been reported as a indicator of periodontal disease progression. In addition, it has been reported that peptides with L-proline at their penultimate position inhibit the activity of dipeptidyl peptidase IV (DPPIV) and inhibit the entry of HIV into permissive CD4+ cell lines. Based on the cleavage specificity of elastase and on proline rich properties of the salivary acidic proline rich proteins (APRP), APRP-elastase digests have a high probability to exhibit DPPIV inhibitory activity.

APRP were purified from human parotid saliva by electroelution from a 9% preparative polyacrylamide gel. Various amounts of APRP were incubated with excess amount of human leukocyte elastase at 37 °C for 30 min. Human parotid saliva in glycin Tris buffer (pH 8.3) was then added as the source of DPPIV. Gly-Pro-p-nitroanilide was used as the substrate of DPPIV. After incubation at 37 °C for 30 min, the reaction was stopped using acetate buffer (pH 4.2). The released p-nitroaniline was detected by differential absorbance at 380 nm. Substrate alone was substracted as background. The effects of APRP, elastase and APRP-elastase-digests on the DPPIV activity were compared. A biphasic DPPIV inhibitory effect was observed by the incubated mixtures of various concentrations of APRP with constant amounts of elastase. The inhibitory effect of APRP-elastase digests on DPPIV activity may explain in part the inhibition of HIV infectivity by salivary secretions.

D4-349 **VIRUS SPECIFIC IMMUNITY IN CYNOMOLGUS MONKEYS EXPOSED TO A LOW DOSE OF SIVsm**

Rigmor Thorstensson, B Mäkitalo, D Böttiger, E Rud[1], G Biberfeld, P Putkonen, Swedish Institute for Infectious Disease Control and Karolinska Institute, Stockholm, Sweden, [1]Health Canada, Ottawa, Canada

Objective: To determine the minimum infectious dose of a macaque cell grown stock of SIVsm (SMM-3) in vivo by the intravenous (IV) and by the intrarectal route (IR) in cynomolgus monkeys and to characterize the virus specific immunity, especially the cell-mediated immunity in monkeys which received a subinfectious dose.

Methods: SIVsm was propagated in vitro on cynomolgus monkey PBMCs.Ten-fold serial dilutions of stock virus were inoculated IV and IR into a total of 18 cynomolgus monkeys. Monkeys were IV given one ml of the stock ranging from 10^{-1} to 10^{-6} (two animals for each dilution) or 3ml of the stock ranging from 10^{-1} to 10^{-3} by atraumatic intrarectal inoculation. Infection was determined by virus isolation and PCR(gag, pol, env, LTR). Virus specific immunity was investigated by ELISA, Western blot, neutralization assay, T-cell proliferation and CTL (gag/pol, nef).

Results: Infection was demonstrated by positive virus isolation and PCR and by seroconversion in all of the monkeys inoculated IV with the virus stock diluted 10^{-1} to 10^{-5} and in one of two monkeys given the 10^{-6} dilution. Two of two monkeys became infected by the IR route given the 10^{-1} dilution and 1/2 became infected given the 10^{-2} dilution.The remaining three IR inoculated animals did not become infected as shown by negative virus isolation and serology but showed SIV specific gag/pol and nef CTL. Studies on neutralizing capacity and T-cell proliferative response are in progress.

Conclusions: A larger cell-free virus inoculum is required for infection over the rectal mucosa compared to the intravenous route. Monkeys exposed to low concentrations of SIV developed SIV specific CTL but no demonstrable viral antibodies. These animals will be rechallenged with a higher dose to determine if the cellular immunity can confer protection against challenge.

D4-350 SYNERGISTIC NEUTRALIZATION OF HIV-1 BY MONOCLONAL ANTIBODIES DIRECTED AGAINST THE V2 DOMAIN, V3 LOOP, AND CD4-BINDING SITE OF gp120, Shermaine A. Tilley and Sujata Vijh-Warrier, Public Health Research Institute, NY, NY 10016

We have recently described[1] a potent neutralizing chimpanzee mAb, C108G, that is directed against a unique, carbohydrate-dependent epitope in the V2 domain of gp120; this mAb is relatively type-specific, recognizing the IIIB strain and certain related clones as well as rare PBMC-passaged monocytotropic isolates. Previous studies by our group and other investigators demonstrated synergistic neutralization of HIV-1 by two-mAb combinations of anti-V3 loop and anti-CD4-binding site (bs) mAbs. In this study, we demonstrate that two-mAb combinations of C108G paired with either of two anti-V3 loop mAbs or either of two anti-CD4-bs mAbs synergistically neutralize both the IIIB and HXB2 strains of HIV-1. Synergism was observed across a range of ratios of these mAb pairs, with greatest synergism being achieved at approximately equipotent ratios. In all cases, synergism was greater against the heterogenous IIIB strain than the essentially homogeneous HXB2 clone, suggesting that strain broadening might be a significant factor in the synergism observed as well as conformational changes that probably occur within gp120 upon binding to these mAbs. In addition, we observed synergistic neutralization of HIV-1_{HXB2} with a three-mAb combination of C108G, anti-V3 loop mAb 0.5β, and anti-CD4 bs mAb 1125H; this synergism was greater than that observed with any of the three two-mAb combinations of these three mAbs as determined by the dose reduction indices of each mAb required to achieve a given level of neutralization. This is the first report of three-way synergistic neutralization of HIV-1 by mAbs directed against the three major neutralization epitope clusters in gp120. Implications for vaccine design and for immunoprophylaxis / immunotherapy with a combination of mAbs will be discussed.

[1]Warrier et al. (1994) *J. Virol.* 68: 4636.

D4-351 VH3 IMMUNOGLOBULINS OF THE NATURAL ANTIBODY REPERTOIRE BIND gp120, ACTIVATE COMPLEMENT AND SEQUESTER gp120 AWAY FROM T-CELLS. Janet Townsley-Fuchs, Lee Goodglick, and Jonathan Braun. UCLA Dept. of Pathology, Los Angeles, CA 90024 The loss of CD4+ T-cells in HIV infection leads to a breakdown of cell-mediated immunity resulting in many opportunistic infections and eventually, death. While the mechanism for the loss of T-cells remains unclear, they can be infected by the virus. The coat protein of HIV, gp120, can directly bind to CD4 and this interaction leads to infection. Recent studies in our laboratory have demonstrated that IgM from non-immune individuals can bind to gp120 by a superantigen mechanism. The present study sought to determine if the binding of gp120 to natural IgM has an impact on the pathogenesis of HIV. We hypothesized that gp120 could form immune complexes, activating complement and causing the virus to be sequestered by phagocytic cells. We have found that IgM in normal human serum can inhibit the binding of gp120 to T-cells by flow cytometry in whole blood. In contrast, ELISAs and flow cytometry have shown that IgM alone cannot directly interfere with the gp120 CD4 interaction. Furthermore, the inhibition of binding in whole blood was dependent on complement. The inhibition was reversible by pre-incubating the blood with anti-complement receptor antibodies. Also, inhibition was not seen when heat inactivated serum was used. These findings indicate that natural immunoglobulins have an innate capacity to bind to gp120 and that this interaction may cause HIV to be taken up by phagocytic cells.

D4-352 QUANTITATIVE DETERMINATION OF HLA-DR MOLECULES INCORPORATED WITHIN HIV-1, Michel Tremblay, Réjean Cantin, Richard Bernier, Guylaine Briand, Ginette Lamontagne and Jean-François Fortin, Unité de Rétrovirologie - Laboratoire d'Infectiologie, Centre de Recherche du CHUL and Département de Microbiologie, Université Laval, Québec, Canada, G1V 4G2.

We specifically investigated the incorporation of cellular major histocompatibility complex class II (MHC-II) molecules within human immunodeficiency virus type 1 (HIV-1) particles grown in two different human CD4+ MHC-II+ cell lines expressing different levels of surface MHC-II. We found that, as previously reported, a very selective incorporation of the HLA-DR determinant and not of the HLA-DP or HLA-DQ isotype has taken place within the budding viral particles. The use of Western blot and HLA-DR specific enzymatic assays indicate that the amount of virally-embedded HLA-DR molecules can quantitatively differ depending of the virus producer cell line. Our results also demonstrate that HIV-1 acquired HLA-DR molecules can represent as much as 18% of the total protein, while the external envelope gp120 protein makes up less than 0.64% of the total protein in the highly purified viral stock. Based on the notion that the mass of an individual HIV-1 particle is 11.6×10^{-16} g, we evaluated that there are appoximately 4388 HLA-DR complexes and 37 gp120 molecules per virion in the purified virus stocks. Thus our data reveal that virally-embedded cellular HLA-DR molecules greatly outnumber the external virus envelope gp120 protein. These observations extend beyond already published results and suggest that the biological functions of the budding virions may vary depending of the virus producer cell line.

D4-353 CROSS-CLADE NEUTRALIZATION OF PRIMARY HIV-1 ISOLATES BY ANTI gp120 AND ANTI gp41 HUMABS AND TETRAMERIC CD4-IgG2, Alexandra Trkola [1], Dennis R. Burton[2], Carlos F. Barbas III[2], Graham P. Allaway[3], Paul J. Maddon[3], Hermann Katinger[4], and John P. Moore[1], [1] Aaron Diamond AIDS Research Center. New York University School of Medicine, New York, NY 10016, [2] Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037, [3] Progenics Pharmaceuticals Inc., Tarrytown, NY 10591, [4] Institute of applied Microbiology, University of Agriculture, 1190 Vienna, Austria

Despite the relative insensitivity of PBMC-grown primary viruses to neutralization in PBMC-based assays, it is apparent that these viruses can be neutralized by sera from HIV-1 infected humans, especially those who are long-term non-progressors. Furthermore, we have also shown that antibodies to conserved, discontinuous epitopes on gp120 can show considerable cross clade reactivity, at least in binding assays. To assess whether there are conserved neutralization epitopes, we are undertaking an exploration of the ability of selected reagents to neutralize primary isolates from clades A-F and O. The reagents in our test panel were the anti-gp120 HuMAbs IgG1b12 and 2G12, the anti gp41 HuMAb 2F5 and a tetrameric form of CD4-IgG2. We selected up to three viruses from each clade for our test panel. The 90% neutralization titers of reagents against the different viruses were estimated in a PBMC-based assay. Results available to date indicate that each reagent possessed significant neutralizing activity against HIV-1 isolates from within and outside clade B, albeit with a considerable range of potencies. Thus, a HIV-1 vaccine based on humoral immunity that might be effective against multiple clades is not impossible, provided that we learn more about these broadly reactive antibodies and how to induce them.

D4-354 CONTINUOUS PROPAGATION OF REV- AND RRE-INDEPENDENT HIV-1 MOLECULAR CLONES IN PRIMATE CELLS: POTENTIAL CANDIDATES FOR ATTENUATED HIV-1 STRAINS, Antonio Valentin, Andrei S. Zolotukhin*, George N. Pavlakis, and Barbara K. Felber,* Human Retrovirus Pathogenesis Group*, and Human Retrovirus Section, National Cancer Institute-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, MD 21702-1201

Molecular clones of human immunodeficiency virus type 1 (HIV-1) were constructed containing either 37 point mutations in the Rev responsive element (RRE) that do not affect the overlapping *env* reading frame or both a mutated RRE and two mutations that eliminate Rev. The mutations in RRE were shown to remove both negative and Rev-inducible positive effects of RRE on gene expression (1). Upon insertion of a *cis*-acting element of simian retrovirus type 1 (SRV-1) into these clones, both RRE(-) and Rev(-)RRE(-) clones were expressed efficiently. Virus stocks produced after transfections of these molecular clones in human cells were infectious upon cell-free transmission, replicated about 5-10 times less efficiently than wild-type virus, and were propagated continuously for more than 8 months in human peripheral blood mononuclear cells (PBMCs) (2) as well as in macrophages. Growth characteristics and sequence analysis of these viruses after long-term culture demonstrated that no RRE(+) or Rev(+) revertants developed. Therefore, the presence of this SRV-1 element permits efficient virus expression independent of the Rev/RRE regulatory system. The SRV-1 element has properties similar to the recently described element of MPMV (3). Our data demonstrate that the Rev protein is required only as a component of the Rev/RRE positive regulatory system, and, once replaced by appropriate cellular factors, Rev is not essential for virus replication in vitro. High titer virus stocks were also produced in cell lines. These stocks were used to infect chimpanzee and pig tail macaque PBMC. It was found that PBMC from both species support the SRV element-mediated virus growth. The replacement of the Rev/RRE regulatory axis may generate viruses with altered biological properties and it may allow the production of attenuated nonpathogenic HIV-1 strains. The produced strains also give an opportunity to study the effects of Rev/RRE at the virus level.Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-46000 with ABL.

1. G. Nasioulas, *et al., J Virol* 68, 2986-2993 (1994).
2. A. S. Zolotukhin, A. Valentin, G. N. Pavlakis, B. K. Felber, *J Virol* 68, in press (1994)
3. M. Bray *et al., Proc Natl Acad Sci USA* 4, 1256-1260 (1994)

D4-355 HIV-1 IIIB ENV/REV-SPECIFIC CTL ACTIVATION IN HIV-INFECTED SUBJECTS FOLLOWING DIRECT RETROVECTOR ADMINISTRATION, John F. Warner*, Gloria Peters*, Mike Irwin*, Carol-Gay Anderson*, Nancy Sajjadi*, Kieron Kowal*, Jay Merritt*, Doug Jolly* Steve Mento*, and Jeff Galpin**, Viagene, Inc. 11055 Roselle St., San Diego, CA 92121; **Shared Medical Research Foundation, Tarzana, CA 91356

The cytotoxic T lymphocyte (CTL) response plays an important role in controlling the severity and duration of viral infections. Gene-based vaccines, using non-replicating retroviral vectors (retrovectors) 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 a murine retrovector encoding the HIV-1 IIIB env/rev proteins and examined this vector for the ability to stimulate CTL responses in different animal models and humans. The retrovector consistently activates $CD8^+$, major histocompatibility complex (MHC)-restricted anti-HIV-1IIIBenv/rev CTL responses in mice, Rhesus macaques and baboons. Most importantly, HIV-infected human subjects exhibit an increase in $CD8^+$, MHC-restricted CTL responses against HIV-1 env/rev expressing target cells following three intramuscular direct vector injections. These studies demonstrate the ability of a retrovector encoding the HIV-1 IIIB env/rev proteins to provide an effective means of inducing or augmenting CTL responses. The results of these animal studies and Phase I clinical trials will be discussed.

D4-356 **DECREASED T CELL RECEPTOR (TCR)/CD3 SURFACE EXPRESSION ON HIV-1 INFECTED T CELLS IS CORRELATED WITH AN INCREASE IN TAT REGULATED TRANSCRIPTIONAL ACTIVITY**, Karen E. Willard-Gallo[1], Steven M. Wolinsky[2], and Bruce K. Patterson[2], [1]International Institute of Cellular and Molecular Pathology, Brussels, BELGIUM and [2]Northwestern University Medical School, Chicago, IL

We have previously shown that HIV-1 and HIV-2 can modulate expression of the T cell receptor (TCR)/CD3 complex on the surface of the human $CD4^+$ T cell line, WE17/10, by specific interference with CD3-γ gene transcripts. We have been investigating possible interactions between the regulation of retroviral and CD3-γ gene transcription that could account for this phenomenon. We have found that the quantity of intracellular viral p24 antigen increases in parallel with a decrease in surface TCR/CD3 complexes. Further evidence that a correlation exists between virus and TCR/CD3 expression comes from $TCR/CD3^+$ HIV-1 infected WE17/10 cells exposed to extracellular Tat protein. The Tat-treated infected cells decrease their surface receptor expression in a time and dose-dependent manner.

To determine whether this phenomenon is selectively due to the interaction of Tat with specific host cell factors, WE17/10 cells were infected with a tRNA-driven, double copy TAR decoy, DCTAR, and positive infectants were selected. TCR/CD3 surface expression in both control and TAR expressing cells decreases in the presence of extracellular Tat; however, a more significant decrease in surface receptor complexes occurs in the presence of Tat plus TAR, with a 33% decrease in TCR/CD3 surface density detectable after four days. This Tat-induced loss of receptor expression resembles the transition from $TCR/CD3^{hi}$ to $TCR/CD3^{lo}$ observed early after HIV infection of WE17/10 cells, where the $TCR/CD3^{lo}$ infected cells have become functionally anergic. Evidence provided by this *in vitro* model that TCR/CD3 surface expression is linked to Tat-regulated transcriptional activity suggests that the level of surface receptor expression may be related to the early loss of the antigen activation pathway observed in HIV-seropositive individuals.

D4-357 ANTI-HIV HUMAN MONOCLONAL ANTIBODY VARIABLE REGION GENE USAGE, Adam Wisnewski, Lisa Cavacini, Gillian Kingsbury, David Scadden, Marshall Posner, Human Monoclonal Antibody Laboratory, Department of Hematology/Oncology, N.E. Deaconess Hospital, Boston MA 02115

Restricted antibody heavy chain variable region (VH) gene usage related to HIV pathogenesis has been proposed thus the present study investigates human monoclonal antibodies (HMabs) which have been derived from HIV-infected patients and subjected to natural selection *in vivo*. VH usage and somatic mutations were analyzed by cloning and sequencing two anti-HIV HMabs. HMab F240, which recognizes a linear gp41 epitope comprising aa 592-606 (BH10) (George Lewis, personal communication) utilizes a VH3 family gene with strongest homology (85%) to the VH3.3-11 germline gene. F240 is the second gp41 HMab determined to use VH3 family genes, refuting the hypothesis of HIV-induced VH3 depletion, at least with regard to gp41 antibodies. A second HMab F285, which binds cell surface expressed HIV envelope antigens, utilizes a VH1 family gene and demonstrates the strongest homology (87%) with VH1.51P1, a germline gene also used by two different anti-gp120 HMabs. Usage of a VH1 family gene by F285 supports the notion of positive preferential VH1 family gene usage by HIV envelope antibodies previously proposed based on serological data, suggesting either a regulatory basis for expression or the importance of framework (FR) regions in HIV envelope antigen binding. Both HMabs demonstrated extensive somatic mutations compared to likely germline progenitors. The mutation frequency for F240 was 13%, 7%, 14%, 23%, and 15% in FR1, CDR1, FR2, CDR2, and FR3 respectively. The mutation frequency for F285 was 3%, 13%, 7%, 27%, and 17% in FR1, CDR1, FR2, CDR2, and FR3. In all, 71% and 66% of the nucleotide changes in F240 and F285 resulted in amino acid changes indicative of an antigen driven selection process. Further comparison of the F240 and F285 somatic mutations and V-region gene usage with other functionally distinct HMabs will define the structure/function interactions between human antibodies and HIV envelope antigens.

D4-358 LIGATION OF CD28 CAN MEDIATE AN INCREASE IN SPLICED VIRAL RNA SPECIES IN PBLs FROM DONORS CHRONICALLY INFECTED WITH HIV-1, Justin G.P. Wong, Molly D. Smithgall, and Omar K. Haffar, *Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.*

Efficient replication of HIV-1 in T-lymphocytes is thought to depend upon cellular activation events resulting in both induction of virus from infected cells as well as *de novo* infection of target cells. Activation of T-cells is achieved through signals generated following ligation of TcR/CD3 complex, together with second signals mediated through ligation of accessory molecules such as CD28.

We have previously demonstrated that ligation of CD28 is necessary for efficient replication of HIV-1 in anti-CD3-stimulated PBMC cultures, and that *de novo* infection of anti-CD3 stimulated PBMC targets can be inhibited by abrogating the interaction between CD28 and its counter-receptor B-7 on monocytes.

In this study, we used an assay based on reverse transcriptase mediated PCR (RT-PCR) to assess the role of CD28 in the induction of viral RNA. We demonstrate that anti-CD28 stimulation of freshly isolated PBLs from donors chronically infected with HIV-1 is able to mediate an increase in levels of spliced viral RNA species compared to levels expressed by unstimulated PBLs. This increase results from transcriptional activation of the viral LTR rather than stabilisation of existing RNA, since pre-treatment of cells with actinomycin D abrogates the response. CD28 mediated transcriptional induction is not dependent upon protein synthesis, however, suggesting the involvement of pre-formed transcriptional factors. Furthermore, transcription is sensitive both to the tyrosine kinase inhibitor herbimycin A and to cyclosporin A, consistent with known pathways of CD28 signalling. These results, together with results from our previous studies, suggest that direct interference with CD28 co-stimulatory pathways may modulate the course of HIV-1 infection.

D4-359 IMMUNOLOGIC ANALYSIS OF VARIABLE AND CONSERVED REGION DELETED HIV-1 gp120 GLYCOPROTEINS. Richard Wyatt, Chris Nixon, Molly Accola, Elizabeth Desjardins, Regina Allen and Joseph Sodroski. Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, MA 02115

During the course of HIV-1 infection, neutralizing antibodies are elicited against various elements of the exterior envelope glycoprotein of HIV-1, gp120. In most HIV-1 infected individuals, two classes of neutralizing antibodies directed against gp120 are elicited: strain-restricted and broadly cross-reactive antibodies. Strain-restricted antibodies are most commonly directed against linear determinants within the gp120 third variable region or V3 loop. This class of antibodies has been relatively easy to generate in both primate and nonprimate animal systems. The second variable (V2) loop of gp120 is also a target for strain-restricted neutralizing antibodies. The broadly-neutralizing antibody class contains a major subset of antibodies which recognize conformationally-dependent, discontinuous epitopes overlapping the discontinuous CD4 binding site on gp120. We have previously demonstrated that deletion of the major variable loops V1, V2 and V3 (ΔV1/2/3 glycoproteins) better expose the gp120 CD4 binding region to this subset of neutralizing antibodies. Here we report on the effects of removing conserved gp120 C1 and C5 residues. These apparently immunodominant epitopes elicit nonneutralizing antibodies commonly isolated from HIV-1 infected individuals. Using these antibody probes, we have continued to modify ΔV1/2/3 by deletion of C1 and C5 nonneutralizing epitopes. We have utilized CD4 binding site antibodies to monitor the extent conserved residues could be eliminated without compromising recognition by these conformationally-sensitive antibodies. In addition, another subset of more broadly neutralizing antibodies, termed CD4 induced antibodies (i.e. 17b and 48d), do not recognize ΔV1/2/3 as originally constructed. We have been able to restore 17b and 48d recognition of ΔV1/2/3 by making more conservative variable loop deletions. These modifications may alter gp120 immunogenicity by focusing the immune response to retained conserved neutralizing epitopes while eliminating potentially immunodominant nonneutralizing conserved residues.

D4-360 INFECTION OF A CERVIX-DERIVED EPITHELIAL CELL LINE WITH HTLV-I, Vanaja R. Zacharopoulos, and David M. Phillips, The Population Council, 1230 York Ave, NY, NY 10021
Transmission of HTLV-I may involve transfer of virus across an intact epithelium. Using an in vitro model we have previously presented evidence that epithelial cells, derived from the human gut, are targets of HTLV-I infection, and that HTLV-I infected T-cells are the primary source of infection, as opposed to cell-free virus. We observed that when the HTLV-I transformed lymphocytes (MT-2 cells) were added to the epithelium, the T-cells adhere, polarize into pear-shaped cells, and secrete virus unidirectionally into the region of contact between the T-cell and epithelial cell. Epithelia then take up virus, incorporate provirus, and subsequently produce new virions (*J Virol* 66:4601,'92). In order to study the possibility that heterosexual transmission of HTLV-I occurs via a similar mechanism involving cell-mediated infection of intact epithelia, we added MT-2 cells to cultures of a CD4 negative epithelial cell line, MS751, derived from the human cervix. A fluorescence-based adhesion assay was used to quantitate attachment of MT-2 cells to the epithelia. Infection was measured by ELISA and PCR. A week after addition of MT-2 cells, productively infected MS751 cells produced 60pg of HTLV-I p24 antigen per 10^6 cells over a 24h period. Adherence of MT-2 cells to the epithelial monolayer, an apparently crucial step in infection, could be blocked by the enzymes α- and ß-mannosidase and α-L-fucosidase, and sulfated polysaccharides. Other enzymes including neuraminidase and N-glycosidase F, other sugars, and blocking antibodies to adhesion molecules including ICAM-1, LFA-1 and LFA-3 did not block adhesion. Productive infection of the epithelium was blocked by sulfated polysaccharides. The data suggests that a sugar moiety on the epithelium, possibly mannose or fucose, may be involved in adhesion of HTLV-I infected T-cells to cervix-derived epithelia. The observation that sulfated polysaccharides block both adhesion to the epithelium and productive infection of the epithelium suggest that a sulfated polysaccharide might be effective as the active ingredient in a vaginal formulation which could be used to inhibit sexual transmission of HTLV-I.

D4-361 PREFERENTIAL TRANSMISSION OF CELL-ASSOCIATED VIRUS AND EVIDENCE FOR SELECTION OF HIV-1 IN THE NEW HOST FOLLOWING SEXUAL CONTACT. Tuofu Zhu, Ning Wang, Daniel S. Nam, Yunzhen Cao, Andrew Carr*, David Cooper*, 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.

Studies on the genotype of HIV-1 in seroconvertors to date have suggested that there is selection certain viral variants in sexual transmission. However, it is not clear where the selection occurs and whether the transmission is primarily via HIV-infected cells or free virions. To address these questions, we have compared HIV-1 gp120 sequences in 8 acute seroconvertors with those found in PBMC, plasma, seminal cells and seminal plasma of the corresponding transmitters. Four cases involve homosexual transmission from male to male, whereas the remaining 4 cases involve heterosexual transmission from male to female. In addition to comparison of nucleotide sequences, we have used a single-strand heteroduplex mobility assay (ssHMA) as a complement to large-scale sequencing to compare the overall composition of the quasispecies in each transmission pair. The transmitted viruses are genetically closer to those found in seminal cells or PBMC of the transmitters, and viruses closely related to the transmitted virus are more frequently detected in transmitters' seminal cells or PBMC. Transmitted viruses are typically homogeneous in that 6 of 8 seroconvertors harbored only one viral population, whereas 2 seroconvertors had 2 to 3 related variants. The transmitted virus in 7 of 8 seroconvertors represents a minor variant in samples from the corresponding transmitters, while in one case the transmitted virus is the major variant in the transmitter. There is evidence for compartmentalization of virus in that variants found in genital secretions may be different from those in blood. Our findings suggest that sexual transmission of HIV-1 may be more often mediated by cell-associated virus, and that selection of certain HIV-1 variants following sexual transmission probably occurs in the new host.

Maternal-Child Transmission, Pediatric AIDS, Tropism, Animal Models, Co-factors, Cohort Studies

D4-400 **HIV-1 INFECTION OF HUMAN ADULT OLIGODENDROCYTES** Andrew Albright, Julie Strizki, Janet Harouse, Michael O'Connor, and Francisco Gonzalez-Scarano, *Depts. of Neurology and Neurosurgery, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6146*

Although unquestionably its etiologic agent, the precise role of HIV-1 in the development of HIV Dementia (HIVD) is not well understood. Microglia, the resident brain macrophage, is the only CNS cell type that is consistently reported as being infected with HIV-1 *in vivo*. However, recent *in situ* PCR studies have suggested that other cells may harbor HIV sequences. Some pathological findings common in HIVD like myelin pallor and demyelination, could be explained by a direct infection of oligodendrocytes, or by interaction of HIV-1 gp120 with their cell surface. Although oligodendrocytes do not express CD4, their membranes are rich in galactosylceramide, a potential alternative HIV receptor.

We prepared 99%-pure oligodendrocyte cultures from human brain tissue obtained from temporal lobe resections. These cells were exposed to HIV-1, and infectivity was assayed by detection of p24 antigen, PCR amplification, or co-cultivation with permissive CD4 positive cells. Two weeks after infection with two HIV-1 strains (IIIB and BaL), but not with others (89.6), we detected low levels of p24 in the culture supernatants, and were able to amplify HIV-1 DNA. Direct co-cultivation of the infected oligodendrocytes with CEMx174 cells indicated the presence of infectious virus; however, no infectious virus was detected in the oligodendrocyte *supernatant* absent co-cultivation.

These results indicate that oligodendrocytes can harbor the HIV-1 provirus, but in common with many infections of neural cells, the level of virus production is low. Current experiments are investigating the potential interactions between oligodendrocytes and microglia that might synergistically increase HIV-1's effect on oligodendrocytes.

D4-401 **GENETIC DIVERSITY AND SUB-TYPING OF HETEROSEXUAL HIV-1 TRANSMISSIONS IN ENGLAND**

CATHERINE ARNOLD[1], PETER BALFE[2] AND JONATHAN P. CLEWLEY[1]

Virus Reference Division, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT[1], and Department of Virology, University College and Middlesex School of Medicine, Windeyer Building, Howland Street, London[2].

The number of European and North American heterosexually transmitted HIV infections is increasing. Injecting drug users and bisexual men may be a source of infection for heterosexual transmission and the HIV-1 epidemic may be spreading from them into the general population. To gain insight into heterosexual transmission in England we investigated two transmission events by sequencing several single gp120 molecules from each of the six implicated individuals. The epidemiological linkage of these cases was confirmed by their relative *env* sequence similarity, compared with unlinked sequences from the Los Alamos database. Comparison of their sequences with genetically sub-typed sequences revealed that one male had transmitted HIV-1 of sub-type B (typically associated with Europe/ U.S.A.)and the other male had transmitted sub-type D (typically associated with infection in Africa). Though, to date, HIV-1 infection in the U.K. has been mainly of sub-type B, and transmitted homosexually and by injecting drug use, this laboratory has found in England evidence of genetic sub-types A,B,C and D. In Africa the epidemic is genetically more diverse (sub-types A-I and O present) and is mainly heterosexually transmitted. The possibility of a heterosexually transmitted epidemic in the U.K may be increased by the introduction of strains of HIV-1 sub-types other than B, e.g. the African sub-types. Our detection of the presence of sub-types A-D and of non-B heterosexual transmission in England demonstrates that these introductions have already taken place.

D4-402 **Hematopoietic Suppression by HIV-1: The Role of Stroma** Ingrid Bahner, Karen Kearns, Donald B.Kohn, Division of Research Immunology and BMT, Childrens Hospital, Los Angeles CA 90027

Hematopoietic suppression in ARC and AIDS, i.e. anemia, neutropenia and thrombocytopenia is a well documented syndrome. The physiologic basis for this suppression is not completely understood. When we evaluated the isolation of CD34+ cells from AIDS patients, we found that patients with a CD4 count of less than 50 showed a tenfold reduction in number of CD34+ progenitor cells as compaired to HIV-1 negative control marrows. Additionally, these cells showed an 80-95% impaired proliferation potential compared to HIV-1 negative CD 34+ isolates. Infection of normal human Long Term Bone Marrow Cultures (LTBMC) with a monocytotrophic strain of HIV-1 caused a marked decrease in hematopoietic cell production and the numbers of clonogenic progenitors. Growth of human marrow in LTBMC either using a cloned stroma line (S17) or in the absence of a stromal layer (supported by cytokines IL3/IL6/SCF) prevented the adverse effects of HIV-1 infection, implying a causal role for infection of the human stroma. Significantly, normal human stroma transduced with retroviral vectors encoding anti-HIV-1 genes (*trans*-dominant rev, RRE decoy or hammerhead ribozyme) also did not suffer loss of hematopoietic support function when infected with HIV-1. When we infected a normal stroma cell layer directly with HIV-1 we were not able to establish a productive infection at an MOI lower than 0.1. However, we were able to rescue the virus from cultures infected with lower MOIs eight days after the initial infection by cocultivation of nonadherent hematopoietic cells. These data indicate that HIV-1 is able to directly infect the stromal component of bone marrow. To further elucidate the mechanism of HIV-1 mediated hematopoietic suppression we are currently investigating the HIV-1 gene products and cellular cytokines produced in HIV-1 infected LTBMC.

D4-403 PERSISTENT AND PRODUCTIVE HIV_1 INFECTION OF THYMIC EPITHELIAL CELLS WITHOUT ANY CELL DEGENERATION *IN VITRO*

Françoise Barré-Sinoussi, Joséphine Braun, Marie-Thérèse Nugeyre, Maurice Rothe, Hélène Ohayon, Pierre Gounon and Hélène Valentin, Institut Pasteur, Paris, France.

The dramatic depletion of circulating $CD4^+$ T cells in AIDS may result from an early invasion of lymphoïd tissues by HIV_1. Among such tissues, the thymus which is the primary organ for T cell maturation was shown to be infected and altered in seropositive individuals and in animal models. Studies both *in vivo*, using the SCID-hu mouse, and *in vitro*, have indicated that subsets of immature and mature thymocytes are susceptible to HIV infection. HIV was also found within thymic epithelial (TE) cells but productive infection of these cells has not yet been definitively demonstrated. The purpose of the present study was to address this issue by using a well characterized TE cell culture obtained from thymic explants. Using two laboratory HIV_1 strains with distinct phenotypes (HIV_1/LAI and HIV_1/NDK), we first found that both viruses can enter into TE cells through a mechanism depending on CD4 molecule expression as indicated by the detection of CD4 mRNA by RT-PCR and by the blockage of viral entry by CD4 mAb.
However, we were able to easily demonstrate an active viral replication into TE cells only with the highly infectious HIV_1/NDK strain. The infection of TE cells by HIV_1/NDK was clearly productive as shown by the high level of virion-associated RT activity in cell-free supernatants and by the morphologic pattern seen in electron microscopy. No detectable TE cell death was observed following their productive infection which was found to occur in about 30% of the cells, by *in situ* hybridization and to persist for up to 60 days in culture. Those data strongly suggest that the destruction of TE cells reported in infected thymuses is not related to their productive infection but rather involves other mechanisms such as intact functions of thymocytes. However, the productive and persistent infection of TE cells that, according to our results can depend on the infecting HIV_1 isolate may have significant implications for the potential role of these cells as a viral reservoir for the rapid spreading of HIV in the thymus.

D4-404 NEUTRALIZATION OF FELINE IMMUNODEFICIENCY VIRUS *IN VITRO* IS AFFECTED BY NUMEROUS VARIABLES AND *IN VIVO* HAS LITTLE IF ANY PROTECTIVE EFFECT, Mauro Bendinelli, Donatella Matteucci, Stefania Lombardi, Carlo Garzelli, Daniela Del Mauro, Claudia Massi, Paola Mazzetti, Patrizia Bandecchi, and Franco Tozzini, Retrovirus Centre and Virology Section, Department of Biomedicine and Department of Animal Pathology, University of Pisa, I-56125 Pisa, Italy.

It is becoming increasingly evident that antibody-mediated neutralization of lentiviruses is a complex phenomenon. Neutralization of FIV is no exception. In our hands inhibition of FIV by antibody was influenced by a number of variables, including virus isolate and passage history, type of cells used to measure the residual infectivity, and type of medium used to propagate the host cells. Under certain circumstances antibody-dependent enhancement of FIV infectivity was also observed. This makes it difficult to predict the role neutralizing antibodies may play in host protection during infection in vivo. So far we have been unable to protect cats by preincubating FIV in vitro with neutralizing antisera or by pre-immunizing with vaccines that elicited good levels of neutralizing antibody.

D4-405 LEISHMANIA: A POTENT CO-FACTOR IN THE PATHOGENESIS OF HIV INFECTION, Richard Bernier[1], Salvatore J. Turco[2], Martin Olivier[1] and Michel Tremblay[1], Unité de Rétrovirologie - Laboratoire d'Infectiologie, Centre de Recherche du CHUL, Département de Microbiologie, Université Laval, Québec, Canada, G1V 4G2[1] and Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536[2]

The most prominent clinical feature of the acquired immunodeficiency syndrome is the development of several opportunistic infections. There are some opportunistic protozoan parasites in HIV infection. One of them, *Leishmania*, will likely become of major importance in individuals infected with HIV because the distribution of both agents overlap in numerous parts of the world. Furthermore, they can infect and replicate within the same target cell, the macrophage. In this study, we demonstrate that both the protozoan parasite *Leishmania* and one of its major surface molecule, lipophosphoglycan (LPG), can induce HIV-1 expression in two cell lines of monocytoid origin latently infected with HIV-1 (OM-10.1 and U1). Indeed, we have found that the purified LPG of *Leishmania donovani* incubated with undifferentiated OM-10.1 and differentiated U1 cells can induce HIV-1 expression, whereas the protozoan parasite induces HIV-1 expression only in differentiated U1 cells. We observed that treatment of U1 cells with various concentrations of LPG (1, 5 and 10 μM) resulted in a dose-dependant secretion of TNF-α. These results were confirmed by showing that polyclonal anti-TNF-α antibodies can efficiently suppress the LPG-mediated induction of HIV-1 expression. We conclude that the major surface constituent of the protozoan parasite Leishmania (LPG) induces the secretion of TNF-α that will next function in an autocrine manner resulting in the induction of HIV-1 expression. Our results indicate that the protozoan parasite *Leishmania* can be viewed as a potential co-factor in the pathogenesis of AIDS.

D4-406 **Biological Characterization of F and B HIV-1 subtypes of recent seroconvertors from the São Paulo area. Brígido, L.F.M.; Sabino, E.; Oliveira,M.I.; Rossini,M.; Orii,N.; Duarte,A.J.S.**

Retrovirus laboratory, Virology Service , Adolfo Lutz Institute and Allergy & Immunology Service, Faculty of Medicine, USP, São Paulo, S P, Brasil

Three HIV-1 subtypes (B,C,F) have been detected, by different groups, in Brazil. Although the great majority of isolates can be classified as B, new subtypes may have been more recently introduced. This may be suggested in the WHO sampling of recent seroconvertors, where no F subtype were detected in previous samples (91, 92 and 93) ,whereas in the last evaluation, in 1993, 20% (4/20) isolates of recent seroconvertors could be classified as F subtype. Although these numbers are too small, its potential implications for vaccine strategies warrant further evaluation. We have used the WHO protocols to cultivate, study the biological characteristics and perform Hetero Duplex Mobility Assay in isolates from consecutive sampled patients with unknown time of seroconvertion (n=6). All were subtyped as B , most isolates grow before 2 weeks in primary cultures, and there was a good correlation for isolates host range and syncytia inducing with clinical staging and CD4 counts. For the recent seroconvertors analysed up to now (n=4), two B and two F subtypes have been detected. These isolates were all positive before two weeks in primary culture, and none grow in cell lines or induced syncytia. This may suggest either that they have a similar host range and pathogenic potential or that these methodology is not sensible enough to distinguish their " in vitro" behavior.

(Supported in part by Fogarty Foundation)

D4-407 **CELLULAR FACTORS ASSOCIATED WITH THE SPECIFICITY OF HIV-1 FUSION**

Christopher C. Broder, Ofer Nussbaum, Yu Feng, and Edward A. Berger, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, 20892 USA

We have employed a newly developed vaccinia-based *LacZ* reporter gene activation assay to analyze two aspects of cell-cell fusion specificity mediated by HIV-1 env/CD4 interaction: a) The requirement that CD4 be expressed on a human cell type. We have used the *LacZ* gene activation assay to confirm our previous finding that animal cells expressing human CD4 are rendered competent for fusion with env-expressing cells by formation of transient hybrids with human cells. This result suggests the presence of a human-specific accessory fusion factor(s). Our results do not support the proposal by another group that the human CD26 antigen plays a direct role in env/CD4-mediated fusion. b) The selective tropism of different HIV-1 isolates for T cell lines vs. primary macrophages. We have found that the tropism of different HIV-1 isolates for CD4+ cell lines vs. primary macrohages is associated with highly cell-type specific fusion activity of the corresonding vaccinia-encoded envs. Envs from both types of isolates mediate fusion with PBMCs, consistent with the ability of both types of isolates to infect these cells. The fusion specificities of the two classes of envs for distinct CD4+ partner cell types are maintained independent of the cell type used to express the envs. We are investigating the possible involvement of cell type-specific accessory fusion components in this cell type tropism.

Complementary approaches are being applied to identitfy accessory fusion factors, including membrane vesicle transfer strategies to analyze the biochemical properties of these components, as well as cell microinjection techniques to possibly detect relevant mRNAs.

D4-408 MECHANISMS OF INEFFICIENT INFECTABILITY OF CEM-CCRF CELLS, Robert W. Buckheit, Jr. and Carol Lackman-Smith, Virology Research Group, Southern Research Institute-Frederick Research Center, Frederick, MD 21701

A more complete understanding of the cellular factors which influence the infectability of HIV-1 in human cells will be useful in elucidating pathogenic features of infection and suggesting potential therapeutic strategies to interfere with the initial infection and spread of HIV in an infected individual. Two CEM cell lines have been studied which are highly divergent in their ability to be infected with HIV-1. CEM-SS cells become rapidly and efficiently infected with HIV-1 and die within one week of infection, exhibiting high levels of virus production and cytopathic effects. CEM-CCRF cells become infected much less efficiently with virus production delayed until three to four weeks post-infection and little detectable cytopathic effects observed. The burst of virus production observed at this time is similar to that seen in CEM-SS cells during the first week. Both cell lines become chronically infected and produce high levels of infectious virus for long periods of time. CEM-SS and CEM-CCRF cells express similar levels of cell surface CD4 and bind comparable amounts of infectious virus when infected at the same MOI. The mechanism of differential infectability appears to be a result of inefficient fusion and virus internalization in the CEM-CCRF population of cells or that the pool of infectable cells in the population is quite small. In order to further investigate these possibilities we have derived a large panel of single cell clones from the CEM-CCRF parent line and have examined HIV-1 infectability in twenty of these CEM-CCRF subpopulations. Among this group of clones, seven (35%) were able to support HIV replication with infection kinetics either similar to (2-4 weeks) or highly delayed (>10 weeks) relative to the parent cell line. Virus was detected by RT activity assay and by PCR amplification of proviral DNA. The remaining clones (65%) were cultured for at least 100 days and appear to be uninfectable by HIV. Further analysis of cell surface markers and other investigations into the total lack of infectability of 65% of the clones are in progress and will be presented.

D4-409 **GENETIC DETERMINANTS OF MACROPHAGE TROPISM IN AN HIV-1 PRIMARY ISOLATE ARE INDEPENDENT OF THE ENVELOPE V3 REGION.**
Ronald G. Collman, A. Srinivasan & Frances M. Kim. University of Pennsylvania School of Medicine & Thomas Jefferson University, Philadelphia, PA 19104

HIV-1 isolates vary in their ability to infect and replicate in macrophages, and several groups have mapped the genetic basis for macrophage (M)-tropism to regions of *env* that include the third hypervariable (V3) region. We recently described the isolation and molecular cloning of a primary isolate (89.6) which is highly M-tropic yet differs from other M-tropic strains studied in that it is cytopathic (syncytia-inducing; SI) in T cells. Sequence analysis of the V3 loop of strain 89.6 showed that it differs markedly from M-tropic non-syncytia-inducing (NSI) isolates and is more similar to the highly charged sequences of non-M-tropic SI strains. Genetic mapping of this virus was done using chimeras generated with the prototype non-M-tropic strain HXB2. Replacement of a 2.7 kb region of HXB that contains *env*, as well as the second exons of *tat* and *rev*, with corresponding sequences from 89.6 conferred the M-tropic phenotype, but insertion of the 89.6 V3 region along with V4/V5 sequences did not. Conversely, placement of HXB sequences that included V3 into 89.6 did not impair its ability to replicate in macrophages. Thus, in this naturally occurring isolate the V3 region is not a determinant of macrophage tropism and other regions of strain 89.6, likely within *env*, confer this property. This suggests that: (1) macrophage tropism either is defined by structural determinants resulting from complex interactions among multiple *env* regions rather than V3 sequence-specific requirements, or else there are multiple mechanisms by which different strains may establish productive macrophage infection; (2) factors other than M-tropism alone may be responsible for selection of the less charged "M-tropic consensus" V3 patterns described, and; (3) because the HXB V3 loop supports productive macrophage infection in the background of 89.6, phenotypic characterization of V3 sequences should be considered specific to the viral context in which they are placed.

D4-410 MATERNAL HIV RNA LEVELS ARE DIRECTLY RELATED TO PERINATAL TRANSMISSION RISK AND SIGNIFICANTLY REDUCED BY ZDV TREATMENT.
Ruth Dickover, Steve Herman, Eileen Garratty, Lorenz von Seidlein, Pamela Boyer and Yvonne Bryson, Depts of Pediatrics and Ob-Gyn UCLA School of Medicine, L.A. Ca 90024 and Roche Molecular Systems, Somerveille N.J. 08876.

Studies have shown that ZDV treatment during pregnancy, delivery and of the infant significantly reduce perinatal HIV transmission which could be related to reduction of maternal virus load and/or prophylaxis in the infant. We therefore, quantitated HIV levels using DNA and RNA (Roche) PCR in 87 infected mothers at delivery including 49 women followed during gestation (mean = 13 wks). Transmitters (n=20) had significantly higher HIV DNA (276±173 vs. 43±68 copies/μg PBMC DNA, $p<0.0001$) and RNA (198,806±192,003 vs. 8,858±9,510 copies/ml plasma, $p<0.0001$) levels at delivery and lower CD4 counts (417±236 vs. 633±305 / μl, $p = 0.01$) than non-transmitters (n=67). Women with plasma HIV levels > 50,000 copies/ml at delivery were more likely to transmit HIV than those with < 50,000 copies/ml (16/18=89% vs. 4/69=8%, $p<0.0001$). HIV levels in 20 non-transmitters treated with ZDV (mean =5.3 wks) showed a 3-17 fold reduction in RNA levels (mean = 6.4 fold) with a mean initial copy number of 50,060 reduced to 7,838 copies/ml plasma by delivery. Untreated non-transmitters (n=5) maintained low plasma HIV levels (338-13386) during gestation and at delivery (x=5486). In contrast, 8 of 10 transmitters showed 3-20 fold (x=8 fold) increase in HIV RNA levels late in gestation. These studies indicate that maternal viral load (as measured by quantitative PCR) is directly related to the risk of perinatal transmission and the mechanism of ZDV efficacy may relate to the observed reduction of maternal viral load during pregnancy.

D4-411 ACCELERATED DISEASE PROGRESSION PRODUCED BY FELINE IMMUNODEFICIENCY VIRUS INFECTION, Lauri J. Diehl, Candace Mathiason-DuBard, Lynne L. O'Neil, Leslie A. Obert, and Edward A. Hoover, Department of Pathology, Colorado State University, Fort Collins, CO 80523

Feline immunodeficiency virus (FIV) infection in cats results in a disease syndrome similar to that caused by HIV-1 in humans. Experimental FIV infection results in a transient acute phase flu-like illness followed by a prolonged asymptomatic period during which there is a progressive decline in CD4+ T cells and a terminal symptomatic phase. The similarities between FIV- and HIV-related disease make FIV infection of cats an interesting animal model of lentiviral immunodeficiency disease, however, the prolonged asymptomatic phase has hindered FIV studies. To overcome this limitation, we have developed an infection strategy which results in a rapid and consistent disease course and an approximate 60% mortality rate within 8 weeks PI. A high titer, cell-free plasma pool was prepared by serial plasma passage of FIV-C PGammer3213 virus from cats during the acute phase of infection and used to infect 8-, 12- and 16-week old cats. Infected cats developed lymphadenopathy, diarrhea, oral ulceration, and decreased growth rate by 3 to 4 weeks PI. The onset of clinical disease was coincident with or immediately followed peak plasma viremia which also occurred at 3 weeks. Decreased CD4+ cell numbers occurred by 2 weeks PI and became progressively more severe. All 8-week old and approximately 60% of 12- and 16-week old cats progressed to intractable disease characterized by anorexia, wasting, and intermittent diarrhea. Peak plasma virus titers in terminal animals were 1-2 logs higher than those of survivors. Severe thymic atrophy was observed in terminal cats, as was lymphoid depletion and increased macrophages in lymph nodes. In situ hybridization revealed viral RNA in all lymphoid organs examined. These studies indicate that accelerated FIV disease can be produced under experimental conditions and rate of virus replication and and clinical progression are directly related.

D4-412 CHARACTERIZATION OF dUTPase DEFICIENT FIVs, John H. Elder[1], Danica L. Lerner[1], Tom R. Phillips[2] and Pamela C. Wagaman[1], [1]Department of Molecular Biology; [2]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

FIV encodes the enzyme dUTPase (DU) within the *pol* gene. The enzyme is thought to serve as a regulator of host cell dUTP levels during reverse transcription, reducing the potentially mutagenic misincorporation of uracil into viral DNA. In addition, cleavage of dUTP by DU produces dUMP, the major substrate for dTTP synthesis.

A mutant of the FIV-PPR strain has been prepared that contains an in-frame insertion in DU (ΔDU-FIV). In vitro analyses of wild type and DU deficient FIVs indicate that ΔDU-FIV was able to grow with delayed kinetics on T lymphocytes, but failed to grow productively on primary macrophages. Wild type FIV-PPR grew well on both cell types. The results are consistent with the notion that the DU mutant virus can grow sufficiently where DU function can be "borrowed" from the host cell, but does not grow well in environments where endogenous levels are reduced.

In vivo tests of the DU mutant have been initiated, with the purpose of determining the relative time course of infection, tissue distribution, and host immune response to the virus, as compared to wild type FIV. After one year of infection, the results show the following: 1) All four animals infected with DU deficient FIV became viremic, with seroconversion occuring at approximately the same rate as with animals infected with wild type FIV; 2) The virus burden is reduced in the DU deficient animals, compared to wild type FIV, with prominent reduction of virus copy number in spleen and salivary gland; 3) Early in the course of infection, a substantial increase in mutation frequency was found in FIV sequences from macrophages isolated from animals infected with DU deficient FIV as compared to macrophages from animals infected with wild type FIV. Increased mutation rate of ΔDU-FIV over wild type FIV was also noted in non-adherent PBMCs. A predominant increase in A to G conversion was noted, consistent with incorporation of uracil during reverse transcription, with subsequent misincorporation of guanine during second strand synthesis. ΔDU-FIVs continue to infect and affect the CNS, in spite of attenuated growth in macrophages.

D4-413 PLACENTAL MACROPHAGES DISPLAY HIGHLY SELECTIVE SUSCEPTIBILITY FOR HIV-1 STRAINS

Warwick R. Fear, Hassan Naif, Anthony Cunningham and Alison Kesson, Department of Virology, CIDM, Westmead Hospital, Westmead NSW 2145, Australia.

Macrophages from human term placentae were isolated and cultured *in vitro* to investigate their susceptibility to HIV-1 infection and possible role in vertical transmission. After 10 days *in vitro* culture the cells displayed a macrophage phenotype, expressing non-specific esterase, acid phosphatase, HLA-DR, HLA-ABC, CD45, CD14 and intracellular CD68. The cells were negative for myeloperoxidase and placental alkaline phosphatase on day 1 of culture. CD4 was expressed at very low levels and was confirmed by flow cytometry antibody blocking studies. CD4 mRNA could be detected in the cells by RT PCR. Exposure of placental macrophage cultures to cell-free monocytotropic HIV-1 isolates $HIV_{Ba\text{-}L}$ and $HIV_{JR\text{-}FL}$, and two clinically derived tissue-macrophage-tropic isolates (m.o.i. = 0.1) resulted in productive infections first detectable by day 7 (p24 Ag ELISA). Progeny virion production continued to increase for at least 21 days. Kinetics studies of reverse transcription revealed minus strand cDNA at 8 hours post-infection and a complete cDNA transcript at 3 days, sometimes up to 7 days, post-infection. However inoculation of 10 blood-derived HIV-1 isolates resulted in rapid appearance of extracellular p24 antigen without concomitant synthesis of cDNA, suggesting that these were not true infections. By the same molecular and biological criteria some of these blood-derived isolates could be shown to productively infect freshly elutriated human monocytes. If confirmed, such data suggests differential growth in blood monocytes and tissue macrophages.

D4-414 A POTENTIAL ROLE FOR MITOCHONDRIA IN CELL-DEATH INDUCED BY CYTOPATHIC HIV-1 ISOLATES.

Serene E. Forte, S. Mohan, J.L. Sullivan, and M. Somasundaran, Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA. 01605

Our laboratory has been involved in understanding the mechanisms of HIV-1 induced cytopathicity and its role in $CD4^+$ T-cell depletion in vivo. We have recently demonstrated that high levels of viral RNA localized in mitochondria of $CD4^+$ T-cells acutely infected with cytopathic (CP) laboratory-adapted HIV-1 isolates (HTLV-IIIB, RF, NL4-3). Localization of viral RNA in mitochondria was associated with decreased mitochondrial viability and increased cell death. In contrast, viral RNA was not localized to the mitochondria of $CD4^+$ cells chronically infected with HIV-1. These data strongly suggest a potential role for mitochondria in HIV-1 induced cell death (Somasundaran, M., et al (1994) J. Cell Biol 126:1353). This novel mechanism may possibly be involved in depletion of $CD4^+$ T-cells in vivo. To that aim, we phenotyped viral isolates from HIV-1 infected individuals. Two of the six viral isolates were found to be cytopathic (CP) in acute infection of PHA-stimulated seronegative PBLs. The other four viral isolates were non-cytopathic (NCP). CP isolates were recovered from symptomatic HIV-1 infected individuals and NCP isolates were recovered from asymptomatic HIV-1 infected individuals. We further determined whether CP viral isolates affected mitochondrial viability. This was analyzed using a mitochondrial specific dye, rhodamine 123. By day 12, cells infected with a CP viral isolate showed a significant reduction in mitochondrial viability when compared to uninfected cells. Greater than 80% of the cells in culture were dead as determined by trypan blue staining. Similar analyses on $CD4^+$ cells infected with NCP viral isolates demonstrated that mitochondria were functionally viable and greater than 90% of the cells in culture were viable. Currently, we are isolating biological clones of the CP and NCP viral isolates. These clones will be used to determine the presence of viral RNA in mitochondria of cells infected with CP isolates. These studies may lead to a better understanding of the mechanisms of CD4 depletion in vivo and the design of novel anti-viral therapy.

D4-415 LOSS OF HIV-1 VIREMIA WITH PERSISTENT SPECIFIC IMMUNITY IN A WOMAN AND HER CHILD.

Lisa M Frenkel[1,3], Joan E Nichols[2,4], Larry E Wagner[1], Ashley T Haase[5], Norbert J Roberts, Jr[1,2,4], [1]Depts. of Peds and [2]Med, Univ. of Rochester, Roch, NY 14642; and [3]Dept. of Peds, Univ. of Washington, Seattle, WA 98195; [4]Depts. of Med and Microbiol and Immunol, Univ. of Texas Medical Branch, Galveston, TX 77555; [5]Dept. of Microbiol, Univ. of Minnesota, Minn, MN 55455.

We observed two individuals, a woman and her child, who were HIV culture-positive and subsequently became culture- and PCR-assay-negative. Peripheral blood mononuclear leukocytes (PBMCs) from the woman and child were culture-positive on 3/91 and 6/91, and on 3/90, 5/90 and 9/90, respectively. The *env* V3 DNA sequences varied 2% between the woman's isolates, 2-4% between the child's isolates and 2-4% between the woman's and child's isolates. The woman's isolates varied 10% and the child's isolates varied 8-10% from the North American consensus HIV-1 sequence. Subsequent PBMC cultures of the woman were negative on 7/91 and 1/92, and the child's cultures were negative on 7 occasions at regular intervals between 12/89 and 1/93, including cultures of CD8-depleted PBMCs. The woman's PBMCs were PCR-negative for HIV *gag* and *pol* on 7 occasions from 9/92 to 8/94, and the child's PBMCs were PCR-negative on 9 occasions from 2/92 to 2/94. Both the woman and child have had normal CD4 and CD8 values since 1989, have been healthy over the past 5 years, and have not had lymphadenopathy since 1992. In 1994, PBMCs from both the woman and child recognized HIV antigens in lymphocyte proliferation assays. Also, in 1994 both the woman's and child's PBMCs contained precursor cytotoxic T lymphocytes (CTL) that recognized HIV antigens, including *gag*, *pol*, and *env* products. In 9/94, lymph nodes from the woman were obtained and will be examined for HIV. Determinations of HIV epitopes recognized by the woman and child, and sequencing of their HIV-1 genome are continuing. CTL responses to HIV are believed to play a role in preventing progression to AIDS, and studies such as these may help identify important features of immunologic defense.

D4-416 LABORATORY MEASUREMENTS OF HIV SPECIFIC CELLULAR IMMUNITY DO NOT CORRELATE WITH VIRAEMIA IN CHILDREN.

Karin Froebel, Marian Aldhous, Mark Armitage, Myra Arnott*, Jacqueline Mok**. HIV Immunology Unit, University of Edinburgh Dept of Medicine; *Dept of Medical Microbiology, and **Infectious Diseases Unit, Edinburgh, UK.

Cytotoxic T lymphocyte (CTL) activity is believed to be a major means by which the cellular immune system suppresses HIV, and maintains clinical stability. We describe the laboratory and clinical findings of 2 children who were infected with HIV through verrtical transmission, one of whom has viraemia despite vigorous HIV specific cellular immunity, and the second of whom has undetectable level of virus, but no cellular immunuity. The first child is 78 months old, and has had HIV viraemia since the age of 24 months. In 1994, the CD4 count plummetted to 110 (9%), and virus levels rose further, with an SI strain, despite HIV specific cytotoxic T lymphocyte activity, and effective CD8 cell mediated, non-lytic suppression (NLS) of HIV. The second child is 109 months old. Since immunological monitoring began, 20 months ago, it has had undetectable plasma antigen levels, negative virus culture results, and has increased its CD4 count from 540 to 900 (28 -> 33%). CTL activity against HIV gag, tat pol and env has been negative throughout. Both children have been clinically well throughout. Both were infected by their mothers who belong to the Edinburgh IVDU cohort, and who are believed to have been infected with a similar strain of HIV (the childrens' viruses have not been sequenced).

These results present a conundrum for the clinician and for the immunologist. Either the laboratory measurement of CTL and NLS does not correspond to *in vivo* cellular immunity, or virus levels are not determined by these mechanisms. More work needs to be done to establish the laboratory assays that truly correspond to effective *in vivo* anti-HIV immunity.

D4-417 GENETIC VARIATION OF THE *env* GENE IN HIV-1 INFECTED CHILDREN, Shanthi Ganeshan, Ruth E. Dickover*, Yvonne J. Bryson*, and Steven M. Wolinsky, Department of Medicine, Northwestern University, Chicago, IL 60611, *- Department of Pediatrics, University of California, Los Angeles, CA 90024.

Perinatal human immunodeficiency type 1 (HIV-1) occurs in approximately 13-40% of infants born to HIV-1 infected mothers. Different rates of disease progression have been found among HIV-1 infected children. The objective of this study was to characterize and compare virologic and biologic differences between HIV-1 infected infants followed from birth, who exhibit different rates of disease progression.

Blood samples were obtained from 2 rapid and 2 slow progressors at 4 time points. Plasma free viral RNA and cell-associated DNA were quantified by PCR in these samples. To characterize the distribution of genetic variants, we amplified, cloned and sequenced 10-15 randomly selected clones from the V3-V5 region of the envelope gene. Viral sequences were edited using MASE and similarity matrices and phylogenetic analyses were performed. Preliminary data suggests that slow progressors exhibit lower proviral and viral burden, and greater genetic variation than rapid progressors over time. On an average, there seems to be 8-10% variation in the slow progressors whereas only a 3-6% variation is seen in the rapid progressors. The sequence data provides insights into HIV-1 pathogenesis in children and facilitates the development of new vaccine strategies.

D4-418 ANTIBODY REACTIVITY TO ELLELDKWASLWNC, A GP41 PEPTIDE, IN CHILDREN PERINATALLY INFECTED WITH HIV-1 WITH VARYING SURVIVAL TIMES. R. Geffin*, M. Melenwick*, A J. Conley¶, S. Lai§, C. Hutto*, and G. B. Scott*. *Department of Pediatrics and §Biostatistical Working Group Comprehensive AIDS Program, University of Miami School of Medicine, Miami, FL, and ¶Department of Antiviral Research, Merck Research Laboratories, West Point, PA.

Previous studies performed in our laboratory have shown that antibody reactivity to the viral proteins gp120, gp160 and p24 are correlated with survival of children perinatally infected with HIV-1. Even though antibodies to all three proteins were correlated with survival, antibodies to gp160 were the most significant. To investigate whether antibodies to specific regions in the gp41 molecule were associated with survival, a peptide, containing the sequence ELLELDKWASLWNC, located between aa 659-672 of HIV-1 BH-10 isolate, was used. This peptide may be important for viral infectivity because a monoclonal antibody to a smaller portion of it, ELDKWA, was able to neutralize divergent HIV-1 variants, as well as primary virus isolates. Sequential sera from 29 children were tested for antibody reactivity. Of these children, 14 were alive by the end of the study (median age= 75.5 months), and 15 had expired (median age= 39 months). A median number of 3 sequential samples/child were used. Antibody reactivity to the peptide was tested by ELISA, and specific reactivity to the peptide was calculated by subtracting the O.D. values obtained in wells not coated with the peptide from the O.D. obtained in corresponding wells coated with the peptide. By analyzing the antibody reactivity over time in this group of children, a significant association was found between the levels of antibody reactivity to the peptide, and CD4+ lymphocyte numbers, ICD p24 antigen, and disease status. Early virus isolates from 6 of the 12 children that had no detectable antibodies to the peptide were sequenced in the gp41 region corresponding to aa 627 and 675. Of these 6 children, 3 had identical sequences as the peptide used in the ELISA, indicating that these children have not developed antibodies to this particular epitope. The remaining three children had virus quasi-species that contained changes in the epitope, and thus, their antibody reactivity to that region is not known.

D4-419 SELECTIVE INFECTION BY SIVmac239 *in vitro* AND INTERFERON-MEDIATED SUPPRESSION OF VIRAL REPLICATION IN RHESUS MONKEY TROPHOBLASTS, Thaddeus G. Golos, Kimberly J. Johnson, Lisa A. Krugner-Higby, Maureen Durning and Kevin T. Schultz[1]. Wisconsin Regional Primate Research Center and [1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison WI, 53715-1299

The prenatal transmission of primate lentiviruses to the fetus during intrauterine development is poorly understood, but prepartum maternal/fetal transmission is likely to involve infection of the placenta as a primary step. We compared the infection of two components of the maternal-fetal interface, primary cultures of rhesus monkey syncytiotrophoblasts (STB), and extravillous cytotrophoblast (EVTB) cell lines developed in our lab. In general, SIVmac251 and SIVmac239 appear to replicate equally well in primary syncytiotrophoblast cultures. However, SIVmac239 was consistently (at least 5-fold) more infectious for EVTB than SIVmac251, in spite of a higher TCID on CEMx174 cells for the SIVmac251 stock. RT-PCR demonstrated that the EVTB lines do not have CD4 mRNA, suggesting the route of infection in EVTBs is CD4-independent. SIV replication in STB.was potently suppressed by interferons α or γ, but a number of other cytokines were without effect. Interferons did not influence replication in EVTB.

The inefficient transfer across the placenta may mean that there are viral determinants which influence placental infection and subsequently transmission to the fetus. We have begun to examine the specific viral variants infecting placental cultures *in vitro*, to determine whether viral clones with enhanced tropism for placental infection *in vivo* can be found. We have PCR-amplified cDNAs for the V1-V3 region of the *env* gene from SIVmac251- and SIVmac239-infected cultures of primary STB, EVTB, and CEMx174 cells. While significant differences were seen between the SIVmac239- and SIVmac251-derived clones, only one minor difference between trophoblasts and CEMx174 cells was seen: a D to P substitution at residue 258, in SIVmac251-infected STB. The variation seen *in vitro*, in general, resembles that seen *in vivo*, since substitutions and deletions were predominantly clustered in the V1 and to a lesser extent, the V2 region. By contrast, the V3 region is highly conserved in CEMx174 and STB-derived clones. Additional differences may be identified in other elements of the *env* gene or the LTR, both under further study.

D4-420 ANALYSIS OF HIV-1 V3 ENV SEQUENCES PROVIDES EVIDENCE FOR CASES OF BOTH *IN UTERO* AND PERIPARTUM TRANSMISSION IN KENYAN MOTHER-INFANT PAIRS. Anton Scott Goustin, Viera Zacharova, Vladimír Zachar, Charanjit S. Bambra, Aggrey Nyongo, Henrik Hager, Uffe Koppelhus and Peter Ebbesen. Wayne State University, Detroit, MI 48220 USA; Academy of Sciences, Bratislava, Slovak Republic; Kenyatta National Hospital and Institute for Primate Research, Nairobi, Kenya; Danish Cancer Society, Aarhus, Denmark.

The human immunodeficiency virus type 1 (HIV-1) is transmitted from seropositive mothers to their infants at a rate that varies between 11 and 60%. The timing and route of transmission is poorly understood, with studies supporting both in utero transmission and peripartum transmission. We have used reverse transcriptase-polymerase chain reaction (RT/ PCR) to amplify HIV-1 viral RNA from maternal and cord blood plasma taken from mother-infant pairs admitted to Pumwani Maternity Hospital in Nairobi, Kenya. Nested PCR with primers JA9/JA12 (stage 1) and JA10/JA53 (stage 2) followed by cloning of ~325 bp PCR products in pGEM3Z generated 360 useful clones of which 240 have been fully sequenced using automated Taq cycle sequencing on the ABI Model 370A instrument. Four mother-infant pairs have been fully analyzed. In three of these cases, the data demonstrate a typical pattern that has been described by others (Scarlatti *et al., Proc. Natl. Acad. Sci. USA* 90, 1721 (1993)) in which the maternal and neonatal *env* sequences are quite similar, marked by a narrowing of quasispecies diversity in the neonate compared to the mother. This pattern is suggestive of a recent transmission event, near the time of delivery. In the fourth pair (M689/C689), neonatal quasispecies diversity exceeded maternal quasispecies diversity, suggesting a period of time lapsed between the vertical transmission event and sampling (at delivery). Only 1 out of 20 cord-derived *env* sequences in pair 689 (C689-12) resembled the consensus maternal sequence, suggesting that this fetal quasispecies derived from the mother prior to delivery, perhaps representing a viral species outnumbered by a second quasispecies evolving in the fetus during gestation. We have recently quantitated interferon (IFN) in maternal and cord blood, and detected its presence in placental trophoblasts from this pool of patients (Ebbesen *et al., J. Interferon Res.*14, in press (February 1995)); these four mother-infant pairs represent cases in which IFN levels were low. In addition, we have also identified five HIV-1-seropositive mothers from Nairobi in which IFN levels were elevated; DNA sequence from these pairs is underway and will also be presented. Research supported by grants from the NIH (HD27444) and Danish Cancer Society.

D4-421 VIRUS LOAD IN TISSUES OF FELINE IMMUNODEFICIENCY VIRUS INFECTED CATS, Kathleen A. Hayes and Lawrence E. Mathes, Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210

The focus of this study was to determine the distribution and amount of virus throughout tissues of experimentally FIV-infected cats. The study included 3 control cats and 8 cats infected with the Mount Airy, Maryland isolate of FIV (FIV-MD). Nucleic acids were extracted from mesenteric lymph node, spleen, thymus, bone marrow and brain which were snap frozen at necropsy. We performed a standard nested PCR for FIV using tissue homogenates as the source of DNA template. Reactions for the PCR were standardized to contain equivalent amounts of total DNA with input amounts of 500 ng and 100 ng/reaction, respectively. At 500 ng/reaction, PCR products were detected after amplification and Southern blot analysis in lymph node of 8/8, in thymus of 8/8, in spleen of 7/8, in bone marrow of 3/3 and in brain of 5/8 FIV-infected cats. At 100 ng/reaction, amplified products were detectable in lymph node of 1/8, in thymus of 6/8, in spleen of 3/7, in bone marrow of 2/3 and in brain of 1/8 FIV-infected cats. These results suggested that the greatest amount of viral DNA is within the thymic tissue followed possibly by bone marrow. Our current work involves the localization of virus in the tissues of these cats using in situ PCR and PCR in situ hybridization techniques. Preliminary results showed virus present at a high level in the choroid plexus. Infected cells within lymph node were localized mainly within cortical nodules and paracortical regions. This work will facilitate the evaluation of virus load as an endpoint for determining the effects of antiviral therapy.

D4-422 HIV-1 SUBTYPES IN POTENTIAL VACCINE TRIAL SITES OF THE WHO Belinda L. Herring[1], Chantapong Wasi[2], Eric L. Delwart[3], James I. Mullins[1], Saladin Osmanov and the WHO Network for HIV Isolation and Characterisation[4], [1]Department of Microbiology, University of Washington, Seattle, [2]Department of Microbiology, Mahidol University, Bangkok, [3]Aaron Diamond AIDS Research Institute, New York University, New York,[4]Vaccine Development Unit, Global Programme on AIDS, World Health Organisation, Geneva.

Geographic clustering of HIV-1 subtypes has become apparent as greater numbers of viruses are analysed throughout the world. As part of the *WHO vaccine development and evaluation strategy, a collaborative study* under the auspices of the WHO Global AIDS program was initiated to isolate and characterize HIV-1 strains from geographical regions seen as possible vaccine trial sites. Isolates from 5 countries: Brazil (35), Thailand (125), Rwanda (15), Burundi (7) and Uganda (16), were analyzed. Envelope sequence subtypes were determined using the heteroduplex mobility assay (HMA) after amplification with envelope specific primers. Amplified unknown samples were mixed with fragments generated from known subtype references and their mobility through acrylamide observed. Fast moving heteroduplexes with a subtype reference indicated classification of the unknown to that subtype. A total of 35 samples from Brazil were analysed, 83% (29) were B , 6% (2) were C and 11% (4) were F. 7 samples were analyzed from Burundi, 6 were C subtype and 1 was a D subtype. From Uganda 16 samples were analyzed and both A and D subtypes were found to be present; 7 were A subtype (44%) and 8 were D subtype (50%). One sample did not fall within the known subtypes. All 15 samples analyzed from Rwanda were subtype A. The final group of samples from Thailand can be divided into two: firstly 100 samples from IV drug users in the Bangkok metropolitan area were analysed. In this group, 2 samples were HIV-1 antibody negative, 2 additional samples were PCR negative and the remainder were either subtype B (77/96, 80%) or E subtype (19/96, 20%). A second group of 27 samples, where most infections were acquired through heterosexual contact, were analysed. In this group the prevalence of E subtype was higher (20, 74% versus 7, 26% of B subtype) compared to the IVDU group. When stratified by time of infection the prevelance of subtype E amongst the IVDU group has increased over time from 3% in 1989 to 43% in 1992-93. Subtype determinations using HMA correlated 100% with determinations made by serology and sequence analysis of the V3 loop region (for the Thai IVDU group). Therefore it has proved to be both a specific and sensitive method for following patterns of virus spread.

D4-423 FUNCTIONAL ANALYSIS OF NEF ALLELES DERIVED FROM LONG-TERM SURVIVORS OF HIV-1 INFECTION. Yaoxing Huang, Linqi Zhang, and David D. Ho. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

Studies have shown that infection of rhesus macaques with a nef deleted SIV results in a low level of viremia and lack of disease progression. Given the similarity of this clinical profile to that observed in long-term survivors (LTS) of HIV-1 infection, we sought to examine the nef alleles in 10 patients who are clinically healthy and immunologically normal despite more than 12 years of infection. We have previously shown that there is no gross deletion nor sequence abnormality within nef in the 10 cases studied, although a substantial interpatient sequence diversity has been observed. To functionally access these nef alleles, we have developed a new technique termed site-directed gene replacement. Using this method, we have precisely replaced the nef gene of an infectious molecular clone of HIV-1 (HXB2) with the nef alleles derived from LTS. Growth properties of these chimeric viruses have been assayed, demonstrating that the nef alleles derived from LTS do enhance viral replication as does the wild-type HIV-1 nef. This suggests that sequence and functional abnormalities within nef are not likely to be a common explanation for the non-progression in the LTS of HIV-1 infection.

D4-424 GENETIC AND PHYLOGENETIC ANALYSIS OF HIV TYPE 1 IN GABON, Wouter Janssens, Leo Heyndrickx, Katrien Fransen, Eric Delaporte, Martine Peeters, Jean-Luc Perret, Crepin Atende, Peter Piot and Guido van der Groen. Department of Infection and Immunity, Institute of Tropical Medicine, Antwerp, Belgium.

Objective
To examine the genetic variation of HIV-1 isolates in Gabon.

Design
Phylogenetic comparison of 17 HIV-1 strains isolated from patients in Gabon with previously docum
ented HIV-1 strains of different geographic origin.

Methods
To sequence a 900 base-pair fragment of the *env* gene containing V3, V4, V5 and the beginning of gp41. Phylogenetic analysis was done with the software package TREECON.

Results
Gabonese HIV-1 strains were classified in 5 group M subtypes: A (n = 7), C (n = 2), D (n = 2), F (n = 1) and G (n = 4), and in group O (n = 1). Intra-genotype genetic distances for the Gabonese strains were on average 10.1 % (A), 7.1 % (C), 8.9 % (D), 10.1 % (G). The pattern of V3 loop octameric sequences was for group M subtype A: HIGPGQF (n = 4), RIGPGQTF (n = 2), RIGIGRGQVF; subtype C: RIGPGQTF, RIGPGQAF; subtype D: PIGLGQAL, HIGPGQAL; subtype F: RIGPGRVI; subtype G: KFGTGRVL (n = 2), HIGPGQAL, RIGPGQTF; group O: KIGPMAW.

Conclusion
These findings on a limited number of HIV-1 isolates suggest extensive *env* gene diversity of HIV-1 strains in Gabon.

D4-425 EVOLUTIONARY CHANGES AND DISTRIBUTION OF HIV-1 SUBTYPES FROM INJECTING DRUG USERS IN BANGKOK, THAILAND. M.L. Kalish[1], C-C Luo[1], A. Baldwin[1], G. Schochetman[1], T.D. Mastro[2], C. Wasi[3], N. Young[2], S. Vanichseni[4], H. Rubsamen-Waigmann[5], H. Von Briesen[5], J.I. Mullins[6], E. Delwart[6], B. Herring[6], J. Esparza[7], W.L. Heyward[7], and S. Osmanov[7]. [1]The Centers for Disease Control and Prevention, Atlanta, Georgia, USA. [2]The HIV/AIDS Collaboration, Nonthaburi, Thailand. [3]Siriraj Hospital, Bangkok, Thailand. [4]Bangkok Metropolitian Administration, Bangkok, Thailand. [5]Georg-Speyer-Haus Chemotherapeutisches Forschungsinstitut, Frankfurt on Main Germany. [6]Standford University School of Medicine, Standford, California, USA. [7]Global Programme on AIDS, World Health Organization, Geneva, Switerland.

We describe the genetic diversity in and distribution of HIV-1 envelope subtypes from 84 injecting drug users (IDUs) in Bangkok, Thailand - a potenital study population for HIV-1 vaccine efficacy trials. Direct sequencing of the C2-V3 *env* gene yielded 345 nucleotides for phylogenetic analysis using a neighbor-joining algorithm. One (1%) of the 84 specimens were "typical" subtype B viruses, 69 (82%) were genetically distinct subtype B' viruses and 14 (16%) were subtype E strains. Persons infected for >4 years were almost exclusively (97%) infected with subtype B' viruses. In contrast, only 9 (56%) of 16 persons infected for <2 years had subtype B' viruses; the other 7 (44%) were infected with subtype E viruses. Pair-wise intra-subtype differences increased within subtypes E and B' from an average of 3.5% in 1991 to 5.3% and 4.3%, respectively, in 1994. These results indicate that the genetic diversity of the HIV strains among Bangkok IDUs is increasing and that subtype E is accounting for an increasing proportion of new infections. This study demonstrates the importance of ongoing monitoring of HIV-1 strains in a population being considered for vaccine trials.

D4-426 ANALYSIS OF FACTORS GENERATING VIRAL HETEROGENEITY IN PATIENTS INFECTED WITH HIV-1 A.H.Kaplan, J.S.Sinsheimer, Y.L.Yang, W.Lech, H.Ly, Y.Chee, G.Wang, M.Wang, L.Patrone, K.Dorman, and D.McCrae. Departments of Medicine, Microbiology & Immunology, and Biomathematics, UCLA School of Medicine, Los Angeles, CA Infection with HIV-1 is characterized by the generation of a population of virus with considerable genetic heterogeneity. The ability of this virus to produce viral variants which are capable of evading immune surveillance, resisting anti-retroviral drugs, and demonstrating tropism for a broad array of cell types undoubtedly plays an important role in viral pathogenesis. The observed heterogeneity results from the interplay of three different factors. First, the error-prone viral RT introduces approximately three errors/round of reverse transcription; the variability is proportional to the number of rounds of replication. Second, immune surveillance generates heterogeneity by providing a selective advantage to escape variants. Third, selection for viral replication favors those variants which are able to meet the requirements of the viral life cycle most efficiently. In contrast to the two other mechanisms, this factor decreases variability. We have used three approaches to analyze the contributions of each of these forces. First, we have sequenced the coding domains of p6, the protease, and the V4 region of envelope from 14 patients. Quantitative DNA PCR was used to measure viral burden. By comparing variability in these patients with viral burden, we can estimate the impact of viral replication on heterogeneity. Second, we have analyzed the published HIV sequences from patients for which CD4 cell counts are available. We are evaluating the ratio of synonymous to non-synonymous nucleotide substitutions at various stages of disease. We predict that late in disease as immune surveillance declines, more of the variability will represent RT errors. Finally, we have evaluated sequence heterogeneity in an HIV-1 infected person with Bruton's agammaglobulinemia. We have found that, in the absence of antibody selection, the viral DNA recovered from this person was almost completely homogeneous.

D4-427 THE UNIQUE NATURE OF *NEF* QUASISPECIES FROM A LONG-TERM SURVIVOR OF HIV INFECTION: IMPLICATIONS FOR FUNCTION/COMPARISON WITH OTHER ISOLATES

H.W. Kestler, D. Premkumar*, M. Hirsch+, Xue-Zhong Ma, J.R. Salkowitz, and B.K. Chakrabarti; Department of Molecular Biology, The Cleveland Clinic Foundation, Cleveland, OH 44195; *Case Western Reserve University, Cleveland, OH 44106; +Massachusetts General Hospital, Boston, MA 02114.

Forty years ago a vaccine strategy to combat poliovirus was developed based on the discovery that some people escaped disease due to the fact that they harbored attenuated virus. Today we employ a live virus vaccine that contains a few point mutations in the gene encoding the surface protein of the virus. Thus, a dramatic decrease in the pathogenic potential of virus is observed with genetic changes that appear to be subtle. A proportion of people infected with Human Immunodeficiency Virus (HIV) remain asymptomatic despite having been infected for long periods of time. We are studying such subjects in search of natural attenuated HIV.

We have focused on the *nef* gene of HIV to look for evidence of attenuated virus. The *nef* gene was shown to be required for induction of AIDS. Simian Immunodeficiency Virus (SIV) deleted in *nef*, while infectious, fails to sustain high viral loads necessary for the induction of AIDS in infected Rhesus monkeys. Our initial focus on these subjects has been on the *nef* gene since it is known to be involved with pathogenesis. One subject was found to harbor open *nef* reading frames containing a short in-frame deletion. Interestingly, the subject's *nef* genes bore a signature point mutation: a cysteine at amino acid 139 was found in all the quasispecies. This sequence was compared to the sequence data base for AIDS and human retroviruses at Los Alamos. Several isolates from asymptomatic individuals appeared to be closely related to the *nef* quasispecies of this individual. Furthermore, the extra cysteine was found in CIV, the chimpanzee lentivirus that is very similar to HIV. Chimpanzees do not develop AIDS. We also observed the presence of multiple cysteines in the *nef* gene of African green monkey virus, SIVagm. The seroprevalence of SIVagm is very high in the wild and there is no known disease associated with this virus. The pathogenic virus isolated from Asian macaques, SIVmac, encodes a *nef* that has very few cysteines. We are expanding our observations to other long-term healthy survivors of HIV and we are investigating the structure of *nef* containing different numbers of cysteines.

D4-428 **Genetic Selection of the Human Immunodeficiency-1 Virus in Mother to Child Transmission**

Srisakul C. Kliks[1], Christopher H. Contag[2], Heather Corliss[1], Diane D. Wara[3] and Jay A. Levy[1].

Cancer Research Institute[1], Department of Pediatrics[3], School of Medicine, University of California, San Francisco, CA 94143[1,3] Microbiology Department, School of Medicine, Stanford University, CA 94305[2]

Previous study on vertical transmission of HIV-1 in San Francisco Bay area comparing paired viral isolates from 6 transmitting mothers and their infants and isolates from 12 non-transmitting mothers indicated that efficiently replicative virus that was resistant to maternal serum-neutralization was selective for the transmission. The selection appeared to have occured prior to or near the time of birth as indicated by the similarity or difference respectively in the viral susceptibility to maternal serum-neutralization in each mother/infant pair.

In the present study, we further assessed the genetic characteristics of the viral isolates from this cohort. Based on the heteroduplex mobility analysis of the C2-V5 viral *env* region, the non-transmitting viruses appeared to be more heterogenous than those of the transmitting mothers and the infants. In addition, although the signature V3 sequences of the viruses from all the mothers were distinct from one another, the sequences of non-transmitters showed divergence from those of the transmitters. In comparing the V3 sequences (8 clones per isolate) between the paired mothers and infants, common V3 clones were found in both mother and infant among pairs with concordance in the serum-neutralization while none was found in the discordant pairs. The findings suggest immunological impact on genetic selection of the transmitting virus.

D4-429 **NTera-2 HUMAN NEURONAL CELLS AS A MODEL FOR HIV-1-INDUCED NEUROTOXICITY.** Dennis L. Kolson, Carlos E. Llanes, Frances M. Kim, and Ronald G. Collman. Departments of Neurology and Medicine, University of Pennsylvania School of Medicine. Philadelphia, PA 19104

The NTera-2 human cell line is a neuronally-committed teratocarcinoma derivative which, upon exposure to retinoic acid, differentiates into a post-mitotic neuronal phenotype with features of mature neurons including neurofilaments, microtubule-associated proteins 1&2 (dendrites), GAP-43 (*axons*), and glutamate receptors. NTera-2 cells can be grown in large numbers as >95% pure neurons, and we are utilizing them to study direct and indirect effects of HIV-1 on neuronal function and survival that may be relevant to the pathogenesis of the AIDS Dementia Complex (ADC). First, we determined the susceptibility of NTera-2 cells to infection with several HIV-1 isolates. Both undifferentiated and differentiated cells can be infected with the T lymphocyte-tropic strain IIIB, resulting in highly restricted non-productive infection. In contrast, NTera-2 cells could not be infected by macrophage-tropic strains 89.6 & SF162. Secondly, we have developed a multi-cell co-culture model in which NTera-2 cells are maintained in mixed cell culture with primary human peripheral blood monocyte-derived macrophages (MDM) and primary astrocytes derived from rat brain. In this mixed cell model the MDM remain susceptible to productive infection with macrophage-tropic strains of HIV-1, and differentiated human neurons can be maintained in contact with infected macrophages and astrocytes. Such a three-cell culture system mimics cell-cell interactions within the brain that are believed to be critical to development of neuronal dysfunction in ADC. Finally, to address neuronal function we have developed methods to examine one of the critical features of neurons, neurotransmitter enzyme activity. NTera-2 cells express multiple molecular forms of the neurotransmitter enzyme acetylcholinesterase (AChE) that resemble those found in developing CNS neurons. One of these AChE forms is released by NTera-2 cells into the medium as occurs in neurons within the CNS in vivo. This novel feature of NTera-2 cells may provide a useful marker for functional integrity of neuronal cells, as well as an indicator of neuronal viability. Thus, the NTera-2 neuronal cell line and our multi-component cell co-culture system will be a valuable model with which to examine functional and toxic effects resulting from HIV-1 infection of macrophage cells and direct or indirect mechanisms involving neurons and other glial cells.

D4-430 IDENTIFICATION OF TWO NEW HIV-1 SUBTYPES: GENETIC AND PHYLOGENETIC ANALYSIS OF HIV-1 ISOLATES FROM PATIENTS IN CYPRUS. Leondios G. Kostrikis[1], Evis Bagdades[2], Yunzhen Cao[1], Lin Qi Zhang[1], Dimitrios Dimitriou[2], and David D. Ho[1], The Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, N Y, U.S.A[1]; The AIDS Clinic, Nicosia General Hospital, Nicosia, Cyprus[2]

DNA sequences encoding the C2-C4 envelope glycoprotein gp120 of human immunodeficiency virus type 1 (HIV-1) were amplified by polymerase chain reactions (PCR) from uncultured peripheral blood mononuclear cells obtained from twenty five HIV-1 seropositive patients from Cyprus. Using heteroduplex mobility assay (HMA), all amplified products were studied genetically and compared to sixteen previously characterized HIV-1 strains belonging to subtypes A through F. HMA results revealed that HIV-1 gp120 sequences from sixteen of our patients were of subtype B of HIV-1, whereas one isolate was subtype C. However, gp120 sequences from eight patients had no obvious similarities to the known subtypes. DNA sequencing and phylogenetic analyses confirmed the results by HMA and placed the eight undefined HIV-1 isolates into two distinct groups, equidistant from all other known subtypes within group M of HIV-1 (A through H). Further characterization of viruses from these new subtypes is now in progress.

D4-431 **LTR- AND CELL TYPE-SPECIFIC ACTIVITY RESULTING FROM IN VIVO DIVERSITY OF HIV-1 LTR CIS-ACTING REGULATORY SEQUENCES.** **Fred C. Krebs[1], Maureen M. Goodenow[2], and Brian Wigdahl[1], [1]Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA 17033, and [2]Department of Pathology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL 32610**

The development of human immunodeficiency virus type 1 (HIV-1) genomic heterogeneity within infected individuals during the course of the infection generates HIV-1 quasispecies with distinct but related genomic sequences. Nucleotide substitutions, deletions, and insertions in the long terminal repeat (LTR) can create, alter, or destroy transcription factor binding sites and potentially affect gene expression in a cell type-specific manner by altering binding sites for trans-acting factors specific to a variety of cells susceptible to HIV-1 infection. We have initiated investigations into the relationship between HIV-1 LTR diversity and the regulation of viral gene expression in cells of lymphoid and nervous system origin previously identified as targets for HIV-1 infection. Peripheral blood samples were obtained from two families in which both the mothers and three children in each family were infected with HIV-1. LTR sequences were amplified from each sample, cloned into chloramphenicol acetyltransferase (CAT) vectors, and sequenced. Transient expression analyses demonstrated that the PCR-amplified LTRs had activities which varied significantly above the basal activity of the LAI LTR in U-373 MG cells (an astrocytoma cell line) and in Jurkat cells (a CD4-positive lymphocytic cell line). Significant differences in activity were found among LTRs isolated from different individuals, as well as between LTRs isolated from the same individual. While LTRs which demonstrated the highest activities in U-373 MG cells also yielded high activities in Jurkat cells, the LTRs were generally more active in Jurkat cells when compared to the LAI LTR. To investigate the relevance of base changes within the cloned LTRs to their activities during transient CAT expression, electrophoretic mobility shift assays were performed. Using a probe specific to a single base change within a high activity cloned LTR, we have demonstrated cell type-specific differences in DNA-protein complex formation using U-373 MG and Jurkat cell nuclear extracts. While these results do not yet support a single base change as the cause of divergent LTR activities, they suggest the possibility that LTR sequence variation and the cell type in which the LTR is functioning may have significant impact on LTR function.

D4-432 SPECIFIC MUTATIONS IN ENV AND VPR, BUT NOT IN VPU, DISTINGUISH VIRUSES CIRCULATING AMONG IV DRUG USERS AND HOMOSEXUAL MEN Carla Kuiken, Marion Cornelissen, Jaap Goudsmit, Human Retrovirus Laboratory of the University of Amsterdam

Studies of the evolution of the 290-basepair V3 region in 138 participants in the Amsterdam Cohorts have shown that highly consistent risk group-related distinctions exist in this region. V3 sequences from 12 hemophiliacs had characteristics of those found in the homosexual population, which would be consistent with an epidemiological origin. The same distinctions were also found in sequences from other European countries, as well in some, but not all, sequence sets from the United States. To further investigate the nature of this distinction, two other genes, vpr and vpu, were sequenced from 33 individuals (16 homosexuals, 17 drug users). Results showed that the distinction is not limited to the V3 region. Both vpr and vpu showed a number of mutations that were risk group-associated with varying consistency. In vpr, three mutations were found to be significantly associated with risk group; these were spread evenly over the gene. In contrast, the mutations that were found in vpu were all located in the 3' end of the gene, which also encodes the envelope protein. The uneven spread may indicate that the pattern of risk group related mutations in the HIV-1 genome is not random (as might be expected when it would result from a chance outgrowth of a particular variant) but rather that the differences are conserved in some genes but not in others, which in turn is suggestive of a functional difference. The fact that both vpr and env play a role in determining the capacity of the virus to infect macrophages, suggests that the mutations may give the virus some form of adaptive advantage in the different environments.

D4-433 LONG-TERM NON-PROGRESSION ASSOCIATED WITH A DISTINCT PATTERN OF HIV-1 GP120 SEQUENCE DIVERGENCE, T. H. Lee[1], W. K. Wang[1], M. F. McLane[1], K. Mayer[3], G. Seage[4], C.C. Hsieh[2], and M. Essex[1]; Departments of Cancer Biology[1] and Epidemiology[2], Harvard School of Public Health, Boston, MA 02115; [3]Infectious Disease Division, Brown University AIDS Program, Memorial Hospital of Rhode Island, Pawtucket, RI; and [4]Department of Epidemiology and Biostatistics, Boston University School of Public Health, Boston, MA

gp120 sequence divergence over time is characterized by excessive non-synonymous to synonymous nucleotide substitutions, suggesting that divergence is influenced by selection pressures. Few long-term HIV-1-positive individuals (infected for more than 8-10 years) maintain normal levels of CD4-positive cells and remain clinically asymptomatic. We hypothesize that selection pressures in such long-term non-progressors (LTNP) are qualitatively different from those in progressors, and predict a distinct pattern of intrasubject gp120 sequence divergence in LTNP. We compared patterns of gp120 sequence divergence among homosexual men followed in a community health center in Boston since 1985. Subjects included 2 LTNP and 6 progressor controls. Sequence divergence of viruses isolated from progressors corresponded with the currently accepted gp120 model in that relatively more changes were observed in variable regions than in constant regions. In contrast, viruses isolated from LTNP had disproportionately more changes in constant regions than those from progressors. These results support our hypothesis and suggest that anti-viral strategies resulting in strong selection pressures on constant regions of gp120 can be more beneficial than those targeting variable regions.

D4-434 **LOW LEVELS OF SURFACE CD4 ARE CRITICAL FOR HIV-1 *IN VITRO* INFECTION OF ALVEOLAR MACROPHAGES.** Sharon R Lewin[1]*+, Secondo Sonza[1], Lou Irving[2], Christine F McDonald[2], John Mills[1] and Suzanne M Crowe[1]. National Centre for HIV Virology Research, Macfarlane Burnet Centre for Medical Research, Yarra Bend Rd., Fairfield, Victoria, Australia 3078[1], Dept Respiratory Medicine, Heidelberg Repatriation Hospital, Heidelberg, Victoria, Australia[2].

The CD4 glycoprotein is the major cellular receptor for HIV. It has been reported by us and other groups that CD4 surface expression of monocytes decreases with time in culture while their susceptibility to HIV-1 increases . Our aim was to investigate whether this phenomenon occurs in macrophages that have differentiated *in vivo* by investigating CD4 expression and HIV-1 infection of alveolar macrophages (AM).

Using fluorescent activated cell sorter (FACS) analysis of CD4 expression by anti-Leu-3a labelled directly or indirectly with fluorescein isothyocyanate (FITC) or allophycocyanin (APC), we found that CD4 was expressed at low but detectable levels, despite the very high background autofluorescence well described in AM. This finding was supported by the detection of CD4 mRNA in AM using RT-PCR. T cell contamination of mRNA extracts of AM was excluded by amplifying in parallel with primers to the constant region of the T cell receptor.

Despite this low level of surface CD4, AM can be infected with the macrophage tropic HIV-1 strain, Ba-L. Infection of AM was documented by rising reverse transcriptase concentrations in culture fluid over time and *de novo* appearance of HIV cDNA (*gag* sequences detected after PCR amplification). On the other hand, monocytes, on the day of isolation, although expressing high levels of CD4 were significantly less permissive to HIV-1 infection. In separate experiments, using recombinant soluble CD4 or anti CD4 antibody, HIV infection of AM was completely inhibited.

We conclude that differentiation of monocytes into macrophages *in vivo* appears to be necessary for successful HIV-1*in vitro* infection of cells of the monocyte/macrophage lineage. CD4, although expressed at exremely low levels on the surface of these cells appears to be critical to viral entry.

+SRL is a recipient of an NH&MRC postgraduate medical scholarship

D4-435 COMPARISON OF TWO METHODS FOR THE DETECTION OF SYNCYTIUM-INDUCING HIV STRAINS.

Corinne Liesnard*, Marie-Luce Delforge, Michel Tchetcheroff, Joëlle Eykmans, Claire-Michèle Farber, Jean-Paul Van Vooren.
AIDS Reference Laboratory - Erasme Hospital-Université Libre de Bruxelles-Brussels-Belgium.

Several reports have suggested an association between the presence of syncytium-inducing (SI) phenotype and a more advanced clinical stage and/or a more rapid progression to AIDS. For determination of the SI phenotype some authors used direct cocultivation of patient peripheral blood mononuclear cells (PBMC) with the MT-2 cell line, others inoculated MT-2 cells with the supernatant obtained from the coculture of patient PBMC with healthy donors PBMC.

We have compared these two methods on 279 heparinized blood samples from 88 HIV infected patients followed for a 13 months period. One hundred and twenty seven (45.5%) isolates were found NSI and 107(38.4%) were found SI with both methods. Twelve (4.3%) were SI with the direct coculture method alone and 33(11.5%) were SI with the supernatant method alone, giving a sensitivity of 78% and 92% respectively.The median times to syncytium detection were comparable : 17 days in the direct coculture method and 19 days in the supernatant method. This time can be reduced to 14 days for the latter. One hundred and fifty three sequential samples were obtained from 30 patients with SI strains. The results confirmed that the recovery of a SI phenotype by only one of the methods reflected well the presence of this phenotype in a given patient.

In conclusion,both methods are easy to perform. In our hands, the supernatant method was more rapid and had a better sensitivity. The passage of the virus on donors PBMC did not select a phenotypically different strain compared to the direct coculture with MT-2 cells.

D4-436 **INTRA-HOST HIV-1 EVOLUTION IS POSITIVELY CORRELATED TO THE LENGTH OF IMMUNOCOMPETENT PERIOD**

Vladimir V. Lukashov, Carla L. Kuiken and Jaap Goudsmit

Human Retrovirus Laboratory, Academic Medical Centre, University of Amsterdam, The Netherlands

In order to investigate the HIV-1 intra-host evolution and its relation to AIDS pathogenesis genomic RNA sequences (290 bp) encoding the V3 region of the envelope glycoprotein gp120 were determined in 44 patients at seroconversion and 5 years later. Significantly higher levels of nonsynonymous substitutions were accumulated by virus strains in patients who did not develop AIDS during this period (non-progressors, n=31) compared with those in progressors (n=13) (0.051 and 0.031, respectively, p=0.01). Numbers of synonymous substitutions were similar in both groups (0.018 and 0.025, respectively, p>0.1). The ratio of nonsynonymous to synonymous substitutions was 2 times higher in non-progressors (p=0.02). The quantity of nonsynonymous substitutions was not significantly associated with the level of virus RNA in serum, p24 antigen production or virus phenotype. There was no indication for the existence of specific transmissible or pathogenic virus strains. During the follow-up period 21 patients (12 progressors and 9 non-progressors) developed immunodeficiency (defined as $CD4^+$-cell count drop below 200 per μl). The numbers of nonsynonymous substitutions after 5 years in these patients correlated strongly with the duration of the immunocompetent period of infection (correlation 0.64, R squared 0.41, p=0.001), while there was no correlation with the number of synonymous substitutions (p=0.4). Our data suggest that host surveillance is a main driving force of intrapatient virus evolution.

D4-437 DEPLETION OF LYMPHOID CELLS BY CELL-TO-CELL TRANSMISSION OF FELINE IMMUNODEFICIENCY VIRUS IN THE PRESENCE OF AZT, Lawrence E. Mathes, Deborah Rumpf and Kate Hayes, Center for Retrovirus Research, The Ohio State University, Columbus, Ohio 43210

A number of mechanisms have been proposed to account for the loss of CD4 cells in HIV infected individuals including direct virus killing, apoptosis induction by cell-to-cell contact, and syncytium formation. In spite of the cytopathic consequence of HIV infection, CD4 cell levels remain relative stable throughout most of the latent period before the emergence of AIDS. This pattern of CD4 stability suggests that the stem cell population remains virus negative and therefore there is a renewable capacity for this cell type in HIV infected individuals. AZT therapy of AIDS patients is an attempt to protect the rejuvenated CD4 cell population from infection by cell free virus. However, based on in vitro studies, AZT may not be effective in preventing cell-to-cell transmission of HIV to CD4 cells.

In this study, feline immunodeficiency virus (FIV), a small animal model for lentivirus infection, was used to evaluate the efficacy of antiviral agents against cell-to-cell transmission. Either cell-free virus or FIV-infected 3201 cells were inoculated into uninfected 3201 cells or into cat PBMC cultures both pretreated and grown in the presence various concentrations of AZT. Control 3201 cells infected with cell-free FIV turned virus positive with approximately 30% cell death over a period of 21 days in culture. PBMC cultures infected with FIV showed >90% cell death by 14 days in culture. Cell-free FIV inoculated AZT treated cells at concentrations of $\geq$ 1 ug/ml remained virus antigen negative with cell growth curves comparable to uninfected cells. When lethally x-irradiated FIV-infected 3201 cells were mixed with uninfected 3201 cells at a ratio of 1:8 or 1:1 the uninfected cells converted to FIV antigen positive by day 10 of culture. AZT even at doses of 40 ug/ml was not able to prevent virus conversion of the uninfected 3201. These results suggest that the dynamics and/or mechanisms of virus transmission from cell-to-cell are different from that of cell-free virus.

D4-438 ENVELOPE SEQUENCE DIVERSITY OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 AND DISEASE PROGRESSION IN A LONGITUDINAL STUDIED COHORT, Richard A. McDonald, Douglas L. Mayers, Raymond C.-Y. Chung, Kenneth F. Wagner, Deborah Birx, Nelson L. Michael and the RV43 Study Group, Walter Reed Army Institute Research, 13 Taft Court, Rockville, MD 20850.

We sought to define the relationship of HIV-1 sequence diversity to disease progression. Previous studies have suggested that the degree of sequence variation within gp120 correlates inversely with disease progression. We have analyzed the evolution of sequences from a portion of gp120 from four patients. These clones were derived by nested PCR amplification of a portion of gp120 encompassing the C1 through C3 regions. All the patients were being treated with AZT. Sequences were analyzed at two time points, separated by two to three years. Two patients experienced significant CD4 depletion during this time while the other two patients had stable CD4 counts and clinical courses. Because of the blinded design of this study, we are currently unable to match sequence data with clinical data. However, based on the degree of diversity within the regions sequenced thus far, our preliminary findings show that the data sort into two distinct groups. We are extending this study to include 5 patients who experienced significant CD4 depletion and 5 nonprogressors. The laboratory and clinical data on all ten patients will be presented at the meeting.

D4-439 **PHARMACOKINETICS OF P24 ANTIBODY (Ab) IN HIV⊕ PREGNANT ♀ & NEWBORNS RECEIVING HYPERIMMUNE HIV IMMUNOGLOBULIN (HIVIG) IN ACTG PROTOCOL 185.** L Mofenson[1], J Lambert[2], C Fletcher[3], ER Stiehm[4], J Moye[1], W Meyer[5], G Nemo[1], B Mathieson[1], G Hirsch[6] for the ACTG 185 Team. [1]NIH, Bethesda MD [2]Johns Hopkins, Baltimore, MD [3]Baylor ACTU, Houston TX [4]UCLA, Los Angeles CA [5]Maryland Medical MetPath, Baltimore MD [6]Westat, Rockville, MD

BACKGROUND: As of 10/18/94, 49 HIV⊕ pregnant ♀ have enrolled in ACTG 185, a controlled (IVIG), blinded trial of HIVIG to reduce perinatal HIV transmission. HIVIG/IVIG infusions (200 mg/kg) are given monthly to HIV⊕ ♀ starting at 20-30 wks gestation; 1 infusion is given to the newborn by 12 hrs of age. **METHODS:** In ♀, quantitative serum p24 Ab (HIVAB, Abbott Laboratories) was measured at pre- & 1hr, 1, 3, 7,14 & 28 d post-infusion; in infants, measurement was at pre- & 1 hr, 1, 7, 14 & 28 d post-infusion. **RESULTS:** Data were available for 11 ♀ after 1st HIVIG infusion, 10 after 2nd, 9 after 3rd, & 3 after 4th infusion & for 4 newborns. A 2 compartment model best fit the data. Table shows mean value (% coefficient variation) for ♀ and newborns for p24 Ab terminal half-life (T½); distribution volume (V_d); total body clearance (CL); & p24 Ab titer at 1 hr & 28 d post-infusion.

Dose	No.	T½	V_d	CL	1 hr	28 d
♀: #1	11	15 d (60%)	71 ml/kg (36%)	4 ml/kg/d (46%)	13,961 (49%)	3,250 (131%)
♀: #2	10	15 d (44%)	70 ml/kg (40%)	4 ml/kg/d (56%)	48,602 (208%)	4,445 (91%)
♀: #3	9	30 d (105%)	126 ml/kg (66%)	4 ml/kg/d (50%)	15,726 (52%)	3,349 (84%)
♀: #4	3	37 d (79%)	190 ml/kg (38%)	5 ml/kg/d (66%)	10,195 (43%)	3,107 (77%)
New-born	4	30 d (54%)	164 ml/kg (33%)	5 ml/kg/d (58%)	13,913 (60%)	3,382 (62%)

CONCLUSIONS: PK evaluation of HIVIG administered to HIV⊕ pregnant ♀ & newborns demonstrates stable drug disposition. Overall CL was similar in all infusions; some increase in V_d and T½ was seen in pregnant ♀ after the 2nd infusion, possibly secondary to increasing gestational age. Interpatient variability in PK was observed, but is consistent with data in pregnant ♀/newborns for other immunoglobulin products.

D4-440 HIV-1 GENOMIC RNA VARIATION AT SEROCONVERSION IN A RWANDESE MOTHER-CHILD COHORT. Mulder-Kampinga GA[1,2], Simonon A[3], Kuiken C[1], van de Perre P[3], Goudsmit J[1]. Human Retrovirus Laboratory[1], Department of Obstetrics & Gynecology[2], Academic Medical Center, The Netherlands. AIDS Reference Laboratory, Kigali, Rwanda[3]

Objective: To analyze the HIV-1 virus population at time of seroconversion and transmitted by breast feeding in a cohort of Rwandese mothers and their children (P. vd Perre et al., N Engl J Med, 1991; 325:593-598).

Materials and Methods: RNA was isolated from 8 mothers and 6 children of the first seropositive sample, obtained within 3 to 6 months after a seronegative sample, or from a preseroconversion sample (one mother). The mothers were infected heterosexually. In 5 of the 6 pairs, mother and child seroconverted during the same three months period after delivery. cDNA was generated for the V3 and $p17^{gag}$ region and amplified by nested PCR. 10 to 14 clones were sequenced. Mean nucleotide variation introduced by experimental misincorporations was found to be 0.5% and 0.2% for the V3 and gag region, respectively.

Results: In 4 women and 3 of their infected children a homogeneous V3 population was observed with a mean intra-sample nucleotide variation of 0.5 to 0.7% (maximum: 1.4 to 1.8%). These mothers and children had seroconverted within the same time period and the V3 consensus sequence of the children was identical to the one found in the mother. In 4 mothers a more heterogeneous V3 sequence population was found (mean variation: 1.4 to 9.0%, maximum 3.3 to 24.3%). Two of their infected children contained a homogeneous population (mean: 0.0 to 0.6%), resembling either a major or minor maternal variant. In the other child, the V3 population was also rather heterogeneous (mean variation 2.2%) and many polymorphic positions were shared with the mother. In 3 of the 4 mothers and in the child, the variants observed within one sample as well as in the samples of the mother and child were clearly genetically linked (Neighbor-joining analyses). However, in one mother two distinct V3 populations were found which clustered with different HIV-1 subtypes (type A and C). The co-existence of these two V3-populations was still detectable at 24 months after seroconversion. For the p17 region only type A sequences were found (mean intra-sample variation of 0.4%). This suggest a recombination event between the gag and the env region of two HIV-1 subtypes. The A-subtype was transmitted to the infant. In one other mother we observed a combination of A subtype V3 sequences with C subtype gag sequences. The V3 and p17 sequences of the other mothers and children belonged to clade A. Variation in gag, examined in one mother and 2 children with a homogeneous V3 population and 3 mothers and one child with a heterogeneous population, was in general higher in persons with a heterogeneous V3 population (mean: 0.3 to 0.5% and 0.4 to 2.0%, respectively).

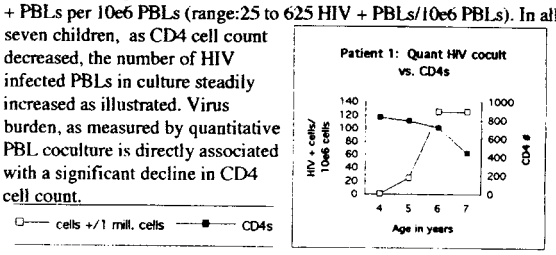
Conclusions: The observation of a heterogenous V3 sequence population at time of seroconversion, as demonstrated by the observation of multiple point mutations and recombination events, suggests that in this African cohort transmission of multiple *env* variants is not uncommon.

D4-441 LIMITED VARIABILITY AND COPY NUMBER OF HIV-1 IN HUMAN ALVEOLAR MACROPHAGES, Koh Nakata, M. Weiden, T Harkin, D. Ho, and WN Rom. Division of Pulmonary and Critical Care Medicine, and Bellevue Chest Service, NYU Medical Center and Aaron Diamond AIDS Research Center, New York, NY.

Alveolar macrophages (AM), a potential reservoir for HIV infection, may have special HIV genotype characteristics to achieve their viral burden. We evaluated HIV proviral DNA integrated in human AM obtained by bronchoalveolar lavage and peripheral blood monocytes (PBM) from ten HIV patients who had normal chest radiographs. Using a sensitive quantitative PCR, the HIV copy number in AM and PBM were low ranging from 0 to 20.3 copies per 10^5 cells. 4 patients with $CD4^+$ cells<200/mm^3, had more than 3 copies per 10^5 cells in both AM and PBM. Two patients with $CD4^+$ cells>200/mm^3 and two patients with $CD4^+$ cells<10 mm^3 had <3 copies/10^5 AM or PBM. There was no difference in HIV copy number between AM and PBM. To evaluate diversity of HIV gp120, we obtained env DNA sequences (V3-V5, 600-650 b.p.) from 5 patients with CD4<200mm^3 using nested PCR followed by M13 cloning and sequencing (4 to 12 clones from each sample). From the nucleotide sequence data of 60 clones including more than 38,000 bases, we calculated intra-patient genetic similarity between two different clones using Clustal V program. The mean genetic similarity in AM was 98.0±0.42 (n=57), which was significantly higher than that in PBM (97.2±0.40 (n=31), p<0.001), suggesting that variability of HIV-DNA in AM was relatively limited. Mean similarity between AM and PBM was much lower than those in AM or PBM (95.3±0.23 (n=105), p<0.001). DNA heteroduplex mobility assay of the PCR products from the same patients supported these data; bands from AM clustered compared with those from PBM and banding patterns were clearly different between AM and PBM in 3 of 5 patients. Two other patients with CD4<20/mm^3 demonstrated a homogeneous banding pattern in AM with a single predominated band which had the same mobility as the major band in PBM. These results suggest that the HIV genotype infected in AM is more restrictively selected than those in PBM. These data are consistent with an AM microenvironment in which host defense mechanisms reduce HIV infection *in vivo*.

D4-442 A PROSPECTIVE EVALUATION OF VIRUS LOAD IN VERTICALLY HIV-INFECTED PEDIATRIC LONG TERM SURVIVORS. K Nielsen, L Wei, R Dickover, E Garratty, YJ Bryson. Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024

In vertically HIV infected children, progression of HIV-1 disease tends to occur more rapidly than in adults. There are children, however, who survive for many years with HIV infection without disease maintaining stable CD4 counts. We identified a cohort of seven perinatally infected children all eight years of age or older who have had sequential virologic and immunologic evaluations over the past five years. At a mean entry age of 6.1 years (range: 4 - 10 years), all children had stable and relatively high absolute CD4 cell counts (mean: 600/mm3). HIV-1 quantitative peripheral blood lymphocyte (PBL) cocultures were performed sequentially over the years. Patient PBLs were isolated from heparinized whole blood following centrifugation on Ficoll-Hypaque gradient, serially 5-fold diluted and cocultured with previously PHA stimulated donor PBLs, RPMI-1640 medium, fetal bovine serum, L-glutamine, penicillin/streptomycin, and IL-2. Over five years absolute CD4 numbers declined by a mean of 58.4 % (range: 30.9 - 80.4 %). Concurrently, the number of HIV infected PBLs in culture per 1 x 10e6 PBLs increased from a mean of 8 HIV+ PBLs per 10e6 PBLs (range: 1 to 25 HIV+ PBLs/10e6 PBLs) to a mean of 254 HIV + PBLs per 10e6 PBLs (range:25 to 625 HIV + PBLs/10e6 PBLs). In all seven children, as CD4 cell count decreased, the number of HIV infected PBLs in culture steadily increased as illustrated. Virus burden, as measured by quantitative PBL coculture is directly associated with a significant decline in CD4 cell count.



D4-443 APOPTOSIS INDUCTION VIA VARIABLE REGION 5 OF THE FeLV-C_{Sarma} ENVELOPE GLYCOPROTEIN: A FELINE MODEL OF RETROVIRAL APOPTOSIS INDUCTION, Phipps, A.J., Hartke, J.R., Mathes, L.E., Rojko, J.L.,Dept. of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210.

Isolates of FeLV that contain a C_{Sarma} variable region 5 (CVR5) like motif (LCKKTQKGHKGTHYL) in the envelope (SU) protein are cytosuppressive for, and induce apoptosis in a variety of human and feline hemolymphatic cells. These isolates are not cytosuppressive for, nor induce apoptosis in feline fibroblastoid cells. FeLV isolates with an $A_{Glasgow}$ variable region 5 (AVR5) like motif (LCNKTQQGHTGAHYL) or other $C_{nonSarma}$ variable region 5 motifs do not induce apoptosis in any human or feline cell line. Multimeric 15-mer synthetic peptides (Multiple Antigenic Peptides, MAPs) corresponding to the VR5 regions of the FeLV-$A_{Glasgow}$ and FeLV-C_{Sarma} surface unit gp70 were constructed in order to determine the minimal pathogenic motif. Two determinants are required for killing of susceptible hemolymphatic cells *in-vitro*: a $C_{Sarma\text{-}like}$ VR5 motif and a multimeric presentation. Furthermore, addition of anti-CVR5 antibodies prevents CVR5-MAP cytosuppression *in-vitro*. Intracellular free calcium levels were also measured by real time confocal microscopy with the fluorescent indicator INDO-1. Susceptible hemolymphatic cells exposed to CVR5-MAP had elevated intracellular calcium levels within 1 to 10 minutes post exposure, while cells exposed to AVR5-MAP did not demonstrate any perturbations in intracellular calcium levels. We were able to identify a single 15 amino acid region of the FeLV SU glycoprotein which causes apoptosis and cytosuppression *in vitro*. The lack of effect of the 15 amino acid region from the relatively non-pathogenic virus and the dramatic effect of the peptide from the pathogenic virus suggests that the VR5 region containing a C_{Sarma} like motif is responsible for the subtype characteristic erythroid aplasia and immunosuppression. The susceptibility of various human as well as feline hemolymphatic cell lines suggests similar amino acid sequences in other retroviruses may be responsible for apoptosis and cytosuppressive properties such as CD4+ T-Cell depletion via apoptosis in HIV infected individuals.

D4-444 IMMUNOLOGIC FACTORS IN THE MATERNAL TRANSMISSION OF HIV INFECTION, Susan Plaeger, Saul Bermudez, Nina Harawa, Jeanne Bertolli, Yvonne Bryson, Maryanne Dillon and Deborah Wafer, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024.

The factors involved in the transmission of HIV infection from mother to child are complex and not yet fully understood. We are characterizing immunologic factors in relation to viral parameters in HIV+ pregnant women. The focus of the present study is on the role of activated CD8 T cells as a mechanism for immunologic control of HIV infection in women, thus lowering viral burden and risk of transmission. We present here data from 40 women whose infants' infection status is now known; five of the infants are HIV-infected. A control group of HIV seronegative women also has been studied. The function and cell surface phenotype of CD8 cells, as well as serologic markers of immune activation were examined. Focusing on the third trimester and immediate post-partum period, we found that, compared to non-transmitting women, the transmitting mothers had 1) no difference in total CD8 cell numbers, 2) similar levels of serum ß2 microglobulin but elevated neopterin, 3) no difference in the proportion of CD8 cells that were HLA-DR+/CD38+, but a decreased proportion of CD8+/$CD11_a$/CD3+ cells, 4) diminished ability of CD8 T cells to suppress HIV production in autologous CD4 cell cultures. These data suggest that putative mechanisms of immunologic protection by CD8 T cells are deficient in women who transmit infection to their infants. Potential therapeutic strategies in HIV+ pregnant women might, thus, be directed toward immunomodulatory as well as antiviral modalities.

D4-445 CORRELATION BETWEEN THE MAGNITUDE OF VIRAL LOAD IN EARLY INFANCY AND SURVIVAL AMONG PERINATALLY HIV-INFECTED CHILDREN, Henry Pollack, Nina Arlievsky, Mona Rigaud, Aditya Kaul, Keith Krasinski, William Borkowsky, Department of Pediatrics, NYU Medical Center-Bellevue Hospital Center, New York, N.Y. 10016.

Between 10-30% of perinatally infected infants will have rapid progression of disease and demise before 2 years of age. In adults, early inability to control viral replication, as demonstrated by persistently elevated viral load following sero-conversion, is associated with rapid CD4 lymphocyte decline and poor outcome. We examined the correlation between the viral load, assessed with quantification of p24 antigen, in the first 6 months of life in 58 HIV-infected infants followed prospectively. The median concentration of p24 antigen in the first 6 months of life in 15 infants who died before 2 years of age was 228 pg/ml compared to 14 pg/ml in 26 infants who survived beyond 2 years of age, $p<0.05$. Of 7 infants who survived beyond 6 years of age, the median p24 antigen concentration was 0 pg/ml. Proportional survival to 2 years of age, measured by product limited analysis, of infants with >100 pg/ml of p24 antigen in the first 6 months of life was 38% compared to 92% of infants with <100 pg/ml, $p<0.01$. These results suggest that the magnitude of viremia in early infancy is a predictor of clinical outcome; those infants who are unable to control viral replication early in the course of perinatal HIV-infection do poorly. Whether this is determined by the mode of transmission, the inital viral inoculum, the biologic properties of the virus, the host response or a combination of these factors remains to be determined.

D4-446 **HIV-1 TROPISM FOR T-LYMPHOID CELL LINES: ROLE OF DISTINCT ENVELOPE DETERMINANTS**

Lee Ratner and Alejandro Carrillo, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St Louis, MO 63110

Although several groups have shown that the V3 loop of the HIV-1 gp120 *env* glycoprotein is a minimal determinant necessary for entry into macrophages, our studies indicated that a second determinant in gp120 was necessary for infection of T-cell lines. Initially we had narrowed this second determinant to a 100 amino acid (aa) region downstream of V3 which contains the V4 loop, C4 domain, and a portion of the V5 loop. Site-specific mutagenesis was used to identify 2 aa within C4, which when combined with a T-cell line tropic V3 loop, were sufficient to confer infection of T-cell lines. These results suggest a 2-determinant model for establishing infection of T-cell lines, perhaps mediated via an interaction of the V3 loop with the C4 domain.

The combination of T-cell line-tropic V3 and V4 loops also resulted in productive infection of T-cell lines, although peak RT levels were significantly delayed. Sequence analysis of viral DNA derived from the infected cells revealed a secondary reversion in the V1 loop of gp120 with an asparagine to aspartic acid conversion. Similarly, combination of the V1 change with a T-cell line tropic V3 loop (but not T-cell line-tropic V4 loop) also resulted in delayed kinetics of replication, again suggesting a compensatory reversion. These findings suggest that infection of T-cell lines depends on conformation-specific interactions between the V1, V3, and V4 loops.

D4-447 **DISTRIBUTION OF HIV-1 SUBTYPES A, C, AND D IN UGANDAN REGIONAL COMMUNITIES.** Janice Riley, Gary Pestano, Karlene Hosford, Culette Francis, [Peter Mugyenyi, [+]Peter Kataaha, and William M.O. Boto. Department of Biology and Chemistry, The City College of New York, NY 10031; [Joint Clinical Research Centre, Kampala, [+]Nakasero Blood Bank and Department of Medicine, Makerere University, Kampala, Uganda.

The goal of the present study was to assess the relative seroprevalence of the HIV-1 phylogenetic clades A, B, C and D in Ugandan localities. Synthetic peptides comprising the putative PND in the V3 loop of new HIV-1 clones were tested in ELISA for reactivity with asymptomatic sera obtained from five regionally representative communities in the country. Irrespective of the geographical origin of the donors, the majority of the test sera gave intense and most prevalent reactivity with the novel North American clone RT3.6 (Group B), the Ugandan clone UG045 (Group C) and the reference clone RMA (Group F). The ELISA data segregated the test sera into discrete subsets: one subset reacted preferentially with BRT3.6; and another subset showed comparable reactivity with CUG045 and FRMA. However, a third subset of the sera cross-reacted with BRT3.6, CUG045 and FRMA. The results from ELISA inhibition assay indicated that CUG045 and FRMA express closely related antigenic specificities in the V3 loop. A surprising observation was that the clones AUG06c and DUG23c from the prevalent Ugandan phylogenetic clades A and D, respectively, were not as reactive with the indigenous sera. These observations raise several possibilities. The PND residues in CUG045 and BRT3.6 appear to be well conserved in the HIV-1 subtypes which account for the majority of infections in the selected Ugandan locales. Secondly, the patterns of seroreactivity observed for this epitope are not consistent with the phylogenetic classification of the Ugandan HIV-1 subtypes.

D4-448 *IN VITRO* DIFFERENTIATION OF $CD34^+$ BONE MARROW DERIVED CELLS INTO MATURE T LYMPHOCYTES AND NATURAL KILLER CELLS,

M. Rosenzweig, D.F. Marks and R.P. Johnson. Division of Immunology, New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772.

The precise mechanisms associated with CD4 lymphocyte depletion in HIV infection remains poorly understood. Retroviral infection of T cell progenitors may result in interruption or disruption of T cell development, and this may exacerbate the characteristic CD4 lymphopenia. Perturbation of lymphocyte ontogeny may occur in bone marrow derived progenitor cells or in the thymus. Thymic abnormalities have been reported, but the specific role of thymic HIV-1 infection in the development of immune dysfunction has not been determined. Specific effects of HIV on bone marrow derived lymphoid progenitors remains controversial.

Lymphoid differentiation of committed hematopoietic stem cells requires interaction with both cellular and soluble factors present in the thymic microenvironment. We have established a non-human primate model that supports the differentiation of autologous and allogeneic T cell progenitors in culture. Thymic stromal monolayers were prepared from the adherent cell fraction of collagenase digested fetal or neonatal thymus. After 10-14 days, $CD34^+$ bone marrow-derived cells cultured on thymic stromal monolayers yielded $CD3^+CD4^+CD8^+$, $CD3^+CD4^+CD8^-$, $CD3^+CD4^-CD8^+$ and $CD3^{lo}CD8^{lo}CD16^+$ cells. No cells expressing the CD3 or CD16 markers were detectable in the original purified population of $CD34^+$ cells, and no $CD3^+$ lymphocytes were observed in cultures of CD34 - depleted cell fractions on thymic monolayers.

These data indicate that rhesus thymic stromal monolayers can provide the necessary cellular and soluble factors required for differentiation of $CD34^+$ progenitor cells into both T lymphocytes and natural killer cells. This model will be used to further elucidate mechanisms of T lymphocyte ontogeny as well as to study the functional consequences of SIV infection of hematopoietic stem cells on their ability to undergo lymphoid differentiation.

D4-449 **VARIATION IN ENV GENE SEQUENCE AND PERINATAL TRANSMISSION OF HIV-1**

Rousseau, Christine[1], Leslie Louie[1], Ming Lee[1], Jeanette Just[1], Haynes Sheppard[2], Elaine Abrams[3], Zena Stein[3], Mary-Claire King[1]. [1]University of California at Berkeley; [2]California Dept of Health Services; [3]Columbia University, New York.

This study was undertaken to examine viral genetic factors that may explain why infected mothers transmit HIV-1 to their infants in only 13-40% of their pregnancies. We report here our preliminary results for the influence of viral genetic risk factors in perinatal HIV-1 transmission.

The study population was drawn from 2 prospectively followed cohorts - Harlem Hospital Center in New York City and the Bay Area Perinatal AIDS Cohort in San Francisco. Blood samples were collected from the participants and genomic DNA was extracted. We have samples from 26 transmitting mother-infant pairs and 115 mothers who may or may not have transmitted HIV-1. Nested PCR was used to amplify the variable regions V3, V4 and V5 of envelope gene. These PCR products were cloned and 10 clones per participant were sequenced. We will sequence the regions of interest for all transmitting mother-infant pairs and a subset of non-transmitting mothers. Preliminary data is presented for the HIV-1 sequences collected to date.

Among the ten clones sequenced from one mother who transmitted the virus to her infant (T), there is 98.9% (+/- 0.77%) similarity. Among seven sequences cloned in her infant, the sequences are identical to each other and the mother's consensus sequence. Among twelve clones sequenced from the mother who did not transmit the virus to her infant (NT), there is 98.3% (+/- 0.8%) similarity. HIV sequences tend to fall under five categories entitled A, B, C, D, and E. Sequences of two mothers, T and NT, are most similar to consensus sequence of category B. NT has an average viral sequence similarity of 97% to category B. T has an average viral sequence similarity of 89% to category B consensus sequence. Phylogenetic tree analysis corroborates this data. Further analysis of mother infant pairs may reveal particular signature patterns of V3 and/or the V4 and V5 sequence that correlate with increased risk of infection and/or increased protection from infection. Study of viral genetic factors associated with HIV-1 transmission can lead to further understanding of factors that influence an infant's susceptibility to infection with HIV-1, a mother's ability to transmit HIV-1, or disease progression among infected infants.

D4-450 INITIAL LENTIVIRAL-HOST INTERACTIONS AT THE SINGLE LYMPH NODE LEVEL: A STUDY USING MAEDI-VISNA VIRUS INFECTION OF SHEEP, Douglas J. Roy, Barbara A. Blacklaws, Prue Bird, David R. Sargan* and Ian McConnell*, Department of Veterinary Pathology, Edinburgh University, Summerhall, Edinburgh, Scotland EH9 1QH, *Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, England CB3 0ES

Lentiviruses establish persistent infections despite a sustained immune response mounted by the host. It is probable that the early immunological and virological events occurring during initial lentiviral infection play a crucial role in the establishment of persistence. In HIV little is known of the initial stages of infection and induction of immunity due to the subclinical nature of the infection. Uniquely in the sheep the response of individual lymph nodes to viral challenge can be continuously monitored via cannulation of afferent and efferent lymphatic vessels. This model system has been used to better define the virus-host interactions which take place during initial lentivirus infection. The appearance of cell associated virus, and viral DNA and RNA, were monitored in relation to the development of the immune response within single lymph nodes draining the subcutaneous site of maedi-visna virus infection. A peak of infected cells was detected between day 7 and 14 p.i. and was seen both in isolated lymph node cells and in cells exiting in the efferent lymph. This peak coincided with the appearance of T cell proliferative responses, the presence of precursor CTLs directed against the virus, and neutralising antibody. A wave of CD8+ lymphoblasts exits the node at this time and MVV precursor CTLs are prominent within this population. A sustained decrease in virus production was seen following the development of the cellular immune response. However, low numbers of infected cells could always be isolated at subsequent points, and the number of cells at later time points containing viral DNA increased to relatively high levels. In addition, rev and pol gene transcripts were readily detectable indicating the establishment of a reservoir of cells in a latent or restricted state of replication. We also found evidence for the requirement of rev synthesis prior to the initial peak in virus production. T cell responses to MVV therefore occur during periods when infectious virus isolation was maximal, but cellular immunity may act to control the level of infection from day 18 onwards.

D4-451 AUTOLOGOUS NEUTRALIZING ANTIBODY RESPONSE AND VIRUS PHENOTYPE IN PROGRESSION OF DISEASE OF HIV-1 INFECTED CHILDREN; Gabriella Scarlatti*, Vida Hodara#, Claudia Colognesi*, Alberto Beretta*, Jan Albert**, and Eva Maria Fenyö#. Laboratory of Immunobiology of HIV, S.Raffaele Scientific Institute, Milan, Italy; #Microbiology and Tumorbiology Center, and **Dept. of Clinical Virology, Karolinska Institute, Stockholm, Sweden.

The bimodal pattern of disease progression observed in HIV-1 infected children born to seropositive mothers was suggested to be due to mode of transmission, as well as phenotype of the transmitted virus. Furthermore, the presence of neutralizing antibodies against lab strains in young children was indicative of slow disease progression. We have studied the development of neutralizing antibodies to autologous viruses (NT) and the replicative pattern of the virus isolates of HIV-1 infected children with slow and fast progression of disease.

Samples were obtained from HIV-1 infected children from first months of age and followed up to 53 months of age or untill death. Six children were classified as fast progressors and 8 as slow progressors according to their clinical stage and CD4+ cell decline. The sera of the children were tested for NT against their own isolates (2 to 4 isolates for each child) obtained simultaneously or at follow-up. Furthermore, the replicative capacity of the isolates from the children were tested in cell lines of T-lymphoid and monocytoid origin (Jurkat, Jurkat tat, CEM, U937 and MT-2).

A persistent autologous NT through the whole follow-up was evident only for the 3 slow progressors tested. Although NT were not always detected with virus and serum obtained simultaneously, NT always developed during follow-up. On the contrary, the 2 fast progressors tested never developed NT. Six of 8 slow progressors harboured a slow replicating virus through the whole follow-up period (31 to 53 months). The virus of the remaining 2 slow progressors showed a switch from slow to fast replication when the children reached an age of 37 and 48 months. Half of the fast progressors harboured a rapid replicating, syncytium-inducing virus from early age on. However, the remaining 3 children, who died before 1 and 2 years of age, never developed a rapid virus.

Our data show that the presence of a rapid virus isolate in HIV-1 infected children from early age on is prognostic of fast progression of disease. However, fast progression can occur also in the presence of slow virus isolates. Moreover, fast in contrast to slow progressors seem unable to develop a neutralizing antibody response to their own virus. In conclusion, the interplay between virus and host factors seems to be of crucial importance for progression of HIV-1 disease in children.

D4-452 **INDUCTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 BY HERPES SIMPLEX VIRUS TYPE 1; ROLE OF NF-κB AND ICP0; Susan L. Schafer[1], Pierre Beauparlant[2], Richard Bitar[2], John Hiscott[2], and Paula M. Pitha[1], Oncology Ctr., Johns Hopkins University[1], Baltimore, MD USA; Lady Davis Institute McGill University[2], Montreal, CANADA**

Our previous studies have shown that expression of HIV-1 provirus was enhanced in cells co-infected with the Herpes virus. We have shown that HSV-1 induces the HIV-1 provirus by both an NF-κB-dependent and -independent mechanisms. HSV-1 stimulation of the NF-κB complex occurs approximately eight hours after infection; using NF-κB-specific antibodies, we have found that HSV-1 increases binding of p50 and p65 and c-rel to an HIV-1 NF-κB probe. Northern blot analysis shows a transient increase of p65 and p50 mRNAs in HSV-1-infected cells. Transfection of HIV-1 LTR CAT into cell lines, which overexpressed individual NF-κB proteins, showed increased expression in cell lines overexpressing p52, p100, and c-rel after HSV-1 infection, while HSV-1-mediated stimulation of HIV-1 LTR CAT was reduced in lines overexpressing p105 and IκBα and IκBγ.

Strong synergism, of HIV-1 LTR transactivation was observed between ICP0 and Tat. In the presence of ICP0, Tat transactivation can occur in the absence of TAR binding site. This transactivation, which is specific for HIV-1 LTR, can also be demonstrated by using GAL-4 model system. Analyses of ICP0 regions, which are essential for this transactivation, are being examined as well as a direct interaction between ICP0 and Tat protein.

D4-453 *Abstract Withdrawn*

D4-454 RESTRICTED SEQUENCE VARIABILITY OF THE HIV-1 V3 LOOP *IN VIVO*. *P. Simmonds[1], E.S. Hughes[1]; W.J. Livingstone[1], Y.K. Donaldson[1]; E.C.Holmes[2]; H.K. Brown[3], J.E. Bell[4]*: DEPARTMENTS OF [1]MEDICAL MICROBIOLOGY, [3]MEDICAL STATISTICS, [4]PATHOLOGY, UNIVERSITY OF EDINBURGH, EH8 9AG, [2]DEPARTMENT OF ZOOLOGY, UNIVERSITY OF OXFORD, OX1 3PS, UK.

The relationship between sequence variation in the *env* gene with the cell tropism and cytopathology *in vivo* of HIV was investigated in post-mortem tissue samples from an extended cohort of 49 HIV-infected individuals who either died of AIDS, or for unrelated reasons while asymptomatic. Infection of non-lymphoid tissue such as brain, spinal cord and lung was confined to those with AIDS. V3 loop sequences generally showed highly restricted sequence variability and a low overall positive charge of the encoded amino acid sequence compared with those of standard laboratory isolates of HIV-1, and was equivalent to the restriction observed in isolates with a non-syncytium inducing (NSI) and macrophage-tropic (MT) phenotype *in vitro*. p24 antigen was detected by immunocytochemical staining in the germinal centres within lymphoid tissue, and in lung and brain tissue of the AIDS patients where we observed multi-nucleated giant cells (syncytia) even though the V3 loop sequences of these variants resembled those of NSI isolates *in vitro*. These studies indicate that a lack of syncytium forming ability in established T cell lines does not necessarily predict this property in primary target cells *in vivo*. Furthermore, variants with V3 sequences characteristic of NSI/ macrophage-tropic isolates form the main population in a wide range of lymphoid and non-lymphoid tissues *in vivo*, even in patients with AIDS.

We are currently investigating the contribution of sequence variation in the V1 and V2 hypervariable regions to *in vivo* phenotype. In contrast to V3, this region is highly variable *in vivo* with no evidence for reproducible differences between asymptomatic and symtomatic individuals, nor between lymphoid and non-lymphoid tissue. The finding of completely different populations of V1/V2 sequences in different parts of the same organ suggests that much of the observable variation in the this region confers no change in the tropism of the virus.

D4-455 EXPOSURE OF THE THIRD HYPERVARIABLE REGION ON INTACT HIV-1 VIRIONS DISPLAYING MACROPHAGE- AND T-CELL LINE-TROPISM, Leonidas Stamatatos and Cecilia Cheng-Mayer, Aaron Diamond AIDS Research Center, New York, New York 10016.

We previously reported an association between V3 loop conformation of monomeric gp120 and HIV-1 cellular tropism, and proposed that the mechanism by which the V3 loop determines tropism is by regulating the type and/or the extent of conformational changes that the gp120 molecule undergoes upon virus-cell association. To test this hypothesis, the binding patterns of anti-V3 loop human monoclonal antibodies (MAbs) towards intact and detergent-disrupted virions displaying macrophage- and T-cell line-tropism (HIV-1_{SF162} and HIV-1_{SF2} respectively), in the presence or absence of sCD4, were evaluated using immunochemical approaches; sCD4 being used as surrogate for cellular CD4. In the absence of sCD4, the binding pattern of the MAbs used is similar between intact virions, lysed virions and soluble gp120 molecules obtained from transient transfection of COS-7 cells. However, the extent and affinity of MAb binding towards intact virions is decreased. These differences are particularly pronounced in the case of the macrophage-tropic HIV-1_{SF162} virus, whose V3 loop appears to be occluded on the virion surface. Receptor binding of the virions resulted in the exposure of some, but not all V3 loop epitopes examined. Of interest is the observation that the rate and extent of sCD4-induced exposure of the V3 loop epitopes vary depending on the viral tropism. For example, the exposure of the epitope recognized by MAb 391-95D, which we previously reported to be associated with macrophage-tropism, is enhanced upon binding of the macrophage-tropic HIV-1_{SF162} virus , but not the T- cell line-tropic HIV-1_{SF2} virus to sCD4. A dual-tropic recombinant virus between HIV-1_{SF162} and HIV-1_{SF2} displays an intermediate degree of exposure of this particular epitope. The sensitivity to neutralization by these MAbs and the binding patterns of these MAbs towards additional HIV-1 isolates, recombinant and mutant viruses that display different cellular tropisms are being examined to confirm these preliminary observations and to correlate specific conformational changes within the V3 loop to viral tropism and infectivity.

D4-456 **INFECTION OF HUMAN MICROGLIA WITH ACUTE HIV-1 ISOLATES** Julie M. Strizki, Andrew Albright, Michael O'Connor, George Shaw* and Francisco Gonzalez-Scarano. *Depts. of Neurology and Neurosurgery, University of Pennsylvania School of Medicine, Philadelphia, PA 19104. *Dept. of Medicine,University of Alabama School of Medicine, Birmingham, AL 35294.*

HIV dementia (HIVD) is a serious consequence of HIV infection, affecting approximately 30% of all AIDS patients. Currently, the phenotype and the origin of neuroinvasive viruses is poorly understood. Histopathological evidence suggests that microglia are the primary cell type infected in the brain, and are postulated to be partially or completely responsible for the neuronal dysfunction associated with HIVD. To investigate the neuroivasiveness of viruses obtained during acute infections, we tested HIV-1 isolates obtained at the time of the initial viremia for their ability to replicate in brain microglia.

Microglia prepared from human brain obtained from temporal lobe resections, and monocyte derived macrophages (MDM) were infected with primary clinical isolates from six individuals. Virus replication was assayed by the production of p24 antigen in the culture supernatant and by PCR amplification of proviral DNA. Five of the six primary isolates replicated well in MDM. In contrast, only two of the isolates, both of which replicated in MDM, grew to high titer in human microglia. These findings suggest that macrophage tropism is a prerequisite, but not in itself sufficient to confer microglial tropism. Furthermore, they indicate that potentially neuroinvasive viruses are present at the time of initial infection with HIV-1.

Sequential passaging of the two primary isolates in microglia was associated with a concomitant increase in peak p24 antigen production during infection, suggesting adaptation to this cell type. This phenomenom may correspond to the genetic selection that been observed in HIV infected brains *in vivo*, and could contribute to HIV neuropathogenesis. The genetic implications of these observations are currently under investigation.

D4-457 CELL MEDIATED IMMUNITY IN HIV-POSITIVE LONG TERM SURVIVING BLOOD TRANSFUSION RECIPIENTS, John Sullivan, Wayne Dyer, Jennifer Learmont, Andrew Geczy, Caroline Farrell and Lynne Cook, NSW Red Cross Blood Transfusion Service 153 Clarence St Sydney NSW 2000 Australia.

By June 30th, 1994, 130 individuals with transfusion acquired-HIV have been traced by the NSW Red Cross Blood Transfusion Service Lookback Tracing Program. Specific donors and time of donation have been clearly identified for 108 of these individuals. While the majority have now died from AIDS or HIV-associated complications, 41 remain alive some 9-14 years after infection, displaying a wide range of clinical symptoms ranging from full blown AIDS to asymptomatic and effectively disease free.

In the present study the in-vitro cell-mediated immune response of a number of HIV-positive long term surviving transfusion recipients has been assessed in response to mitogens, alloantigens and recall antigens. All HIV-positive individuals displayed a proliferative response and IL-2 production in response to mitogens and alloantigens while recall responses to two strains of flu virus were greatly reduced in individuals with low CD4 counts and advanced disease, compared to asymptomatics with stable CD4 counts. In addition we were unable to demonstrate significant proliferation or IL-2 production in response to a range of published env and tat HIV-derived peptides. HLA typing of the asymptomatic, stable CD4 group did not demonstrate an obvious sharing of HLA alleles or haplotypes.

These results suggest that theories of immune collapse predicting a progressive loss of recall, alloantigen and mitogen responses are an over-simplification and it is probable that long term survival in the face of infection with HIV is a result of a complex interplay between host and virus.

D4-458 ROLE OF THE *TAT* AND *VIF* GENES OF CAEV IN THE VIRUS REPLICATION IN VITRO AND IN VIVO,

M. Suzan[1], A. Harmache[1], M. Bouyac[1], C. Hiéblot[1], P. Russo[2], C. Vitu[2], F. Guiguen[3] and R. Vigne[1], 1: INSERM U372, BP 178, 13276 MARSEILLE Cedex 09, France, 2: CNEVA, 105 route des Colles, 06410 Biot, France, 3: Ecole Vétérinaire, 1 Av. Bourgelat, Marcy l'Etoile, 69280 Lyon, France.

The effect of the *tat* or *vif* gene deletion on CAE virus replication was studied in vitro on primary goat synovial membrane (GSM) cells and blood derived macrophages, and in vivo by intra-articular injection of goats with wt, *tat-* or *vif-* CAEV DNA or viral supernatants. The *tat-* CAE virus displays a slightly delayed growth kinetics and lowered infectious titer on both cell types. Quantitative PCR and RT-PCR analyses revealed no difference in the various steps of a single replication cycle. In vivo inoculation of *tat-* CAEV DNA or virus resulted in the seroconversion of the goats. Virus isolation could be realised from differentiated blood macrophages, synovial fluid or mammary secretions. These animals have been challenged with a virulent preparation and 4 months later we still cannot detect the wt CAE virus in the blood derived macrophages or in cells present in mammary secretions. The *vif-* CAE virus exhibited a marked phenotype characterized by a slow and low replication in GSM cells and in differentiated blood macrophages. Quantitative PCR and RT-PCR analyses of the different steps within one replicative cycle revealed that the defect introduced by the *vif* deletion is located at the very late steps of the replicative cycle since three fold less RNA was found associated with the *vif-* particles than with the wt particles produced at 24h post-infection. This effect was increased after a second round of replication. In vivo inoculation of *vif-* CAEV DNA did not result in the infection of the goats as demonstrated by the absence of seroconversion, no virus isolation from blood derived macrophages or synovial membrane cells, and no induction of arthritis in the inoculated joints. These results would suggest an essential role for the *vif* gene of CAEV in the replication in vivo and so on the onset of infection and pathogenesis.

D4-459 A CLINICAL AND MOLECULAR CHARACTERIZATION OF LONG-TERM SURVIVAL IN PEDIATRIC HIV PATIENTS

CL Vavro[1], C R Jackson[1,2], MH St. Clair*[1], KN Pennington[1], ER Lanier[1], ME Valentine[2], R E McKinney[2]. [1]Burroughs Wellcome Co., RTP, NC; [2]Duke University Medical Center, Durham,NC.

Eighteen HIV infected children greater than eight years of age were studied. All children were infected via vertical transmission or neonatal transfusion. Based on a clinical impression of "wellness", they appeared to separate into two groups. Decrements of CD4+ decline were calculated using linear regression. The "well" group had CD4+ slope ranges of +1.47 to -1.53. The "well" group was further divided into 2 subgroups with CD4+ slope ranges of +1.47 to -0.85 (group 1) and -1.25 to -1.53 (group 2) respectively. The clinically progressive group had a negative CD4+ slope range of -1.78 to -8.06 (group 3). Viral phenotype and in vitro antiviral resistance for select members of each group have been determined. The well group as a whole had lower AZT IC 50's and predominently NSI phenotype while the clinically progressive group had AZT IC 50's greater than 1 µm and SI viral phenotype. Group 2 had resistance profiles higher than Group 1 and included some patients whose virus was determined to be SI. Virus from select members of the intermediate group (Group 2) changed from NSI to SI, correlating with a change in the CD4+ slope. The presence of AZT resistance and SI phenotype correlated with clinical progression in pediatric patients. Patient virus will be characterized by a molecular analysis of the CD4 binding region and nef coding sequence .

D4-460 **HYPER IGE IN HIV-INFECTED CHILDREN WITH SLOW OR RAPID DISEASE PROGRESSION**

A.Viganò, L.Crupi, D. Bricalli, N.Principi and M. Clerici*

Cattedra di Pediatria IV, H.L. Sacco, Università degli Studi di Milano.

**Cattedra di Immunologia, Università degli Studi di Milano.*

Background: hyper IgE is an indicator of poor prognosis in HIV-infected (HIV$^+$) adults and children. Vertically-acquired HIV infection can result in different clinical outcomes. **Objectives:** to evaluate hyper IgE in children infected vertically with HIV with slow or rapid disease progression and to analyze possible correlations between hyper IgE, IL-4 production and CD4$^+$ depletion. **Methods:** 31 HIV$^+$ children were evaluated for skin prick test (SPT) responses to common airborne and food allergens, individual and family history of atopy, IgE levels, CD4$^+$ counts. and IL-4 production by TPHA-stimulated PBMC. All HIV$^+$ children had a follow-up of 1 year for IgE levels and CD4$^+$ counts. **Results:**

Clinical status[1]	*Age (yrs)*[2]	*IgE (KIU/l)*[2]	*IL-4 PHA stimulated production (pg/ml)*[2]	*Pts with hyper IgE*[3]
P-1B	8.7	43	143.1	0/7
(n=7)	(2.9)	(16)	(38)	(0%)
P-2A	5.6	102	254.4	4/10
(n=10)	(1.3)	(52)	(125.8)	(40%)
P-2B-F	7.4	369	298.4	10/14
(n=14)	(1.7)	(153)	(128.7)	(71%)

[1]*CDC classification* [2]*Mean (SD)* [3]Fraction *(percentage)*

SPT and history of atopy were negative in all HIV$^+$ children. The incidence of hyper IgE was directly correlated with the clinical stage and with the mean concentration of IL-4. There was a significant association between persistent hyper IgE and severe decline.(≥ 30% over 1 year) in CD4$^+$ counts. (p=0.002). **Conclusions:** symptomatic vertical HIV infection is associated with hyper IgE and increased IL-4 production. A persistent IgE hyperproduction is correlated with a severe CD4$^+$ depletion.

D4-461 Primary HIV-1 Infection during Pregnancy associated with Transmission of SI phenotype and Rapid Loss of CD4 Cells in Mother and Infant.

Lorenz von Seidlein, E.Richard Stiehm, Eileen Garraty, MaryAnn Dillon, Ruth Dickover, Paul Krogstad, Yvonne Bryson; *UCLA School of Medicine, Los Angeles, CA*

Primary infection of HIV during pregnancy, a period of high viral load in the absence of neutralizing antibody has been associated with a high risk of materno fetal transmission. We report a 15 year old mother who became infected with a SI isolate during early gestation and transmitted an SI isolate to the infant despite ZDV therapy, followed by rapid CD4 loss in mother and infant.

Five months prior to conception, the mother initiated a sexual relationship with the father, an 18 year old HIV-infected hemophiliac. At 14 weeks gestation, the mother had a negative HIV antibody test, at 18 weeks a DNA PCR was positive and by 25 weeks of gestation she had seroconverted. ZDV therapy was initiated po at 30 weeks gestation, ZDVinfusion was given during delivery and oral ZDV was continued postpartum in mother and infant. As part of the ACTG protocol 185 the mother received Anti-HIV hyperimmune globulin or IVIG monthly prior to delivery; the infant received the same product immediately after delivery. Maternal viral load was high by quantitative coculture (3125 IUPM), plasma culture (>1000 TCID/ml) and RNA PCR (110 000 copies/ml) at 6 months gestation. Despite a significant reduction in viral load by the time of delivery (25 IUPM by quantitative coculture, negative plasma culture and 13 000 copies/ml by RNA PCR), the infant was infected inutero as shown by positive culture and PCR at birth. Virus isolates from father, mother at 6 months gestation and the infant were SI phenotype in MT2 cells and susceptible to ZDV in vitro. The maternal CD4 count rapidly declined from 254 to 96 cells/mm^3 over 7 months period as did the infant's CD4 count (4942 at birth to 116 cells/mm^3 at 12 weeks of age). The decline in CD4 count was accompanied by a burst of viral replication in the infant, RNA PCR increased from 15 871 copies/ml to 770 000 copies/ml by 12 weeks of age.

This unique case described rapid CD4 decline in a mother infant pair associated with an SI HIV isolate. The fact that the mother transmitted HIV to her infant despite a significant reduction in viral load and antiretroviral therapy suggests inutero transmission prior to 6 months of gestation.

D4-462 LACK OF VERTICAL TRANSMISSION OF A MOUSE RETROVIRUS, BM5ECO Nancy Wade, Emily Scheuremann and Lorraine Flaherty, Department of Pediatrics, Albany Medical College and Division of Molecular Genetics, Axelrod Institute, New York State Department of Health, Albany, New York, 12208

Maternal/infant transmission of HIV continues to be the major source of infection in children. In order to better understand transplacental transmission of a retrovirus, we wanted to determine if the mouse retrovirus, BM5Eco, could be transmitted from mother to fetus. BM5Eco is a nonpathogenic ecotropic retrovirus and is one of the viruses involved in producing a murine immunodeficiency (MAIDS) The virus alone does not cause disease, but virus can be recovered by culture and can be detected by RT-PCR. C57Bl/6 females were injected with 10^7 p.f.u. of BM5Eco i.p. during pregnancy. Embryos were delivered by Caesarean section at day 18 of gestation. RT-PCR was performed on total RNA extracted from individual pools of spleen, thymus and liver from each embryo and from maternal spleen. Primers were designed to the gag p-12 region of the viral genome. The primers have been shown to amplify a 180 bp segment from virally infected cells from spleen, thymus and lymph node. In nine pregnancies, 54 embryos were negative for viral sequences. In 5 pregnancies in which the mother was injected less than six days prior to C-section, 31 embryos were negative. In 3 additional pregnancies in which the mother was injected >20 days prior to C-section, 15 embryos were also negative. We conclude that BM5Eco does not transmit across the placenta even when injected i.p. during pregnancy.

D4-463 SEQUENCE DIVERSITY OF V1 AND V2 DOMAINS OF gp120 FROM HUMAN IMMUNODEFICIENCY VIRUS TYPE 1: LACK OF CORRELATION WITH VIRAL PHENOTYPE. Ning Wang, Tuofu Zhu, and David D. Ho. The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY.

We analyzed by polymerase chain reaction and direct sequencing 57 viral sequences from 47 individuals of North American, Australian and Haitian origin infected with human immunodeficiency virus type 1 (HIV-1), focussing on the V1 and V2 regions of gp120. There was extensive length polymorphism in the V1 region, which rendered sequence alignment difficult. The V2 hypervariable locus also displayed considerable length variations, whereas flanking regions were relatively conserved. Two-thirds of the amino acid residues in these flanking regions were highly conserved (>80%), presumably reflecting their critical contribution to V2 structure or function. We also characterized the syncytium-inducing (SI) properties of the isolates from which we derived sequence information. There was no correlation between V1 or V2 sequences with the viral phenotype, contrary to a previous report (Groenink et al; Science 260: 1513-1516). The sequence heterogeneity described in this study provides information to suggest that it would be most difficult to exploit the V1 and V2 domains for vaccine development.

D4-464 SYNTHETIC MULTIMERIC PEPTIDES DERIVED FROM THE V3 LOOP OF HIV-1 GP120 BIND TO GALCER AND BLOCK HIV-1 INFECTION IN CD4-NEGATIVE MUCOSAL EPITHELIAL CELLS, Nouara Yahi, Jean-Marc Sabatier, Stephen Baghdiguian*, Francisco Gonzalez-Scarano+, Jacques Fantini, CNRS URA 1455, Faculté de Médecine Nord, Marseille, and *INRA-CNRS URA 1184, Université Montepellier 2, France, and Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104-6146, USA.

The glycosphingolipid galactosylceramide (GalCer), which binds gp120 with high affinity and specificity, is a potential alternative receptor for HIV-1 in some CD4-negative neural and epithelial human cells, including the human colonic epithelial cell line HT-29. In the present study, we demonstrate that synthetic multi-branched peptides derived from the consensus sequence of the HIV-1 V3 loop block HIV-1 infection in HT-29 cells. The most active peptide was an 8-branched multimer of the motif Gly-Pro-Gly-Arg-Ala-Phe, which at a concentration of 1.8 µM induced a 50% inhibition of HIV-1 infection in competition experiments. This peptide was not toxic to HT-29 cells, and preincubation with HIV-1 did not affect viral infectivity, indicating that the antiviral activity was not due to a non specific virucidal effect. Using a high performance thin layer chromatography binding assay, we found that multi-branched V3 peptides recognized GalCer and inhibited binding of recombinant gp120 to the glycosphingolipid. In addition, these multibranched peptides recognized GalCer but not ceramides, gangliosides or sphingomyelin in a solid phase binding assay. These data provide additional evidence that the V3 loop is involved in the binding of gp120 to the GalCer receptor, and show that multi-branched V3 peptides are potent inhibitors of the GalCer-dependent pathway of HIV-1 infection in CD4-negative mucosal epithelial cells.

Late Abstracts

HIV-2 SEQUENCE VARIATION IN GUINEA-BISSAU: CORRELATION TO CLINICAL OUTCOME

Jan Albert, Thomas Leitner, Anders Nauclér, Department of Clinical Virology, Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden.

The clinical manifestations of HIV-2 infection in Guinea-Bissau shows an interesting dichotomy in that the highest incidence of infection is seen in comparably old individuals (50-59 years), whereas the mean age of HIV-2 associated AIDS cases is lower (approximately 40 years). One explanation for this could be that the two groups are infected with distinct virus strains which differ in virulence. The purpose of this study was to genetically characterize and compare HIV-2 strains from patients with different clinical manifestations.

Blood samples were obtained from clinically and immunologically well-characterized HIV-2 infected individuals in Guinea-Bissau; ten were asymptomatic and less than 25 years old, 10 asymptomatic and more than 50 years old and 10 had overt HIV-2 associated AIDS. Regions corresponding to the HIV-2 matrix (MA) protein and envelope V3 domain were directly sequenced from uncultured lymphocytes.

All individuals were shown to carry virus strains belonging to subtype A of HIV-2. Phylogenetic tree analyses of MA and V3 sequences did not reveal any specific clustering pattern for sequences from AIDS patients or asymptomatic individuals.

This study shows that all examined HIV-2 infected individuals from Guinea-Bissau carried virus strains which belong to subtype A and which are genetically closely related despite the fact that the clinical manifestations varied widely. Thus, these differences in clinical outcome must be due to as yet unidentified minor genetic differences between different HIV-2 strains and/or host-specific differences.

CD4-INDEPENDENT ENTRY OF LYMPHOCYTOTROPIC STRAINS OF HIV-1 INTO MACROPHAGES MEDIATED BY ENHANCING ANTIBODIES

Heike Trischmann, David Davis and Peter J. Lachmann, Molecular Immunopathology Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, Great Britain

We have used rat antisera to a complete set of overlapping peptides synthesized according to sequences of gp120 and gp41 of HIV to determine their capacity to enhance viral infection.

Predominantly, antisera to all variable (V) regions of gp120 and to conserved parts of gp41 facilitated infection of primary human macrophages with the homologous virus HIV-1 SF2mc. This included V2 and V3 sequences - antisera to which neutralized T-cell infection by the same viral strain. Such dual biological effects were also shown by a mouse monoclonal antibody (SC258) against a neutralizing epitope in V2.

In contrast, heterologous virus infection was mediated by antisera to conserved (C) regions only, particularly to C4 and C5. Infection mediated by heterologous antisera led to more rapid cytopathic effect.

Both viral strains used in this study are naturally T-cell tropic. Therefore, preincubation with certain antibodies can cause a change in HIV tropism. Such antibodies have to fulfill several conditions: 1) they have to bind to cell surface Fc receptors, 2) their binding to the accessible parts of viral glycoproteins has to be impaired, 3) their concentration in relation to the virus has to be low.

The effects described were fully dependent on FcγRIII occupancy and independent of CD4. Implications for disease progression and vaccine development will be discussed.

AMPLIFICATION AND CLONING OF VIRTUALLY FULL LENGTH HIV-1 GENOMES FROM DIVERSE SUBTYPES

Mika Slaminen[1], Jean Carr[1], DS Burke[2] and Francine McCutchan[1], [1]Henry M Jackson Foundation and [2]Division of Retrovirology, WRAIR.

The genome of the human immunodeficiency virus type-1 (HIV-1) is approximately 10 kilo bases (KB) in length. Much of the genetic variability of HIV-1 has been established sequencing of subgenomic fragments due to technical limitations of the PCR technique used to recover HIV-1 genome from primary cells or virus cultures. The few full length genomes that have been sequenced were derived from highly enriched sources by library construction using bacteriophage lambda and most are from genetic subtype B. Five of the eight genetic subtypes of HIV-1 are without a single full-length, sequenced prototype.

We have used recent improvements of the PCR technique to amplify, clone, and restriction map virtually full length (>9KB) HIV-1 genomes from genetic subtypes A through G, inclusive, using DNA from virus cultures. Genomic clones were confirmed by Southern blot hybridization with HIV-1 specific probes. Restriction analysis showed great inter-subtype diversity and also molecular diversity within individual isolates, as expected. Sequencing of representative clones is in progress.

Regeneration of infectious molecular clones would require, at minimum, supplementation of the small segment of DNA in the LTR region that is duplicated in the genome and could not be included in the PCR amplicon. A restoration vector that permits regeneration of the 0.15 KB segment, amplified separately from each isolate, is under construction. This will permit selection for infectious molecular clones using *in vitro* transfection procedures.

Studies of HIV-1 evolution, diversity, recombination, and pathogenesis should be greatly enhanced by this strategy for recovery of essentially intact proviral DNA genomes from a full range of HIV-1 variants.