FRONTIERS IN HIV PATHOGENESIS

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Poster Sessions

Acute Infection

Q 001 ANALYSIS OF HIV PROVIRAL BURDEN AND VIRAL EXPRESSION DURING PRIMARY HIV INFECTION, Cecilia Graziosi[1], Giuseppe Pantaleo[1], James F. Demarest[1], Michael S. Saag[2], George M. Shaw[2], and Anthony S. Fauci[1], [1]Laboratory of Immunoregulation, NIAID, Bethesda, MD 20892, [2]University of Alabama, Birmingham.

A significant percentage (50% to 70%) of patients with primary HIV infection experience an acute mononucleosis-like syndrome approximately 3 to 6 weeks after initial infection. This period is associated with viremia (i.e. high levels of p24 antigen and high titers of infectious virus in the plasma). Resolution of symptoms and a precipitous fall in viremia occur within a period of 2-4 weeks and is associated with the emergence of HIV-specific antibody.

In the present study, we have analyzed the temporal aspects of HIV DNA and RNA synthesis in peripheral blood mononuclear cells (PBMC) of HIV infected individuals during primary HIV infection.

Sequential determinations of HIV-1 burden and expression were performed in PBMC isolated from 4 patients. The first sample of PBMC was obtained 1 to 2 weeks after the onset of symptoms, and twice weekly for the first month, then weekly or monthly thereafter. Equal numbers (5×10^4) of PBMC, collected at different time points after the onset of symptoms, were simultaneously analyzed for the presence of HIV-1 proviral DNA by PCR. The frequency of HIV infected cells was estimated by comparing the relative intensity of the PCR signals observed at each time point with those from tenfold serial dilutions of ACH-2, a chronically infected T cell clone containing one proviral copy per cell. By using gag and LTR primer pairs, the estimated frequency of HIV-1 infected cells at the initial time point was 1/500 in PBMC, and no significant changes were observed over time in 3 of 4 patients. In patient #4 however, the frequency of HIV-1 infected cells found with gag primers peaked (1/500) at day 13, and declined significantly, at least 1 log, by day 27. More importantly, in patients #2 and #4 we observed a discrepancy in the frequency of HIV-1 infected cells following amplification with gag and LTR primer pairs. By using LTR primers, the frequency of HIV infected cells was significantly higher (1/2 to 1 log) compared to that observed with primers for gag, indicating that a pool of circulating MC contains incompletely reverse transcribed HIV genome during primary HIV infection.

Total RNA was prepared from the individual PBMC samples collected over time, reverse transcribed and amplified with primer pairs specific for structural (gag, env) and regulatory (tat/rev) mRNAs. In all 4 patients, high levels of HIV-specific messages for both structural and regulatory proteins were observed in the circulating mononuclear cells in the first 2 weeks from the onset of symptoms. A significant decline (several logs) in the levels of virus replication was observed between the second and the third week and by week 4 the levels of virus replication were barely detectable or undetecable.

These results demonstrate that during primary symptomatic infection, HIV genome may exist in an incompletely reverse transcribed (non full-length DNA) and a completely reverse transcribed (full-length) form in infected cells. In addition, high frequencies of HIV infected cells and transient high levels of HIV specific messages for both structural and regulatory proteins are found in PBMCs of patients during primary HIV infection.

Q 002 THE IMMUNE RESPONSE TO ACUTE HIV-1 INFECTION, Richard A. Koup[1], Jeffrey T. Safrit[1], Yunzhen Cao[1], Charla A. Andrews[1], Charles Farthing[2], William Borkowsky[2], Gavin McLeod[3], and David D. Ho[1], [1]Aaron Diamond AIDS Research Center, New York, NY 10016, [2]New York University Medical School, New York and [3]New England Deaconess Hospital, Boston.

Individuals experiencing acute symptomatic infection with HIV-1 exhibit, in their peripheral blood, high viral loads which subsequently and rapidly decline. Definition of the immune mechanisms responsible for this control is important for our understanding of HIV-1 pathogenesis and for the development of immune therapeutic and vaccine strategies. In order to address this area, intensive virologic and immunologic evaluation was carried out during or soon after symptomatic HIV-1 seroconversion in three individuals. The first patient was identified during the symptomatic phase of acute infection before seroconversion. The patient seroconverted 3 weeks later and was followed for >11 months. Serum p24 assays and end-point dilution culture of plasma and peripheral blood mononuclear cells (PBMC) indicated that HIV-1 was decreasing even before seroconversion. HIV-1 was eliminated from plasma and severely reduced in PBMC by 5-8 weeks after presentation. Antibodies capable of neutralizing the patient's own HIV-1 isolates were not detected until 11 weeks after presentation, and no antibody response capable of neutralizing laboratory strains of HIV-1 were detected during the 11 months of follow-up. In addition, no antibodies capable of mediating antibody-dependent cellular cytotoxicity (ADCC) against laboratory strains of HIV-1 were detected. A transient rise in NK cell activity was noted during the symptomatic phase of infection. Cytotoxic T lymphocyte (CTL) precursors specific for HIV gag, pol and envelope antigens were detected 2 days after presentation. The precursor frequency of CTL with all three specificities rose during the first 5 weeks, and then decreased to levels observed in chronically-infected persons by 15 weeks after presentation. HLA class 1-restricted CTL clones specific for envelope and gag were isolated from a blood sample drawn 10 days after presentation.

To confirm the presence of HIV-1-specific CTL early in HIV-1 infection, two other patients who were identified shortly after seroconversion were studied. In both cases HLA class 1-restricted CTL clones specific for HIV-1 envelope antigens were isolated from time points before neutralizing antibody responses were apparent. Specific characteristics of these CTL clones will be presented. These studies indicate that the initial neutralizing antibody response in acute HIV-1 infection is specific for the infecting virus strain and occurs after the virus load has already decreased. The group-specific neutralizing and ADCC responses occur much later in infection. CTL, on the other hand, are apparent in PBMC early during infection, even before seroconversion. These CTL are HLA class 1-restricted, and rises in the precursor frequency of these CTL correlate well with reductions in virus load. Virus-specific CTL are likely to represent the major immune response capable of controling HIV-1 replication during acute infection.

Q 003 *IN VIVO* USE OF A CD4-SPECIFIC MONOCLONAL ANTIBODY IN SIVmac-INFECTED RHESUS MONKEYS. Keith A. Reimann*, Linda C. Burkly[+], Richard L. Cate[+] and Norman L. Letvin* *New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772 and [+]Biogen, Inc., Cambridge, MA 02142

Monoclonal antibodies (mAb) specific for CD4 are potent inhibitors of HIV replication *in vitro*. We employed rhesus monkeys and the simian immunodeficiency virus of macaques (SIVmac) to explore the safety and potential usefulness of an anti-CD4 mAb *in vivo* as an AIDS therapy. The anti-CD4 mAb 5A8 efficiently inhibits HIV and SIVmac *in vitro* replication and virus-induced cell fusion. This antibody binds to D2 of the CD4 molecule and inhibits virus replication at a post-virus binding step. A single 3 mg/kg infusion of mAb 5A8 into normal rhesus monkeys coated all circulating and lymph node CD4 cells for 4-6 days. CD4 cells were not cleared from circulation nor was the CD4 molecule modulated from the lymphocyte surface. In fact, administration of mAb 5A8 resulted in a 50-100% increase in absolute number of circulating CD4 cells. With repeated administrations in normal rhesus monkeys, CD4 lymphocytes remained coated with mAb for >9 days. PBL of these monkeys retained normal *in vitro* proliferative responses to mitogens and these animals generated normal humoral responses *in vivo* to tetanus toxoid. When administered to monkeys infected with SIVmac, an increase in circulating CD4 cells was also seen. Infected monkeys infused with a mAb with an irrelevant specificity showed a transient, marked increase in SIVmac provirus in PBL as measured by a quantitative polymerase chain reaction technique. In contrast, anti-CD4-treated infected monkeys showed either a decrease or no change in PBL SIVmac provirus during the treatment period. Loss of cell coating with mAb in both normal and infected monkeys corresponded to the appearance of anti-mouse Ig antibodies. Thus, administration of the anti-CD4 mAb 5A8 achieved CD4 cell coating without severe immunosuppression. Anti-CD4 mAb may exert a specific antiviral effect in AIDS virus-infected individuals.

Q 004 THE USE OF QUANTITATIVE COMPETITIVE PCR (QC-PCR) AS A MEASUREMENT OF ANTIRETROVIRAL DRUG ACTIVITY, M.S. Saag[1], M. Piatak[2], Jr., J.C. Kappes[1], S.J. Clark[1], K.C. Luk[2], L.M. Yang[2], B.H. Hahn[1], E.A. Emini[3], J.D. Lifson[2], and G.M. Shaw[1], [1]University of Alabama at Birmingham, Birmingham, AL 35294, [2]Genelabs Incorporated, Redwood City, CA 94063, and [3]Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Current clinical trials of antiretroviral agents often utilize laboratory studies, such as CD4 counts, HIV-1 p24 antigen levels (both regular and acid-dissociated), quantitative cell cultures, and levels of infectious HIV-1 in plasma, to assess the relative antiretroviral activity of drugs used in the treatment of HIV-1 infection. These so-called "surrogate markers" of drug activity offer the advantage of earlier determination of endpoints in clinical trials, thereby decreasing the time of development of new therapeutic approaches. However, many of these markers may be present at low or nondetectable levels, especially in early asymptomatic disease, thereby limiting their utility. Standard PCR techniques have shown promise as a surrogate marker of viral burden, but are generally only semiquantitative because of variability in the efficiency of amplification and the use of external quantitative standards. Accurate measurement of plasma virion-associated RNA by Qunatitative-Competitive-PCR (QC-PCR) because of its use of internal standards and methods to enhance recovery of viral RNA, offers the potential of direct virologic assessment in virtually all HIV-1 patients, regardless of clinical stage or CD4 count (see Piatek, et al, this volume). In this study, we applied QC-PCR to a subset of existing specimens obtained in a recently conducted comparative study of L-697,661 at three different doses versus zidovudine. Antiretroviral therapy resulted in a prompt and reproducible 4.8-fold reduction in viral RNA levels for zidovudine treated patients and a dose-related 1.6 to 5.2-fold reduction in L-697,661 treated patients. HIV-1 virion RNA levels returned to baseline in L-697,661 treated patients who developed resistance to the drug within 6 weeks.

Based on the high sensitivity (> 98%) of this assay, even in early asymptomatic patients (CD4 > 500; Piatek et al), the dynamic range of response to antiretroviral therapy, the ability to utilize stored plasma specimens in a retrospective (batched) fashion, and the high degree of reproducibility of this assay, we conclude that QC-PCR is a promising surrogate marker of antiretroviral activity in clinical trials of HIV-1 infection.

Q 005 IMPLICATIONS OF PERSISTENT PLASMA VIREMIA IN ACUTE AND EARLY-CHRONIC INFECTION IN HIV-1 NATURAL HISTORY AND PATHOGENESIS. G. Shaw, M. Saag, S. Clark, J. Kappes, B. Hahn, L.-M. Yang, K.-C. Luk, M. Piatak, and J. Lifson. The University of Alabama at Birmingham, Birmingham, AL, 35294 and Genelabs Inc., Redwood City, CA, 94063.

The acute retroviral syndrome associated with primary HIV-1 infection is characterized by clinical signs of immune activation, multi-system organ dysfunction, and high levels of viremia, p24 antigenemia, and proviral burden. Clinical symptoms associated with acute HIV-1 infection and measures of viral burden and replication generally decline in association with seroconversion. A central paradox of AIDS involves the early and progressive development of immunologic abnormalities, including CD4 cell depletion, lymphocyte hyporesponsiveness to recall antigens, and oligoclonal/polyclonal B-cell activation, at a time when conventional assays of virus replication and burden in blood suggest minimal levels of viral replication throughout much of the disease course. Comparative analysis of viral sequences in blood and lymphoid tissue suggests that the latter compartment represents a preferential site of viral replication. We have utilized a new method for accurately quantifying virion-associated RNA in plasma (Quantitative Competitive-Polymerase Chain Reaction, QC-PCR; see Piatek et al., this volume) along with quantitative plasma virus culture, regular and ICD-p24 Ag measurements, and CD4+ cell counts to examine the dynamics of viral replication during the acute and early-chronic stages of infection. In 6 patients studied sequentially for 1-3 years, infectious virus in plasma reached peak titers as high as 10,000/ml and p24 Ag levels as high as 5405 pg/ml within 14 days of symptom onset (CDC stage I). In 5 of 6 patients, infectious plasma viremia cleared completely by 14 to 46 days of illness, whereas in only 2 of 6 patients had p24 Ag cleared by day 75. Measurement of ICD-p24 Ag did not increase the sensitivity of p24 Ag detection until at least 112 days after symptom onset, and then, only in 2 patients. In contrast to all other virologic markers assayed, QC-PCR determined virion RNA levels were positive in all patients at all time points. Peak viral RNA levels in acute infection ranged from $3.6x10^5$ to $2.2x10^7$ copies/ml plasma (corresponding to $1.8x10^5$ to $1.1x10^7$ virions/ml), fell by approximately 100-fold with resolution of the acute syndrome, and then persisted in the range of 10^3 to 10^5 virions/ml plasma. Total virion levels measured by QC-PCR approximated virus titers that were calculated from circulating p24 Ag levels (assuming 1 pg p24 Ag is equivalent to approximately 10^3 to 10^4 virions) and exceeded titers of infectious virus by 1,000 to 10,000-fold. We have extended these analyses to include 59 additional consecutively studied patients at all stages of infection and with CD4 counts ranging from 0 to 1080/mm^3. The results of these studies reveal persistent circulation in the plasma of virtually all patients of moderate to high virus loads that previously have gone undetected by conventional culture techniques. The pathophysiologic implications of persistent plasma viremia and the potential utility of plasma virion-associated RNA as a new and direct measure of the *in vivo* activity of novel chemotherapeutic and immunotherapeutic regimens will be discussed.

Viral Load; Treatment Effects

Q 006 EFFECT OF ANTIVIRAL TREATMENT AND DISEASE STATE ON HIV VIRUS LOAD. Mark Holodniy, VA Medical Center, Palo Alto, CA, and Stanford University, Stanford, CA.

Quantification of HIV viral load has become increasingly important in the management of HIV disease. Current measurements of HIV disease activity include indirect measurements such as CD4 count or P24 antigen or direct measurement of viral load utilizing quantitative culture techniques to assess plasma on PBMC viremia or PCR techniques to assess plasma virion associated RNA on PBMC chromosomal/episomal forms of DNA or mRNA. In addition to peripheral blood, viral load in lymph node tissue or other body compartments are now starting to be assessed.

PCR has been widely applied to the detection of HIV proviral DNA from PBMC for diagnosis of HIV infection. Quantification of HIV RNA or DNA by PCR techniques has also been reported utilizing internal or external reaction standards. Quantification has demonstrated a wide spectrum of copy number across stage of disease, but generally increased copy numbers of both DNA or RNA have correlated with advancing disease. However, in order to assess whether quantitative changes are meaningful, it is necessary to determine constancy of signal over a short term observation period. Precision data to date suggests that inter and intra assay variability are low and hence values obtained from representative samples are reproducible. As these assays become standardized, it becomes important to understand whether nucleic acid copy number fluctuates overtime in a patient who is clinically stable. It appears that upon weekly sampling, copy number for both plasma RNA and PBMC DNA appear constant. There may, however, be diurnal fluctuation in some patients. Certain infections, particularly Herpes viruses may elevate viral load acutely. Clinical progression of HIV disease, likewise may elevate viral load levels. These variables will need to be accounted for in assessing antiviral response.

The response to antiviral therapy is only now beginning to be studied by PCR techniques. In general, proviral DNA and plasma RNA copy number are decreased in subjects receiving antiretroviral therapy, although this may be dependent on the length of therapy. When measured over time after initiation of therapy, plasma HIV RNA levels decrease more acutely than proviral DNA levels. Several small studies have reported responses to nucleoside analogues or immunomodulatory agents. Responses are certainly not uniform in all subjects. Large scale evaluation of these techniques is currently ongoing in some ACTG trials. Finally, sites other than blood, such as lymph nodes and semen, have shown quantitative changes in viral load in response to antiviral therapy. Studies are ongoing to see whether this response is concordant with blood viral load.

Q 007 STUDIES ON VIRUS LOAD AND SEQUENCE IN HIV TRANSMISSION, Andrew J. Leigh Brown, Lin Qi Zhang, Pamela Robertson, Alexander Cleland, Edward C. Holmes and Peter Simmonds, University of Edinburgh, Edinburgh, Scotland.

There are dramatic differences between the viral population in primary HIV infection and that associated with later stages in the disease. We have detected viral titres of the order of 10^7 particles per ml. in both hemophiliacs and patients infected by heterosexual contact, whereas viral titres found after several years of infection may be lower than 10^2 per ml. in some asymptomatics. Furthermore, in primary infection, regions of the *env* gene usually characterised by high levels of variability have been found to be completely monotypic. An average of 26 sequences of the V3 region and 19 of the V4 region were obtained from each of 5 patients in acute HIV infection, by consensus sequencing of cDNAs isolated by limit-dilution. 1) No variation in either the amino acid or the nucleotide sequences was detected within any of the plasma samples in these regions. The within-patient frequency of variable nucleotide sites in the V3 region was less than 2×10^{-4} (*cf:* more than 1/200 in the plasma of a long-term infected patient). 2) DNA from HIV provirus present in PBMC samples within 6 months after seroconversion was also monotypic and identical to the plasma sequence found before seroconversion. (3) Despite the complete lack of variation in *env*, variation was detected in the p17 region of the *gag* gene in 2/4 plasma virus samples from primary infections and 5/5 post-seroconversion provirus samples. The average frequency of variable sites was approximately 2×10^{-3} (*cf:* 3×10^{-3} in late infection), and about 3/4 of the substitutions occurred at silent nucleotide sites.

We propose that the HIV infection is frequently initiated by a pool of virus particles from which *env* gene variants which are best adapted to establishing an infection are selected. The V3 loop itself appears to be a major target of selection: in 2 epidemiologically unlinked infections the amino acid sequences, but not the nucleotide sequence, were identical to each other and to the "global consensus". In addition, 3/4 haemophiliacs infected following exposure to the same batch of factor VIII were found to have identical proviral V3 sequences in samples taken 6 months after infection.

Early Events

Q 008 **NON-CD4 MOLECULES ASSOCIATED WITH HIV BINDING AND ENTRY**, Jacques Fantini, David G. Cook, Neal Nathanson, Jeffrey Peterson, Steven L. Spitalnik, and Francisco Gonzalez-Scarano, Departments of Neurology, Microbiology and Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA 19104-6146 and University of Provence, Marseille, France

Previous studies have implicated galactosyl ceramide (galactocerebroside, GalCer) in HIV-1 binding and entry into neural cells[1], and independently, into the colonic adenocarcinoma derived cell line HT29[2]. To extend these studies, in which antibodies against GalCer were shown to inhibit HIV-1 entry and infection, we subcloned the HT29 colonic adenocarcinoma cells into high and low expressors of GalCer and assessed (1) their HIV-1 infectability and (2) their ability to bind recombinant HIV-1 gp120 both in culture and in lipid extracts.

Single cell clones of HT29 cells were obtained and three clones representing high expression of GalCer (clone A7), medium expression (clone D4) or low expression (clone D9) were tested for their ability to support replication of three HIV-1 strains, IIIB, NDK or 89.6. There was a clear correlation between the level of GalCer expression, as detected by either immunofluorescence or HPTLC, and productive infection with either HIV-1(*IIIB*) or HIV-1(NDK). HIV-1(89.6), a macrophage-tropic strain, did not infect any of these cell lines. HIV-1 infection in the HT29-A7 cells, which was easily detectable by assay for p24 in the supernatant or by immunofluorescence, was inhibited by an anti-GalCer monoclonal antibody. Recombinant gp120 bound to lipid extracts of HT29 and its GalCer expressing clones, but barely or not at all to extracts of HT29-D9 or to the non-infectable Caco-2 cells, another intestinally derived, GalCer negative line. These results were confirmed with pseudotype viruses that expressed the HIV-1 glycoproteins on Cocal virus (a rhabdovirus).

1. Harouse et al, Science 253, 320, 1991
2. Yahi et al, J. Virol. 66, 4848-4854

Q 009 STRUCTURAL ANALYSIS OF THE HIV-1 ENVELOPE GLYCOPROTEIN gp120, John P.Moore[1], Marcus Thali[2], Bradford A.Jameson[3], George K.Lewis[4], Quentin J.Sattentau[5], and Joseph Sodroski[2]; [1]Aaron Diamond AIDS Research Center, New York, [2]Dana-Farber Cancer Institute, Boston, [3]Jefferson Cancer Institute, Philadelphia, [4]University of Baltimore, [5]Centre d'Immunologie de Marseille-Luminy.

We are analysing the structure of HIV-1 gp120 immunochemically. Thus site-specific monoclonal antibodies (Mabs) to linear epitopes are used to determine which regions of the glycoprotein are exposed on the surface of the native, recombinant protein, and which are buried in the interior and exposed only after denaturation of the protein. The epitopes of these Mabs are determined using gp120 point mutants and peptide binding assays. By this method, we can show that most of the constant regions of gp120 are not exposed on the protein surface, or only poorly so. Some constant regions near the N- and C-termini that are exposed on the recombinant protein, are hidden on oligomeric gp120 expressed on the surface of infected cells, probably because they are occluded by gp41 or other gp120 molecules. Most variable regions of gp120 are exposed on the gp120 surface, but in many instances Mab binding to these regions is poor, perhaps because of shielding carbohydrate moieties. We are using antibody mapping data to prepare computer models of individual gp120 regions.

The binding of Mabs to one domain can be affected by mutations in another region of gp120. Thus the binding of two Mabs to C4 is abolished by a V3 loop mutation, while four other Mabs binding to the same C4 peptide are unaffected by the V3 change. Conversely, mutations at the base of the V3 loop can increase binding of Mabs to discontinuous epitopes elsewhere in gp120. Thus there may be a functional interaction between the V3 and C4 domains of gp120. Other preliminary studies suggest an interaction between regions of the C1 and C2 domains that may be important for overall gp120 configuration. By mapping the epitopes for Mabs to discontinuous epitopes, we are gaining an understanding of the inter-domain interactions in gp120. We will also present data on the epitopes for 7 neutralising and non-neutralising Mabs to the V1/V2 loop structure on gp120.

Tropism; Co-Factors

Q 010 ASPECTS OF THE MOLECULAR PATHOGENESIS OF AIDS, Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

I will report progress in three areas of HIV research in our laboratory.

1) New results on the immune pathogenesis of AIDS that may provide additional explanation for the T4 cell decline, the eventual CTL and NK cell decline and abnormalities, and the abnormality of lowered antigen activation of T cells.

2) Pathogenesis of Kaposi's sarcoma (KS). Recent results implicate Tat, not only a growth factor for KS spindle cells, but as having properties of a prototypic angiogenic factor. These results and data on the Tat receptor will be summarized.

3) The results on inhibition of HIV replicaiton by antisense and by gene therapy approaches, and of KS by antisense and by angiogenesis inhibition will also be summarized.

The KS experiments described here have chiefly been conducted by B. Ensoli and G. Barillari of this laboratory, and A. Albini (Genoa); the anti-HIV studies by M. Klotman, J. Lisziewicz, D. Sun, and J. Smythe of this laboratory.

Regulation

Q 011 HIV-SPECIFIC T HELPER CELL RESPONSES IN EXPOSED, SERONEGATIVE, INDIVIDUALS.
Mario Clerici[1], Jay A. Berzofsky[2], and Gene M. Shearer[1], [1]Experimental Immunology and [2]Metabolism Branches, National Cancer Institute, NIH, Bethesda.

The immune response to HIV after infection and in vaccine trials is characterized by the production of antibodies and the development of cell mediated immunity - including both helper and cytotoxic T lymphocyte responses. Assays for serum antibodies are routinely used to detect infected individuals, and to assess the potential of vaccines. However, hundreds and probably thousands of indiividuals have been exposed to HIV, but have not seroconverted. In collaboration with investigators from several centers, we have tested the PBMC of seronegative individuals who are at significant risk for HIV infection or are known to have been parenterally exposed to HIV for specific T helper cell (TH) activity. These TH responses were assessed by HIV antigen-stimulated interleukin 2 (IL-2) production to synthetic peptides corresponding to five gp160 *env* determinants and to *pol*. A TH response was considered positive if two or more peptides stimulated IL-2 production by at least a four-fold increase in IL-2 units compared to unstimulated PBMC from the same donor. These seronegative, at-risk individuals who exhibited *env*--specific TH responses included: a) 68% of exposed gay men; b) 45% of IV drug users; c) 75% of exposed health care workers; and d) 38% of newborn infants of HIV-infected mothers. The infants who were subsequently found to be seropositive were not among those who exhibited *env* TH responses at birth. Our results of HIV-exposed individuals who are T cell reactive but antibody-negative resembles the early rgp160 vaccine trials, in which low dose immunization resulted in T cell responses but not antibody production. Our findngs suggest an important role for cell mediated immunity in protection against AIDS and in early diagnosis of exposure to HIV, and have potential implications for the design of efficient AIDS vaccines.

Q 012 **HIV-1 TAT AS AN EXTRACELLULAR REGULATOR OF HIV-1 ACTIVITY, Ofra Weinberger[1], Adriana Marcuzzi[1], Jay Dobkin[2], and Christian A. Thomas[1], [1]Department of Physiology and Cellular Biophysics and [2]Department of Medicine, Columbia University, New York, NY.**

An understanding of HIV-1 infection must involve consideration of the direct effects of viral gene expression in infected cells, as well as transcellular effects on surrounding uninfected cells. HIV-1 *tat* expression alters the regulation of a number of cellular genes, e.g. interleukin-1β and fibronectin, in *tat*-expressing cells, and also mediates transcellular activation of HIV-1 LTR-directed gene expression in cocultured cells. Transcellular activation is TAR-dependent, proceeds with rapid kinetics, and at ratios as low as 1 HIV-expressing cell to 10,000 target cells. Expression of *env* in addition to *tat* facilitates transcellular activation. In contrast to HIV-mediated fusion and infection, the events that follow CD4-gp120 binding in transactivation do not require the gp120-neutralizing domain. We hypothesized that this "transcellular activation" may contribute to activation of the HIV-1 provirus in latently infected cells.

Transcellular activation is evident with a variety of donor or recipient cell lines and with HIV-1 infected PBMC from healthy donors, as well as with freshly isolated PBMC from HIV-1 infected patients in various stages of the disease. Thus, transcellular activation occurs with both primary isolates as well as with laboratory adapted strains of HIV-1. Furthermore, in cocultures of infected cells with "indicator cells", i.e. various cell types transfected with LTR-CAT, activation of the HIV-1 LTR occurs in cells that are not infectable with HIV-1, indicating that transmission of viral particles is not required for this activity. Transcellular activation of surrounding cells thus appears to be a distinct component of the events that follow infection with HIV-1. To determine whether transcellular activation can result in the activation of a latent or a defective provirus, cocultures were established of *tat*-expressing U937 cells with CEM cells into which a *tat*-defective HIV-1 provirus had been transfected. Viral replication was activated in these cocultures, as determined by syncytia formation and increased culture RT activity. We examined the ability of cells expressing transcriptional activators of DNA tumor viruses to activate viral promoters in cocultured cells. The results suggest that transcellular activation may be a general mechanism that is not unique to HIV-1 *tat*.

Transmission

Q 013 MATERNAL-FETAL TRANSMISSION OF SIV IN THE MACAQUE. Michael Murphey-Corb, Billie Davison-Fairburn, Gary B. Baskin. Tulane Regional Primate Research Center, Covington, LA 70433.

We have previously shown that inoculation of 10 pregnant rhesus monkeys with SIV/DeltaB670 induced 2 abortions coincident with rapid disease progression in the female. The remaining neonates were delivered 10 days prior to term by Caesarian section and found to be uninfected. More recently, 5 other pregnant females were similarly inoculated, and one female inoculated prior to conception, and then allowed to vaginally deliver to access the contribution of vaginal delivery in promoting perinatal transmission. Four other females were inoculated with a nonpathogenic infectious clone of SIV (SIVmacBK-28) and allowed to vaginally deliver. Of the females which had vaginally delivered infants, 5 of 6 became immunodeficient during their pregnancies. Of these, 2 delivered SIV-infected infants: one stillborn and one livebirth that lived 7 days. All 4 animals inoculated with the SIV clone BK-28 delivered uninfected offspring.

Histopathological examination of placental tissue from both abortuses and the 2 infected infants revealed lesions within the decidua and/or chorion. Interestingly, only one of 8 placentas from the 10 day pre-term Caesarian deliveries exhibited placental pathology whereas 6 of 8 placentas recovered after vaginal deliveries exhibited lesions. Taken together, these data suggest that in the rhesus monkey placental lesions induced either by opportunistic infections early during gestation or as a result of placental senescence at term may promote entry of SIV into placental tissue. Additional factors are also required, including pathogenicity of the infecting strain of SIV and active progressive disease in the mother during pregnancy.

Five pregnant cynomolgus monkeys were also inoculated with SIV/DeltaB670 during various stages of gestation to examine maternal-fetal transmission in this species. All 5 females developed severe thrombocytopenia and anemia. Two fetuses died in utero. One fetus was severely autolysed. SIV was identified by PCR in the placenta and aminonic fluid, but not the fetus, in the second. A third female aborted at 111 days gestation, 61 days PI. This fetus was also PCR negative. A fourth female delivered a full-term infant that lived for 24 hours before dying of pneumonia and funisitis, indicating possible placentitis; this infant was PCR positive. The fifth infant appeared clinically normal at birth, but was PCR positive. Ultrasonography showed possible intrauterine growth retardation of several of the infants. These data suggest that SIV/DeltaB670 is highly pathogenic in cynomolgus monkeys, and induces placental insufficiency and fetal demise in pregnant females.

Q 014 GENETIC VARIATION AND HIV TRANSMISSION. Gerald Schochetman, Laboratory Investigations Branch, Division of HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, GA 30333.

For viral infections of humans, molecular biologic techniques to analyze viral genetic sequence information have been used to study viral transmission from person to person, within communities, and between countries. Requisite to such studies is the existence of viral genetic variation; the greater the variation, the greater the ability of the method to distinguish strains of the virus. For a virus with significant genomic variation, identification of strains with a high degree of genetic relatedness may imply an epidemiologic linkage between persons infected with these strains. HIV is known to undergo a rapid genetic evolution, such that HIVs from different individuals are found to be genetically distinct. The extent of genetic diversity of HIV strains in a region is a function of the length of time that it is present in the population. In the current talk I will discuss the use of PCR followed by DNA sequencing to study potential person to person transmission of HIV in cases of public health importance. I will also discuss the genetic variation of HIV from geographically distinct regions of the world and the difficulty of using this technology in regions where the virus has recently been introduced. In these areas (e.g., Thailand) there exists a relatively small inter-person HIV genetic variation making tracking of transmission more difficult.

Pathogenic Mechanisms

Q 015 PROGRAMMED CELL DEATH AND AIDS PATHOGENESIS, Jean Claude Ameisen, Hervé Groux, Jérôme Estaquier, Thierry Idziorek, André Capron, Unité INSERM U167-CNRS 624, Institut Pasteur, Lille, France

Programmed cell death (PCD) is a physiological cell suicide process that plays an essential role in embryonic development, in particular in shaping the immune and nervous systems, and in adult tissue renewal. Unlike cell degeneration or necrosis, PCD can be induced or prevented by the withdrawal or the addition of activation signals.

We have proposed[1] that most immunological and non-immunological defects observed in HIV-infected individuals could be related to the inappropriate induction of PCD in various cell populations including CD4+ T cells and neurons, as a consequence of indirect interference of HIV with inter- and intracellular signalling. This model postulated that PCD could provide an explanation for both the early dysfonction and the late depletion of CD4+ T cells that lead to AIDS, and that modulation of cell signalling may prevent T-cell death.

Experimental support for this model has been provided by observations from our laboratory and others, indicating that T cells from HIV-infected people are abnormally programmed to undergo PCD, in particular in response to *in vitro* stimulation[2-4]; that *in vitro* HIV infection of CD4+ T cells leads to PCD[5,6]; and that CD4 cross-linking by gp120 plus anti-gp120 antibodies leads in normal human CD4+ T cells to PCD in response to T-cell receptor (TCR) stimulation[7] (for a review, see Ref. 8).

Results presented here will concern

1°) the *in vivo* relevance to pathogenesis of *in vitro* T-cell PCD: the correlation, in simian models of HIV and SIV infection, between abnormal T-cell programming for PCD, and the *in vivo* pathogenic nature of the lentiviral infection; simian models include HIV-1-infected chimpanzees, SIV-infected african green monkeys, and macaque rhesus infected either with pathogenic or non pathogenic strains or clones of SIV;

2°) the mechanisms of HIV-mediated T-cell PCD induction: the capacity of HIV to prime *in vitro* normal human CD4+ T cells - in conditions that neither interfere with TCR-mediated T-cell proliferation, nor require productive T-cell infection by HIV - for PCD in response to subsequent TCR restimulation; and the nature of HIV gene products involved in this process;

3°) the cellular mechanisms involved in the induction and prevention of *in vitro* T-cell PCD.

Significance and implications of these findings will be discussed in the context of other observations suggesting that T-cell PCD may also participate into extrathymic tolerance and into the physiological regulation of the immune response.

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Q 016 IMMUNOPATHOGENIC MECHANISMS OF HIV INFECTION, Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Following initial infection with HIV, wide dissemination of virus as detected by high levels of plasma viremia occurs. During this time period, high levels of virus replication are associated with the retrafficking of lymphocytes to various lymphoid organs. Following this acute infection, there is an immune response to HIV which is associated with the curtailment of plasma viremia and the initiation of a state of clinical latency. During this period of clinical latency, there is very little detectable virus in the blood as measured by plasma viremia or as mononuclear cells which express HIV DNA or RNA. By examining the lymphoid tissue and peripheral blood in a number of HIV-infected individuals at various stages of disease, we have demonstrated that even early in the course of infection there is sequestration of virus in the lymphoid tissues with trapping of large amounts of extracellular virions in the germinal centers of lymph nodes by follicular dendritic cell (FDC). During the clinically latent phase the virus continues to replicate and the FDC network begins to involute gradually leading to a "burned-out" stage during which time the FDC can no longer effectively filter and trap HIV. Concomitant with this phenomenon, HIV spills over into the peripheral blood and is manifested as accelerated viremia and ultimately advanced HIV disease. In addition, we have investigated the role of the thymus in HIV infection using the SCID-hu mouse model, which consists of a SCID mouse into which human fetal thymus and liver are placed and human thymopoiesis subsequently occurs. HIV infection of the SCID-hu mouse thymus results in infection of the thymocytes, first manifested by infection of the CD4 single positive subset, followed by infection of the CD4/CD8 double positive cells and the CD8 single positive cells. Additionally, disruption of the thymic microenvironment, with thymic epithelial (TE) cell degeneration and direct HIV infection of the TE cells, is observed. HIV induced depletion of intrathymic T cell precursors and the disruption of the nurturing thymic microenvironment have implications for further understanding of the immunopathogenesis of HIV infection.

Q 017 INHOMOGENEITY OF HIV PROVIRAL COPY NUMBER AND SEQUENCE COMPLEXITY IN SPLEENIC WHITE PULPS, Rémi Cheynier[1], Eric Pelletier[1], Brigitte Autran[2], and Simon Wain-Hobson[1], [1]Unité de Rétrovirologie Moléculaire, Institut Pasteur, Paris, [2]Laboratoire d'Immunologie Cellulaire et Tissulaire, Hôpital Pitié-salpétrière, Paris.

We have continued our work on HIV-1 infected spleens from two patients one of whom (S) presented with Castleman's syndrome (accompanied by IL-6 over production) while the second (L) presented a marked CD8 lymphocytosis. Spleenectomy followed on from untreatable thrombocytopenia. Over 100 individual splenic white pulps were microdissected from the material and treated with proteinase K and lysis buffer. HIV sequences corresponding to the envelope hypervariable regions VI+V2 were amplified. These regions were most useful as they exhibited substantial length variation which constituted an alternative marker, albeit of lower resolution, for sequence variation. All 54 of the white pulps examined from patient S were strongly positive for HIV. In addition there was considerable length variation. Amplified material was cloned from four white pulps and 20 clones sequenced. Extraordinary sequence complexity was encountered - up to 15% at the amino -acid level within a single white pulp.There was no sequence common to all four white pulps. In the case of the second spleen (patient L) only 9/40 white pulps harboured HIV. There were many fewer proviruses within these nine than was seen for patient S as judged by the intensity of the bands and length polymorphisms, suggesting more restricted replication of HIV-1. Sequencing of cloned products from these white pulps is in progress. While limited to only two cases it is fascinating to note the extensive HIV sequence variation associated with general stimulation of the immune system (patient S). This lends support to the notion that immune system reactivity and HIV burden are linked. It is perharps not prudent to attribute directly the paucity of HIV in spleenic white pulps to the CD8 lymphocytosis (patient L). An analysis of the complexities of the T cell repertoires will be attempted.

Animal Models

Q 018 ANALYSIS OF NOVEL NEUTRALIZING DETERMINANTS ASSOCIATED WITH RECEPTOR-BINDING FOR SIVAGM, Jonathan S. Allan, Evelyn Whitehead, Mary Short, Kevon Shuler, Raymond Dunham, and Patrick Kanda, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX 78228.

The process of viral entry is theorized to include a complex series of events involving the modulation of viral envelope. The attachment step of gp120 binding to CD4 has been well-characterized whereas the events that follow are largely unknown. Naturally-occurring viruses may provide clues to both the pathogenesis for HIV-1 and may aid in answering fundamental molecular mechanisms in viral entry. We have previously shown that soluble forms of CD4 (sCD4) act on SIVagm, a virus from African green monkeys, by enhancing in vitro infectivity while its effects are to block HIV-1 infection. Although the requirements for virus binding to sCD4 are similar for both SIVagm and HIV-1, the ability of sCD4 to activate SIVagm and enhance infectivity indicates that sCD4 may mimic the natural event of virus binding to cellular CD4 and may therefore be useful as a model to study post-receptor binding events in viral fusion.

To determine the exact nature of receptor-mediated induction of envelope conformations that are important in entry, we have generated and screened a series of monoclonal antibodies for their ability to specifically inhibit infection of sCD4-treated SIVagm. Our previous findings indicated that significant cryptic neutralizing epitopes exist which are exposed upon sCD4 binding and activation. Monoclonal antibodies specific for these epitopes might allow for a detailed analysis of envelope determinants that might function not only as neutralizing determinants that could be novel targets in immunotherapies but also might function in viral penetration subsequent to gp120-CD4 binding. One monoclonal antibody that we have identified, differentially binds to and blocks sCD4 treated SIVagm infection. Antibodies made to one strain of SIVagm(tyo) also recognize other SIVagm viruses and additionally bind to and neutralize sCD4-treated SIVmac251, a virus that is highly related to a human virus HIV-2 and whose envelope is only 50% related to SIVagm. A broadly reactive, neutralizing antibody may provide a means for treating widely differing lentiviral infections. There is still much that must be understood before we can accurately assess the efficacy of combination therapies that include both sCD4 in conjunction with these novel neutralizing antibodies.

Cryptic neutralizing domains within the SIVagm envelope map to the V3-like loop. This region has been shown to function in HIV-1 viral entry by as yet unknown processes. Comparison of the V3-like loops from SIVagm, SIVmac and HIV-2 indicate that this region is relatively highly conserved among these viruses in contrast to HIV-1 and suggests that all of these viruses may have similar if not identical methods for achieving viral entry. It is likely that the overall mechanisms for viral entry will necessitate receptor-mediated activation of the viral envelope as a crucial step for initiating subsequent events culminating in viral fusion.

Q 019 FURTHER DEVELOPMENTS IN THE M. NEMESTRINA MODEL. L. Corey, L. Frumkin, M. Agy and W. Morton, University of Washington, Seattle, WA

Since the initial report in Science magazine, an additional 14 Macaque nemestrina have been challenged with HIV-1; 13 of these 14 have subsequently been infected. A comparison trial utilizing cell-free virus (IIIB) in previous chimpanzee studies indicated that the infectious M. nemestrina dose is from 100-500 chimpanzee infectious doses. To date, peak viral replication appears to be from 2-6 weeks post inoculation; all animals remain PCR positive and seropositive for extended time periods (20-40 weeks post inoculation), but HIV-1 has not been isolated in PBMC after week 24. The M. nemestrina continue to be easily infected with HIV-1. To date no evidence of long term disease has been manifest. Studies to utilize in vivo passage of virus to develop disease models are being initiated. The model appears to be quite useful for measuring protection against acute infection, as it allows one to evaluate the neutralization of HIV-1 isolates with varying V-3 loop regions.

Q 020 INTERACTION OF AN ACUTELY LETHAL SIVsmm WITH SIMIAN LYMPHOCYTES, Patricia N. Fultz[1], Rebecca Schwiebert[1], Liya Su[1], Binli Tao[1], Edward Hoover[2], Zimra Israel[2], Norman Letvin[3], and Zheng Chen[3], [1]University of Alabama at Birmingham, Birmingham, AL, [2]Colorado State University, Fort Collins, CO, [3]Harvard Medical School, Southborough, MA.

SIVsmmPBj14 is unique among the primate immunodeficiency lentiviruses in that it induces an acutely lethal disease characterized by extensive gut-associated lymphadenopathy and large numbers of SIV-infected lymphocytes and monocyte/macrophages in both lymph nodes and the peripheral circulation. Also unlike other lentiviruses, smmPBj14 has the ability to induce proliferation of and to replicate in resting pig-tailed macaque lymphocytes in vitro, suggesting that this virus may encode or function as a superantigen, a hypothesis currently being tested. In vivo and in vitro analysis of biologic and molecular clones of smmPBj14 suggest that these latter properties correlate with the virus' virulence. The genetic basis for the above and other phenotypic differences between smmPBj14 and its parent virus, SIVsmm9, are being determined by analysis of molecular and biologic sequential virus isolates obtained from macaque PBj between the time of initial infection and recovery of smmPBj14 (14 mos later). Preliminary results indicate that significant changes occurred between 6 and 10 months after infection. Because smmPBj14 can activate and induce lymphocytes to proliferate, we tested whether the virus could enhance transformation of PBMC from a mangabey monkey that was naturally infected with STLV-I. Following Con A stimulation and superinfection of the PBMC with smmPBj14, a continuous interleukin-2-independent mangabey T-cell line was established. This cell line expresses proteins encoded by both SIV and STLV-I, produces large numbers of retroviral particles (many of which have an abnormal morphology), and exhibits unusual properties in vitro, which are suggestive of interactions between the glycoproteins encoded by both viruses. In parallel experiments with smm9, a T-cell line expressing only STLV-I was established. Although there was no selective pressure other than the presence of an unrelated retrovirus in the co-infected cell line, extensive genomic mutations accumulated in smmPBj14. Macaques have been inoculated with the co-infected cell line, or with infectious virus recovered from it, to assess the pathogenicity of the virus(es).

Q 021 MACROPHAGE-TROPIC, NON-CYTOPATHIC HIV ISOLATES SHOW ACCELERATED CD4 T CELL DEPLETION IN HU-PBL-SCID MICE, Donald E. Mosier[1], Richard Gulizia[1], Bruce Torbett[1], and Jay A. Levy[2], [1]The Scripps Research Institute, La Jolla, CA 92037 and [2]UCSF, San Francisco, CA 94143

Isolates of HIV-1 or HIV-2 differ in their biologic behavior in cell culture with regard to cell tropism, replication rate, ability to induce cytopathic changes, and modulation of CD4 expression. We have compared a series of molecularly cloned HIV isolates that differ in these properties for their ability to cause deletion of CD4 T cells *in vivo*, using SCID mice transplanted with human PBL (hu-PBL-SCID mice) as the animal model. Hu-PBL-SCID mice are highly susceptible to infection with all isolates of HIV-1 or HIV-2 examined, and infection with the HIV-1_{IIIB} strain leads to rapid CD4 T cell depletion. The following isolates were used to compare *in vitro* properties with *in vivo* CD4 T cell depletion: HIV-1_{SF33} - T-cell tropic, high replication, cytopathic, CD4 modulation; HIV-1_{SF162} - MØ-tropic, low replication, non-cytopathic, no CD4 modulation; HIV-1_{SF2} - T-cell tropic, medium replication, cytopathic, CD4 modulation; HIV-1_{SF13} (a later isolate from the same patient as SF2) - T-cell and MØ-tropic, high replication, cytopathic, CD4 modulation; and HIV-2_{UC1} - MØ-cell tropic, low replication, non-cytopathic, no CD4 modulation. Hu-PBL-SCID mice were infected with 100 tissue culture infectious doses of each of these HIV, and CD4-, CD8-, CD3-, and CD45-positive human cells were enumerated by flow cytometry at 2 and 4 weeks after infection. In addition, the extent of viral replication was determined using quantitative PCR analysis of proviral copy number and measurement of plasma p24 core antigen concentrations. In three replicate experiments, the macrophage-tropic, non-cytopathic isolates (HIV-1_{SF162} and HIV-2_{UC1}) induced the most rapid and profound CD4 T cell depletion, and the most highly cytopathic strain *in vitro*, HIV-1_{SF33}, induced the least and slowest CD4 T cell depletion. The relative ability to induce CD4 T cell death was UC1>SF162>SF13>SF2>SF33. This ability was not correlated with the extent of viral replication, as the relative viral burden at 4 wk after infection was SF33>SF162>SF13=SF2 (UC1 was not determined). Antibody and CTL responses to HIV have not been detected within 4 wk of infection, so CD4 T cell depletion appears to be a consequence of viral infection and not immune destruction of infected cells. We conclude that the behavior of HIV isolates in tissue culture may not predict their pathogenic potential *in vivo*, and suggest that macrophage-tropism may be a marker for a more highly cytopathic effect (either directly or indirectly mediated) for human CD4 T cells in HIV-infected individuals. (Supported by NIH grants AI29182 to DEM and AI24999 to JAL).

Immune Response to HIV

Q 022 GENETIC AIDS RISK-GROUP-DEPENDENT SEPARATION AND ANTIGENIC AIDS RISK-GROUP-INDEPENDENT DIVERSIFICATION OF HIV-1 VARIANTS DURING THE CURRENT AIDS EPIDEMIC, Jaap Goudsmit, Els Hogervorst, Gabriël Zwart, Mathijs Tersmette, Jean-Jacques de Jong, and Carla Kuiken, Department of Virology, University of Amsterdam, Academic Medical Centre, Amsterdam, the Netherlands

Aim: to analyze the genotypic and phenotypic variation as defined by amino acids crucial for neutralization sensitivity and virus biology in vitro, within the third variable domain of HIV-1 gp120 during the course of the HIV-1 epidemic in three AIDS risk-groups: IV drugusers, hemophiliacs and homosexual men (1982-1992).
Method: a 276 bp fragment, including gp120 V3, was amplified by RT-PCR using RNA isolated from sera of a total of 75 seroconverters at the moment of seroconversion and, in case of disease progression, 5 years later (CD4 < 200) and subsequently directly sequenced. Antigenic variation was assessed by using the same sera in sequence matched peptide-binding assays and in a transfection inhibition assay using infectious molecular clones chimeric for the patients' own V3 domain.
Results: phylogenetic analysis showed that the V3 domains of the viruses circulating in the three AIDS risk-groups were indistinguishable on the basis of amino acid sequences and nucleotides occupying the first and second codon positions. This suggests that the mode of transmission does not greatly affect the amino acid composition of the V3 loop, indicating that ancestral information has been lost. However, the IVDU group could be separated from the other two groups on the basis of overall nucleotide changes, number of silent changes and nucleotides at the 3rd codon. The most distinguishing nucleotide was the 3rd in the Gly codon at the tip of the V3 loop (GPGR). C in 28/32 IVDU sequences versus a G or A in 12/12 hemophiliacs and 30/30 homosexual men sequences. V3 coding sequences associated with a syncytium inducing (SI) virus phenotype were only present in 4/75 (3%) seroconverters at the moment of seroconversion. Antigenic variation was highly dependent on variation at position 308 (H, P or N). A temporal increase in variation was observed at this position, indicating antigenic diversification. Preliminary studies indicate that fixation of a changed aa at position 308 occurs at similar frequency after 4-5 years of individual HIV-1 infection as in the population, indicating a relationship between these two types of variation.
Conclusion: genetic AIDS risk-group-dependent separation as well as antigenic diversification independent of the AIDS risk-group occurred during the 10 year course of the current AIDS epidemic in Amsterdam, the Netherlands.

Variation

Q 023 STRUCTURE/FUNCTION RELATIONSHIPS OF THE HIV-1 ENVELOPE GP120 IN DETERMINING HOST CELL TROPISM, CYTOPATHICITY, AND SENSITIVITY TO sCD4 NEUTRALIZATION, Cecilia Cheng-Mayer, Leonidas Stomatatos, Atsushi Koito, and Albrecht Werner, Cancer Research Institute, University of California, San Francisco, San Francisco, CA 94143-0128

Studies of recombinant viruses generated between HIV-1 isolates that show differences in their ability to infect T-cell lines or primary macrophages, to induce syncytia formation in the infected cells, and in their sensitivity to sCD4 neutralization have identified a 160 amino acid StuI/MstII fragment of env gp120, encompassing the V3 loop, as a major determinant for these biological properties. However, to achieve full biological phenotypes, additional regions of env gp120, e.g., the V2 domain, are required.

Within the StuI/MstII region, both amino acid (aa) changes within and outside of the V3 loop affect the infectivity, host range, cytopathicity, and sCD4 neutralization sensitivity of HIV-1. The amino acid changes that confer tissue tropism are different than those that confer sCD4 neutralization sensitivity, suggesting that the two biological properties are regulated by different mechanisms. Furthermore, the effects of aa changes in the V3 loop can be compensated by aa changes outside of the loop indicating an interaction of the V3 loop with either regions of env gp120. Taken together, our results suggest that the structure and/or accessibility of the V3 loop, play an important role in determining the biological properties of an HIV-1 isolate.

The exact mechanism (s) by which the V3 loop determines HIV-1 biological properties is presently unknown, but recent data reported by others indicate that V3 participates in the conformational changes of gp120 that are induced upon binding to sCD4. V3 loop sequence variation among different strains could alter the structure of the loop in such a way as to modulate these conformational changes in a strain-specific manner, and thus influence the infectivity, host range, cytopathicity and sCD4 neutralization sensitivity of HIV-1. In this regard, we found that the conformational changes in gp120 that are induced upon binding to sCD4 are strain-specific and that aa changes both within and outside of the V3 loop affected the structure of env gp120.

Q 024 GENETIC AND BIOLOGICAL VARIATION OF HIV-2, Feng Gao[1], Ling Yue[1], Robert J. Biggar[2], Alfred E. Neequaye[3], David D. Ho[4], Paul M. Sharp[5], George M. Shaw[1], and Beatrice H. Hahn[1], [1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, [2]Viral Epidemiology Section, National Cancer Institute, Bethesda, [3]Department of Medicine, University of Ghana, Accra, Ghana, [4]Aaron Diamond AIDS Research Center, New York, [5]Department of Genetics, Trinity College, Dublin, Ireland.

HIV-2 virulence properties are known to vary significantly and to range from relative attenuation in certain populations to high level pathogenicity in others. Differences in clinical manifestations may, at least in part, be determined by genetic differences encoded in the genomes of the infecting virus. Evaluation of the full spectrum of HIV-2 genetic diversity is thus a necessary first step toward understanding its molecular epidemiology, natural history of infection, and biological diversity. Since the first description of HIV-2, several additional strains have been reported. All of these, however, represent virus isolates that were propagated in tissue culture prior to their genetic analysis. Hypothesizing that such isolates represent only a subset of a much larger and genetically more complex group of viruses, we used nested PCR to amplify HIV-2 viral sequences directly from uncultured peripheral blood cells of five individuals (originating from Liberia, Ghana and Sierra Leone), from whom virus isolation attempts were repeatedly unsuccesful. Sequence analysis of LTR, *gag*, *pol*, and *env* regions identified two viruses (HIV-2_{223B} and HIV-2_{PA}) to be highly divergent from all existing HIV-2 strains, two others (HIV-2_{GH2} and HIV-2_{GH4}) to be closely related to HIV-2_{D205} (a previously described HIV-2 variant), and a fifth virus (HIV-2_{FO784}) to be more closely related to simian immunodeficiency viruses infecting sooty mangabeys and macaques than to viruses of human derivation. These results indicate that tissue culture is highly selective for only a subset of viruses and that genetic variation studies based on tissue culture amplified viruses grossly underestimate the extent of HIV diversity. These results also suggest that certain HIV-2 strains may represent "ill-adapted human viruses" resulting from a recent simian/human cross-species transmission. To follow up on this possibility, we have extended our analyses of HIV-2_{FO784} and have PCR amplified, cloned and sequenced nine partially overlapping regions of its genome. Although the majority of PCR derived clones were defective, we were able to identify at least one clone per amplified region to contain uninterupted open reading frames. This has allowed us to compile the sequence of a complete FO784 provirus with intact and correctly positioned *gag*, *pol*, *vif*, *vpx*, *vpr*, *tat*, *rev*, *env* and *nef* genes. Moreover, sequence analysis revealed no apparent alteration of the FO784 LTR which contained all major enhancer and regulatory elements previously identified for HIV-2/SIV_{SM}/SIV_{MAC} viruses. Finally, phylogenetic comparisons of the full-length provirus confirmed the close genetic relationship of HIV-2_{FO784} to simian immunodeficiency viruses. Reconstruction of a complete FO784 provirus is currently underway to obtain transfection competent molecular clones for *in vitro* and *in vivo* (animal model) analysis.

Q 025 HIV ENVELOPE V3 LOOP VARIATION AND PATHOGENESIS, Gerald Myers and Bette Korber, HIV Sequence Database and Analysis Project, Los Alamos National Laboratory, Los Alamos, NM 87545

At least five distinct sequence subtypes, or clades, of HIV-1 can be identified from samples taken from the major centers of the pandemic. It remains to be seen whether these "cladistically" defined subtypes represent distinct phenotypes, differing in their properties of pathogenicity and transmissibility. To explore this matter, we have turned our attention toward "phenetic" modes of analysis of HIV envelope V3 loop (PND) protein sequences: sequences are compared using an amino acid class covering algorithm that evaluates chemical similarities as well as identities. The findings from this analysis are of a piece, for the most part, with the results of the cladistic analysis -- the predominant picture is one of V3 loop divergence. However, we noted through the phenetic analysis some cases of sequence parallelism, that is, the occurrence of unexpectedly similar V3 sequences among otherwise highly dissimilar HIV-1 subtypes. In particular, some African-derived V3 loop amino acid sequences of the cladistically determined A and C subtypes were found to be virtually identical to certain V3 loop sequences of nucleotide sequence clade B. This manifestation of evolutionary parallelism can be explained as sequence convergence or, simply, as a lack of divergence. Support for the latter interpretation comes from comparative analyses of HIV-2 envelope V3 sequences, which indicate that stable lineages of V3 loop sequences are a common phenomenon associated with less pathogenic or nonpathogenic HIV-2 infections. These findings further suggest that the broad classification of viral phenotypes into syncytium-inducing (SI) and nonsyncytium inducing (NSI) forms has some relevance for global surveillance and, possibly, vaccine strategies.

Q 026 MOLECULAR BIOLOGY AND CLINICAL SIGNIFICANCE OF HIV-1 BIOLOGICAL PHENOTYPE, Hanneke Schuitemaker, Matthijs Tersmette, Maarten Koot, Martijn Groenink, Ron A.M. Fouchier, Linde Meyaard, Han Huisman, and Frank Miedema, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

HIV-1 isolates show variation in syncytium inducing (SI) capacity, replication rate and cell-tropism. Emergence of SI variants in an individual usually preceded increases in viral load and accelerated CD4 cell decline. The incidence of NSI/SI conversion declined at lower CD4+ cell numbers and NSI/SI conversion was rare following AIDS diagnosis. Previously, we showed that changes in phenotype correlated with increased positive charge at fixed amino acid residues (11 and 28) of the V3 loop. Exchange of V3 between related infectious molecular HIV-1 clones with different SI capacity, however, revealed that SI capacity was only partially conferred to NSI variants by the V3 domain. The phenotype is converted to wild type SI if both V1-V2 and V3 are exchanged. Similar results were obtained by reciprocal exchange from a NSI env gene fragment to a SI background. Analysis of a large panel of primary isolates demonstrated that the NSI/SI conversion was often preceded by the acquisition of an additional potential glycosylation site in the V2 domain.

Determinants of monocytotropism were analyzed and revealed restrictions at entry and post-entry levels. In this latter case, viral entry did not result in establishment of productive infection since only partial proviral DNA species were synthesized, suggestive for an insufficient support of the RT process from both viral and cellular factors.

Monocytotropic variants play an essential role in AIDS pathogenesis. Functional impairment of antigen presenting cells due to HIV infection may be the basis for the induction of early T cell-dysfunction in humans. Additional evidence for this was obtained from studies on three long term HIV-infected healthy chimpanzees. From these animals, no monocytotropic HIV variants could be isolated, compatible with complete insusceptibility of chimpanzee monocytes for HIV infection in vitro. APC function of the monocytes derived from these animals was normal and evidence for T cell abnormalities (anergy nor apoptosis) could not be obtained. The incapability of HIV to infect chimpanzee monocytic cells (APC) therefore may be the basis for the absence of induction of systemic immune dysfunction in these animals.

Acute Infection; Viral Load; Detection Methods, Therapeutics

Q 100 NEUROLOGIC DAMAGE AND IMMUNE MEDIATORS IN AIDS, Cristian L. Achim, Eliezer Masliah, Melvyn P. Heyes*, Rebecca Wang, Don Miners and Clayton A. Wiley, Departments of Pathology and Neurosciences, UC San Diego, La Jolla, CA 92093-0612 and *SAB, LCS, NIMH, Bethesda, MD 20892

Neurologic disease is a frequent complication in AIDS patients. The pathologic process underlying most of the focal neurologic deficits is opportunistic infection of the central nervous system (CNS). A subset of AIDS patients have diffuse neurologic symptoms that in a severe form are defined as AIDS dementia complex (ADC). At autopsy, brains from patients with ADC show the histopathologic picture of HIV encephalitis (HIVE) that is characterized by abundant infection and activation of CNS macrophages without detectable neuro-glial infection. How CNS macrophage infection by HIV leads to synaptic damage and neuronal loss is unknown. Likely candidates to mediate this process are macrophage associated immune factors. We studied viral burden and immune activation within the CNS of 43 AIDS patients without significant opportunistic infection. Fresh frozen autopsy material was analyzed for presence of β-2M, TNFα, IL-6, TGFβ, LTB4, PGD2 and QUIN using antigen capture ELISA and gas chromatography. Viral burden was quantified using semi quantitative PCR, HIVp24 antigen capture assay and immunocytochemistry. Although the correlation between intraparenchymal and CSF levels of HIVp24 was good, there was no association between intrathecal virus and brain macrophage activation factors. The correlation between intraparenchymal levels of HIV proteins and nucleic acids was excellent indicating a high inter reliability among the assays used. Most of the viral load was localized in the deep structures and correlated with elevated levels of immune factors, especially TNFα, QUIN and β-2M. Presence of TGFβ correlated inversely with TNFα levels and viral burden. Synaptic damage and neuronal loss correlated with viral burden and presence of specific immune factors. The distribution of neurologic damage, viral and immune factors suggests that neocortical damage follows basal ganglia involvement. (This work was supported by the California Universitywide AIDS Research Program).

Q 101 IDENTIFICATION OF DRUG-RELATED GENOTYPIC CHANGES IN HIV-1 FROM SERUM USING SELECTIVE POLYMERASE CHAIN REACTION. Barry D. Anderson, Takuma Shirasaka and Hiroaki Mitsuya, Experimental Retrovirology Section, Medicine Branch, National Cancer Institute, Bethesda, MD 20892.

We attempted to detect the presence of known drug-related HIV-1 *pol* gene mutations by selective polymerase chain reaction (PCR) of both proviral DNA and viral RNA isolated from patients (pts) with AIDS or ARC receiving antiretroviral therapy. Cell lysates of 7-day-cultured peripheral blood mononuclear cells (PBM) from pts before and after receiving alternating AZT/ddC therapy for 15-41 months or ddI monotherapy for 12-26 months were subjected to initial PCR amplification of the proviral *pol* gene followed by selective PCR at positions 70, 74, 215 and 219. HIV-1 virions from serum samples obtained at the same pre- and post-therapy time points were isolated by ultracentrifugation and subjected to RNA extraction, reverse transcription, and *pol* gene amplification (RT-PCR), followed by selective PCR.

For all the eight pairs of pre- and post-therapy proviral DNA samples we examined, results obtained by the selective PCR method for identifying wild or mutant genotypes at the four sites completely agreed with results of nucleotide sequencing conducted independently. RT-PCR combined with the selective PCR step allowed the detection of *pol* gene mutations from virions isolated from patient serum. In some cases the viral RNA genotypes varied with those of the proviral DNA. This was noticed particularly when sequential samples from a patient who had switched antiretroviral drugs were examined.

We conclude that (i) selective PCR of cultured PBM lysates successfully detects the presence of proviral *pol* gene mutations, (ii) RT-PCR in combination with selective PCR may be of use to define mutations in the *pol* gene of viral RNA isolated from serum (or plasma), and (iii) drug-related mutations may be detected earlier in virions in serum (or plasma) than in proviral DNA in PBM. Thus, genotypic information obtained from HIV-1 virions in serum (or plasma) may better reflect the actively replicating viral population and provide an earlier indication of drug-related changes during the therapy of HIV-1 infection.

Q 102 **SUPPRESSION OF SURFACE CD4 EXPRESSION BY CLINICAL ISOLATES OF THE HIV nef GENE FROM AIDS PATIENTS.** Stephen Anderson[1], D. Shugars[2], R. Swanstrom[2], and J.V. Garcia[1], [1]Dept. of Virology & Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38101, [2]Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599.

The nef gene of the Human Immunodeficiency Virus (HIV) was originally described as a negative regulator of transcription from the viral LTR promoter. This observation has since been disputed and the function of Nef remains uncertain. In vivo experiments have indicated that an intact Nef is necessary for disease progression in macaques infected with SIV, suggesting a role for Nef in the pathogenesis of AIDS. We have previously shown that expression of the nef gene in CD4+ cells results in a decrease in CD4 cell surface expression. Since previous experiments were performed with a nef gene derived from a laboratory strain of HIV-1 (SF2), it was important to determine whether nef genes obtained directly from clinical isolates of HIV were also able to suppress surface CD4 expression. The nef genes of HIV isolated from two patients were cloned into the retroviral expression vector, pLXSN, and introduced into human and mouse T cell lines by retroviral mediated gene transfer. Cells of both the murine thymoma line, AKR 1G1, and the human Szezary cell lymphoma line, HUT-78, were shown by Western blot to express Nef. The Nef 233 and Nef 248 proteins have slightly different molecular weights and both are significantly smaller than SF2 Nef. Both Nef 233 and Nef 248 were able to suppress surface CD4 expression in murine and human T cell lines to the same extent as the SF2 Nef as determined by flow cytometry. Similar results were also obtained with an artificially synthesized nef gene encoding a consensus nef sequence derived from in vivo isolates, suggesting that the ability to suppress CD4 expression is a common property of Nef. Comparing the protein sequences of different Nef isolates should aid in the identification of domain(s) of Nef which are involved in its effect on CD4 expression.

Q 103 THE KINETICS OF THE FIRST TEMPLATE SWITCH AND INHIBITION BY NUCLEOSIDE ANALOGS, Eric J. Arts and Mark A. Wainberg, McGill AIDS Centre and the Department of Microbiology and Immunology, Montreal, Quebec, Canada, H3T1E2

Reverse transcription begins with the binding of the human immunodeficiency virus reverse transcriptase (HIV-RT) to $tRNA^{lys3}$ forming a transcriptional complex on the primer binding site found 3' of the HIV RNA long terminal repeat. Reverse transcription, primed by $tRNA^{lys3}$, proceeds to the end of the R region producing the (-) strong-stop DNA strand. Upon RNase H digestion, the R of the (-) strong-stop DNA anneals to the R of the second HIV RNA template to complete production of (-) HIV DNA. To study the kinetics of this process, we have developed an endogenous cell-free reverse transcription assay using $tRNA^{lys3}$, HIV-RT and HIV RNA involved in the first template switch. The use of $tRNA^{lys3}$ over a deoxy-oligonucleotide primer increased the efficiency of template switching by 3 fold. Interestingly, decreases in the overlap in the R region only had a slight effect on template switching but greatly decreased priming from the primer binding sequence. Therefore, the R region appears to have a stabilizing effect on the transcriptional complex.

Quantitative PCR amplification of viral DNA in nucleoside analog-pretreated, HIV-exposed CD4-positive cells revealed a preferential chain termination immediately following the first template switch. By using the cell-free reverse transcription assay, it was shown that 3'-azido-3'-deoxythymidine (AZT-TP) can inhibit the synthesis of products after template switching but only when the reaction is primed with $tRNA^{lys3}$. Furthermore, this inhibition appeard to be controlled by the rate of polymerization and template switching. Stalling occurs during the annealing of the DNA R region to the second HIV RNA template which may permit triphosphorylated nucleoside analogs to bind to HIV-RT, possibly displacing the nucleoside triphosphate. The analog may then be incorporated opposite to its pairing partner immediately following continuation of polymerization.

Q 104 CHARACTERIZATION OF THE CD8+ POPULATION IN HIV-1+ LYMPH NODES BY DOUBLE AND TRIPLE COLOUR IMMUNOFLUORESCENCE. Margarita Bofill, Nicola Borthwick, Wendy Gombert and George Janossy, Department of Clinical Immunology, Royal Free Hospital and Medical School, Rowland Hill Street, London NW3 2PF, England.
Lymph nodes from HIV-1 infected patients show an infiltration of CD8+ lymphocytes into both the paracortical (PC) areas and the germinal centres (GC). It has been suggested that these CD8+ cells are cytotoxic effectors and are contributing to the breakdown in the structure of the lymph node observed in AIDS. To further investigated this lymph node sections from 18 HIV-1+ patients and 7 HIV-1- controls were investigated for their expression CD45RO, CD28, bcl-2 and TIA-1, an antibody specific for cytotoxic cell granules. Double and triple colour IF studies were performed using both conventional epifluorescence microscopy and confocal microscopy. There was an inverted ratio of naive (CD45RA) to primed (CD45RO) CD8+ cells in HIV-1+ patients due to an increased percentage of CD45RO+ lymphocytes (74.6±3.21) compared to (22.3±3.04) in the control group. More than 95% of the CD8+ cells infiltrating the GC expressed CD45RO. A small proportion (18.3±3.64) of these also weakly expressed CD45RA. Of the CD8+CD45RO+ primed population in the GC of HIV-1+ patients 87.6±6.15% were TIA-1+ and expressed CD28 at levels similar to the control group (83.2±3.52). Such CD8+CD45RO+TIA-1+CD28+ cells are not seen in GC of HIV-1- controls but a small percentage (32.0±7.26) of the CD8+ cells in the PC areas were found to have this phenotype. A high proportion of the CD8+ cells infiltrating the lymph node had diminished or undetectable levels of bcl-2 (23.0±3.63% compared to 65.0±3.91%). This data indicates that the CD8+ lymphocytes infiltrating the lymph nodes of HIV-1 infected patients are short lived, primed, cytotoxic cells that probably regenerate very quickly.

Q 105 PROPHYLACTIC AND POST-EXPOSURE EFFECTS OF DIDEOXYINOSINE (DDI) ON HIV-ASSOCIATED PATHOLOGY IN HIV-1 INFECTED HUMAN FETAL THY/LIV IMPLANTS IN SCID-HU MICE. Mark L. Bonyhadi*, Linda Rabin*, Suzan Salimi*, Daniel A. Brown**, Jon Kosek**, Joseph M. McCune*, and Hideto Kaneshima*. *SyStemix, Inc., 3400 W. Bayshore, Palo Alto, CA, 94303;**Veterans Administration Hospital, Palo Alto, CA.
Individuals infected by the human immunodeficiency virus type 1 (HIV-1) exhibit progressive depletion and severely reduced function of peripheral CD4+ helper T lymphocytes. A variety of mechanisms have been proposed to account for the observed loss of CD4+ T cells, including virally mediated cytopathic killing of infected cells, deleterious infection of T cell precursors and HIV-mediated indirect killing of uninfected cells. The latter mechanisms may be especially important in the setting of fetal and pediatric HIV infection, a time during thymic ontogeny when T lymphopoiesis is most active. We have previously shown that implants of human fetal thymus and fetal liver in the SCID-hu mouse form a conjoint organ which is functionally and structurally similar to normal human fetal thymus, that this "Thy/Liv" organ supports long-term human T lymphopoiesis *in vivo*, and that the structure is permissive for HIV-1 infection. We have used this surrogate *in vivo* model to evaluate the effect of HIV-1 infection on T lymphopoiesis in the human fetal thymus. When Thy/Liv implants were inoculated with primary patient isolates of HIV-1, spread of viral infection, increased viral replication and a progressive depletion of CD4-bearing thymocytes were observed. Thymocytes from infected Thy/Liv implants exhibited flow cytometric and morphological profiles consistent with that of cells undergoing programmed cell death. The effect of antiviral agents on the progression of HIV-associated pathology following infection in SCID-hu Thy/Liv mice was also evaluated. When ddI was administered prior to direct intrathymic injection of HIV-1, at six weeks post-inoculation, thymocytes were negative for p24 by ELISA and the CD4 and CD8 expression pattern in the thymus was normal as assessed by flow cytometry. When administration of ddI followed virus inoculation by one week, virus replication was effectively suppressed and thymocyte CD4 and CD8 expression appeared normal. However, when ddI administration began two or three weeks after HIV inoculation, p24 values increased and CD4-bearing thymocytes were depleted. These observations suggest that the SCID-hu Thy/Liv model may be useful not only for the evaluation of HIV-induced pathogenesis, but also for assessing the activity of various compounds in suppressing pathogenesis.

Q 106 NUCLEOSIDE DERIVATIVES WITH LABILE AND/OR FLUORESCENT 3'-BLOCKING GROUPS, AND THEIR ANTI-HIV ACTIVITIES

Kevin Burgess,[1] Andrew Civitello,[2] Larry Donahue,[2] Richard Gibbs,[2] Michael L. Metzker,[2] and Ramesh Raghavarchari.

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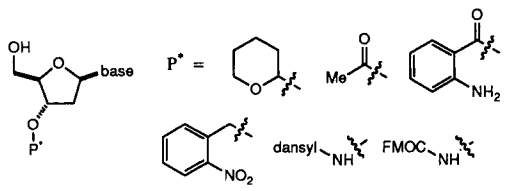
A series of 3'-protected nucleoside derivatives have been synthesized as potential inhibitors of a model *reverse transcriptase*. The structures of the compounds are represented by the generalized diagram below:



Some of the 3'-protecting groups were designed to be labile under physiological conditions, while others were equipped with fluorescent tags to facilitate identification of the base-analogs incorporated into growing DNA strands.

Triphosphate derivatives of these materials were taken with a primed DNA strand in the presence of reverse transcriptase and several polymerases, hence the tolerance of these enzymes to changes at the 3'-position was accessed. Cytotoxicity data, and the activity of these compounds with respect to cell lines infected with HIV-1 were also obtained.

The nucleoside derivatives presented above are also being used to develop new methodology for DNA sequencing without gel electrophoresis.

Q 107 HIV-SPECIFIC CELLULAR IMMUNITY IN PRIMARY HIV-1 INFECTION, Elizabeth Connick, *Stephen Clark, *Michael S. Saag, Robert T. Schooley, Tyler J. Curiel, Box B-168 University of Colorado Health Sciences Center, Denver, CO 80262; *University of Alabama, Birmingham, AL 35294.
Primary HIV-1 infection is characterized by high titer viremia which dramatically declines after 3 to 12 weeks. This decrease often precedes the appearance of neutralizing antibodies (ab), suggesting that cellular immunity may be responsible, at least in part, for this decline. We have examined HIV-1 specific cellular immune responses in 3 subjects with acute infection to help address this question. Peripheral blood mononuclear cells (PBMC) were used as effector cells, and EBV-transformed B cells as target cells in 51Cr release assays. Antigen expression was induced by infection of target cells with recombinant vaccinia encoding HIV-1 env or gag, or the *E. coli* lac control. Cytotoxic T cell lines were derived from PBMC in the presence of irradiated allogeneic feeder cells, IL-2 and anti-T-cell ab. Gag-specific, HLA-restricted killing was detected in PBMC from 2 of 3 subjects by 12 weeks after infection or seroconversion. HLA-unrestricted env killing was detected in PBMC from all 3 subjects, coincident with development of anti-HIV ab. HIV env- or gag-specific cell lines were derived from PBMC of 2 of 3 subjects. Of interest, two env-specific cytotoxic T cell lines derived from one patient at the time of acute infection were CD3+,CD4+,CD8-. Two additional patients with recent HIV infection are currently under investigation. These data demonstrate that cellular immunity to HIV gag and env occurs early in HIV-1 infection, and may therefore play a role in terminating acute viremia.

Q 108 EVALUATION OF THE BIOLOGICAL PROPERTIES OF A SERIES OF SEQUENTIAL HIV-1 ISOLATES FROM A PATIENT WITH RAPID CD4+ T-CELL DECLINE. Ruth I. Connor and David D. Ho, Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, NY 10016.

CD4+ T-cell decline has been associated in HIV-1 infected individuals with an increase in viral burden and the emergence of a more cytopathic viral phenotype. To evaluate specific virologic changes which may contribute to this phenomenon, we studied in detail sequential isolates from a patient exhibiting a precipitous drop in CD4+ T-cells. This patient had a significant increase in viral burden prior to CD4+ T-cell decline, coinciding with a switch from a non-syncytia inducing (NSI) to a syncytia-inducing (SI) viral phenotype. Analysis of sequential isolates in PBMC cultures showed a progressive increase in the kinetics of viral replication from early (NSI) to late (SI) isolates. Differences in the levels of HIV-1 p24 measured in culture supernatants could be detected as early as 24-48 hrs after infection. In further experiments, DNA was extracted from PBMC 4, 8, 12, and 24 hrs after infection and analyzed by PCR using primers which detect late products of reverse transcription. Our results indicate an increase in both the rate of appearance and the levels of complete or nearly-complete double-stranded HIV-1 DNA in PBMC infected with SI isolates taken at the time of CD4+ T-cell decline. These findings suggest changes in the viral phenotype may reflect differences in efficiency in the early stages of the viral life cycle. Studies are now underway to evaluate this possibility using a large series of phenotypically characterized biological clones derived from the original sequential isolates.

Q 109 PROVIRAL LOAD IN THE EVALUATION OF THE EFFICACY OF AZT AND DDI MONOTHERAPY, Brian Conway, Doreen S. Ko, Nanci Foss, D. William Cameron, Lionel G. Filion and Francisco J. Diaz-Mitoma, Department of Medicine, Ottawa General Hospital and Department of Microbiology and Immunology, University of Ottawa, Ottawa, Canada.

It is proposed that virologic assays be used for the evaluation of the efficacy of antiretroviral therapy. We have measured circulating proviral load by quantitative PCR in 19 patients treated with AZT (median 13mo), 8 of which were continued on AZT and 11 changed to ddI. Of those maintained on AZT, proviral load increased in 5 (26-1670%) over 6-12mo, corresponding with the development of opportunistic infections (2 cases) and decreasing CD4 cell counts (9-103 cells/μl). Proviral load decreased in 3 patients (25, 29, 92%), with no opportunistic infections and increasing CD4 cell counts in 2 cases (44, 77 cells/μl). Of those changed to ddI, proviral load increased in 4 (164-935%), over 6-12mo, with no opportunistic infections and decreasing CD4 cell counts in 2/4 (18, 174 cells/μl). Proviral load decreased in 7 patients (39-97%), with one minor opportunistic infection and increasing CD4 cell counts in 7/7 (9-159 cells/μl). In 5 patients, an initial rise in CD4 cell count was lost over 2-9 months. This latter phenomenon was accompanied by a secondary increase in proviral load ($>100\%$).

In association with other clinical and virologic assays, it appears that proviral load will be a useful marker of the efficacy of antiretroviral therapy.

Q 110 EFFECTS OF RETINOID COMPOUNDS ON THE PROLIFERATION OF KAPOSI SARCOMA CELL CULTURES. Jacques Corbeil*, Pat Badel* Eric Rapaport*, Flossie Wong-Staal*, Douglas D. Richman*§ and David Looney*§. Departments of Medicine and Pathology, University of California, San Diego * and Veteran Administration Hospital San Diego §. La Jolla, California 92093-0679.

We assessed the ability of four retinoid compounds; acitretin, isotretinoin, tretinoin and RO 13-7410 (a Roche product) to alter the proliferation of Kaposi sarcoma (KS) cells *in vitro*. Utilizing a standard thymidine uptake assay, these retinoids were employed at concentration ranging from 10^{-9}M to 10^{-5}M to determine the extent of their effect on KS cell growth response. Human umbilical vein endothelial cells (HUVEC), fibroblasts and the smooth muscle cell line (SKLMS) were used as controls. Cells were grown in serum-free medium to minimize the effects of endogenous retinoids normally present in serum.

A significant and reproducible reduction of growth (≈80%) was observed when tretinoin at a concentration of 10^{-5}M was added to the growth media. Similar growth reductions were observed, but to a lesser extent, with RO13-7410 and isotretinoin. However, acitretin, an all-trans nonatetraenoic acid, had no effect on KS cell growth. While, HUVEC and smooth muscle cells behaved in a fashion analogous to KS cells, fibroblasts, interestingly, showed no significant reduction in growth with any of the retinoids. We are presently focusing on the mechanisms of this effect in the hope to provide an effective therapeutic option.

Q 111 QUANTITY OF GENOMIC HIV-1 RNA IN CEREBRO-SPINAL FLUID AND SERUM OF AIDS PATIENTS DOES NOT CORRELATE WITH THE OCCURRENCE OF DEMENTIA. Marion Cornelissen[1], Peter Portegies[2], Marjon Clements[1], Susan Hartman[1] and Jaap Goudsmit[1]. 1 Department of Virology, 2 Department of Neurology, University of Amsterdam, Academic Medical Centre, Amsterdam, The Netherlands.

Aim: Quantification of genomic HIV-1 RNA in serum and CSF of aids patients in order to analyze the correlation between virus load and the development of AIDS dementia complex (ADC). Sequence analysis of the third variable (V3) domain, because this domain has been implicated in determining the HIV phenotype, including fusion capacity and monocytropism in relation to HIV-1 RNA quantity.

Methods: Limiting-dilution RNA-PCR analysis of gag gene sequence with a sensitive virus isolation procedure. Fifty microliter serum or CSF of 20 patients divided in 4 groups were analyzed.

Group 1: 5 patients with ADC and p24 antigen in their CSF.
Group 2: 5 patients with ADC but no p24 antigen in their CSF.
Group 3: 5 patients without ADC and p24 antigen in their CSF.
Group 4: 5 patients without ADC and no p24 in their CSF.

After the reverse transcription reaction the cDNA was diluted and amplified with specific gag primers. One tenth of the first reaction was analyzed with an inner primer pair. Sequence analysis of the V3 domain was possible after cloning of the nested PCR product.

Results and Conclusions: The dilution PCR method is able to amplify a single cDNA copy. The virus load in CSF of group 1 varied from less than $5\ 10^3$ molecules/ml to 10^5 molecules/ml. The virus load in the sera of this group from $5\ 10^5$ to $5\ 10^7$ molecules/ml. The virus load in CSF of group 2 was beneath our detection level. The virus load in the ADC negative group (group 3 and 4) was not significant higher than in the ADC group. No correlation between virus load and dementia was found. The sequence analysis is still in progress.

Q 112 IN SITU/PCR AND EM IN SITU HYBRIDIZATION TO HIV, F.J. Denaro, Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The sensitive and specific identification of viral genomic sequences is of utmost importance for diagnosis and research. We have previously employed the PCR method to detect low levels of HIV in lymphocyte samples (Spector, S.A., Karen Hsia, Frank Denaro, and Deborah H. Spector, Use of Molecular Probes to Detect Human Cytomegalovirus and Human Immunodeficiency Virus. *Clin. Chem.* 35(8): 1581-1587, 1989). In the present study we combine the amplification power of PCR with the histochemical characteristics of colorimetric in situ hybridization. By this technique we identify HIV in the brain (cells are morphology similar to microglia), blood and lymphatic tissue from AIDS patients. The signal to noise ratio of this technique is improved when compared to in situ hybridization without PCR amplification. Research is involved in determining the sensitivity of the technique. In addition, colorimetric probes make possible the ultrastructural identification of HIV gene sequences. Both colloidal gold and DAB may be used for the cytological location of hybridized probe. Pseudo color and gray scale densitometry can give an idea of the relative intensity of hybridization of the probe in the cells. With this technique it may be possible to cytologically identify the temporal dynamics of HIV gene regulation in infected cells. These two sensitivity techniques present the researcher with new avenues for examining the pathophysiology of HIV infection.

Q 113 QUANTITATION OF HIV-1 INFECTION KINETICS, Dimiter S. Dimitrov,[1] Ronald L. Willey,[2] Hironori Sato[2], Lung-Ji Chang[2], Robert Blumenthal[1] and Malcolm A. Martin[2] [1]Section on Membrane Structure and Function, National Cancer Institute, and [2]Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

Tissue culture infections of CD4 positive human T cells by HIV-1 proceed in three stages: 1) a period following the initiation of an infection during which no detectable virus is produced; 2) a phase in which a sharp increase followed by a "peak" of released progeny virions can be measured; and 3) a final period when virus production declines. In this study, we have derived equations describing the kinetics of HIV-1 accumulation in cell culture supernatants during multi-cycle infections. Our analyses indicated that the critical parameter affecting the kinetics of HIV-1 infection is the infection rate constant $k = \ln n/t_i$, where n is the number of infectious virions produced by one cell per infection cycle (of the order of 100), and t_i is the time for one complete cycle of virus infection (typically 3-4 days). Of particular note was our finding that the infectivity of HIV-1 during cell-to-cell transmission is 10^2 to 10^3 times greater than the infectivity of cell-free virus stocks, the commonly used inocula to initiate tissue culture infections. We also demonstrated that the slow infection kinetics of an HIV-1 tat minus mutant is not due to a longer replication time but reflects the low number (approximately 10) of infectious particles produced per cycle. The quantitation of HIV-1 infection kinetics we have described may assist in the interpretation of experiments in which the host range, replicative and cytopathic properties of different virus isolates are compared. This approach may also prove useful when considering the kinetics of HIV-1 infection in infected individuals or when developing mathematical models of AIDS progression and *in vivo* HIV-1 infections by providing the necessary parameters and relationships for such analyses.

Q 114 POTENT INHIBITION OF HIV-1 INFECTION OF MONONUCLEAR PHAGOCYTES BY SYNTHETIC PEPTIDE ANALOGUES OF HIV-1 PROTEASE. Carol S. Dukes[1], Thomas J. Matthews[1], Dennis M. Lambert[2], Geoffrey B. Dreyer[2], Stephen R. Petteway[2] and J. Brice Weinberg[1]. [1]Duke and VA Med Ctrs, Durham, NC 27710 [2]SmithKline Beecham Pharm, King of Prussia, Penn 19406.

The HIV-1 genome encodes a protease which is required for viral processing of precursor polyproteins $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ Inhibitors of HIV-1 protease activity in human lymphocytes have been shown to decrease production of infectious virus. Earlier studies demonstrated that these compounds were less effective in human mononuclear phagocytes (MP) [monocytes (Mo) and macrophages (Mac)], possibly due to MP-mediated degradation of the protease inhibitors (PI). We now report enhanced inhibition of HIV-1 infectivity and protein processing in human MP using a class of PI, hydroxyethylene isosteres, which are nonhydrolyzable substrate analogues of HIV-1 protease. We tested the ability of 8 of these PI to inhibit $HIV\text{-}1_{BaL}$ infection of blood Mo and peritoneal Mac collected from healthy donors. The PI were added only once ~ 1 hr prior to inoculation with virus; every 3-5 days, half the media was replaced with fresh DMEM/10% pooled human serum. Inhibition of infectious virus production was assessed by measuring reverse transcriptase (RT) activity in supernatant media 14 days after infection. The concentration of drug inhibiting infection by 50% (IC_{50}) was calculated in three experiments and ranged from 0.17 μM to 2.99 μM (mean=1.25; SD=0.77). This is in contrast to the IC_{50}'s observed previously using oligopeptide PI which had IC_{50}'s in the 25 μMolar range. Immunoblot analysis of 2 of the PI (SB205700 and SB108922) confirmed inhibition of protein processing of the polyproteins. In control cells, 22% of viral protein p55 was processed to p24 by 24 hrs, and 51% by 48 hrs. In cells treated with the 2 PI (tested at 2 μMolar concentration), p24 processing was inhibited 77% at 24 hrs and 89% at 48 hrs. Thus, these synthetic peptide analogues of HIV-1 protease potently inhibit HIV-1 productive infection of mononuclear phagocytes. Drugs of this class may be useful for the treatment of HIV-1 infection in humans.

Q 115 PROPHYLACTIC AZT THERAPY PREVENTS EARLY VIREMIA AND DELAYS INFECTION IN FELINE IMMUNODEFICIENCY VIRUS INOCULATED CATS, Kathleen A. Hayes, Louis J. Lafrado, Julie G. Ericson and Lawrence E. Mathes, Center for Retrovirus Research, The Ohio State University, Columbus, Ohio 43210

The effect of 2',3'-azidothtymidine (AZT) treatment on early events in lentiviral infection was evaluated using the feline immunodeficiency virus (FIV)-cat model for HIV-1 infection of humans. AZT treatment (30 mg/kg/day by continuous subcutaneous infusion) was initiated 48 hours prior to virus inoculation and continued for 28 days. Transient plasma antigenemia was evident at week two post inoculation (pi) in 6/6 FIV-inoculation controls and was absent in 6/6 AZT treated cats. CD4 and CD8 lymphocyte numbers were consistently higher in the AZT-treated cats than in either the FIV-inoculated or age-matched controls. Further, declines in CD4 and CD8 lymphocytes were seen in the FIV inoculates starting at 10 weeks pi while decreases in CD4 and CD8 lymphocytes in the AZT-treated cats were not apparent until 12-13 weeks pi. CD4/CD8 ratios were lower for the AZT-treated cats than for either the FIV-inoculates or the age-matched controls due to much higher numbers of CD8 lymphocytes in the AZT-treated cats during the first 12 weeks pi. Anti-FIV antibody responses were identical between the AZT-treated cats and the FIV-inoculates. Polymerase chain analysis showed FIV provirus in PBL in 5/6 FIV inoculates and 3/6 AZT-treated cats at 6 weeks pi. By 14 weeks pi, all 12 cats were PCR positive. The results demonstrate that even under optimal conditions, short term prophylactic AZT therapy does not prevent infection. However, immediate post-exposure AZT treatment may be valuable in retarding the early systemic spread of infection as mediated by viremia and delay lymphocyte decline.

Q 116 **DIFFERENTIAL REGULATION OF CYTOKINE mRNA DURING ACUTE INFECTION OF PRIMARY MONONUCLEAR PHAGOCYTES BY HIV-1,**

G. Herbein, S. Keshav, M. Collin, L. Montaner, and S. Gordon, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

Cytokines such as monocyte chemotactic protein (MCP-1), interleukin 1 beta (IL-1B), tumor necrosis factor alpha (TNFa) and alpha interferon (aIFN) may play a role in immunopathogenesis of AIDS. We studied early effects (0.5-48 hours) of monocytotropic (ADA) or lymphotropic (IIIB) strains of HIV-1 on cytokine mRNA expression by a semi-quantitative RT-PCR assay. Three day-old monocyte-derived macrophages were exposed to tissue culture supernatants containing virus (at M.O.I. of 0.05) or control supernatants spin-filtered through 100 MW filters to remove virions and free gp120. ADA strain, but not IIIB, replicated in primary blood monocyte-derived macrophages; soluble CD4 (sCD4) was used to inhibit binding of both strains to macrophages.

We found that cytokine mRNAs were induced differentially depending on virus binding, replication, time of incubation, and the presence of virus-free supernatant. Alpha IFN mRNA was induced only by ADA, but not IIIB, at 12 hours post-infection and was abolished by sCD4. By contrast, IL-1B and TNFa gene expression was induced by both strains 1-3 hours after addition of virus; enhanced expression of both cytokines was inhibited by sCD4. MCP-1 message expression was independent of virus but was induced by virus-free supernatant.

We conclude that binding via CD4 is sufficient to enhance IL-1B and TNFa mRNA, whereas viral replication is required for induction of aIFN mRNA. Therefore, different pathways regulate gene expression of different cytokines by macrophages during initial infection by HIV-1.

Q 117 GENOTYPE AND PHENOTYPE CHARACTERIZATION OF HIV-1 TRANSMITTED BY SEXUAL CONTACT. David D. Ho, Hongmei Mo, Yunzhen Cao, Ning Wang, Daniel S. Nam, Charles Farthing, Richard A. Koup, and Tuofu Zhu. The Aaron Diamond AIDS Research Center and NYU School of Medicine, 455 First Avenue, New York, NY 10016.

HIV-1 sequences from variable regions of gp120, gp41, p17, and nef were obtained following PCR amplification from uncultured peripheral blood mononuclear cells of 4 acute seroconvertors and 3 of the corresponding sexual partners. The viral populations in the transmitters showed varying degrees of sequence heterogeneity in all regions examined, while the viral populations of the newly infected individuals were homogeneous during the early phase of HIV-1 infection. The viruses in each seroconvertor represented a minor population of the viruses in the corresponding transmitter. The newly transmitted HIV-1 isolates appear to have a homogeneous phenotype when analyzed for their biologic properties in vitro. That selective transmission of one homogeneous viral populations occurs during sexual contact has important implications for HIV pathogenesis and vaccine development.

Q 118 **Profound inhibition of HIV-1 replication in cultured cells by ribozymes: converse correlation with *in vitro* activity,**

William James and Paul Crisell , Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, U.K.

Ribozymes can be engineered to cleave HIV RNAs in a sequence-specific manner, providing a potential mechanism for anti-viral therapy. However, ribozymes that work catalytically *in vitro* are frequently disappointing when analysed in cells unless expressed at very high levels relative to their target RNAs.

Here we show that antisense ribozymes with long flanking regions of complementarity (FRC), expressed at modest levels in cells from a retroviral vector, are potent inhibitors of HIV compared to the parental antisense RNA or a cleavage-incompetent point mutant. A family of eleven ribozymes are tested that share a common cleavage site on HIV-1_{HXB2R} mRNA but whose FRCs range in size from 9 to 564nt.

When tested under cell-free conditions, only ribozymes with relatively short FRC showed substantial turnover, in agreement with the observations of others. Those with long FRC cleaved only between one and two molar equivalents of target RNA at equilibrium. However, when tested for HIV-inhibitory potency in cells, only ribozymes with FRC>27nt showed any activity. The derivative with FRCs of 33nt produced more than 10^3-fold inhibition over an extended period. It would seem that, while the optimum FRC length *in vitro* is 6-8nt, it is 33-45nt in the cell.

It is possible that the presence of ssRNA-binding proteins favours the action of long ribozymes in the nucleus and here we report initial experiments to test this possibility.

Q 119 HIGH VIRAL RNA COPY NUMBER AT SEROCONVERSION PERSISTS IN INDIVIDUALS PROGRESSING TO AIDS

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HIV-1 viral RNA load in serum of infected individuals was studied using NASBA (*N*ucleic *A*cid *S*equence *B*ased *A*mplification), a nucleic acid amplification technology capable of selectively amplifying RNA. The amplification is based on the simultaneous enzymatic activity of AMV-reverse transcriptase, RNase H and T7 RNA polymerase. The sensitivity of detection in the NASBA reaction is 10-100 molecules HIV-1 RNA. Quantification of RNA in serum was achieved by co-amplification of a dilution series of in vitro generated RNA, differing only by a 20 nucleotide randomized sequence from the wild-type RNA, thus ensuring equal efficiency of amplification. Differential detection of the wild-type and mutant amplification products was performed using a non-radioactive detection assay. The dynamic range of the quantitative NASBA procedure is 10^2-10^6 initial RNA molecules. Quantification of a virus stock containing 2.9 (± 1.6) x 10^{10} particles/ml determined by quantitative electron microscopy, corresponding to 1.2 (± 0.04) x 10^4 infectious units, yielded 5.5 (± 1.8) x 10^{10} RNA molecules/ml by quantitative NASBA confirming the accuracy of the quantification method.

The HIV-1 RNA load was determined in longitudinal samples obtained from eight p24-antigen negative and four p24-antigen positive individuals, progressing to AIDS after 15-66 months. In all samples HIV-1 RNA was detected, ranging from 2.3 x 10^4 to 5.0 x 10^7 copies/ml serum. High levels of viral RNA persisted in all individuals from seroconversion until AIDS diagnosis, indicating that there is no latency in viral replication.

Q 120 THE RECOMBINANT VIRUS ASSAY: A NON SELECTIVE METHOD TO DETERMINE DRUG SENSITIVITY OF HIV-1 IN HELA CD4+ CELLS.

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The ability to assess the sensitivity of HIV isolates to drugs targeted at the virus encoded enzyme reverse transcriptase is an important aspect of monitoring clinical trails. Protocols used to date have drawbacks, including the selection of sub-populations of HIV during co-culture in heterologous cells to produce virus to assay. Here we report a method that allows RT sequences to be inserted directly into virus able to form syncytia in HeLa CD4+ cells. The RT coding sequence is obtained from PBL DNA using a nested PCR procedure which has sufficient sensitivity to amplify from less than 10 copies of target RT sequence. The RT is then incorporated into the HXB-2D genetic backbone by homologous recombination with an RT deleted proviral clone following electroporation of C8166 cells. Conditions have been determined with respect to the amount of proviral clone, donor RT and T-cells required to allow optimal levels of recombination and virus production within 12-14 days. Recombinant virus was recovered by this method from multiple samples of patients receiving AZT therapy that harboured either syncytium inducing (SI) or non-syncytium inducing (NSI) variants of HIV-1. The sensitivity and genotype of the recombinant viruses was comparable to the drug sensitivity of virus derived by co-culture from SI virus infected individuals. The sensitivity of recombinant virus derived from NSI individuals was comparable to the level of resistance predicted by genotypic analysis of PBL DNA from these patients. The Recombinant Virus Assay should allow analysis of the predominant virus population in patients PBL's and potentially plasma virus, avoiding the problem of analysing virus isolates that have a growth advantage when cultured in heterologous cell lines.

Q 121 A QUINOXALINE DERIVATIVE WITH HIGH INHIBITORY ACTIVITY AGAINST HIV-1 DEFINES A NEW BINDING SITE ON THE REVERSE TRANSCRIPTASE

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Data are presented that describe the activity of a quinoxaline derivative against HIV-1.

It was found that this compound has an IC_{50} value with the reverse transcriptase (RT) of HIV-1 that is among the lowest values so far reported for inhibitors of that enzyme. The substance prevented HIV-1 infection of cells in tissue culture at doses 10^3 - 10^4 fold below the cytotoxic concentration.

The compound was also shown to inhibit the RT molecule of a TIBO resistant virus at submicromolar concentrations.

By culturing HIV-1 *MN* in H9 cells in the presence of increasing amounts of the drug, a viral variant with reduced sensitivity to the compound was obtained.

The genotypic change associated with the emergence of the resistant strain is different from mutations that were previously shown to be responsible for resistance against other nonnucleoside RT inhibitors.

Q 122 INVESTIGATIONS ON MEMBRANE ACTIVE ETHER LIPID ANALOGS THAT ALTER FUNCTIONAL EXPRESSION OF HIV-1 INDUCED GLYCOPROTEINS AND INHIBIT PATHOGENESIS.

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Published data from our laboratories indicated that synthetic ether lipids (amido derivatives of ether phospholipids, EL) and EL-AZT conjugate compounds have selective and potent activity against infectious HIV-1 replication. Investigations were done to better understand the major molecular target(s) for EL and EL-AZT antiviral activity in CEM-SS and H9III$_B$ cells. Data indicate that treatment of cells with 1μM EL or EL-AZT conjugate inhibited HIV-1 induced cell fusion (EL:EL-AZT, 67:51% inhibition). Pretreatment of uninfected CEM-SS cells also blocked cell fusion during coculture with persistently infected H9III$_B$ cells (EL:EL-AZT, 64:91% inhibition). HIV-1 progeny virus particles made in the presence of EL or EL-AZT conjugate were altered in their capacity to bind to (29:15% inhibition) and infect (18:73% inhibition) CD4+ CEM-SS cells. Also, EL or EL-AZT pretreatment of CEM-SS cells blocked subsequent infection, (78 and 72% inhibition) with untreated HIV-1 particles. EL or EL-AZT conjugate treatment of HIV-1 persistently infected H9III$_B$ cells for 2 hrs caused significant inhibition of gp120 expression on the cell surface (85:74% inhibition). However, EL or EL-AZT did not affect functional CD4+ receptor function, membrane fluidity or the capacity of peripheral blood lymphocytes to be activated by phytohemagglutinin. In summary, EL or EL-AZT selectively inhibit HIV-1 functional expression of gp120/gp41 at cell surface membranes and virus induced pathogenesis.

Q 123 TWO INHIBITORS OF TNFα CAN INHIBIT THE PRODUCTION OF TNF PROTEIN WITHOUT INHIBITING INDUCTION OF TNF mRNA

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It has been previously shown that pentoxifylline, a phosphodiesterase inhibitor, can inhibit the production of TNFα protein (1). BRL 61063 also inhibits production of TNFα protein, and is 100- to 1000-fold more potent than pentoxifylline as assessed by TNFα-inhibition in cultured, LPS-stimulated human monocytes. We utilized an LPS-stimulated whole human blood assay to model some of the clinical activity of these two compounds. TNFα protein production kinetics and LPS dose response in whole blood were similar to those previously reported in a cultured human monocyte experimental system. The kinetics of TNFα protein and mRNA production were then measured in whole blood treated with a range of concentrations of pentoxifylline and BRL 61063 prior to LPS stimulation. Protein levels were measured by ELISA and mRNA levels were measured by both Northern blot and semi-quantitative PCR analysis. Although treatment with these compounds resulted in a large reduction in TNFα protein production at one hour post-stimulation, TNFα mRNA levels were not similarly reduced at this time point. TNFα mRNA levels in the absence of LPS-stimulation were not detectable, while substantial levels of TNFα mRNA were always detected at one hour after stimulation, even in treated cultures. BRL 61063 and pentoxifylline treatment resulted in decreased levels of TNFα mRNA measured at three hours after stimulation relative to the LPS-stimulated control, and TNFα protein production was still suppressed. These results suggest that the compounds affect TNFα message accumulation over time, but not transcriptional activation, and therefore it is likely that they exert some of their TNFα protein-inhibition effects through mechanisms other than inhibition of transcription. Since TNFα has been implicated in several aspects of AIDS pathogenesis (enhanced HIV replication, cachexia and dementia), inhibition of TNFα could become an important new therapeutic strategy for HIV disease. Based on the results reported here, care must be taken in interpreting TNFα mRNA levels in clinical samples as a marker of drug efficacy. Further studies are needed to determine the mechanism of action of these inhibitors.

1. Streiter R.M., Remick D.G., Ward P.A., Spengler R.N., Lynch J.P., Larrick J., Kunkel S.L., (1988) Biochemical and Biophysical Research Communications, 155:1230-1236.

Q 124 PHOTODYNAMIC INACTIVATION OF VIRAL FUSION AND INFECTIVITY BY HYPERICIN AND ROSE BENGAL: EFFECTS ON HIV AND OTHER ENVELOPED VIRUSES, John Lenard, Arnold Rabson, Nancy R. Stevenson and Roger Vanderoef, Robert Wood Johnson Medical School (at Rutgers) and CABM, Piscataway, New Jersey.

Hypericin has been under active investigation as a therapeutically useful drug for treatment of AIDS. The mechanism of the anti-viral activity of hypericin was characterized and compared with that of rose bengal. Both compounds inactivate enveloped (but not unenveloped) viruses upon illumination by visible light. HIV and vesicular stomatitis virus (VSV) were photodynamically inactivated by both dyes at nanomolar concentrations. Photodynamic inactivation of fusion (hemolysis) by VSV, influenza and Sendai viruses was induced by both dyes under similar conditions (e.g., I_{50}=20-50 nM for VSV), suggesting that loss of infectivity resulted from inactivation of fusion. Syncytium formation, between cells activated to express HIV gp120 on their surfaces and $CD4^+$ cells, was inhibited by illumination in the presence of 1μm hypericin or rose bengal. Hypericin was found to be ineffective in protecting mice against infection by Friend leukemia virus (FLV). The FLV inoculum was efficiently inactivated, however, upon illumination in presence of hypericin or rose bengal. Hypericin and rose bengal thus exert similar virucidal effects. Both act at least in part, by the same mechanism, namely the inactivation of the viral fusion function by singlet oxygen produced upon illumination, although evidence for additional effects will also be presented.

Q 125 QUANTIFICATION OF HIV-1 IN CLINICAL SAMPLES USING COMPETITIVE PCR AND COLORIMETRIC DETECTION

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Methods for quantification of human immunodeficiency virus type 1 (HIV-1) based on competitive PCR and colorimetric detection were developed. The methods were used to quantify viral content in different types of patient materials, including viral DNA in peripheral blood mononuclear cells and CD4+ lymphocytes as well as HIV-1 RNA in plasma and serum. The CD4+ lymphocytes and HIV-1 RNA were isolated by simple and rapid protocols based on magnetic beads. HIV-1 DNA or cDNA and known amounts of cloned competitor DNA was co-amplified by PCR with nested primers. The competitor DNA contained the same primer binding sequences as the wild type DNA/cDNA, but in addition also the *lac* operator sequence. The ratio of wild type DNA to competitor in the PCR product was determined by a simple colorimetric solid phase assay which is specific for the *lac* operator. The methods presented here can be used for quantification of HIV-1 DNA and RNA in clinical samples and will be useful for monotoring disease progression and treatment effects.

Q 126 DISCOVERY OF INHIBITORS OF HIV PROTEASE IN NATURAL PRODUCT FERMENTATION EXTRACTS USING A HIGH VOLUME FLUORESCENTLY BASED ASSAY, Janet C. Lynn, Paul K. Tomich, Michael J. Bohanon, Miao-Miao Horng, Eric P. Seest, Lester A. Dolak, Grace P. Li, and Joyce I. Cialdella, Chemical & Biological Screening, The Upjohn Company, Kalamazoo, MI 49001

The primary cause of AIDS (acquired immunodeficiency disease syndrome) is thought to be the human immunodeficiency virus (HIV). During virus maturation, HIV protease processes the *gag* polyprotein into four virion core structure proteins and the *gag/pol* polyprotein into protease, reverse transcriptase, ribonuclease H, and integrase. However, if HIV protease is defective or inhibited the maturation and infectivity of the virus is blocked. Thus, HIV protease inhibitors would be important therapeutic compounds in the treatment of AIDS. A rapid, high-throughput, fluorescently tagged substrate (FITC, fluorescein isothiocynate) based assay was used to screen for novel, nonpeptidic inhibitors of HIV protease. We will explain the high volume assay and show a few representative compounds discovered in natural product fermentation extracts.

Q 127 RELATIONSHIPS BETWEEN SUBSETS OF CD4 AND CD8 LYMPHOCYTES, VIRAL LOAD, AND CLINICAL STATUS IN HIV+ HOMOSEXUAL MEN.

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The purpose of this study was to investigate the relationship between viral load and expression of subset markers on $CD4^+$ and $CD8^+$ lymphocytes from HIV^+ homosexual men in the Baltimore MACS study. The subjects enrolled covered the spectrum of HIV-1 infection, from recent seroconverters to persons with AIDS (CDC 1987 definition); some subjects had low $CD4^+$ lymphocyte counts without AIDS or AIDS with high $CD4^+$ lymphocyte counts. Viral load was quantified by culture or PCR of serial 5-fold dilutions of peripheral blood mononuclear cells, beginning with 10^6 cells, and expressed as the highest titer yielding a positive p24 assay or a positive PCR result. T cell sub-subsets were quantified by 2-color flow cytometry using FITC-conjugated antibodies to CD4 and CD8 and PE-conjugated antibodies to CD29, CD45RA, HLA-DR, and CD38.

Compared to persons without AIDS (n=82), persons with AIDS (n=20) had significantly ($p<0.05$) higher expression of CD38 and HLA-DR among $CD4^+$ cells, and CD38 and CD45RA among $CD8^+$ cells. Culture titer ($p>0.05$) and PCR titer ($p<0.05$) were lower in the AIDS group. Among AIDS-free subjects, relative expression of HLA-DR among $CD4^+$ lymphocytes and CD38 among $CD8^+$ lymphocytes increased slightly ($p<0.05$ for CD38/CD8) in proportion to culture titer. Among subjects with AIDS, differences in marker expression in relation to culture titer were seen, but interpretation was precluded by small sample sizes. These preliminary results support the hypothesis that relationships among T cell subset expression, HIV load, and clinical status may be observed as more individuals are studied. Specific changes in subpopulations of T cells, such as $CD38^+$ $CD8^+$ lymphocytes and HLA-DR^+ $CD4^+$ lymphocytes, may correlate with changes in viral burden in HIV^+ individuals.

Q 128 STRATEGIES FOR HANDLING HIV IN THE CLINICAL, RESEARCH, AND PRODUCTION LABORATORY, Linda S. Martin, Jonathan Richmond, and Russ Metler, National Institute for Occupational Safety and Health, Office of Health and Safety, and National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333

Laboratory workers in a variety of settings are at risk for acquiring HIV occupationally. Of the 32 documented occupationally acquired cases of HIV/AIDS, 11 were clinical laboratory technicians and one was a nonclinical laboratory technician. Among these 12 with documented occupational transmission, 8 had percutaneous exposure, 3 had mucocutaneous exposure and 1 had both. Eleven were exposed to HIV-infected blood and one to concentrated infectious HIV. CDC has also received reports of 69 workers with possible occupationally acquired HIV infection; 12 of these were clinical laboratory technicians and one was a nonclinical laboratory technician. In addition, there have been reports of workers who have developed antibodies to simian immunodeficiency virus (SIV) following exposures (primarily percutaneous injuries) in research laboratories.

In 1988, the second edition of the *Biosafety in Microbiological and Biomedical Laboratories* handbook was reprinted to include the Agent Summary Statement for HIV, previously published in CDC's Morbidity and Mortality Weekly Report. An updated third edition, including guidelines for working safely with HIV and other retroviruses in clinical, research, and production laboratories will be published this year. In addition, regulations have been promulgated by the Occupational Safety and Health Administration (Bloodborne Pathogen Standard, 29 CFR 1910.1030).

The term "containment" is used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The elements of containment, including laboratory practices and techniques, safety equipment, and facility design, will be presented. Components of the guidelines and regulations will be featured. Occupational transmission of HIV and other retroviruses to laboratory workers is preventable through rigorous adherence to safety recommendations and the use of engineering controls.

Q 129 HIV-1 RNA QUANTITATION IN CLINICAL LYSATES BY PCR USING Tth DNA POLYMERASE.
N. McKinney, J. Mulder, C. Christopherson, J. Sninsky and S. Kwok, Dept. of Infectious Diseases, Roche Molecular Systems, Alameda, CA 94501.

An assay system has been developed in which HIV-1 RNA in plasma or sera can be quantitated in less than 8 hours. The assay is performed in a single tube and uses a single enzyme, *Tth* DNA polymerase, for both reverse transcription and PCR amplification. UNG (AmpErase®) and dUTP are included for PCR product carryover prevention.

An RNA internal control is spiked and coamplified with each clinical lysate. The RNA internal control is designed so that the amplification efficiency is equivalent to that of HIV-1. The internal control uses the same primer binding sites, yields a product of the same size and base composition, but the internal sequence is shuffled and possesses a distinct probe region for differentiation from HIV-1. Product from amplification of both HIV-1 and the internal control are detected by a colorimetric microwell plate assay using distinct probes. The microwell assay format allows automation and computerized analysis and handling of data.

By spiking and coamplifying a known copy number of the internal control standard with the clinical lysate, adjustments can be made for sample/reaction differences. Following adjustment, the quantity of viral RNA can be extrapolated from an external standard curve generated separately in the same assay.

Q 130 AZT TREATMENT BLOCKS HIV-1 REPLICATION IN ACUTELY-INFECTED CD8+ CELLS, Louis Mercure[a,b], Denis Phaneuf[b] and Mark A. Wainberg[a], Lady Davis Institute - Jewish General Hospital[a] and Department of Infectious Disease, Hôtel-Dieu de Montréal[b], Montreal, Canada H3T 1E2

Different groups have established that normal peripheral CD8+ cells are susceptible to HIV-1 infection. Moreover, it has been shown that these cells are naturally infected in HIV-1 positive individuals.

Peripheral blood lymphocytes from HIV-1 seronegative individuals were infected with 0.02 $TCID_{50}$/cell of III_B strain. Eight days after infection CD8+ cells were sorted out by means of immunomagnetic particles with a yield of at least 95% of purity. These cells were maintained in IL-2-supplemented medium for 3 months without any apparent CPE in the presence of various AZT concentrations (0.4, 0.04 and 0.004 μM). HIV-1 replication in persistently-infected CD8+ cultures was monitored by measuring the amount of p24 Ag in the culture fluid. The infection of CD8+ cells has been demonstrated by two-color flow cytometry with the use of both anti-gp120 (0.5ß) and anti-CD8 (Leu2a) mAbs.

At the highest concentration of AZT, viral replication was completely inhibited. ^3H thymidine uptake has revealed that the antiviral effect of AZT in CD8+ cells correlates with its anti-proliferative activity toward these cells. A time course experiment using a quantitative PCR assay has shown that AZT added 12 h post-infection or after does not greatly reduce HIV-1 load at 0.04 and 0.004 μM, whereas the formation of proviral DNA was completely prevented at 0.4 μM even if AZT is added 24 h after infection.

In conclusion, AZT can block HIV-1 expression in acutely-infected CD8+ cells.

Q 131 DIDEOXYNUCLEOSIDE TRIPHOSPHATES INHIBIT MITOCHONDRIAL DNA POLYMERASE-γ: COMPARISON OF ZIDOVUDINE AND DIDANOSINE. Ralph R. Meyer and William Lewis, Departments of Biological Sciences and Pathology, University of Cincinnati College of Arts and Sciences and College of Medicine, Cincinnati, OH 45267-0529.

Dideoxynucleosides (ddNs) zidovudine (azidothymidine, AZT) and didanosine (dideoxyinosine, ddI) both inhibit human immunodeficiency virus replication and reduce AIDS severity. AZT's limiting side effects include both skeletal myopathy and cardiomyopathy. Limiting side effects of ddI include peripheral neuropathy. Our data with AZT suggest that these side effects may relate to altered mitochondrial replication in skeletal and cardiac muscle tissues. The active toxic moiety of ddNs is believed to be their triphosphorylated derivatives (AZTTP, ddITP). AZTTP interferes with mitochondrial DNA polymerase-γ (DNA pol-γ) and alters mitochondrial (mt-) DNA replication. We determined AZTTP- and ddITP-induced kinetic changes in DNA pol-γ activity in a model of mitochondrial toxicity *in vitro*.

Purified DNA pol-γ was utilized in kinetic reactions *in vitro* with AZTTP or ddITP as an inhibitor. Results showed AZTTP inhibited DNA pol-γ activity by 50% at 1 μM AZTTP. ddITP caused 50% inhibition of DNA pol-γ activity at 5 μM.

Data suggest that a common biochemical mechanism for the toxicity of both AZT and ddI *in vivo* may relate to AZTTP and ddITP inhibition of DNA pol-γ activity in mitochondria with decreased mtDNA in affected organelles. Interference with DNA pol-γ activity by AZTTP, possibly by mtDNA chain termination, may be a pivotal biochemical mechanism of AZT cardiac and skeletal muscle toxicity. By analogy, this biochemical mechanism may be common amongst dideoxynucleosides even though they may not share toxic changes in the same organs.

Q 132 SULFATED POLYANIONS ENHANCE HIV-1 REPLICATION IN MACROPHAGES, Pascal R.A. Meylan, Richard S. Kornbluth and Douglas D. Richman. Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, and Departments of Pathology and Medicine, University of California San Diego.

Dextran sulfate (DS) is a potent inhibitor of HIV replication in lymphocytic cells, apparently by reducing binding of virions to host cells. DS has been shown to block antibody binding to the V3 principal neutralizing determinant of HIV gp120, possibly through electrostatic interactions between DS and the positively charged amino acids in this loop. Since cellular tropism determinants map to the same structure, we examined whether polyanions would have a different effect on the infection of macrophages by HIV-1.

Using the HIV-1BaL isolate, we found that DS (and also the closely related polyanion heparin) enhanced infection in monocyte-derived macrophages and PMA-stimulated THP-1 cells up to 10 fold, and increased the binding of HIV-1BaL to PMA-stimulated THP-1 cells in a flow cytometry assay. This level of enhancement is at least as high as any antibody-mediated enhancement described with HIV. When the effect of DS was tested on 13 primary isolates, enhancement was observed in 5/13, inhibition in 2/13 and no effect in 6/13.

These data are interesting in two respects. First, when DS was administered intravenously to patients infected with HIV, the serum p24 antigen, a marker for viral replication, was found to increase over two weeks in 8/8 patients (Flexner et al, Antimicrob. Agents Chemother., 1991;35:2544). Our observation that DS can enhance HIV infection of macrophages may explain the possible adverse clinical effect of this drug. Second, the overall electric charge of the amino acids of the V3 loop of primary isolates has been shown to correlate with their cytopathicity and tropism. We are currently testing whether the effect of DS on HIV replication can be predicted from the electric charge of the V3 loop of gp120.

Q 133 THE ROLE OF INTERFERON IN HIV DISEASE. Donna Mildvan, Beth Israel Medical Center, New York, New York 10003

An important yet paradoxical role has been recognized for the interferons – both limiting *and* contributing to the pathogenesis of HIV disease. Inteferon (IFN)-α is a potent antiretroviral, with activity *in vitro* and *in vivo* against HIV, and of proven efficacy in HIV-associated Kaposi's sarcoma. However, patients with advanced HIV disease display dysregulation of the endogenous cytokine system. High levels of endogenous IFN have been associated with a poor prognosis and may contribute to the febrile/wasting syndrome characteristic of HIV constitutional disease. Not surprisingly, detectable circulating IFN is associated with reduced tolerance and effectiveness of exogenously administered recombinant IFN treatment -- an intervention which has, therefore, been challenged for patients with advanced HIV disease.

We have explored these questions along several lines. ACTG 068: A Phase I/II Trial of Combination Low-Dose Zidovudine (ZDV) and IFN-α-2A in HIV p24 Antigenemic Patients with Early ARC has demonstrated an acceptable degree of tolerance among 36 treated patients, particularly at the highest combination dose level examined: 600 mg ZDV plus 6 x 10^6 U IFN daily. Using a novel study design that permitted application of the Combination Index Method of Chou and Talalay, *in vivo* antiretroviral synergy with respect to HIV p24 antigen suppression was shown, also favoring the highest dose level. There is, moreover, indication of clinical and CD4+ cell stabilization in this symptomatic patient population. These results, taken together with the demonstration that ZDV but not placebo lowers circulating IFN and triglyceride levels in patients with advanced HIV disease (Mildvan et. al., Lancet 1992; 339:453-6), point to the interpretation that ZDV may enhance the tolerance and response to treatment with recombinant IFN-α via a beneficial effect on the endogenous cytokine network. Thus combination ZDV/IFN-α warrant further development for the treatment of HIV infection.

Q 134 ENHANCED DIAGNOSTIC EFFICIENCY OF THE POLYMERASE CHAIN REACTION (PCR) BY CO-AMPLIFICATION OF MULTIPLE REGIONS OF HIV-1 AND HIV-2, Udaykumar, Alonso Heredia, Vincent Soriano, Jay S. Epstein and Indira K. Hewlett, CBER/FDA, Bethesda, MD 20892.

PCR has been used widely in the diagnosis of both HIV-1 and HIV-2. In most cases, amplification is performed with a single set of primers targeted to a specific gene sequence. This approach suffers the disadvantage that false negative reactions could occur due to mismatches or deletions caused by genetic variation. In such instances, amplification of multiple target sequences may be necessary for greater sensitivity of detection of highly variant strains of viruses. One of the limitations of this approach is sample availability which may be scarce in the case of clinical samples. We have designed a scheme of co-amplification of multiple regions of HIV-1 and HIV-2 in a single reaction, allowing for multiple analysis on a single sample. Primers were designed from the gag, pol and env regions of the viral genomes and the PCR reaction optimized for sensitive and specific detection of their target sequences. The amplified products generated differ from one another by a minimum of 100 base pairs hence distinct bands could be identified after gel electrophoresis. In another set of experiments, primers that amplify gag gene sequences were designed so that the 5' oligonucleotide is common for both viruses. At the 3' end two oligos specific for each viral sequence were used. Further specificity and sensitivity was derived from hybridization with a type specific probe. Both approaches yielded a sensitivity of 1-10 copies for HIV-1 and 1-50 copies for HIV-2. These primer sets were able to identify 4/4 HIV-2 infections in individuals who were dually reactive for HIV-1 and HIV-2 by ELISA. In all cases, no reactivity was seen with the HIV-2 samples using HIV-1 primers. These primers are being used to detect viral sequences in serum using RNA PCR. In conclusion, co-amplification of multiple target sequences with multiple primers may be of practical advantage and clinical utility in the diagnosis and typing of HIV-1 and HIV-2 infections.

Q 135 INACTIVATION OF HIV-INFECTED CELLS IN WHOLE BLOOD BY LYSING/FIXING REAGENTS, Janet K.A. Nicholson, Sandra W. Browning, Sherry L. Orloff, and J. Steven McDougal, Centers for Disease Control and Prevention, Atlanta, GA 30333

Lymphocyte immunophenotyping using the whole blood lysis method is performed by incubating anticoagulated whole blood with fluoresceinated monoclonal antibodies, then lysing the red blood cells. The specimens are then fixed with formaldehyde or paraformaldehyde to 1) stabilize the preparations before analysis on the flow cytometer and to 2) inactivate any potentially infectious microbes. Since many commercially-available lysing reagents also contain fixatives, we tested the ability of the lysing and fixing reagents to inactivate HIV-infected cells in whole blood. Whole blood, spiked with cells from an HIV-positive cell line (H9), was lysed and fixed according to manufacturers' protocols. The cell preparations were then cocultured with T-cell blasts in serial 10-fold dilutions to rescue infectious virus and measure viral titer in a sensitive infectivity assay (capable of detecting 1 H9 cell). All commercial lysing/fixing reagents tested inactivated cell-associated HIV by 3 to 5 logs, while ammonium chloride had little effect. Although an additional incubation with 1% formaldehyde for 30 minutes did not increase the effectiveness of the commercial lysing/fixing reagents to inactivate HIV, it inactivated cell-associated HIV in blood treated with ammonium chloride by 3 to 4 logs.

Q 136 EVALUATION OF *IN VITRO* ZIDOVUDINE RESISTANCE BY DIRECT QUANTITATIVE PBL ASSAY IN HIV INFECTED MOTHERS AND THEIR CHILDREN, Karin Nielsen, Lian Wei, Lisa Frenkel, Sheryl O'Rourke, Audra Deveikis, Margaret Keller, E. R. Stiehm, Yvonne Bryson, Dept. of Pediatrics, UCLA, LAPAC, Los Angeles, CA 90024.

Development of resistance to Zidovudine (ZDV) during the course of HIV infection contributes to HIV morbidity; however the correlation of *in vitro* results with clinical data is unclear. In order to minimize potential artifacts of virus passage and load, we studied *in vitro* ZDV sensitivity using a direct quantitative peripheral blood lymphocyte assay (PBL) in 38 HIV infected patients (9 mother/infant pairs, 3 women, 17 children) receiving ZDV for mean 17.5 months (4-33 mo), compared it to the Consensus ACTG assay and correlated results with clinical status. Ficoll-Hypaque separated PBLs were serially 5-fold diluted ($1x10^6$ to $8x10^3$) into wells with normal PHA stimulated PBLs, IL2, & ZDV: 0.05, 1.0, 5.0, 10.0uM and controls. At 7 days cultures were re-fed with donor PBLs & ZDV and harvested at 14 days for p24Ag. Isolates were defined as very sensitive (VS) if inhibited by ZDV $\leq$0.05uM, sensitive (S) $\leq$1.0uM, moderate resistant (MR) $>$ 1uM $<$5uM, and resistant (R) $>$5uM in the last positive virus control dilution. This assay compared favorably with the ACTG assay in 9/10 patients. Overall, 18 patients had ZDV (VS, S) virus whereas 20 had *in vitro* ZDV (MR, R) virus. Of the 9 mother/infant pairs on ZDV, 6 had concordant results with ZDV (VS, S) virus. 3 infants developed ZDV (R) virus prior to their mothers despite a similar duration of Rx (4,9,17 mo).

	MR, R (n=20)	VS, S (n=18)	P value
Mean ZDV Rx	19 mo.	15.7 mo.	
Failure to thrive/wt. loss	13 (65%)	1 (5%)	0.0001
CNS symptoms	6 (30%)	0	0.02
Recurrent bact. infect.	13 (65%)	5 (28%)	0.02
Opportunistic infect.	6 (30%)	3 (17%)	NS
Change in Rx (dis.prog.)	15 (75%)	0	$<$0.0001

In vitro ZDV resistance may correlate with clinical deterioration. Children may develop ZDV resistance more rapidly than adults.

Q 137 THE USE OF BENZOPORPHYRIN DERIVATIVE AS A POTENTIAL AGENT IN THE TREATMENT OF HIV INFECTION. Janice North and Julia Levy. Quadra Logic Technologies, 520 W 6 th Ave. Vancouver, B.C. Canada.

The discovery of new therapeutic agents for the treatment of AIDS is an obvious high priority. Current treatments using nucleosides, such as AZT, have focused on inhibiting viral replication and thus keeping the viral burden low. Such treatments have generally led to a stabilization of the CD4 population and a subsequent delay in the onset of AIDS. However, toxic side effects and the emergence of AZT resistant strains of HIV impose limitations on the efficiency of such drugs. Other effective treatments are therefore being sought after.

One such potential treatment aimed at reducing circulating infected cells and virus, involves the use of photosensitizer molecules activated by light at an appropriate wavelength. Benzoporphyrin derivative (BPD) is a lipophilic photosensitizer that is activated at 690 nm, which makes it suitable for viral inactivation in blood. Our studies with HIV infected blood have shown that 1. both free and cell-associated virus are photoinactivated using physiological concentrations of BPD and light ; 2. AZT resistant and sensitive strains of virus are equally susceptible to photoinactivation and 3. "activated" leukocytes are selectively eliminated by such treatment.

Therefore the use of photosensitizers, such as BPD, may be a beneficial treatment modality for HIV infected individuals, either alone or as a combination therapy, since it could be effective in both lowering the virus burden and preventing immunological debilitation by eliminating activated cells which both support viral replication and which may also be involved in an autoimmune mechanism thought to accelerates CD4 depletion.

Q 138 QUANTITATIVE HIV-1 RNA PCR ANALYSIS FOLLOWING IN VIVO IMMUNE ACTIVATION IN PATIENTS WITH ARC, Stanislava Ovcak-Derzic and William A. O'Brien, Department of Medicine, West Los Angeles VA Medical Center and UCLA School of Medicine, Los Angeles, CA

Symptoms of AIDS typically occur many years following acute infection. Factors which influence the duration of this clinical latency remain poorly defined, however, in vitro activation of HIV-infected blood CD4+ lymphocytes and macrophages by mitogens or cytokines can markedly increase virus production. We examined the effect of cell activation by influenza immunization by measuring levels of HIV-specific RNA in peripheral blood mononuclear cells (PBMC). Blood samples were obtained on 3 occasions just prior to influenza immunization, and again, 3 to 5 times after immunization. Purified RNA from 3 x 10^5 PBMC was analyzed in parallel with RNA standards for each of 16 patients, and 5 HIV patients who did not receive influenza immunization but had blood samples obtained at similar intervals. There was little baseline variation in viral RNA levels in preimmunization samples or in samples from the non-immunized controls, but a tenfold or greater increase was seen in post-immunization samples in 9 of 16 patients. The peak increase in virus replication was seen at 1 to 2 weeks after immunization, and levels fell to approach baseline values after several months. We have obtained samples from 15 additional patients to extend our analysis, and will follow patients in the VA Immunodeficiency clinic to correlate changes in HIV-1 expression with clinical outcome.

Q 139 QUANTITATION OF HIV-1 RNA IN PLASMA USING A SIGNAL AMPLIFICATION BRANCHED DNA (bDNA) ASSAY. C. Pachl, T. Elbeik, M. Saxer, D. Kern, M. Stempein, S-J. Fong, P. Sheridan, T. Yeghiazarian, P. Neuwald, M. Urdea, M. Feinberg, and J. Todd. Chiron Corp., Emeryville, CA and San Francisco General Hospital, San Francisco, CA.

The level of HIV-1 RNA in plasma has been quantitated using a nucleic acid hybridization assay based on branched DNA (bDNA) technology using signal amplification. Virus is concentrated from 1 ml of plasma, using a benchtop microtrifuge, and a viral lysate is prepared and added to microwells. The RNA target is captured onto the microwell surface, detected via bDNA amplifier molecules and alkaline phosphatase labeled oligonuceotides, and signal is generated using a chemiluminescent substrate.

HIV plasma RNA levels were examined in longitudinal clinical studies. Plasma was analyzed from 12 patients enrolled in the ACTG 175 protocol. Specimens were taken at baseline and 8 weeks after therapy with ddI/AZT, ddC/AZT, AZT or ddI. Consistant values for plasma HIV-1 RNA were observed with two baseline specimens from each patient, and viral RNA load stayed the same or decreased in specimens taken 8 weeks after initiation of therapy. Plasma from 6 patients from another study (non-ACTG) were analyzed by quantitative plasma culture and the RNA assay. HIV-1 RNA was detected in all baseline samples whereas only one baseline sample was detected by plasma culture. An increase in viral RNA copies after 4 to 8 weeks correlated with an increase in plasma virema measured by plasma culture. These results indicate that this HIV-1 RNA assay is a reproducible method for the quantitation of the level of HIV-1 in plasma during the course of antiviral therapy. This method is also amenable to routine laboratory use.

Q 140 EFFECT OF PROTEASE INHIBITOR RO 31-8959 ON EARLY STAGES OF HIV REPLICATION *IN VITRO*,

L.A. Panther, R.W. Coombs, D. Gretch, C. dela Rosa and L. Corey, University of Washington, Seattle, USA.

Introduction: HIV-encoded protease is necessary for the proper processing of structural precursor proteins and maturation of infectious virus progeny. The aspartic protease inhibitor Ro 31-8959 (PI) blocks the activity of this protease and thus the maturation process. Although HIV-encoded protease also cleaves the gag-pol protein product to form functional HIV reverse transcriptase, it is controversial whether PI interferes with early events in HIV replication. We tested the hypothesis that PI interferes with early steps in HIV replication.

Methods: CEM cells were incubated one hour with 1500 $TCID_{50}$ of HIV_{LAI}, washed and cultured in medium with or without 30nM PI. Immediately after infection and on days 2, 3, 4 and 7, aliquots containing $5x10^4$ cells were harvested for DNA and RNA/cDNA PCR analysis. Early HIV DNA transcripts were detected using primers AA55/M667, proviral DNA using *gag* primers SK 38/39 and 2-LTR circular unintegrated HIV DNA using primers U3/U5. RNA was extracted in guanidinium isothiocyanate, reverse transcribed, and the cDNA product was PCR amplified using the *tat* mRNA primers MF8760/MF5869. All PCR products were hybridized with ^{32}P-tagged nested probes, separated on 10% polyacrylamide gel, and detected by autoradiography.

Results: In cultures without added PI, early transcript HIV DNA and *gag* HIV DNA was detected by day 2, *tat* mRNA was detected by day 3, and 2-LTR circular unintegrated HIV DNA was detected by days 3 to 4. In contrast, for cultures treated with PI, there was no detectable early HIV proviral transcript, proviral *gag*DNA, *tat*mRNA or 2-LTR circular HIV DNA after 7 days of culture. PI had no effect on cell viability.

Conclusions: These data suggest that PI may alter HIV replication at more than one stage. The lack of proviral early transcript DNA, *gag*DNA, *tat*mRNA or unintegrated 2-LTR circular HIV DNA suggest PI has an effect prior to reverse transcription. The prospect of PI acting at both early and late stages of HIV replication augments its potential as an effective antiretroviral agent *in vivo*.

Q 141 CHANGES IN VIRAL LOAD IN THE PERIPHERAL BLOOD OF HIV-INFECTED INDIVIDUALS ASSESSED MULTIPLE TIMES OVER ONE MONTH

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Few studies have addressed the changes in the viral load of HIV-infected persons over short periods of time. In this study, short-term changes in cellular viral load were measured using PCR and a liquid phase molecular hybridization system that allows quantitation of the amount of HIV-1 DNA in a sample. To account for variations in the numbers of cells in each sample, values for the amount of HIV-1 DNA were normalized to the amount of total DNA as determined by fluorimetry with Hoechst 33258 dye. These experiments showed 20 of 34 HIV-infected individuals maintained stable HIV-1 DNA levels in their peripheral blood mononuclear cells in two to four serial samples taken at approximately one week intervals. The remaining 14 individuals showed significant changes ($p< 0.05$), ranging from less than 2-fold to greater than 9-fold, in the amount of HIV-1 DNA over the same time period, with 10 individuals having either a consistent increase or decrease, 2 having an increase followed by a decrease, and 2 having a decrease followed by an increase. These changes were confirmed in multiple assays. These decreases or increases may have resulted from the homing or release, respectively, of HIV-infected cells, or bursts of HIV replication. Assessment of correlations with CD4 levels, serum p24, or plasma viremia with the observed changes in cellular viral load may lead to an explanation of the mechanisms involved.

Q 142 **Consistency of cell-free HIV-1 viral load measured by plasma culture and immunocapture-cDNA/PCR.** Jack F. Phillips, Denis Henrard, Joel Gibson, Eric Eggert and Robert W. Coombs. Abbott Laboratories, Abbott Park, IL; University of Washington, Seattle, WA.

Objectives: Determine the semi-quantitative variability of cell free HIV-1 viral load measured by plasma culture and genomic RNA detection. Assess the biological significance of detecting HIV-1 RNA using immunocapture-cDNA/PCR.

Methods: Serial samples were collected from 4 infected individuals over 40-60 days and from one person over a 24 hour period. Plasma HIV-1 titer was determined in real time while HIV-1 RNA levels were measured on batched frozen samples. An average of 5 samples were analyzed for each individual using a 5-fold and a 10-fold end-point dilution protocol for plasma culture and RNA detection, respectively.

Results: The intra- and inter-PBMC donor variability for plasma culture titers was 0.82 and 1.14 log TCID50/ml, respectively. In contrast, immunocapture-cDNA/PCR could reliably and consistently detect a log difference in titer. For the 5 patients analyzed, plasma HIV-1 titers varied by a mean 1.2 log TCID50/ml, while HIV-1 RNA levels were very stable and indistinguisable from baseline measurements for each patient tested. End-point dilutions indicated that the HIV-1 plasma and RNA titers were comparable (2-3 log).

Conclusion: In contrast to plasma titers, HIV-1 RNA detection by immunocapture-cDNA/PCR revealed no significant variation in cell-free viral load. Semi-quantitative measurements of HIV-1 RNA appears to be a good reflection of plasma infectious titer. Therefore, the detection of HIV-1 RNA by immunocapture-cDNA/PCR provides a rapid, sensitive and consistent means of assessing HIV-1 plasma viral load.

Q 143 **HIGH LEVELS OF PLASMA VIRUS DURING ALL STAGES OF HIV-1 INFECTION, INCLUDING ASYMPTOMATIC INFECTION: MEASUREMENT BY QUANTITATIVE COMPETITIVE PCR (QC-PCR),** *M. Piatak, Jr., M.S. Saag, L.-M. Yang, S. J. Clark, J. C. Kappes, K.-C. Luk, B.H. Hahn, G.M. Shaw, and J.D. Lifson, Genelabs Incorporated, Redwood City, CA 94063 and The University of Alabama at Birmingham, Birmingham, AL, 35294*

Competitive Polymerase Chain Reaction (PCR) methods obviate limitations inherent in the PCR that make it non-optimal for quantitative applications. We used Quantitative Competitive PCR (QC-PCR) methods to quantify virion-associated HIV-1 RNA in plasma from 55 infected subjects with different stages of disease, including longitudinal specimens from six patients from the time of acute (primary) infection (CDC Stage I). QC-PCR was used along with processing methods employed to maximize recovery of particle-associated HIV-1 RNA from plasma. QC-PCR readily quantitated HIV-1 RNA in plasma from all subjects at all time points, including asymptomatic patients, providing evidence for ongoing viral replication throughout infection. Determined values ranged from 2,000 to more than 20,000,000 HIV-1 RNA copies per milliliter of plasma (corresponding to from 1000, to more than 10,000,000 virions), levels 10-100 fold higher than have been reported previously in similar patients, using other methods. The highest levels of circulating virus were seen in patients with acute infection or CDC Stage IV disease, with a correlation between QC-PCR determined levels of plasma virus and both disease stage and absolute counts of CD4+ T cells. QC-PCR determined virus levels correlated with, but exceeded by 1,000 to 10,000-fold titers of infectious units determined by endpoint dilution culture. The level of viremia throughout all stages of HIV disease thus appears to be much higher than has been thought previously, and may be composed in part of non-infectious forms of the virus that may contribute to pathogenesis. In addition to shedding light on the pathogenesis of HIV-1 infection, reliable quantitation by QC-PCR of persistent viremia at all stages of HIV-1 infection provides a useful direct virologic endpoint for clinical studies, particularly in early stage disease where conventional virologic markers are often negative.

Q 144 NOVEL ZINC-EJECTING C-NITROSO COMPOUNDS INHIBIT THE INFECTIOUS AND EXPRESSIVE PHASES OF HIV-1 LIFE CYCLE, William G. Rice[1], Catherine A. Schaeffer[1], J. Steven McDougal[2], Sherry L. Orloff[2], Michael F. Summers[3], Terri L. South[3], Jerome Mendeleyev[4] and Ernest Kun[4]. [1]Laboratory of Antiviral Drug Mechanisms, Program Resources Inc./DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702; [2]Immunology Branch, DHA, NCID, Centers for Disease Control, US Public Health Service, Atlanta, GA 30333; [3]Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21228; [4]Octamer Research Foundation, Tiburon, CA 94920.

The HIV nucleocapsid (NC,p7) protein and its *gag*-precursor polypeptide contain two copies of an invariant Cys-X_2-Cys-X_4-His-X_4-Cys (CCHC) sequence that binds zinc and is 100% conserved among all known strains of HIV and other retroviruses. NC plays a critical role in the formation of a functional reverse transcription complex during the infectious phase of the retroviral life cycle, and the NC portion of the *gag* precursor functions in the expressive phase for encapsidation of viral genomic RNA. Recently we reported (Rice et al, *Nature*, In Press) that novel aromatic C-nitroso compounds (in particular 3-nitrosobenzamide, NOBA) react specifically with the CCHC zinc finger motif, causing ejection of zinc from isolated zinc fingers and intact virions and inactivating the infectivity of HIV-1 in human lymphocytes. NOBA has now been shown to inhibit a variety of isolates ranging from the LAV strain of HIV-1 to a lethal variant strain of SIV (SMMpbj), and the inactivation occurred in a concentration and temperature dependent manner. NOBA blocked formation of PCR-detectable proviral DNA but did not block attachment of HIV-1 to lymphocytes or the *in vitro* activity of reverse transcriptase. Addition of NOBA to previously infected lymphocytes blocked the expressive phase production of virus, but NOBA was not inhibitory to the *in vitro* activity of HIV-1 protease. NMR studies demonstrated that NOBA displaced zinc from both zinc fingers in a recombinant form of the intact HIV-1 NC protein and dissociated complexes of the NC protein with tightly bound RNA substrates (oligoribonucleotide equivalent to the psi packaging sequence of HIV-1 genomic RNA). These date indicate that non-toxic levels of NOBA disrupt both the infectious and expressive phases of the HIV-1 life cycle and that the design of zinc-ejecting agents that target retroviral zinc fingers represents a novel approach to the treatment of AIDS.

Q 145 A NOVEL METHOD FOR THE MEASUREMENT OF INTRACELLULAR ZIDOVUDINE TRIPHOSPHATE (ZDV-TP), Brian L. Robbins, Connie McDonald, William Holden, Patricia M. Flynn, Ranga V. Srinivas, and Arnold Fridland, Department of Infectious Disease, St. Jude Children's Research Hospital, 332 North Lauderdale Memphis, TN 38105.

The *in vivo* intracellular pharmacokinetics of ZDV-TP, the active antiviral metabolite of ZDV, is a valuable parameter because it may give insight into the efficacy and eventual loss of antiviral activity with prolonged ZDV treatment. The determination of *in vivo* ZDV-TP concentration in patients is difficult due to the exclusion of radioisotopic methods and the low levels of ZDV-TP produced. We developed a sensitive assay to measure ZDV-TP based on inhibition of HIV reverse transcriptase (HIV-RT) activity. We isolated PBLs from the blood of healthy individuals and incubated them for four hours with labeled ZDV to final concentrations of 0.5, 5, 10 and 50 μM, with and without PHA-P stimulation. Intracellular ZDV-TP concentration was determined in cell extracts using our enzymatic assay and validated by HPLC separation and liquid scintillation counting of the radioactive ZDV-TP. The two methods gave virtually the same answers, resulting in levels of 0.763 pmol/10^6 cells to 5.268 pmol/10^6 cells with 0.5 and 50 μM ZDV, respectively. In unstimulated control PBLs the level of ZDV-TP was about 100-fold lower than with stimulated PBLs. Results for cells isolated from three HIV-infected patients that received a single oral dose of 500 mg of ZDV gave concentrations of 0.18, 0.52, and 0.1 pmol/10^6 cells of ZDV-TP and 0.26, 0.38, and 0.04 pmol/10^6 cells, 1 and 4 hr post ZDV administration, respectively. This assay should prove useful in further studies of ZDV metabolism in patient derived PBLs at the doses of ZDV currently administered. Supported by grants 1R01 AI27652, 1R01 AI31145, Cancer Center Support CORE Grant P30 CA21765 from the NIH and by the American Lebanese Syrian Associated Charities.

Q 146 CHARACTERIZATION OF THE HIV-1-SPECIFIC CYTOTOXIC T LYMPHOCYTE RESPONSE DURING ACUTE INFECTION, Jeffrey T. Safrit, Charla A. Andrews, Alex Lee and Richard A. Koup, Aaron Diamond AIDS Research Center and Departments of Medicine and Microbiology, New York University Medical School, New York, NY 10016

The HIV-1-specific cytotoxic T lymphocyte response was investigated in three individuals during or shortly after acute symptomatic seroconversion to HIV-1. In all three cases, virus-specific CTL were detected earlier than neutralizing antibody responses. In one individual who was identified early, CTL precursors specific for HIV-1 gag, pol and envelope antigens were detected three weeks before seroconversion, and before a drop in virus load had occurred. In two other individuals, CTL were detected after seroconversion but before neutralizing antibodies had appeared. CTL clones specific for HIV-1 gag, pol and envelope have been generated on these individuals. Envelope clones restricted by HLA A31, A32, and B7 have been characterized further. Two HLA A31-restricted clones from the same individual were found to have differing virus strain specificities. Clone R2D3 recognizes IIIB but not RF or MN strains of HIV-1 while clone 3B1 recognizes IIIB and RF but not MN. Both clones recognize the 11 amino acid peptide RLRDLLLIVTR from position 770-780 of the gp41 region of molecular clone HXB2. A change at position 779 in the epitope from T to V abrogates lysis by clone R2D3 but not 3B1. An HLA A3.1-restricted clone (E7.20) has previously been shown by Takahashi et al (PNAS, 1991) to recognize this same minimum epitope. Clone 1E which was generated from another seroconverting individual recognizes amino acids 769-778 of gp41 and therefore overlaps significantly with the region recognized by clones R2D3, 3B1 and E7.20. Finally, an A32-restricted clone was isolated from the same individual as clone 1E and was found to recognize a different region of gp41 which contains a similar amino acid motif to that found in amino acids 769-780, consistent with motif-specific binding of peptides to HLA class 1 molecules. The implications of these findings in relation to acute HIV-1 infection will be discussed.

Q 147 *IN VITRO* ACTIVITY OF TNP-470, A NOVEL ANGIOGENESIS INHIBITOR, IN KAPOSI'S SARCOMA (KS)-RELATED SPINDLE CELL LINES. M. Wayne Saville, Andrea Foli, Samuel Broder, and Robert Yarchoan, Retroviral Diseases Section, Medicine Branch, National Cancer Institute, Bethesda, MD 20892.

Several groups have recently reported that spindle cell lines derived from HIV-1-associated KS lesions may provide a model for the study of this disease. Such lines grow in response to conditioned medium from retrovirally-infected cells and produce both angiogenic and autocrine growth factors. In an attempt to further investigate this area and develop therapeutic strategies, we used previously published methods to derive spindle-cell lines from the pleural fluid of an HIV(+)patient with aggressive pulmonary KS (line KKS-2), an HIV(+) patient with a pleural effusion secondary to lymphoma but without KS (HKS-1), and an HIV(-) patient with a post-surgical effusion (NKS-1). These lines were found to have characteristics similar to those of previously reported spindle lines, including growth in response to hydrocortisone and HTLV-II-conditioned medium, senescence after 14-18 passages, and substantial IL-6 production. Furthermore, they all stained positively for the following histologic markers: non-specific esterase; *Ulex europaeus* lectin; α-smooth muscle actin; factor VIII (vWF); CD34; and keratin. We utilized two of these lines, KKS-2 and HKS-1, to evaluate the activity of TNP-470 (*O*-(chloroacetylcarbamoyl) fumagillol), an angiogenesis inhibitor closely related to the fungal product fumagillin. TNP-470 inhibited the proliferation of KKS-2 and HKS-1 with an IC_{50} of approximately 1 ng/ml. In contrast, TNP-470 did not inhibit proliferation of H9 T cells, or PHA-stimulated mononuclear cell cultures until concentrations of $\geq$1 μg/ml were reached. TNP-470 had no effect on HIV-1 replication in acutely infected H9 cells or elutriated human monocyte/macrophage cultures, as measured by p24 antigen production in culture supernatants. Together with other studies of TNP-470, these data suggest that clinical testing in patients with KS may be warranted. At the same time, the finding that spindle cell lines can be derived from patients without KS or HIV infection suggests that these may represent normal cells under stimulation by growth factors. This is consistent with the notion that at least at certain stages, excessive stimulation by soluble factors may be a central event in the pathogenesis of KS.

Q 148 **Prevention of HIV-1 Infection in Chimpanzees by a Nonnucleoside Reverse Transcriptase Inhibitor,** William A. Schleif[1], Vinod V. Sardana[1], Vera W. Byrnes[1], Krishna K. Murthy[2], and Emilio A. Emini[1]: [1]Merck Research Laboratories, West Point, PA 19486: [2]Southwest Foundation for Biomedical Research, San Antonio, Tx 78228

The HIV-1 specific pyridinone nonnucleoside reverse transcriptase inhibitor L-696,229 has been shown to prevent HIV-1 infection in cell culture (IC_{95} = 0.1μM). The therapeutic potential of this and related nonnucleoside inhibitors is undergoing clinical assessment in HIV-1 infected humans. Initial data suggest that the inhibitor's usefulness as monotherapy is limited by rapid selection for resistant virus. However, viral populations from untreated individuals are generally sensitive to the inhibitor. Accordingly, a study was performed in chimpanzees to evaluate the possible use of the inhibitor for prevention of HIV-1 infection.

L-696,229 was prepared as a liquid oral pediatric formulation and was administered to chimpanzees prior to virus challenge. A single 400mg dose of the inhibitor resulted in peak (one hour post-dosing) plasma levels of 1.0 to 4.0 μM and in trough (six hours post-dosing) levels of 0.1 to 0.5 μM. Three chimpanzees were intravenously inoculated with 100 animal infectious doses of HIV-1, one hour following administration of either the inhibitor (two animals) or a placebo (one animal). The chimpanzees were maintained on four daily 400mg doses of L-696,229 or placebo equivalent. The placebo-treated animal (x291) developed signs of persistent virus infection by 4 weeks, post-challenge. In contrast, the inhibitor-treated animals exhibited either a significant delay in the establishment of infection (16 weeks, x090) or exhibited no infection for up to 24 weeks post-challenge (x342). The latter animal had substantially higher trough plasma levels of inhibitor (approx. 0.5μM) than those exhibited by chimpanzee x090 (approx. 0.1μM). x342 has remained free of HIV-1 infection following withdrawal of inhibitor treatment at 20 weeks. These results establish the potential of the nonnucleoside RT inhibitors as prophylactic agents. The development of inhibitors with improved pharmacokinetic profiles is on going.

Q 149 ANTI-HIV ACTIVITY OF DIDEOXYNUCLEOSIDES IN HUMAN RESTING AND ACTIVATED PERIPHERAL MONONUCLEAR CELLS. Takuma Shirasaka, Wen-Yi Gao, Eiji Kojima, and Hiroaki Mitsuya. Experimental Retrovirology Section, Medicine Branch, NCI, NIH, Bethesda, MD 20892.

We found that azidothymidine (AZT) exerts an extremely potent antiviral activity against HIV-1 as compared to dideoxycytidine (ddC) and dideoxyinosine (ddI) *in vitro* on the basis of molarity when assessed in phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM). The antiviral activity of AZT was about 5-fold and 100-fold more potent than that of ddC and ddI, respectively. These antiviral activities contrast with the recommended daily doses of AZT, ddC and ddI, that are approximately 500 mg, 2.25 mg, and 500 mg, respectively. Studies of intracellular anabolic phosphorylation revealed that AZT was preferentially phosphorylated in PHA-PBM to produce substantially higher ratios of AZTTP (triphosphate of AZT)/TTP than in non-dividing resting PBM (R-PBM). In contrast, phosphorylation of ddC is less efficient in PHA-PBM to produce lower ratios of ddCTP (triphosphate form of ddC)/dCTP than in R-PBM. Ratios of ddATP (triphosphate form of ddI)/dATP were substantially lower in PHA-PBM than in R-PBM. These results suggest that AZT, ddC, and ddI exert disproportionate antiviral effects depending on the activation state of the target cells; *i.e.* ddI and ddC exert antiviral activity more favorably in resting cells than in activated cells, while AZT preferentially protects activated cells against HIV infection.

We further asked whether these nucleoside antiretroviral agents could block the infectivity of HIV-1 in R-PBM by employing the synthesis of R/U5 HIV-1 DNA as an endpoint by using the polymerase chain reaction. We then found that ddI inhibited the synthesis of R/U5 efficiently in R-PBM.

HIV-1 proviral DNA synthesis is known to be initiated in quiescent lymphocytes, which represent the majority of circulating lymphocytes, as efficiently as in activated lymphocytes. Thus, the current data may have practical relevance in the design of anti-HIV chemotherapy, in particular, combination chemotherapy. It is also suggested that dividing, activated cells should not be used alone in the evaluation of antiviral activity of nucleoside analogues.

Q 150 LONG-TERM PERSISTENCE OF ZIDOVUDINE-RESISTANCE MUTATIONS IN THE PLASMA HIV-1 OF PATIENTS REMOVED FROM ZIDOVUDINE THERAPY. Marilyn S. Smith [1], Karen L. Koerber [1], and Joseph S. Pagano [1,2,3]; [1]Lineberger Comprehensive Cancer Center, Departments of [2]Microbiology and Immunology and [3]Medicine, University of North Carolina, Chapel Hill, NC 27599

The emergence of 3'-azido-2',3'-dideoxythymidine (zidovudine)-resistant isolates of human immunodeficiency virus type 1 (HIV-1) from AIDS patients treated with zidovudine has been reported. Here, we examined the sequence of viral reverse transcriptase (RT) by cloning and sequencing non-sibling clones after polymerase chain reaction (PCR) amplification from uncultured leukocytes and from plasma culture DNA. In two patients studied, three resistance mutations (67N, 70R, 215Y) were detected by PCR amplification of RT sequences only from patient plasma cultures and not from the same patients' leukocytes; only the 41L mutation was detected in 2/15 clones from leukocytes in one patient. The differences in distribution of the mutant genotypes from the two sources were highly significant ($P < .001$).

We have identified and followed several patients who discontinued zidovudine treatment. RT sequences were determined by sequencing of cloned PCR-amplified material, usually from plasma culture DNA, from these patients. Multiple characteristic mutations of zidovudine resistance have persisted in six timepoints from four patients for 5 to 15 months (ave. 10.1 mo.) despite the cessation of therapy. In 91 non-sibling clones from these timepoints, the codon 215Y mutation was present in 100%; the 70R, G, or E, 67N, and 219Q mutations were each present in approximately 30% of the clones. Isolates available from two of the patients showed intermediate and highly zidovudine-resistant virus. It is possible that patients removed from zidovudine treatment may be able to transmit zidovudine-resistant or partially-resistant virus to new individuals, e.g., newborns, sexual partners, or health care workers. Those infected with only 215Y-mutated virus may quickly develop highly-resistant virus on zidovudine treatment despite no previous use of zidovudine.

Q 151 LIPOSOME UPTAKE BY HUMAN PRIMARY LYMPHOBLASTS, Sullivan,S.*,Ayers, D.*, Campbell, T.@, Kuritzkes, D.@, Sherrer, J.* and Schooley, R.@*Ribozyme Pharmaceuticals Inc., Boulder, CO; @Division of Infectious Diseases, University of Colorado Health Sciences Center and Veterans Affairs Medical Center, Denver, CO

To facilitate the delivery of anti-HIV ribozymes in either the form of synthetic oligonucleotides or expression vectors, a liposome formulation was developed to deliver these molecules to $CD4^+$ cells. The liposome composition contained a synthetic lipid derivative distearoylphosphatidylethylamidothioacetyl succinimide (DSPE-ATS). Liposome uptake by lymphoblasts was found to be dependent upon this lipid analog. The DSPE-ATS was co-formulated with dipalmitoylphosphatidylcholine and cholesterol. Calcein, a water soluble fluorescien derivative, was trapped inside the liposomes to monitor cell uptake by FACS and the total amount of cell associated calcein was measured by fluorimetry. The liposomes were incubated in complete medium with 10^6 human lymphoblasts. Kinetics of uptake for 100 uM lipid showed that 80% of the cells were labeled after 2 hours and the mean log fluorescence increased linearly over 48 hours. 100 and 200 nm diameter liposomes of the same composition had similar uptake kinetics. The 200 nm diameter liposomes have the capacity to accommodate a larger number of entrapped molecules or a larger molecular weight molecule, such as an expression plasmid. A 25 to 200 uM dose response for the 200 nm diameter liposomes showed a linear relationship between the lipid dose offered and the mean log fluorescence. No cytotoxicity was observed for the 200uM dose. Immunofluoresent staining of the lymphoblasts showed that 55% of the cells were $CD4^+$. Both the $CD4^+$ and the $CD4^-$ cell populations were completely labeled after 24 hours. The liposomes are presently being used to deliver anti-HIV ribozymes and to transfect T-lymphocytes with anti-HIV expression plasmids.

Q 152 PHENOTYPE-RELATED DIFFERENCES IN EFFICACY OF ZIDOVUDINE TREATMENT OF ASYMPTOMATIC HIV-1 INFECTED INDIVIDUALS.

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Previously we reported that in a large cohort study on non-treated HIV-1 infected asymptomatic individuals, appearance of syncytium-inducing (SI) HIV-1 variants in the course of HIV-1 infection is a strong predictive marker for CD4+ T cel depletion and progression to AIDS. Nevertheless eventually about half of infected individuals progressed to AIDS without detectable SI variants, although in these individuals CD4+ T cell counts tended to be higher. In a study on 53 ZDV-treated individuals at high risk for progression to AIDS (CD4+ cell counts between 200 and 500, and/or p24 antigenemia) we observed clinical progression in >90% of persons with SI variants at entry after 3 years follow-up. ZDV treatment did not prevent the emergence of SI variants in 14/40 persons with non-SI variants at entry. In persons who developed SI variants in the course of the study 40% progression had occurred after 3 years follow-up. In contrast after 3 years progression to AIDS had occurred in only 1 out of 26 persons who did not develop SI variants during the study. Comparison of this group of ZDV-treated persons to a group of 96 non-treated individuals with similar characteristics revealed a significant protective effect of ZDV only in persons who did not develop SI variants. This study suggests that additional treatment is needed for HIV-1 infected persons developing SI variants in the course of infection.

Q 153 HIV-1 RNA DETECTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS BY POLYMERASE CHAIN REACTION: ENHANCED SENSITIVITY AFTER MITOGENIC STIMULATION.

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The aim of this study was to investigate whether stimulus-induced upregulation of HIV-1 expression in peripheral blood mononuclear cells (PBMC) could enhance the diagnostic sensitivity of the polymerase chain reaction (PCR). PBMC derived from eleven HIV-1 infected asymptomatic adults were cultured with a stimulus of phytohemagglutinin (PHA) plus phorbol 12-myristate 13-acetate (PMA) for 36 h prior to lysing the cells for PCR. In all eleven patients studied, the intensity of PCR-assisted HIV RNA amplification (RNA-PCR) performed on stimulated cells was significantly ($p<0.001$) higher than that obtained on unstimulated cells. A comparison of conventional PCR-assisted DNA amplification (DNA-PCR) with that of RNA-PCR was made on seven patients. An enhanced sensitivity was also observed following stimulation of cells in DNA-PCR, although the increase of RNA-PCR was greater. This study demonstrates that activation of the PBMC by potent mitogenic stimuli can enhance the number of proviral copies and/or viral RNA expression, and can potentially provide the desired diagnostic information as well as indicate the status of virus infection in HIV-1 seropositive individuals.

Q 154 IN VITRO SUSCEPTIBILITY OF *CRYPTOCOCCUS NEOFORMANS* PREDICTS FAILURE OF PRIMARY THERAPY WITH FLUCONAZOLE IN PATIENTS WITH CRYPTOCOCCAL MENINGITIS AND AIDS, Mallory D. Witt, Robert A. Larsen, Roger J. Lewis, Laurie A. Mortara and Mahmoud A. Ghannoum, Division of Infectious Diseases, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509

Cryptococcal meningitis occurs in 5-10% of patients with AIDS. While amphotericin B is the standard agent for primary (1°) therapy, fluconazole (FLU) is being studied for this indication because of its ease of administration and minimal toxicity. However, failure rates of approximately 60% have been reported with FLU in this setting. We have recently developed a system for determining cryptococcal (CRYPTO) antifungal susceptibilities that is reliable and reproducible. We found that clinical isolates of cryptococci exhibit wide variations in susceptibility to FLU, with MICs ranging from 0.0625 to 16 ug/ml. We hypothesized that failure of 1° therapy with FLU was related to decreased susceptibility of certain CRYPTO isolates. To test this hypothesis, we performed antifungal susceptibility testing on isolates from patients with CRYPTO meningitis and AIDS who were receiving FLU as 1° therapy. We then examined retrospectively the clinical course of these patients (n=29), assigning them to one of two groups based on the success or failure of therapy after 10 weeks. We found a significant increase in the rate of treatment failure in those patients whose isolates had higher MICs for FLU (P = 0.02). Furthermore, a logistic regression model demonstrated that the MIC for FLU and the initial CSF CRYPTO antigen titer were independent predictors of failure. This logistic regression model can also be used to predict the risk of failure of 1° FLU therapy. If only patients with a <10% risk of failure were given FLU, then all 8 of the treatment failures seen in our population could have been avoided, while 17 of 21 of the patients who had successful outcomes with FLU would still have received the less toxic therapy. Thus, the use of FLU susceptibility testing may allow for the prospective identification of patients with a low risk of failure with FLU, allowing them to avoid the toxicity and inconvenience associated with amphotericin B.

Q 155 THE RELATIONSHIP OF HIV-1 PARTICLE NUMBERS TO INFECTIOUS UNITS IN THE PLASMA OF PATIENTS. Ying Wu, Yunzhen Cao, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY 10016.

We used a previously described limiting-dilution culture method to quantify the tissue-culture infectious doses ($TCID_{50}$) of HIV-1 in plasma of infected patients. In addition, we also utilized in parallel a RT-PCR assay recently developed in our laboratory to determine the copy numbers of HIV-1 RNA in the same plasma samples. The infectious titers in plasma ranged from 5 to 625 $TCID_{50}$ ml, whereas the particle numbers ranged from $5x10^3$ to $2x10^4$ per ml. Overall, the ratio of particle number to $TCID_{50}$ ranged from 70 to 250 per $TCID_{50}$. Although it is possible that a large number of infectious virions are required to constitute on $TCID_{50}$, the high ratio of particle number to infectious units found in our study suggests that defective particle formation in vivo is probably a common phenomenon.

Q 156 GENETIC EVOLUTION OF HIV-1 FROM SEROCONVERSION TO DEATH. Tuofu Zhu, Yaoxing Huang, Ning Wang, Ruth Connor, Yunzhen Cao, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, 455 First Avenue, New York, NY 10016.

We have determined the nucleotide sequence of C2-V5 region of HIV-1 gp120 for sequential blood samples from a homosexual man who seroconverted in 1985 and died from AIDS in 1990. Initially, very little sequence heterogeneity was found. As the viral load increased, substantial sequence diversity was noted. Heterogeneity was most prominent in C3, V4, and V5 during the early period, whereas V3 changes were observed in late infection. Overall, genetic diversity increased with time, reaching a plateau at the time of significant CD4 lymphocyte depletion. No decrease in the viral heterogeneity was seen during the final phase of HIV-1 infection. Phylogenetic analysis showed that throughout the course of infection, waves of new quasispecies would appear only to be eliminated and replaced by new populations of viruses. Such analyses nicely illustrate the dynamic nature of the delicate balance between the controlling host immune responses and the ever-changing HIV-1. The viral sequence evolution in this case will be contrasted with that of a long-term survivor of HIV-1 infection.

Early Events; Tropism; Host Factors; Co-Factors, Neuropathology in Humans and Animals

Q 200 **SYNERGISTIC INHIBITION OF HIV-1 ENVELOPE-MEDIATED CELL FUSION BY COMBINATIONS OF CD4-BASED MOLECULES AND ANTIBODIES TO HIV-1 GP120 OR GP41,** Graham P. Allaway, Andrew M. Ryder, Gary A. Beaudry and Paul J. Maddon, Progenics Pharmaceuticals Inc., Tarrytown, New York 10591

CD4-based molecules were tested for inhibition of HIV-1 envelope-mediated syncytium formation when used in combination with neutralizing antibodies to the V3 loop of gp120 or to gp41. Cell fusion was assayed by overlaying C8166 $CD4^+$ T cells on monolayers of Chinese Hamster Ovary cells which stably express HIV-1 gp120/gp41. The cells were incubated with serial dilutions of CD4-based molecules, antibodies, or mixtures of both. Syncytia were counted and the degree of inhibition of cell fusion determined. The Combination Index method was used to analyze synergy, additivity or antagonism.

The CD4-based molecules included soluble CD4 and dimeric fusion proteins based on CD4 linked to human immunoglobulin heavy chains. When assayed in combination with monoclonal or polyclonal anti-V3 loop neutralizing antibodies, these CD4-based molecules generally exhibited additive or synergistic blocking of HIV-1 induced cell fusion, analyzed at higher levels of syncytium inhibition. CD4-based molecules were also tested in combination with an anti-gp41 neutralizing antibody. These combinations were generally synergistic at higher levels of inhibition.

Previous work by other groups found synergy between anti-V3 loop antibodies and antibodies to the CD4-binding domain of gp120. The present study demonstrates that synergy between molecules which block HIV-1 attachment and fusion is a more general phenomenon. Several CD4-based molecules, which inhibit attachment, acted synergistically in combination with antibodies to the V3 loop or to gp41, which block fusion by distinct mechanisms.

These results suggest there would be an advantage to using CD4-based therapeutics and anti-V3 loop or anti-gp41 neutralizing antibodies in a combination therapy to block HIV-1 infection.

Q 201 AN N-GLYCAN WITHIN THE HIV-1 GP120 V3 LOOP IS INVOLVED IN THE MASKING OF V3- AND CD4-BINDING NEUTRALIZATION SITES. Nicole K.T. Back[1], Lia Smit[1], Martin Schutten[2], Jean-Jacques de Jong[1], Wilco Keulen[1], Jaap Goudsmit[1], and Matthijs Tersmette[1].[1]Department of Virology, University of Amsterdam, Academic Medical Centre, Amsterdam, The Netherlands.[2] Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Aim: To analyze the influence of a natural ^{301}N-glycan within the principal neutralization domain V3 on neutralization sensitivity to two V3 and CD4-site directed monoclonal antibodies and sCD4.

Methods: Four HXB2 molecular clones containing HIV IIIB-derived V3 domains were studied. Neutralization sensitivity of the four molecular clones by two anti-V3 MAbs, an human MAb interfering with CD4 binding, and sCD4 was tested. HeLa cells, transfected with a fixed amount of proviral DNA, were cocultivated with C8166 cells in the presence of neutralizing antibodies. In addition, the mechanism underlying the differential neutralization sensitivity was studied by V3 MAb affinity studies using native virion-bound gp120.

Results: Two clones, lacking the ^{301}N-glycan due to different point mutations, were sensitive to the V3-specific 178.1 and 5023 MAbs. In contrast, two clones containing an utilized ^{301}N-glycosylation site, were resistant to these V3 MAbs. Interestingly, similar differences were observed among the two groups of clones with a CD4-binding inhibiting human MAb and sCD4. Three of these clones contained the 178.1 binding site. Binding of 178.1 to a clone with a ^{301}N-glycan was low in comparison with the two clones without glycosylation site in the V3 domain. The binding site of MAb 5023 was conserved in all four clones. Binding of 5023 was also lower for the two clones containing a ^{301}N-glycan.

Conclusion: We demonstrated masking of V3- and CD4-binding neutralization sites by a ^{301}N-glycan. The high degree of conservation of this glycosylation site among V3 sequences in early infection suggests that this particular N-glycan is also used to escape neutralizing antibodies in vivo.

Q 202 **EARLY CELLULAR TARGETS OF FELINE IMMUNODEFICIENCY VIRUS (FIV) INFECTION: AN *IN VIVO* SHIFT IN FIV CELL TROPISM IS ASSOCIATED WITH DISEASE DEVELOPMENT,** Amy M. Beebe, Niels Dua, Peter Moore, Tobie Gluckstern, Gerhard Reubel, Niels C. Pedersen, Satya Dandekar, Departments of Internal Medicine and Veterinary Medicine, University of California, Davis, CA, 95616.

The disease course of feline immunodeficiency virus (FIV) infection in cats closely mimics that of human immunodeficiency virus (HIV) infection in man, with an acute primary disease followed by a long subclinical period and finally an AIDS-like syndrome. Events in the early stage of infection may play a key role in determining the dissemination of viral infection and subsequent disease course. We used *in situ* hybridization and immunohistochemistry to detect viral RNA and examine cell tropism in tissues of experimentally FIV infected cats throughout the acute primary stage of infection. The results are compared to those of a previous study looking at the same parameters in long term FIV infected cats with advanced disease. FIV RNA was detected in cats between three and eight weeks after inoculation. The primary targets were lymphoid organs including lymph nodes, tonsil, thymus, and spleen, and lymphoid tissues in the intestinal tract. This contrasts with the distribution of viral RNA positive cells in lymphoid and nonlymphoid organs in long term FIV infected cats with advanced disease. The distribution of FIV RNA positive cells within infected tissues was different in the early stages of infection also. Most noteably, germinal centers contained most of the positive cells in the lymph nodes, as opposed to extrafollicular distribution of FIV infected cells in cats in the terminal stage of disease. Furthermore, while FIV RNA was rarely present in cells staining with a pan T cell antibody late in infection, it was present both in T cells and in unidentified mononuclear cells early after infection. These results provide evidence for an *in vivo* shift in cell tropism with progression of disease.

Q 203 **MEMBRANE FUSION VIA THE CD4/HIV-1 ENVELOPE GLYCOPROTEIN INTERACTION: A VACCINIA-BASED ASSAY MEASURING SELECTIVE REPORTER GENE EXPRESSION IN FUSED CELLS,** Edward A. Berger, Christopher C. Broder, and Ofer Nussbaum, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892

We have developed a highly sensitive assay for cell-cell fusion mediated by the CD4/HIV-1 envelope glycoprotien interaction, based on the selective expression of a reporter gene in fused cells. Recombinant vaccinia viruses were used to express CD4 and envelope glycoprotein on separate cell populations. In addtion, one cell population expressed vaccinia-encoded bacteriophage T7 RNA polymerase and the other contained the *E. coli* lacZ gene linked to the bacteriophage T7 promoter. Upon mixing of the two cell populations, cell fusion resulted in mixing of the intracellular contents and consequent activation of the *lacZ* gene in the cytoplasm of the fused cells. Beta-galactosidase activity was measured by enzymatic assay of cell lysates or by *in situ* staining. Fusion-dependent enzyme activity was readily detected within 1 hour after cell mixing, and was strictly dependent on the presence of both CD4 and envelope glycoprotein. Sensitivity for detection of fusion was greatly enhanced compared to methods based on quantitation of syncytia. We are employing this assay to study mechanistic features of the CD4/envelope glycoprotein interaction, including analysis of protein-protein interactions mediating membrane fusion, identification of additional co-factors associated with the selectivity of HIV-1 envelope glycoprotein-mediated fusion for specific CD4+ cell types, and analysis of the action of pharmacological agents which interfere with the fusion process.

Q 204 HETEROGENEITY, PLOIDY, AND PROLIFERATION OF SPINDLE CELLS IN HIV-ASSOCIATED AND ENDEMIC KAPOSI'S SARCOMA LESIONS. Peter Biberfeld[1], Ephata E. Kaaya[1,4], Carlo Parravicini[3], Willy Urassa[2,4], Gunnel Biberfeld[2], [1]Immunopathology Lab., Karolinska Institute/Hospital, [2]National Bacteriological Laboratory, Stockholm, Sweden, [3]Departement of Pathology, University of Milano, Italy and [4]Muhimbili University College of Health Sciences, Dar-es-Salaam, Tanzania.

OBJECTIVES: To compare HIV-associated (AKS) and endemic (EKS) Kaposi's sarcoma (KS) with regard to immune phenotype, ploidy and proliferative activity of the so called spindle tumor cells. *METHODS:* 10 EKS and 10 AKS skin or lymphnode biopsies were processed for histopathology, immunohistochemistry and Feulgen based quantitative DNA measurements. HIV serology was performed by ELISA and confirmed by Western blot technique. CD4+ and CD8+ cells were determined by immunoflow-cytometry on patient blood. *RESULTS:* Both EKS and AKS showed the same histopathological features. Spindle cells were heterogeneous by immune phenotype, one expressing CD45+, 68+, the other TE+ CD34+/- defined by their morphology and immunophenotypes. The percentage of cycling cells by cyclin/PCNA immunostaining ranged from 2 to 18. DNA measurement of spindle cells indicated that both types of KS spindle cells have euploid or near diploid DNA content. Low levels of CD4 cells were seen only in AKS patients blood. *CONCLUSIONS:* Tumor spindle cells of EKS and AKS represent the same type of euploid, low to intermediate rate proliferating cells. The ploidy and prolifcration features of such spindle cells are not characteristic of "true" sarcoma clones. If immunodeficiency in EKS pathogenesis is important, its role is probably qualitative and not quantitative. EKS and AKS may represent a category of reactive (cytokine driven ?) lesions with varying potential for autonomous growth.

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Q 205 KINETICS OF HIV-1 ENVELOPE GLYCOPROTEIN-MEDIATED FUSION OF INDIVIDUAL CELLS, Robert Blumenthal and Dimiter S. Dimitrov, National Cancer Institute, NIH, Bethesda, MD 20892

Entry of HIV-1 into cells requires binding of gp120 to cellular CD4, putative conformational changes in the Env protein, and formation of fusion pores between viral and host membranes, which develop into large openings allowing release of the nucleocapsid. As a model system to dissect those events, interactions between HIV-1 Env expressing cells, using recombinant vaccinia, and CD4+ target cells are studied. Formation of fusion pores can be assessed by lipid mixing and cytoplasmic continuity using fluorescent dyes. Formation of multinucleated giant cells was indicative of formation of the large openings which allow nucleocapsid entry. It was found that 50% of the cells exhibited fusion pore formation within 15 to 60 min, whereas 50% syncytia formation is slower and takes hours to accomplish. Those differences could be resolved by taking into account that at least four cell fusion events need to occur before syncytia are observed, which lead to the conclusion of a relatively short lag between pore formation and large opening. That conclusion was corroborated using video microscopy with an image enhanced Nomarski differential interference contrast optics to follow fusion of individual cells in real time. The analysis of the video tape recordings showed several characteristic features of the HIVenv-mediated cell fusion: (i) cells made contact relatively rapidly (within minutes), in many cases by using microspikes to "touch" and adhere to the neighbor cells, (ii) the adhered cells fused after a relatively long "waiting" period, which varied from 15 min to hours, (iii) the morphological changes after membrane fusion, which led to disappearance of the interface separating the two cells, were rapid (within a minute), and (iv) the process of syncytia formation involved subsequent fusion with other cells and not simultaneous fusion of many cells. In some cases it appeared that fusion was accompanied by formation of intracellular vesicles.

Q 206 EFFECT OF PERSISTENT VIRAL INFECTION ON NEUROBEHAVIOR, Michelle D. Brot, Lisa H. Gold, Ilham Polis, Michael B.A. Oldstone, and George F. Koob, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Lymphocytic choriomeningitis virus (LCMV) is a nonlytic murine virus that forms a model system for studying the behavioral correlates of central nervous system virus infections. Newborn or immuno-deprived mice infected with LCMV develop a persistent infection that is characterized by continuous viral production in various organs including the brain. The densest concentration of infected neurons in the brain occurs in the cerebral cortex, hippocampus, and other limbic structures. These experiments explore the functional consequences of LCMV infection by examining the behavior of infected mice, with particular emphasis on activity level and learning parameters.

Neonatal mice were injected with LCMV or vehicle and then tested behaviorally as adults for their ability to learn a Y-maze spatial avoidance discrimination task. In this task, the mouse learns to avoid a shock by running into the non-shocked maze arm prior to the onset of the shock. The number of correct avoidance responses was used as an index of acquisition performance. The virus infected mice showed a deficit in acquisition of the Y-maze discrimination compared to vehicle-injected and non-injected control mice. Following additional training to reach control levels of performance, the infected mice and the controls were injected with the cholinergic antagonist, scopolamine. Scopolamine disrupted the performance of the infected mice significantly more than control performance, suggesting that a cholinergic dysfunction may account for some of the learning deficit. In addition, the locomotor response to a scopolamine drug challenge was evaluated. A high dose of scopolamine produced locomotor hyperactivity in all 3 groups compared to saline, whereas a low dose of scopolamine produced hyperactivity selectively in the virus infected mice. These results also suggest that the infected mice may have an increased sensitivity to cholinergic drugs. Overall, these studies indicate that viral infection can have pronounced behavioral effects in the absence of overt disease and suggest that the changes detected here may be mediated by selective neurochemical action.

Q 207 ROLE OF EARLY EVENTS IN THE VIRAL REPLICATION CYCLE RESPONSIBLE FOR DIFFERENTIAL INFECTABILITY OF TWO HUMAN T CELL LINES BY HIV-1, Robert W. Buckheit, Jr. and Carol Lackman-Smith, Retrovirus Research Section, Southern Research Institute-Frederick Research Center, Frederick, MD 21701

We have been examining differences in the ability of human cell lines to support productive HIV-1 infection to aid in identifying the viral and cellular factors required for efficient infection. Two CEM cell lines have been identified which are highly divergent in their ability to be infected by HIV-1. Both of the cell lines express similar levels of cell surface CD4 and are able to bind comparable levels of infectious virus. Upon infection, CEM-SS cells exhibit massive syncytium formation, produce high levels of infectious virus, and are rapidly killed within 7 days of infection. CEM-CCRF cells infected with the same quantity of infectious virus slowly become 100% infected over the course of 20-30 days, exhibit slight syncytium formation, and a transient decrease in cell viability. These cells become chronically infected with HIV-1 and produce high levels of infectious virus. Utilizing single step infections with HIV-1 we have determined that the inefficient infectivity of HIV-1 in CEM-CCRF cells occurs following binding of the virions to the cell and prior to virus expression. We have determined that the CEM-CCRF cells are also inefficient in forming syncytia with chronically infected CEM-SS cells, suggesting the fusion process may be involved in the inefficient infection of CEM-CCRF cells. We will present the results of our ongoing efforts to identify the viral and cellular factors responsible for differential infectivity of human cells.

Q 208 INCORPORATION OF CELLULAR HOST COMPONENTS CAN INFLUENCE BIOLOGICAL PROPERTIES OF HIV-1, Réjean Cantin and Michel Tremblay, Laboratory of Infectious Diseases, CHUL Research Center, Department of Microbiology, Laval University, Ste-Foy, Quebec, Canada, G1V 4G2

The great majority of enveloped viruses are released from the infected cells via a mechanism of budding through cell membranes. It has been demonstrated that, at this stage, cellular molecules are incorporated into the viral envelope. Few studies have reported that HIV-1 can also selectively acquire cellular antigens such as MHC-II molecules. We will present data which indicate that MHC-II proteins are expressed at the virus surface and that the incorporation of such molecules can influence some basic viral properties of HIV-1.

A B-cell line expressing very high levels of each MHC-II determinant and a cellular clone, originating from this cell line, which has lost the ability to express all MHC-II determinants were transfected with the human CD4. Thereafter, these two cell lines were infected with HIV-1 and incorporation of cellular molecules within the virus envelope was assessed by an enzymatic assay. The rate of virus replication was studied in these two cell lines and we have observed that reverse transcriptase activity and syncytia formation were both increased in cells expressing MHC-II molecules. Infectivity studies were also performed in the presence of an anti-CD4 monoclonal antibody which recognizes the gp120 binding site on CD4. This antibody was much less potent in inhibiting HIV-1 in cells expressing MHC-II molecules. We have next evaluated the level of virus entry and have determined that higher levels of viruses were entering CD4+ cell lines when using virions which have incorporated MHC-II molecules within their envelope.

Altogether these experiments indicate that incorporation of cellular MHC-II molecules into the virus envelope allow a greater rate of virus replication and confer to such virions a diminished sensitivity to inhibition by an anti-CD4 antibody.

Q 209 THE CAPACITY TO REPLICATE IN MICROGLIAL CELLS IS A COMMON PROPERTY OF PRIMARY HIV ISOLATES.
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2. Laboratory of Viral and Molecular Pathogenesis, NIH/NINDS, Bethesda, Maryland.

The main target for HIV-1 infection within the brain is microglial cells. It is yet unknown whether the capacity to replicate in this cell type is only confined to "neurotropic HIV-1 isolates". We have studied the replication of 10 paired HIV-1 isolates from blood and cerebrospinal fluid (CSF) in primary microglial cultures from human brain (Watkins et al., Science 1990). The isolates were obtained from 2 asymptomatic carriers and 3 AIDS patients and had only been passaged in PBMC. Sequences from the V3 region were known for all the isolates. The JrFL strain of HIV-1, which has been previously shown to replicate to high degree in microglial cells, was used in all experiments.

The HIV-1 replication was monitored by p24 antigen assay, PCR and cytophatic changes in cultures. The infected cells were characterized by immunostaining with monoclonal Abs to HIV-1 p17, LDL (macrophage marker) and GFAP (astrocyte marker). Levels of p24 antigen comparable to the ones observed with the JrFL strain, were detected in the cultures infected with 9 of the 10 primary isolates (1.5-12 ng/ml). P17 antigen was regularly associated with LDL positive cells.

Our results indicate that the HIV-1 present in the CSF can replicate in microglial cells and that the tropism of HIV-1 isolates for this cell type may be a general property of HIV-1 isolates.

Q 210 REGULATION OF EXPRESSION OF MHC CLASS I MOLECULES LACKING ASSOCIATION WITH B2-MICROGLOBULIN ON HIV-1-INFECTED CELLS, Sandra Demaria and Yuri Bushkin, Laboratory of Molecular Immunology, Public Health Research Institute, New York, NY 10016.

The regulation of expression of MHC antigens by viruses is common and often modulates the immune response directed at infected cells. We and others have recently shown that activation of human lymphoid cells induces expression of MHC class I heavy chains not associated with β2-microglobulin (β2m-free class I heavy chains) on their surface recognized by specific monoclonal antibodies. Since other cells infected with viruses including HTLV-1$^+$ T cell lines express high levels of β2m-free class I heavy chains, we have analyzed HIV-1-infected H9 cells for the expression of these molecules. We have found that β2m-free class I heavy chains are induced on the surface of H9 cells following infection with HIV-1 strain III_B. In contrast to activated T cells, expression of β2m-free class I heavy chains on H9-infected cells was not accompanyied by the expression of CD25. However, treatment of uninfected H9 cells with the protein kinase C-activator PMA up-regulated the expression of both β2m-free class I heavy chains and CD25. Therefore, different mechanisms may regulate the expression of β2m-free class I heavy chains on HIV-infected cells and normal activated T cells. Since it has been shown that β2m-free class I heavy chains can block activation signals mediated by the T cell receptor, up-regulated expression of these molecules on the surface of HIV-1-infected cells may be involved in the impaired function of CD4$^+$ T cells in HIV-induced pathogenesis.

Q 211 RAPID DECREASE IN UNINTEGRATED HIV DNA AFTER THE INITIATION OF ZDV THERAPY.

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HIV DNA in the peripheral blood mononuclear cells (PBMCs) of HIV infected persons not on antiretroviral therapy usually consists of both the high molecular weight, integrated form (HIV iDNA) and the low molecular weight, unintegrated form (HIV uDNA). Patients on ZDV therapy, however, usually have much less HIV uDNA. The purpose of this study was to determine how rapidly the decrease in HIV uDNA occurred after initiation of ZDV therapy. Nine patients were studied just prior to starting ZDV therapy, and at 1, 4, and 8 weeks after initiation of ZDV therapy. PBMCs were isolated, and the DNA extracted into high and low molecular weight fractions using a modified Hirt procedure. The HIV copy number in both fractions was then determined using a quantitative PCR assay.

The average percentage of HIV uDNA prior to therapy was 42%. After 1 week of ZDV therapy the average percentage of HIV uDNA was 24%. The average percentage of HIV uDNA was less than 5% at 4 and 8 weeks. Thus, the percentage of the unintegrated form of HIV DNA decreased to very low levels within the first 4 weeks after initiation of ZDV therapy. The average half-life of the decrease was approximately 1 week.

Q 212 MUTATIONAL ANALYSIS OF THE HIV-1 GLYCOPROTEIN CLEAVAGE SITE, John W. Dubay, Susan J. Roberts, Hae-Ja Shin and Eric Hunter, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

During transport of the HIV-1 glycoprotein to the plasma membrane the precursor polyprotein is cleaved into the gp120 and gp41 subunits. This cleavage occurs after a sequence of basic amino acids (K-A-K-R-R-V-V-Q-R-E-K-R) that contains two potential cleavage sequences.

We have investigated the role of the two potential proteolytic cleavage sites in glycoprotein cleavage and function. A series of eight mutations that disrupted the dibasic pairs of amino acids at each site individually, as well as combinations that altered both sites, were constructed. Mutation of the REKR sequence to REER had no effect on precursor cleavage or the ability to mediate fusion of HeLa-T4 cells. Infectivity assays using H9 cells confirmed that the mutant glycoprotein was fully functional. This raised the possibility that cleavage was occurring at the KAKRR site. However, studies of additional mutations as well as peptide sequencing of the N-terminus of gp41, confirmed that the REER sequence was being used as a recognition sequence for cleavage of the glycoprotein. Changing the last arginine of the sequence to a serine or altering both sites simultaneously completely blocked cleavage of the precursor polyprotein and abrogated fusion of HeLa-T4 cells, and infectivity of mutant virions. In contrast to previous data concerning cleavage of retroviral glycoproteins, these results indicate that the HIV-1 glycoprotein does not require a dibasic sequence at the cleavage site. This suggests that either an additional enzyme can cleave the HIV-1 glycoprotein, or that the requirement for a pair of basic amino acids at the HIV-1 cleavage site is not as absolute as in other retroviral glycoproteins.

Q 213 Modulation of CD4 expression on primary monocytes.

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CD4 is expressed on T lymphocytes and monocytes. CD4 on T lymphocytes is constitutively expressed, however, it is not known if CD4 expression on primary monocytes can be up or down regulated. Our study focused on the expression of CD4 on monocytes throughout our isolation procedure. We obtained units of whole blood (less than 2 hrs old) from the Ottawa chapter of the Red Cross Society and determined by FACS analysis the level of CD4 expression on CD14+ monocytes prior to the isolation of the mononuclear cells by Ficoll-Hypaque. The monocytes where isolated by a gelatin-fibronectin procedure which takes approximately 2 hrs to complete. Some cultures of monocytes were immediately harvested after their isolation, whereas, others were cultured for 18 hrs to 4 days with medium (10% FCS) only, GM-CSF, M-CSF and LPS. CD4 expression on monocytes was down-regulated within the first 2 hours and completely lost within 18 hours. Cells cultured for 4 days with medium (10% FCS) only, did not express CD4. However cells cultured with GM-CSF for 4 days but not for 18 hrs, did express CD4. We conclude that CD4 on monocytes can be modulated and that this should be considered when ascertaining if various viral strains of HIV are monocytotropic or lymphotropic.

Q 214 SCREENING FOR PSYCHIATRIC MORBIDITY IN AN OUTPATIENT MEDICAL HIV CLINIC , Marc Fishman, Kostas Lyketsos, Anne Hanson, Paul McHugh and Glenn Treisman, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21287

We evaluated 50 consecutive patients with a standardized psychiatric examination at their initial admission intake to an outpatient medical HIV clinic. 54% of patients had a psychiatric disorder other than substance abuse, including a 20% rate of major depression, and a 18% rate of cognitive impairment. We found a high rate of substance abuse disorders, including a 40% rate of comorbid substance abuse in patients who also had other primary psychiatric disorders. Patients who received psychiatric evaluation had a higher rate of attendance at their next scheduled visit than the clinic baseline. We piloted a brief screening instrument to identify psychiatric cases, and found a sensitivity of 81% and a positive predictive value of 71% when compared with our standardized examination.

Q 215 EVIDENCE FOR RESISTANCE TO HIV-1 AMONG CONTINUOUSLY EXPOSED PROSTITUTES. K. Fowke[1], N.J.D. Nagelkerke[2], J.N. Simonsen[1], A.O. Anza[1,2], W. Emonyi[2], J. Bwayo[2], E.N. Ngugi[2], F.A. Plummer[1,2]; [1]Departments of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada; [2]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya.

Objectives: I. To determine if resistance to HIV-1 exists in a well characterized cohort of prostitutes from Nairobi, Kenya.
II. If suggested determine the nature of this resistance.

Methods: A cohort of 263 seronegative (SN) prostitutes were assessed by HIV-1 serology at least bi-annually since 1985. Epidemiologic studies of SN survival and risk/transmission co-factors as well as laboratory analyses of HLA haplotype and CD4 cDNAs were performed. Mathematical models that assumed homogeneity or heterogeneity in susceptibility to HIV-1 infection were fit to the seroconversion (SC) data. The absence of HIV-1 in SN women was confirmed by HIV-1 PCR.

Results: For this study group the average number of unprotected sexual exposures to HIV-1 positive men is 32 per year. Yet despite this intense exposure serological analysis revealed 29 women who have remained persistently SN (PSN) for over 2 years. Survival analysis appears to indicate a decline in risk of SC if SN for two years. Remaining SN for two years was independently associated with a 10 fold reduction in risk of SC (along with duration of prostitution and genital ulcers) and no significant differences in sexual behaviour (number of sex partners, condom use) were noted. The mathematical model that best fits the SC data is a Weibull model which assumes heterogeneity in susceptibility to HIV-1. HLA alleles Aw28 (OR.21, CI95% .08-.57, $p<.01$) and Bw70 (OR.3, CI95% .11-.86, $p<.05$) were associated with PSN while the presence of Aw19 increased the risk of SC (OR 3.2, CI95% 1.3-8.2, $p<.03$). Single strand conformational polymorphism analysis of the CD4 cDNAs from SC and PSN women suggests changes relative to the CD4 clone pT4B. Further analysis of this data will be presented.

Conclusions: A subset of prostitutes highly exposed to HIV-1 are not infected. Epidemiologic and modelling data presented are consistent with a proportion of the cohort being resistant to infection by HIV-1. PSN is associated with HLA alleles Aw28 and Bw70 and seroconversion associated with HLA allele Aw19.

Q 216 ACTIVATION OF HIV-1 GENE EXPRESSION IN MONOCYTIC CELLS BY MHC CLASS II LIGANDS, Ramsay Fuleihan, Nikolaus Trede, Talal Chatila and Raif S. Geha, Division of Immunology, The Children's Hospital, Boston, MA 02115

Monocytes/Macrophages are a prime target of HIV-1 infection and serve as a reservoir for the virus. Engagement of MHC class II molecules on the surface of monocytes and monocytic cells results in transduction of biochemical signals leading to cellular activation and gene expression. We have investigated the capacity of MHC Class II ligands to activate HIV-1 gene expression in monocytic cells. Engagement of MHC Class II molecules by alloreactive T cells, or by the bacterial superantigens, Toxic Shock Syndrome Toxin-1 and Staphylococcal Enterotoxin A, induced HIV-1-LTR driven transcription of a Chloramphenicol Acetyl Transferase construct in the human monocytic cell line THP-1. Engagement of MHC Class II ligands also augmented HIV-1 p24 protein secretion from the HIV-1 infected monocytic cell line U1. The activation of HIV-1 gene expression in monocytic cells by MHC Class II ligands was associated with the induction of NF-κB binding activity. Both MHC Class II mediated-HIV-1 protein secretion and HIV-1-LTR driven transcription were partially inhibited by neutralizing antibody to TNF-α but not to IL-1β. These results suggest that engagement of MHC Class II molecules results in the activation of HIV-1 gene expression in monocytic cells, possibly via NF-κB, and that this activation is augmented by the induction of TNF-α secretion. Activation of HIV-1 infected monocytes/macrophages via MHC Class II molecules may play an important role in the pathogenesis and progression of AIDS.

Q 217 ROLE OF THE HIV-1 gp120 DOMAINS V1, V2, AND V3 IN DETERMINING CYTOPATHOGENICITY. Martijn Groenink, Ron A.M. Fouchier, Silvia Broersen, Catriona H. Baker, Robert C.M. van der Jagt, Frank Miedema, Han G. Huisman, Matthijs Tersmette, and Hanneke Schuitemaker. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory of Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam, The Netherlands.

HIV-1 isolates differ with respect to the syncytium inducing (SI) capacity, replication rate, and cytotropism. Differences in SI capacity can be used as a progression marker to monitor the development of AIDS. To investigate the molecular basis responsible for the differences in SI capacity, fragments of the env gene were exchanged between related infectious molecular HIV-1 clones, which differed in their SI capacity. The envelope glycoprotein gp120 V1-V2 and V3 domains independently influenced the capacity to induce syncytia. However, the wild type SI phenotype could solely be transferred upon a non-SI (NSI) clone by exchange of the V1-V2 and V3 domain. The reciprocal substitutions demonstrated that the identical gp120 domains conferred the wild type NSI phenotype upon a SI clone.

Recently, we demonstrated that the gp120 V3 domain configuration completely correlates with the capacity to induce syncytia. Compared to NSI HIV-1 variants, SI HIV-1 variants contain a V3 loop with a significant higher positive charge, due to amino acid substitutions at fixed positions. However, the above described studies with the chimeric viruses revealed that in addition to the gp120 V3 domain, the gp120 V1-V2 domain is involved in determining differences in SI capacity. Therefore, the sequence analysis of the gp120 V1-V2 domain of a large panel of virus isolates with well defined biological phenotypes was initiated. This analysis showed that compared to stable NSI HIV-1 variants, NSI HIV-1 variants obtained at the moment of a NSI to SI conversion, and SI HIV-1 variants contain an extended sequence in the gp120 V2 domain. The extended sequence in the gp120 V2 domain induces an additional or relocates an existing N-linked glycosylation site.

Q 218 PEPTIDES CORRESPONDING TO VIRAL NUCLEAR LOCALIZATION SIGNALS MAY INHIBIT NUCLEAR TRANSPORT OF HIV-1. Julie A. Gulizia, Michael Bukrinsky, Natasha Sharova, Michael P. Dempsey, Trevor Stanwick, Mario Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-5120

Integration of retroviral DNA with the host cell genome and establishment of the provirus is an obligate step in the life cycle of retroviruses. Events leading to provirus establishment include: binding of the virus to the CD4 receptor, fusion with the cell membrane, entry of the viral particle, formation of the viral pre-integration complex and entry of this complex into the nucleus. The pre-integration complex is a high molecular weight nucleoprotein containing the viral RNA, in various stages of reverse transcription, and the virus-encoded integrase which remains associated with the viral cDNA. After entry into the nucleus, integrase directs integration of viral DNA with host DNA. Thus, integration of the viral genome with the host genome is dependent on targeting of the viral pre-integration complex to the nucleus.

Our laboratory has evidence that nuclear transport of the pre-integration complex of HIV-1 is a rapid and active process which is independent of cell division. In addition, we have made preliminary steps to identifying the viral components which mediate the active transport of the viral pre-integration complex. Previous studies with other viral nucleophilic proteins suggests that these nucleophilic properties are mediated by a nuclear localization sequence (NLS) usually at the N-terminus of the protein. In addition, nuclear transport of nucleophilic proteins can be blocked by competition with excess nuclear localization sequences in peptide form.

Our studies present evidence that the nuclear transport of HIV-1 pre-integration complexes can be specifically and effectively inhibited in the presence of excess peptides containing nuclear localization sequences of nucleophilic viral proteins such as SV40 T antigen and HIV-1 matrix antigen. These studies identify new targets for intevention of HIV-1 replication.

Q 219 HIV-INDUCED LOSS OF CD44 EXPRESSION IN MONOCYTIC CELL LINES, 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 have found that HIV-1 infection of monocytic cell lines results in a new adhesion phenotype. Whereas uninfected cells grow as single cell suspensions, HIV-infected cells grow as large aggregates. The expression of adhesion molecules was investigated because adhesion plays an important role in HIV infection of cells. Among the adhesion molecules studied, CD44 was almost completely depleted from the surface of HIV-infected cells. CD44 is a polymorphic, glycosylated transmembran protein highly expressed on hematopoietic, epithelial and endothelial cells. CD44 mediate lymphocyte homing and binds to hyaluronate acid and fibronectin. Immunoprecipitation with mAb confirmed the loss of CD44 from the surface of infected cells. In addition, loss of surface CD44 was not due to formation of internal complexes or release into the culture supernatant. Soluble CD44 was not detected in culture supernatant from HIV-infected cells. Northern blot analysis showed an altered mRNA pattern in HIV-infected cells. The high molecular weight CD44 mRNA (7.0 kB) was lost from infected cells and the low molecular weight CD44 mRNA (1.2 kB) remained. We have previously shown that anti-CD44 mAb induces homotypic adhesion in CD44+ cell lines. In this report, we show that homotypic adhesion of the HIV-infected cells occurs through a different mechanism than anti-CD44 mAb induced aggregation. The homotypic adhesion in infected cells was CD18-mediated, but anti-CD44 mAb induced homotypic adhesion in uninfected cells was CD18-independent. The change in adhesion phenotype and the loss of CD44 from the surface of HIV-1-infected monocytic cells may alter further transmission and immune function of these cell.

Q 220 **HIV-1 gp120 RECEPTOR ON CD4-NEGATIVE BRAIN CELLS ACTIVATES A TYROSINE KINASE**

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HIV-1 infection in the human brain leads to characteristic neuropathological changes, which may result indirectly from interactions of the envelope glycoprotein gp120 with neurons and/or glial cells. We therefore investigated the binding of recombinant gp120 (rgp120) to human neural cells and its effect on intracellular signalling. We show that rgp120, besides binding to galactocerebroside (GalC) or galactosyl-sulfatide, specifically binds to a protein receptor of a relative molecular mass of approximately 180 kDa present on the CD4-negative glioma cells D-54, but not on Molt4 T lymphocytes. Binding of rgp120 to this receptor rapidly induces a tyrosine-specific protein kinase activity leading to tyrosine phosphorylation of 130 and 115 kDa proteins. The concentrations of intracellular Calcium was not affected by rgp120 in these cells. Our data suggest a novel signal transducing HIV-1 gp120 receptor on CD4-negative glial cells, which may contribute to the neuropathological changes observed in HIV-1 infected brains.

Q 221 2-LTR CIRCULAR DNA AND HIV-1 REPLICATION. Karen J. Hsia and Stephen A. Spector. University of California, San Diego, La Jolla, CA 92093.

The role of the 2-LTR circular DNA in HIV-1 induced pathogenesis is not clearly understood. To determine if there is a relationship between the HIV-1 2-LTR circles and productive viral replication *in vitro*, we infected 2 neuronal cell lines, SK-N-MC and SK-N-SH, with the LAV strain of HIV-1. Virus production, assayed by HIV-1 p24 antigen in culture supernatant, was compared in the neuronal cells and CEM cells for 8-weeks. The presence of 2-LTR circular DNA was determined by PCR amplification of a DNA fragment spanning the 2-LTR junction. The HIV-1 2-LTR circles were not detected in the SK-N-SH cells which failed to support a productive infection. However, a low level of the 2-LTR circles was observed in the SK-N-MC cells which showed a > 100-fold less HIV-1 replication when compared with HIV-1 infected CEM cells. The kinetics of HIV-1 replication in CEM and SK-N-MC were different; while virus production peaked at 7 days post-infection in CEM cells, it took approximately 3-4 weeks to peak in the SK-N-MC cells. In order to examine the importance of viral entry, HIV-1 pseudotyped with an amphotropic murine retrovirus was used for infection. With pseudotype infection, there was an enhanced production of infectious virus and an enhanced level of 2-LTR circles in both SK-N-MC and SK-N-SH cells. However, the amount of virus produced in SK-N-SH cells was significantly lower than that from SK-N-MC cells, indicating that factors other than "virus entry" could also determine the extent of virus replication. The kinetics of infection by pseudotyped HIV-1 in SK-N-MC cells was similar to that in LAV-infected CEM cells, suggesting that the kinetics of virus replication was related to the efficiency of virus entry. These studies suggest that the presence of HIV-1 2-LTR circular DNA may indicate a productive infection of host cells, and may be a useful marker to evaluate increased viral replication.

Q 222 CYTOKINE REGULATION OF LYMPHOCYTE BINDING TO BRAIN ENDOTHELIAL CELLS FROM RATS WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, Stephen J. Hudson and Yee Hon Chin, Department of Immunology and Microbiology, University of Miami School of Medicine, Miami, FL 33101

Brain microvascular endothelium is morphologically different from other endothelia, in that it has continuous tight junctions and low level of intracellular pinocytic vesicles, leading to a high transendothelial resistance. Lymphocyte migration into the central nervous system under normal conditions is therefore rather limited. However, in HIV encephalitis and disease models such as experimental allergic encephalomyelitis (EAE), there are considerable increases in cellular migration across the cerebral endothelium. Although the precise mechanisms for the selective entry of lymphocytes into the CNS is unclear, we hypothesize that cytokines released within the microenvironment may play important regulatory roles in the adhesion of brain endothelial cells (EC) for lymphocytes, which represents the first step in the entry of lymphocytes into the CNS. We have isolated and cultured brain EC from Lewis rats and tested the capacity of proinflammatory cytokines to enhance their adhesiveness for thoracic duct lymphocytes (TDL). Cultures were >85% pure as assessed by morphology and for expression of Factor VIII related antigen. Pretreatment of brain EC at first passage with cytokines such as murine TNF significantly increased their adhesiveness for TDL in a time- and dose-dependent manner. Importantly, pretreatment of brain EC with transforming growth factor-beta 1, the cytokine recently shown to prevent EAE and other autoimmune disorder *in vivo*, significantly decreased the baseline adhesiveness for TDL. In studies now in progress, we have isolated and cultured brain EC from Lewis rats with EAE and tested the responsiveness of these cells to murine IL-1, IL-4, TNF and TGF-beta 1. The results should provide insights into the process by which lymphocytes adhere to EC and subsequently migrate into the brain parenchyma.

Q 223 ROLE OF THE C-TERMINAL DOMAIN OF THE SIV/HIV TM PROTEIN IN GLYCOPROTEIN INCORPORATION, CELL FUSION AND INFECTIVITY, Eric Hunter, Patrick Johnston, John Dubay, and Karl Salzwedel, Center for AIDS Research and Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

The host range specificity of SIV appears to be modulated by the cytoplasmic domain of its envelope glycoprotein. In order to define further the role of this region we have created a series of C-terminal truncation mutants. These truncations result in transmembrane (TM) proteins of 163-207 amino acids, (a.a.) relative to a wild-type 354 a.a. protein. The truncated *env* genes, expressed from an SV40 vector in COS cells, were transported and processed normally. Syncytial assays demonstrated that truncated TM proteins as short as 191 a.a. induced syncytium formation in a manner comparable to wild-type in a variety of T-cell lines, including CEMX174, C8166 and HUT78.

To examine the effect of these truncations on infectivity and host range specificity, proviral constructs were created. The wild-type and mutant viruses with TM proteins of 191-207 a.a. efficiently infected and replicated in CEMX174 cells, whereas only virus containing the 207 a.a. TM protein efficiently infected HUT78 cells. These results contrast those with HIV-1 where cytoplasmic domain truncations drastically reduce virus infectivity on a variety of cell types. Delayed kinetics of infection were noted for virions containing TM proteins of 193 and 354 a.a.. Thus, the tm207 truncation which spontaneously occurs during growth of SIV in HUT78 cells confers a novel growth advantage in this cell line. Complementation studies using an *env* defective HIV provirus demonstrated that the SIV glycoproteins could efficiently complement HIV entry into permissive (CEMX174) cells and that only the tm207 and tm193 glycoproteins could complement HIV for entry into HUT78 cells. These studies suggest that the defect in infectivity of wt SIVmac239 is at an early attachment/entry stage of the life cycle. Biochemical analyses of virus released from CEMX174 cells suggest that truncation of the cytoplasmic domain enhances glycoprotein incorporation into virus and that this may play a role in facilitating entry into HUT78 cells.

Four chimeric HIV-1 *env*-gene constructs which encode the extracellular domain of either the *wt* or a cleavage-defective gp160 fused to a C-terminal glycosyl-phosphatidylinositol (GPI)-attachment signal have been constructed. The proteins were transported intracellularly at a rate similar to that of the *wt* HIV-1 glycoprotein and could be cleaved from the cell surface with PI-PLC. However all four glycoproteins were defective in mediating both cell-cell and virus-cell fusion as determined by syncytium formation in HeLa-T4 cells and *trans*-complementation of an *env*-defective HIV genome. The requirement for a protein anchor in the biological function of the HIV glycoprotein will be discussed.

Q 224 ENHANCEMENT OF HIV-1 REPLICATION BY CIGARRETTE SMOKE CONSTITUENTS: INVOLVEMENT OF NF-KB, PK-C AND TNF-alpha, Bharat Joshi, Jay Epstein, Gary Riordan, Michael Norcross and Indira K. Hewlett, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

In earlier studies, we had shown that cigarette smoke constituents such as benzo(a)pyrene (BP) and NNK, a derivative of nicotine, could induce HIV replication in chronically infected U1 cells. Virus production was enhanced more than 20 fold for p24 antigen production and HIV RNA in cells. Viral particles visualized by transmission electron microscopy were enhanced at least 10 fold in treated cells. Phorbol ester, TPA served as a positive control in these experiments. We have further examined the role of certain cellular factors that may be involved in viral induction by these compounds. Band shift assays for NF-kB revealed the induction of NF-kB activity within the first 2 hours and persisted for 48 hours in treated cells. Increased protein kinase C (PKC) activity, associated with activation of NF-KB, was observed within a few minutes post treatment with both compounds. Tumor necrosis factor alpha (TNFa) levels were elevated in supernatants of treated cells within 12 hours of treatment, followed by a decline in 72 hours. Our results suggest that cigarrette smoke constituents may exert their effect on HIV-1 replication by enhancement of NF-kB activity via phosphorylation by PKC. Virus production may be subsequently upregulated by synthesis of TNFa. We are currently investigating the role of nuclear proto-oncogenes and other cytokines in this induction process with a view to identifying signal transduction mechanisms that are operational in HIV replication and pathogenesis.

Q 225 MOLECULAR BASIS OF SIVmac NEUROPATHOGENESIS, Kodama T, Kawahara T, Ferber N, Desrosiers R*, and Axthelm M, Oregon Regional Primate Research Center, Beaverton, OR 97006 and *New England Regional Primate Research Center, Southborough, MA 01772

Rhesus macaque Mm155-88 exhibited severe encephalopathy and lymph node and lung pathology characterized by the presence of giant cells 27 months after infection with molecularly cloned T-cell tropic SIVmac239. A variant SIVmac155/NT was isolated from brain tissue in primary rhesus macaque brain cell cultures. 155/NT exhibited a profound ability to form syncytium in primary brain cultures, and microglial cells were identified as the major target cell using DiI-Ac-LDL labeling. Rhesus macaque peripheral blood monocytes and PHA-stimulated lymphocytes were used to isolate SIVmac155/MT and /TT variants, respectively, from lymph node. 155/TT exhibited strict T-cell tropism in culture. Both 155/NT and 155/MT were tropic for peripheral blood macrophages in culture, however, the 155/NT brain isolate had a much stronger ability to replicate in brain cell cultures. 155/NT also replicated in human cell lines, whereas SIVmac155MT did not. Viral DNA sequences spanning the gp120 of SIVmac were amplified from genomic DNA of Mm155 brain and lymph node by PCR. The V3 cysteine loop sequences of lymph node derived env clones were considerably variable; in contrast, those of brain derived env clones were well conserved. The brain derived env clones consisted of two sequence variation patterns; one (BR type II) with conversion of threonine residues to isoleucine at both env amino acids (a.a.) 370 and 373, and the other (BR type I) with parental threonine residue at these positions. The BR type I sequence pattern was also present as a minor population in lymph node derived env clones, whereas the BR type II pattern was only observed in the brain derived env clones. Env sequences of the cell type-specific variants were also analyzed by PCR. 155/TT had the same amino acid substitution pattern in the V3 loop region as those of Mm155 lymph node derived env clones. Surprisingly, 155/NT showed conversion of threonine to isoleucine at both a.a. 370 and 373, identical to the BR type II sequence pattern observed in the brain derived env clones. In contrast, 155/MT had the BR type I sequence pattern containing parental threonine residue at a.a. 370 and 373. Tissue- and variant-specific amino acid changes were also identified in the loop II region of gp120. These data indicated that there were at least two SIV variants present in Mm155 brain; one is peripheral macrophage tropic and the other is uniquely tropic for brain cells.

Q 226 ROLE OF THE V1, V2 DOMAIN OF HIV-1 ENVELOPE GP120 IN MACROPHAGE TROPISM.

Atsushi Koito, Jay A. Levy, and Cecilia Cheng-Mayer, Cancer Research Institute, Department of Medicine, UCSF, San Francisco, CA 94143.

The V3 region of the HIV-1 envelope glycoprotein appears to play a critical role in determining tropism for T cell lines and primary peripheral blood macrophages. However, previous studies of recombinant and mutant viruses indicated that substitutions within the V3 loop alone were not sufficient to confer full macrophage tropism on the HIV-I SF2mc, T-cell line tropic virus. A DraIII/StuI fragment of 78 amino acids, encompassing the V1 and V2 domains were necessary for high level of virus production in primary macrophages. To evaluate the role of V1, V2 domains of gp120 in virus entry, we generated recommbinant viruses that contain either the V1 and V3 domains, or the V2 and V3 domains of HIV1SF162mc (macrophage tropic) on HIV-1SF2mc genomic back ground.

Recombinant virus containing the V1 and V3 domains of SF162mc was detected in supernatant of transiently-transfected COS cells, but the viral particles produced were non infectious in peripheral blood mononuclear cells (PBMCs) . The other recombinant virus containing V2 and V3 domain of SF162mc grew to level comparable to that of wild type SF2mc in PBMCs. Replication in primary macrophages and biosynthesis of these recombinant virus envelope glycoproteins are under investigation. These studies may map the viral determinant of macrophage tropism more precisely.

Q 227 LONG-TERM, NONCYTOPATHIC, PRODUCTIVE INFECTION OF THE HUMAN MONOCYTIC LEUKEMIA CELL LINE THP-1 BY HIV-1_{IIIB}

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A long-term, noncytopathic, productive infection of the monocytic leukemia cell line, THP-1, with human immunodeficiency virus type 1 (HIV-1_{IIIB}) was established. Both infected cells (THP-1/HIV-1_{IIIB}) growing in suspension, and uninfected, phorbol 12-myristate 13-acetate (PMA)-treated THP-1 cells, that are adherent, showed ultrastructural characteristics of differentiated cells. PMA-treated THP-1 cells could not be productively infected with HIV-1_{IIIB}, although a low level of p24 antigen was detected in the supernatant. Our interpretation of this result is that the non-specifically bound virions were eluted into the culture supernatant and the decrease of p24 level reflected the release of virus from the cell surface, rather than p24 production. THP-1/HIV-1_{IIIB} cells produced virions mainly by budding at the plasma membrane. These cells retained the ability to differentiate into macrophage-like cells, capable of releasing of the virus for extended periods of time (e.g. 40 days). PMA-mediated differentiation of THP-1/HIV-1_{IIIB} cells modified the pattern of virus localization. Twenty four hours after PMA treatment extracellular mature viral particles adjacent to the cell membrane were observed. After 21 days in culture, however, the virions accumulated in intracellular vacuoles. THP-1/HIV-1_{IIIB} cells may served as a useful model system for *in vitro* testing of antiviral drugs encapsulated in liposomes and taken up by phagocytosing cells, as well as for studies on HIV-infected macrophages.

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Q 228 ALTERATIONS IN THE CYTOPLASMIC TAIL OF SIV TRANSMEMBRANE MOLECULE ASSOCIATED WITH INCREASED INFECTIVITY AND SURFACE ENV EXPRESSION.

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We have described the characterization of biological variants of SIV derived from the BK28 molecular clone of SIVmac. In contrast to the parental virus (NC-MAC), the variants (termed CP-MAC.1 and .2) infected Sup-T1 cells with rapid kinetics, induced CD4 downmodulation, and exhibited a highly stable association of their SU and TM glycoproteins. In addition, compared to NC-MAC, CP-MAC exhibited a marked increase in the level of surface envelope on infected cells (by FACS) and numbers of envelope spikes on virions (by EM). Sequence analysis of the *env* genes demonstrated that CP-MAC.1 and .2 differed from NC-MAC by 2 and 3 amino acids, respectively, in SU, and 6 amino acids in TM. Both CP-MAC viruses contained a Tyr to Cys substitution six amino acids from the membrane-spanning domain in the cytoplasmic tail of TM. Of interest, a Tyr in this position is conserved among all HIV-1s, HIV-2s and SIVs published to date. Monoclonal antibodies raised against cytoplasmic domain peptides containing a Tyr (but not a Cys or Ala) were found to react by immunofluorescence microscopy with NC-MAC-, HIV-2/ROD- and HIV-2/ST- but not CP-MAC-infected cells, suggesting a structural alteration in the cytoplasmic tail of CP-MAC TM. 2D-NMR analysis of peptides from this region indicated that a Tyr (but not a Cys) in this position contributes to the formation of a stable type 1 ß-turn, similar to structural motifs that mediate rapid endocytosis of the LDL receptor and LAP via clathrin-coated pits. As described in the accompanying abstract (Sauter, M. et al), we have evidence that the envelope glycoproteins of NC-MAC but not CP-MAC are constitutively endocytosed through coated pits on chronically infected Sup-T1 cells, and propose that this process is directed by the Tyr-containing ß-turn in the TM tail. Thus, the increased level of envelope on infected cells and spikes on virions exhibited by CP-MAC could result from the Tyr to Cys mutation, disrupting endocytosis of this molecule. Mutagensis experiments in progress will determine the contribution of this Tyr to Cys alteration to envelope expression on cells and virions, as well as the increased infectivity of CP-MAC.

Q 229 DOUBLE STRANDED STRONG-STOP DNA AND HIV REVERSE TRANSCRIPTION, Peng Li, Alice J. Stephenson, Lara J. Kuiper and Christopher J. Burrell, National Centre for HIV Virology Research, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, Australia

Using a synchronized one-step HIV infection model, we report the detection in acutely infected cells of a novel double stranded (ds) strong-stop HIV DNA with a discrete length of ~650 base pairs, commencing from the beginning of the U_3 region of the HIV LTR. The plus-strand of this ds strong-stop DNA possesses the primer binding site (PBS) sequence and appears to be synthesized prior to the completion of the synthesis of its complementary minus-strand. In contrast, the minus-strand of the ds strong-stop DNA lacks the PBS sequence after RNaseH digestion. We proposed that a transient free plus-strand strong-stop DNA is released from its template by displacement synthesis and subsequently used as template for the synthesis of its complementary minus-strand. The proposed transient free plus-strand strong-stop DNA may also mediate the second template switch. An alternative model for HIV reverse transcription will be presented.

Q 230 NERVOUS TISSUE LESIONS IN CATTLE INFECTED WITH THE BOVINE IMMUNODEFICIENCY VIRUS, D. Gene Luther, William J. Todd, Ron Snider, and Matthew A. Gonda. Department of Veterinary Science, Louisiana Agriculture Center, Department of Pathology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, and Program Resources, Incorporated, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702.

Holstein dairy cattle infected with the Bovine Immunodeficiency Virus exhibit neurological signs of depression, ataxia, anorexia, and decreased milk production. These clinical signs progress to coma and death in the infected animals. The animals studied were naturally infected and had been sero-positive for Bovine Immunodeficiency Virus for a period of at least three years prior to development of clinical signs. Some of the affected animals had chronic mastitis and chronic foot-rot problems that failed to respond to traditional treatments. Although it was difficult to isolate the Bovine Immunodeficiency Virus from peripheral blood, the infection was easily transmitted when small quantities of blood were inoculated into Bovine Immunodeficiency virus sero-negative calves. Necropsy of the cattle that died with the above clinical signs showed neurological lesions and inflammation of the brain and spinal cord. The microscopic examination revealed wide spread damage of neurons in the brain and spinal cord. The neuron pathology was thought to be caused by vascular lesions. The vascular lesions include proliferation of vascular endothelium and infiltration of the vessel walls with mononuclear and occasionally polymorphonuclear cells. These neurological lesions in animals infected with Bovine Immunodeficiency Virus are similar to the neurological lesions reportedly associated with Human Immunodeficiency Virus.

Q 231 DEPRESSION IS NOT PREDICTIVE OF MEDICAL OUTCOMES IN HIV INFECTION

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All participants seropositive for HIV on entry to the Multicenter AIDS Cohort Study (N = 1,809) completed the Center for Epidemiologic Studies Depression Scale (CES-D) at baseline and at biannual follow up visits over seven years. In this study participants were categorized as depressed or not depressed based on baseline CES-D scores using several definitions. The effect of baseline depression on medical outcomes was assessed using AIDS and death as outcomes in survival analyses, and CD4 slopes as dependent variables in regression analyses. No differences were found between depressed and non-depressed study participants on any of the outcome variables. Our results contradict a recently publicized report which suggested that depression predicts an accelerated CD4 decline among gay men in San Francisco. Differences in the two study results are likely due to confoundings of symptom reports, and demographic variables.

Q 232 PATHOGENESIS OF ENCEPHALITIS CAUSED BY SIVmac

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SIVmac is a lentivirus which causes an immune deficiency syndrome in Asian macaques that is very similar to AIDS in man. There are two broad classes of disease due to SIV: (1) encephalitis and pneumonia associated with replication of the virus in macrophages, and (2) immune suppression characterized by lymphoid depletion and secondary opportunistic infections and associated with replication of the virus in CD4+ lymphocytes. A non-neurovirulent, strictly lymphocyte-tropic strain of SIV, SIVmac239, was passed four times sequentially in rhesus macaques. During the sequential passages, the virus developed the ability to replicate to high titers in macrophages. Animals inoculated with this passaged virus developed clinical signs of neurological disease and had typical SIV encephalitis with perivascular infiltrates of macrophages and giant cells. In situ hybridization alone and in combination with immunohistochemistry was performed on CNS tissue and revealed abundant viral replication in the perivascular macrophages and giant cells. The infiltration of macrophages in this area suggested that cells were entering the neuropil through the blood-brain-barrier. In order to evaluate morphological features of the blood brain barrier, tissue sections were stained for GFAP to identify astrocytes and Factor VIII-related antigen to detect endothelial cells. GFAP staining demonstrated enlargement of the astrocytic foot processes and separation of the foot processes from the endothelial basal lamina, suggesting loss of blood-brain-barrier integrity.

Q 233 MACROPHAGE TO T CELL TRANSMISSION OF HIV-1 IS REGULATED BY CELL SURFACE STRUCTURES THAT INTERACT IN THE NORMAL IMMUNE RESPONSE, Dean L. Mann and Robert Nalewaik, Immunogenetics Section, Laboratory of Viral Carcinogenesis, NCI-FCRDF, Frederick, MD 21702

The primary reservoir for HIV-1 in the infected host are cells of the monocyte macrophage (M/M) lineage. These cells serve as the primary antigen presenting cells in imitating an immune response by presenting antigens in the form of peptides by HLA class II molecules (HLA DR, DQ, DP). T helper cells are engaged in this response thru the T cell receptor - CD3 complex which contain the α and β chains that together recognize the specific antigen presented. Other cell surface markers such as LFA-3 on M/M and CD2 on T cells also interact in this process. We investigated the role of these cell surface structures in M/M to T cell transmission of HIV-1. M/M's were isolated from PBL's from HIV-1 seronegative individuals by surface adherence and infected with HIV-1 BAL. Autologous peripheral blood T cells were exposed to the infected M/M's for 24 hours in the absence of added mitogens or cytokines, removed and cultured with noninfected autologous M/M's for 7 days. Virus produced during the last 24 hours of culture was measured by determining the level of p24 in culture fluids with an antigen capture assay and cell proliferation by ^{3}H thymidine incorporation. Antibodies to CD3 and TCR$\alpha\beta$ stimulated T cell proliferation and caused a two-fold increase in T cell infection over controls. Antibodies to LFA-3, CD2, and HLA-class I reduced T cell infection 20-25% while antibodies to TCR $\gamma\delta$ had no effect. Antibodies to a nonpolymorphic determinate on HLA-DR reduced T cell infection by 90% as did antibodies to the specific HLA-DR alleles on the antigen presenting cell. Antibodies to specific HLA-DP and HLA-D alleles had no effect. These results together with our recent observation that HLA-DR is selectively concentrated in the HIV-1 virion suggests a model for HIV-1 T cell infection that recapitulates the normal immune processes involved in antigen presentation.

Q 234 HIV-1 OR HTLV-1 AND SPUMARETROVIRUS CAN BE EXPRESSED CONCOMITANTLY FROM THE SAME CELL

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Although the widespread infection of subhuman primates with spumaretroviruses has been well-documented, the coinfection/ coexpression of these viruses concomitant with other retroviruses has not been investigated. Insight into viral interference, transactivation and pseudotyping, relative to spumavirus infection, could significantly impact on studies of SIV pathogenesis and perhaps vaccine development. We sought to determine if spumavirus can be expressed concomitantly with other retroviruses from the same cell. A cell line chronically expressing HIV-1 (H9/HIV-1_{LW-PBM}), a human fetal thymus- derived cell line transformed by and expressing HTLV-1 (FTh/TK), and a human embryonic kidney cell strain (HEK) were used as targets for spumavirus infection. Two isolates of spumavirus, the chimpanzee-derived FV-6, and 87013 (from a rhesus macaque) were used. Reverse transcriptase assay and microscopy were used to monitor spumavirus infection.

As was seen in the spumavirus-infected HEK cells, many extremely large giant cells with large numbers of nuclei were seen in the spumavirus-exposed H9/HIV-1 and FTh/TK cell cultures. Electron microscope examination revealed the presence of both spumavirus particles and HIV-1 or HTLV-I in these cell populations. Moreover, both types of particles were observed budding from the same cell. Interestingly, virus particles were seen budding from only certain regions of the cell membrane; in these regions, both types of particles were usually seen. Atypical particles were also observed, some of which had the appearance of HIV-1 core surrounded by the characteristic spumavirus envelope. Immunoelectron microscopy is currently being used to determine the viral protein composition of these particles.

Q 235 A CD4-LIKE IMMUNOREACTIVITY IN THE PRIMATE BRAIN: DISTRIBUTION AND CELL TYPES,

Sheilendra S. Mehta and F.J. Denaro, Department of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The development of animal models for AIDS research is of central importance. With the development of such models it will be possible to study the pathophysiology of AIDS, test drugs, and develop vaccines. The macaques have become an important animal for HIV research. It has been shown that these animals may develop an AIDS-like disease process (Agy MB, et al. *Science* 257(5066):103-106, 1992). This is particularly true with regard to the development of CNS pathology. With this in mind, we are investigating the brains of macaca nemestrina, macaca fuscata, macaca mulata, and macaca fasicularis for the distribution of CD4-like proteins and other lymphocyte markers (pan T, B, Mac, etc.). Presently, we have examined the cortex, white matter, and basal ganglia and found lymphocytes which stain with the antibodies used. However, the type of immunocytochemical staining depended to some degree on the commercial source of a given antibody type. For example, monoclonals to CD4 which were used to stain frozen tissue, stained the membranes of lymphocytes. Occasionally a very light cytoplasmic staining could also be noted in neurons. On the other hand antibodies which can identify CD4 and CD8 cells in paraffin processed tissue displayed, in addition to what was observed above, some light staining of oligodendrocytes. It is not known if these CD4 positive lymphocytes correspond to human-like helper cells even though there may be considerable homology between human and primate CD4 molecule. Additional immunocytochemical analysis is under way on primate lymph nodes and may help to clarify such questions. Cytoplasmic staining of CD4 requires further elucidation. In addition, other mechanisms may be involved in HIV uptake in cells. Further work is also needed to characterize the anatomical loci of CD4 in the primate brain and immune system.

Q 236 HIV INFECTION OF HUMAN BRAIN CAPILLARY ENDOTHELIAL CELLS OCCURS VIA A NOVEL RECEPTOR-MEDIATED PATHWAY.

Ashlee V. Moses and Jay A. Nelson, Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, OR 97201.

HIV has been implicated in the etiology of AIDS-associated neurological dysfunction. Brain autopsies from AIDS patients reveal that the principle cellular targets for HIV in the CNS are cells of the macrophage lineage and the endothelial cells lining the brain capillaries. Human brain capillary endothelial (HBCE) cells possess structural characteristics (a low degree of pinocytic vesiculation, specialized transport systems and the capacity to form tight junctions) that regulate the flow of substances across the capillary walls and facilitate formation of the blood-brain barrier (BBB). These properties distinguish HBCE cells from endothelial cells of other organs but may make them susceptible to unique manifestations of HIV infection. For example, HIV infection of HBCE cells may compromise BBB integrity and contribute to neurologic dysfunction by disrupting the barrier function or selective permeability of the endothelium. Infected cells may also constitute a direct route of entry of HIV into the CNS. We have established pure primary cultures of HBCE cells and are using this system as an *in vitro* model to define the mechanisms and consequences of HIV infection. We have previously shown significant HIV infection of HBCE cells but not endothelial cells from other organs. The infection persists in culture and is productive yet non-cytopathic. HIV infection of HBCE cells could not be blocked with antibodies to the two known HIV receptors: the CD 4 molecule and the glycolipid galactocerebroside. This implicates the presence of a new HIV receptor which may be important for entry into the CNS. MABs raised against defined epitopes on gp120 were used in a series of neutralization experiments. A MAB directed against an epitope within the CD4-binding domain was unable to neutralize HIV infection of HBCE cells. Conversely, a MAB directed against a highly variable epitope on gp120 (amino acids 308-331) allowed a 75% reduction in infection efficiency and a MAB directed against an epitope on the immunodominant hypervariable loop neutralized infection by up to 90%. The ability to neutralize infection of HBCE cells with MAB against defined epitopes on gp120 suggests that infection is mediated by a specific receptor and is not as a result of a non-specific uptake mechanism. We are currently investigating the nature of the cellular receptor for HIV on HBCE cells.

Q 237 **FACTORS ASSOCIATED WITH SELECTIVE FUSOGENIC ACTIVITIES OF HIV ENVELOPE GLYCOPROTEINS FOR SPECIFIC CD4+ CELL TYPES,**

Ofer Nussbaum, Christopher C. Broder, and Edward A. Berger, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892

The ability of the HIV-1 envelope to mediate CD4-dependent membrane fusion is dependent on additional features of the CD4+ cell. One form of fusion selectivity is the restricted ability of human CD4 to mediate fusion when present on a variety of human cell types, but not on animal cell types. Using transient animal x human cell hybrids, we previously obtained evidence that human cells contain an additional component(s) which can render CD4+ animal cells fusion competent (Broder et al., Virology, *in press*). As one approach to identifying the human-specifc accessory component, we have been developing systems to transfer membrane vesicles, and solubilized components derived from them, to animal cells expressing human CD4. We express CD4 and HIV-1 envelope glycoprotein using vaccinia vectors, and assay cell fusion by the expression of beta-galactosidase selectively in fused cells (one fusion partner contains the *E. coli* lacZ gene linked to the bacteriophage T7 promoter and the other contains vaccinia-encoded T7 RNA polymerase). Preliminary evidence suggests that animal cells expressing human CD4 can be rendered fusion-competent by transfer of components from membrane vesicles derived from human cells, but not from animal cells. We are applying similar approaches to study a second form of fusion selectivity, namely the cell-type tropisms of different HIV-1 isolates. To this end we are preparing vaccinia recombinants expressing envelope glycoproteins from several T-cell-tropic and macrophage-tropic HIV-1 isolates, and are developing assay systems to study their ability to mediate cell fusion selectively with different CD4+ human cell types.

Q 238 FRESH HUMAN BLOOD CONTAINS PRESUMPTIVE DENDRITIC CELLS THAT EXPRESS CD4. Una O'Doherty, Paul Cameron, Iris Kopeloff*, Stuart Gezelter, Michael Peng, Melissa Pope, Nina Bhardwaj, and Ralph M. Steinman. Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021, and *Department of Dermatology, The New York University Medical Center, New York, NY 10016.

Current procedures for purifying dendritic cells from human blood begin by culturing a T cell-depleted fraction of peripheral blood mononuclear cells (PBMC) for 1 - 2 days. At that time, monocytes are selectively removed by adhering them to gamma-globulin coated plates, and dendritic cells are enriched by flotation on columns of metrizamide. When these dendritic cells are stained with mAbs and analyzed by flow cytometry, they are negative for CD4 as well as CD3, TCR α/ß, CD14, CD16 and CD19, but strongly positive for MHC class II. We previously showed that HIV-1 does not enter these cultured dendritic cells, but when they are pulsed with virus they can transmit the virus efficiently to CD4+ T cells.

We have begun to study the antigenic phenotype and functional properties of dendritic cells freshly isolated from human blood. These DC have a higher buoyant density so enrichment on metrizamide columns is not feasible. To purify fresh DC, PBMC are depleted of T cells by E rosetting, and monocytes by adherence to gamma globulin coated plates. Dendritic cells are further enriched by panning with a cocktail of phycoerythrin labeled antibodies against CD3, CD14, CD16, and CD19. "Cocktail nonadherents" are stained with anti-HLA-DR-FITC, and presumptive dendritic cells (cocktail negative, DR positive) are further purified by cell sorting. On staining, these sorted presumptive dendritic cells are positive for CD4, but negative for TCR α/ß and CD3. After 36 hours of culture, the fresh DC develop the characteristic morphology and strong immunostimulatory capacity of cultured DC. We are currently investigating whether these CD4+ DC can be infected with HIV-1.

Q 239 ASSOCIATION OF HOST CELL-SURFACE PROTEINS WITH HIV, Rimas J. Orentas and James E.K. Hildreth, Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In addition to the virus encoded surface protein, gp120/41, the lipid envelope of HIV is also thought to contain a number of host cell-surface proteins. Our laboratory has developed a novel binding assay to detect the presence of these proteins. The assay is carried out by fluid-phase adherence of MAb specific to cell surface proteins in a 96-well plate, capturing virus from the supernatant of infected cells by this MAb, and subsequently solubilizing the captured virion by the addition of detergent. The viral lysate can then be assayed for p24 concentration, as a measure of viral capture. Although the inclusion of HLA antigens in retroviral envelopes has been described by others, (J. Azocar and M. Essex, 1979, H. Gelderblom et al., 1987) we have demonstrated that HIV also includes adhesion receptors such as LFA-1, CD44, and CD43 (sialophorin), as well as CD71 (the transferrin receptor), and CD63 to an equal or greater degree than class I MHC and class II MHC, depending on the cell type used to produce HIV. While the host glycoproteins included in the virion appear to be a reflection of the host cell membrane from which the virus has budded, the specific inclusion of CD63 and the specific exclusion of CD45 demonstrates that protein inclusion within the viral membrane is to some degree a selective process. Recent descriptions of anti-cell surface protein responses in vaccine trials, the possibility that host receptors participate in the biology of HIV by acting as alternate receptors, and anti-self reactivity in AIDS, all call for a more detailed description of the proteins which reside in the HIV envelope.

Q 240 TRANSACTIVATION OF THE VESICULAR STOMATITIS VIRUS MATRIX PROTEIN EXPRESSION INHIBITS HIV-1 REPLICATION

Soon-Young Paik, Akhil C. Banerjea and Manfred Schubert, Laboratory of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, MD, 20892

While studying the mechanism of cytopathogenesis caused by the vesicular stomatitis virus (VSV) matrix protein M, we also expressed the M protein together with the infectious DNA clone, pNL4-3, in HeLa T4 cells. We observed a dramatic reduction in the amount of HIV-1 replication as indicated by a reduction in viral p24 antigen release and syncytia formation. The inhibition of HIV-1 replication was, however, not specific for HIV-1. The expression of other genes from RNA polymerase II promoters was also inhibited. The precise mechanism of this inhibition is unknown. Thus, in addition to its pathogenic effect on the cytoskeleton, the VSV M protein was also able to inhibit cellular transcription including HIV-1 gene expression.

To control the cytopathogenesis caused by the matrix protein, we have placed a temperature sensitive (ts) M protein under control of the Tat inducible HIV-1 LTR. Stable HeLa T4 cell lines were selected which harbor this ts M gene and which allowed to selectively express the cytopathic M protein in HIV-1 infected cells after transactivation by the HIV-1 Tat protein. This inducibility of gene expression eliminated cytopathic effects normally associated with the expression of this gene in uninfected cells. When these cells were transfected with the infectious HIV-1 clone, pNL4-3, the replication of HIV-1 was drastically inhibited only at the permissive temperature of the M protein (32°C). Despite the transactivation of M expression, HIV-1 replicated to high levels at the nonpermissive temperature for M (40°C). Since M protein kills the cell, any cell which contains a functional M protein expressed after induction by HIV-1 would also be killed at the permissive temperature for M. This would not only prevent the replication of HIV-1 but, at the same time, the cell would also be cleared from the cell population. Such an elimination could potentially protect the entire HIV-1 target cell population from further infections and virus spread.

Q 241 CONSTRUCTION OF INFECTIOUS HIV-1 PROVIRUSES BEARING VARIOUS REPORTER GENES, Vicente Planelles, Sheila Stewart, Yiming Xie, David Camerini, Françoise Bachelerie, and Irvin S.Y. Chen, Departments of Medicine and Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024

The CD4 antigen expressed on T-lymphocytes and macrophages is the receptor for HIV. Several lines of evidence, however, demonstrate that surface expression of CD4 is not sufficient to determine cellular susceptibility to HIV infection. Ancillary cellular factors might work in conjunction with CD4 to deterrmine susceptibility to infection. To date, the identity of these putative ancillary molecules remains unknown. Identification and cloning of putative accessory molecules involved in HIV infection will require sensitive methods for quantitating infection and also for separating infected and uninfected cells. To address these issues, we have engineered the HIV-1 genome to express two reporter genes, the enzyme luciferase, and the mouse lymphocyte surface antigen Thy-1. Viruses in which either of these two genes were substituted by the *nef* open reading frame were constructed and found to be replication-competent. In addition, human PBLs infected with these recombinant viruses expressed detectable amounts of the reporter molecule, suitable for very sensitive quantitation, in the case of luciferase, and for isolation of infected cells by immunological methods, in the case of Thy-1.

Using viruses which carry these repoter genes, we intend to study the requirements for permissiveness for HIV-1 at the molecular level. Additionally, these viruses can be used as probes to screen expression libraries in search for genes encoding putative ancillary factors.

Q 242 **PERSISTENT HIV-1 (MN) INFECTION OF MAMMARY EPITHELIAL CELLS *IN VITRO***, Kimber L. Poffenberger, Gary Riordan, Sherwin Lee, Jay Epstein, and Indira Hewlett, Division of Transfusion-Transmitted Disease, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

Different organs and cell types are affected by HIV-1 infection of the host and may serve as reservoirs for virus during the stage termed clinical latency and beyond. In HIV seropositive mothers, virus can be detected in breast milk and studies of mother-to-child transmission suggest that HIV can be transmitted through breast milk. Thus, the epithelial cells lying along the mammary ducts are exposed to HIV in these seropositive women and may be infected.

This work describes our studies with mammary epithelial cell lines isolated from both normal breast and breast carcinomas. Infection has been monitored by p24 antigen assay, DNA PCR analysis, and FACSCAN immunofluorescence for up to 6 months post-infection. After an initial burst of virus production, the infected cells present very low levels of p24 antigen and very few copies of viral DNA. These levels appear to fluctuate in a cyclic manner over time. Infectious virus is not recovered from the culture media of these cells, but H9 cells can be infected when co-cultured with ME monolayers. Cells from both normal and carcinoma tissue have a similar profile of infection. We will report on these and further studies evaluating the infection of ME cells by primary virus strains.

Q 243 TARGETING SPECIFIC HOST AND VIRAL GENES TO CELLS OF THE CENTRAL NERVOUS SYSTEM: TRANSGENIC STUDIES, Glenn F. Rall, Lennart Mucke, and Michael B.A.Oldstone. Department of Neuropharmacology, The Scripps Research Institute, La Jolla CA 92037.

Viruses can cause neuronal injury by three distinct paths. Viruses such as herpes simplex and polio infect and replicate in neurons of the central nervous system (CNS), causing direct lytic injury. Other viruses, like human immunodeficiency virus (HIV), cause neuronal dysfunction indirectly; i.e., without the virus infecting neurons. Finally, viruses such as lymphocytic choriomeningitis virus (LCMV) can alter neuronal function without direct lysis by establishing persistence in neurons. In this instance, although LCMV actively replicates in neurons without causing cell death, significant alterations in neurotransmitter RNA levels are detected. For LCMV or any noncytolytic virus to establish persistence, it must also perfect strategies to evade host immune recognition. Neurons are deficient in the expression of the major histocompatibility molecules (MHC) necessary for immune recognition, while other CNS cells (astrocytes, oligodendrocytes) express little or no MHC, indicating that these cells can serve as a harbor for non-cytolytic viruses. In order to understand the principles by which viruses can persist in CNS cells, we have developed transgenic mice that express MHC genes in cells of the CNS using cell-specific promoters. Here we report successful in vivo expression of a MHC class I molecule (murine D^b) in neurons. It has been shown that transfection of a neuronal cell culture system with the D^b gene allows MHC class I heavy chain expression and permits CTL to lyse virally infected neurons. Using the neuron-specific enolase (NSE) promoter, the MHC class I molecule D^b was expressed in neurons of transgenic mice and the spliced transgene-derived D^b mRNA was detected by RNA PCR in brains of transgenic mice. Protein expression was shown by attachment of hippocampal neurons from transgenic mice on cover slips coated with a conformation-dependent monoclonal antibody directed against the D^b molecule. Neuronal expression of MHC class I glycoprotein was not detrimental since uninfected transgenic mice housed in microisolator cages for as long as 1 year have neither clinical disorders nor an enhanced death rate when compared to non-transgenic littermates. In preliminary studies, when such D^b-expressing neurons are persistently infected with LCMV in vivo, activated CTL given peripherally (intra-peritoneally) home to the CNS and cause disease. Other transgenic lines have been created in which the LCMV-NP is expressed in neurons using the NSE promoter. Studies with these mice as to neurotransmitter defects and of doubly transgenic mice that express both D^b and LCMV-NP in neurons are in progress.

Q 244 **THYMOTROPISM AND THE RELATED IMMUNE DYSFUNCTION OF THE HUMAN IMMUNO-DEFICIENCY VIRUS**, Michael Rosenzweig, Elizabeth Bunting and Glen Gaulton, Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104. U.S.A.

The role of the thymus in HIV infection is still undefined. The thymus is the primary site of T lymphocyte maturation and development and is thus a milieu with an extremely high concentration of T lymphocytes. T lymphocytes undergo both maturation and selection processes whereby immature, double negative cells initially acquire both CD4 and CD8 proteins and are referred to as double positive cells. These cells then mature into single positive (CD4 or CD8) T cells that enter the circulation. The thymus has been shown to be a site of HIV infection. Both double negative, double positive and single positive cells are infected by HIV, however, the consequences of this infection on T cell maturation cascades are not known. We have established human neonatal thymic organ cultures in an attempt to elucidate the effects of HIV and its gene products on T cell development. Organ cultures were incubated with HIV gp120 for varying time intervals in order to assess perturbations that the viral protein may cause in developing thymocytes. We evaluated the effects of gp120 on the surface expression of CD4, CD8 and T cell receptor (TCR) proteins. Our results showed that gp120 alters the surface expression of both CD4 and TCR proteins, which may influence thymic maturation and selection events. Biochemical alterations in thymocytes in response to gp120 were also assessed. Initial results showed that gp120 induces a dissociation of the tyrosine kinase, *lck* from the CD4 protein. This supports previous findings that gp120 may cause aberrations in the cellular signaling cascade. These studies provide evidence that HIV directly influences the thymic micro environment, and thus may influence T lymphocyte maturation and developmental events.

Q 245 CONDOM USE AMONG SEX WORKERS IN PUERTO RICO Santos M., Alegría M., Vera M., Rivera C., Robles R., Burgos M., Graduate School of Public Health, Medical Sciencie Campus, University of Puerto Rico, Río Piedras, PR 00935

ABSTRACT:

Puerto Rico has one of the highest incidence of AIDS in the United States with 50.7 reported cases per 100,000 residents (CDC, 1992). Heterosexual contact corresponds to 13% of the AIDS cases in Puerto Rico. This paper examines condom use practices and HIV infection among women who work in prostitution. Data was collected from 176 sex workers in different sites throughout the island. Seven outreaches were conducted to recruit a total of 93 women who work in the streets and 83 from brothels. They were interviewed using a structured questionnaire and tested for their HIV and other STDs status. The mean age of the women who participated in our study is 31 years, 58.2% have been married one or more times, and 88.2% are from the Caribbean or Latin America. Only 37.3% had received a high school degree. When comparing the brothel group with the street population we observed no significant differences in terms of age, educational level, age they began working in prostitution or time working in profession. However, significant differences were found in terms of condom use. All brothel respondents indicated that they used condoms when having penis-vagina penetration while only 52.7% of the street population reported this practice. When having oral sex 93.4% of the brothel population reported using a condom while only 21.3% of the street population reported this practice. The practice of regularly using condoms for sexual relations was started for the vast majority during the last two years. Most prostitutes (71.8%) negotiated condom use at the beginning of the conversation with the client, while 11.7% discussed it just before having sexual relations. Overwhelmingly, women (84%) brought the condoms rather than their customers (11.7%). With respect to condom use only 11.1% of those who are HIV positive report using condom always or almost always during oral sex in contrast to the HIV negative women where 72.9% report that practice. There is a great need for intervention aimed at altering behavior related to unprotected sexual practices specially among street sex workers. Most of these women are also drug users in which intervention must consider an integral approach that includes health, social, economical, and psychological factors to deal with this population.

Q 246 *Abstract Withdrawn*

Q 247 SIV ENVELOPE GLYCOPROTEINS ARE CONSTITUTIVELY ENDOCYTOSED FROM THE SURFACE OF CHRONICALLY INFECTED CELLS.

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We have described the derivation of highly cytopathic variants of SIVmac (CP-MAC) from Sup-T1 cells chronically infected with the BK28 molecular clone of SIV (NC-MAC). Sequence analysis of has identified a Tyr to Cys mutation in the cytoplasmic tail of CP-MAC in a region that, by 2D-NMR analysis, exhibited striking similarity to structural motifs that direct clathrin-mediated endocytosis of cell surface molecules. The steady state distribution of TM on Sup-T1 cells chronically infected by NC- and CP-MAC viruses was evaluated by immunofluorescence and confocal laser microscopy on permeabilized cells using an anti-TM mAb. For NC-MAC, a punctate intracellular pattern of TM expression was noted, with little detectable on the cell surface. In contrast, CP-MAC TM expression on the cell surface was markedly increased, with a paucity of intracellular punctate staining. To directly determine if envelope glycoproteins of SIV were constitutively endocytosed, anti-TM and anti-SU mAbs were bound to NC-MAC infected cells at 37°C. The anti-SU mAb was seen in a peripheral intracellular compartment within 5-10 min, but by 30 min had concentrated in a central location, likely in lysosomes. In contrast, the anti-TM mAb remained in a peripheral organelle even after 2 hrs, indicating that TM and SU are endocytosed together, but are differentially sorted to distinct cytoplasmic compartments. To determine if the endocytosis of mAbs was mediated by clathrin coated pits, immunoelectron microscopy was performed on cells incubated at 37°C with anti-TM mAbs. For NC-MAC infected cells, coated pits were abundant and stained intensely with anti-TM, while pits were rarely found on CP-MAC infected cells, and those that were seen showed no detectable anti-TM mAb. Efforts are in progress to develop assays to quantitate endocytosis of the SIV envelope glycoproteins. Expression of the SIV envelope and chimeric cell-surface proteins containing the TM cytoplasmic tail will be used to evaluate the structural elements of the SIV tail that are involved. We propose that the ability of SIV to direct the endocytosis of its envelope glycoproteins and reduce expression of these molecules on the surface of infected cells may represent an important mechanism for establishing a persistent infection *in vivo* utilized by SIV and perhaps other members of the lentivirus family.

Q 248 **A POST-ENTRY, PRE-INTEGRATION BLOCK TO HIV-1 REPLICATION IN SOME RODENT CELL LINES.**

J.H.M. Simon[1], G.A. Schockmel[2], A.F. Williams[2] and W. James[1]. [1]Sir William Dunn School of Pathology, and [2]MRC Cellular Immunology Research Unit, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom.

With the exception of three rabbit cell lines[1], non-primate cells are resistant to infection by HIV-1. This block to infection cannot be overcome by expression of human CD4, and has been localized to a post-binding step, probably fusion.

Viral entry and full reverse transcription have been demonstrated in two human CD4-expressing rodent cell lines. However, HIV cannot be recovered after cell-free infection of these cell lines, but is recovered when the cell lines are transfected with HIV DNA, indicating a post-reverse transcription but pre-integration block to HIV replication. This block is not universal among rodent cells, since HIV can be recovered from other rodent cell lines with which HIV has been force-fused.

The results show that the block to HIV-1 entry is not present in all rodent cell types and that in some rodent cell lines, there is a post-reverse transcription, pre-integration block to HIV replication. This second block reveals an essential interaction with the host cell during productive infection by HIV which may provide a suitable target for chemotherapeutic intervention. The molecular and genetic basis of this block will be discussed.

1. Kulaga, H., Folks, T.M., Rutledge, R. & Kindt, T.J. *Proc Natl Acad Sci U S A* **85**, 4455-9 (1988).

Q 249 **EFFECT OF ANTIBIOTICS ON HIV REPLICATION AND HIV-INDUCED CYTOPATHICITY**, Mohan Somasundaran, Kevin S. Byron, Christopher Zarozinski and John L. Sullivan, Dept. Pediatric Immunology, Univ. Massachusetts Med. Center, Worcester, MA 01605.

Normal peripheral blood lymphocytes and T-cells were infected with HIV-infected patient isolates and cloned lab isolates of HIV. Viral replication was assayed by following RT-activity and p24 protein in the culture supernatants. Intracellular expression of viral RNA was determined by in situ hybridization using a biotinylated genomic probe. HIV-induced cytopathicity in infected cell-cultures was determined by dye-exclusion and MTT assays. We observed that viral replication increased by 30 to 80 fold in cultures treated with non-cytotoxic concentrations of tetracycline or its analogues. Unexpectedly, these cultures were protected from lysis. ACH2 cultures, induced by TPA for viral expression, were also protected from cell-death by tetracycline. In vitro CAT-assays suggested that the antibiotics did not have a direct effect on HIV-LTR. Northern blots of total RNA from infected cells did not show any temporal changes in the expression of regulatory and/or structural messages. We have recently demonstrated the presence of viral RNA in mitochondria of infected cells. Further studies suggest a correlation between tetracycline-induced changes in mitochondrial viral RNA and cytopathicity. The mechanism of augmentation of viral replication and the possible effect of antibiotics in localizing viral RNA to mitochondria is being pursued.

Q 250 HIV-1 cDNA FORMATION AND PROCESSING IN MONOCYTES AND MACROPHAGES, Secondo Sonza, Anne L. Maerz, John Mills and Suzanne M. Crowe, AIDS Pathogenesis Research Unit, Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Melbourne 3078, Australia.

Peripheral blood monocytes on the day of isolation (MC), and monocyte-derived macrophages (MΦ) after 1-7days in suspension culture, were infected with HIV-1_{Ba-L} (100ng p24 / 10^6 cells). The extent of reverse transcription from viral genomic RNA was determined at various times post infection (pi) by PCR using primer pairs which amplify the first 100bp of the 5'-LTR, a 278bp sequence in the *vif/vpu/env* region and a 115bp *gag* segment (Zack *et al, Cell 1990, 61: 213-222).* In MΦ, cDNA synthesis was readily detectable by hybridization within 12hr of infection with each of the three primer pairs. No cDNA was detected in MC, even 7days after infection. Differentiation and maturation, therefore, appear to be important in the susceptibility of these cells to infection with HIV-1.
To determine if, and when, proviral DNA then becomes integrated into the MΦ genome, we have developed a novel PCR method which utilises the existence within mammalian genomes of ubiquitous repeat sequences. Using sets of outward-facing primers to the *Alu* repeats, the most abundant of these repeat sequences, and to the HIV-LTR, infected MΦ DNA was amplified at different times pi and hybridized to an LTR-specific probe. Preliminary results with this procedure suggest that proviral DNA may become integrated within a few days of infection. To confirm and extend these results, *Alu*-LTR amplified DNA is being cloned and sequenced.
HIV replication in MC appears to be blocked prior to reverse transcription. In MΦ, however, the reverse transcription and integration steps proceed normally.

Q 251 CENTRAL NERVOUS SYSTEM IMPAIRMENT IS ASSOCIATED WITH HIGH REPLICATING, SYNCYTIA INDUCING HIV-1 ISOLATES IN CEREBROSPINAL FLUID (CSF). Stephen A. Spector, Peter P. Reinhardt and the HIV Neurobehavioral Research Center, University of California, San Diego, La Jolla, CA 92093.
To date, the role of HIV-1 in the development of neurocognitive disorders in infected individuals remains unclear. In the current study, we evaluated if neuropsychologic impairment in HIV-1 infected individuals was correlated with specific properties of their CSF viral isolates including: rate of replication, cell tropism and ability to induce syncytia. Virus isolated from CSF was cultured in monocyte-derived-macrophages and MT-2 cells to determine cytotropism. Rate of replication was evaluated in peripheral blood mononuclear cells. Study participants were examined for abstraction, attention, learning and memory capabilities, verbal and psychomotor skills, and motor and sensory functioning. Individuals identified as harboring high replicating, syncytia inducing CSF HIV-1 isolates were more likely to have neuropsychologic impairment. While only 2 (12%) of 17 HIV infected persons with normal neurobehavioral status carried syncytia inducing isolates, 10 (48%) of 21 neuropsychologically impaired patients had syncytia inducing isolates in the CSF ($p \leq 0.02$) and 15 (71%) of 21 were high replicating isolates. Of the 5 individuals with advanced neuropsychologic impairment 3 (60%) had syncytia inducing isolates in their CSF and all 5 isolates were high replicating. These data indicate that neuropsychologic dysfunction in HIV-1 infected individuals correlates with high replicating, syncytia inducing isolates in the CSF and that the presence of such strains may be a marker for the development of HIV-related CNS disease. Longitudinal studies are in progress to determine the ability of phenotypic and genotypic properties of CSF HIV-1 strains to predict the development of neurobehavioral disorders.

Q 252 CALMODULIN - HIV ENVELOPE GLYCOPROTEIN INTERACTIONS. Ranga V. Srinivas, Department of Infectious diseases, St.Jude Children's Research Hospital, Memphis, TN 38101.

The carboxy-terminal domain of the envelope glycoproteins of human and simian immunodeficiency viruses (HIV-1, HIV-2 and SIV) contain regions that can fold into amphipathic helical (AH) segments. These AH segments closely resemble the positively charged calmodulin (CaM)-binding AH peptides found in CaM-regulated enzymes. Consistent with this idea synthetic peptides corresponding to gp160 AH segments bind to CaM, and inhibit CaM-regulated enzymes. Also, HIV gp160 & gp41, but not gp120 (which lacks the putative AH segments) bind to CaM. Analysis of wild-type and transmembrane-protein (TM-protein) truncated envelope glycoproteins suggest that the carboxy-terminus of gp41 is required for CaM binding. CaM-antagonists were found to inhibit HIV-induced cell fusion. Also, the CaM-antagonists exerted a greater anti-proliferative effects on HIV-infected lymphoblasts, as compared to uninfected cells. In HIV-infected cells, the putative CaM-binding regions of gp160 are located intracellularly, and may therefore interact with the cytosolic CaM, thus disrupting CaM-regulated cellular functions. We have investigated the subcellular distribution of CaM in uninfected and HIV-infected lymphocytic cell lines. We propose that the interactions of HIV envelope proteins and CaM may play a role in HIV pathogenesis.

This work was supported by grants from American Foundation for AIDS Research and the American Lebanese Syrian Associated Charities. Michael LaPuente provided expert technical assistance.

Q 253 IN VITRO INTERACTION OF HUMAN TONSILLAR LYMPHOCYTES AND FOLLICULAR DENDRITIC CELLS (FDC) WITH HIV-1, -2 AND SIV Ingrid Stahmer[1], Alexander Vovoedin[1], Roberto De Mesquita, Rigmor Thorstenson[2], Gunnel. Biberfeld[2], Peter. Biberfeld[1]. [1]Immunopathology Lab., Karolinska Hospital/Institute, Stockholm; [2] National Bacteriological Laboratory, Stockholm.

AIMS: To study defined tonsillar lymphocyte subsets and follicular dendritic cells.(FDC) with regard to their interaction with and susceptibility to HIV-1, -2 and SIV infection., particulary with regard to cytopathic and/or stimulating effects of virus-cell interaction.
MATERIAL AND METHODS: Human tonsils from children undergoing tonsillectomy were enzymatically digested and cell suspensions were further fractionated into B-/ T-lymphocytes and FDC by use of an immunomagnetic procedure (MACS). The cell fractions were cultured in the presence of different retroviruses and studied by immunohistochemistry, PCR and electron microscopy.
OBSERVATIONS: The cultured cell fractions showed differences in cellaggregation, proliferation and viability, depending on the type of retrovirus used for infection.
The permissivness and reactivity of B-/ T-lymphocytes and FDC to the different retroviruses will be discussed.

Q 254 EFFECTS OF MUTATIONS IN THE V3 REGION ON THE ENVELOPE STRUCUTRE AND FUNCTION, Leonidas Stamatatos and Cecilia Cheng-Mayer, Cancer Research Institute, UCSF, San Francisco, CA 94143-0128. We are studying the structure/ function relationships of the envelope glycoprotein of HIV. Specifically, we are examining how the structure of the V3 region of env gp120 affects the overall conformation and the biological activity of that protein. We have previously shown that mutations within and flanking the V3 region of two clinical isolates (SF2 and SF13) affected the infectivity, host cell tropism and pahogenicity of the virus. The wild type and mutant env genes were subcloned and transiently expressed in cos-7 cells. The processing of gp160 into gp120 and gp41 of the mutant glycoproteins, as determined by immunoprecipitation and Western blotting, was compared to that of the wild type. A syncytium assay was used to quantitate the fusion potential of the expressed mutant envelope glycoproteins.

Preliminary results show that the processing of mutant glycoproteins was comparable to that of wild type. However, certain mutations, both within and outside of the V3 region, appear to affect the proper association of env gp120 with gp41. In such cases, gp120 was present predominantly in the cell supernatant and not in the cell lysates, and syncytia were not observed. Other mutations resulted in a gradual loss of gp120 from the cells, but no syncytia were observed even at early times when significant amounts of gp120 were still associated with gp41.

Our data indicate that mutations within the V3 loop can perturb the overall conformation of gp120 and result in loss of biological activity. We are in the process of examining in more detail the changes in the conformation of gp120 that correlate with loss of biological activity.

Q 255 **REPLICATION OF A MACROPHAGE-TROPIC STRAIN OF HIV-1 IN A HYBRID CELL LINE, CEMX174, SUGGESTS THAT CELLULAR ACCESORY FACTORS ARE REQUIRED FOR ENTRY,** Kelly Stefano[1,2], Ronald Collman[3], Dennis Kolson[1,2], James Hoxie[3], Neal Nathanson[2] and Francisco Gonzalez-Scarano[1,2], Departments of Neurology[1], Microbiology[2] and Medicine[3], University of Pennsylvania Medical Center, Philadelphia, PA. 19104-6146.

To investigate the mechanism underlying one aspect of the cellular tropism of HIV-1, we used a macrophage tropic isolate, HIV-1(89.6), and screened its ability to infect a number of continuous cell lines. HIV-1(89.6) was able to replicate robustly in a T-cell/B-cell hybrid line, CEMx174, while it replicated modestly or not at all in either of its parents, one of which is the CD4-positive line CEM(E). Transfection of a molecular clone and a virus uptake assay both provided strong evidence that the block to HIV-1(89.6) replication in the CEM(E) line lies at the level of cellular entry. These results were complemented by preparing a CD4-expressing cell line based on the B-cell parent, 721.174, and demonstrating that it is permissive for productive HIV-1(89.6) replication. Based on these experimental findings, we speculate that there exist cellular accessory factor(s) which facilitate virus entry and infection in CD4-positive cells. Furthermore, these cellular accessory factors may be quite virus strain-specific, since not all macrophage-tropic strains of HIV-1 were able to replicate in the CEMx174 hybrid cell line. This experimental model provides a system for the identification of one or more of these putative cellular accessory factors.

Q 256 A 55-65Kd CELLULAR PROTEIN IS RECOGNIZED BY A MONOCLONAL ANTI-IDIOTYPIC ANTIBODY PREPARED AGAINST THE HIV-NEUTRALIZING, V3 SPECIFIC MONOCLONAL ANTIBODY 110.4, Elaine Kinney Thomas, Roberta J. Connelly, Maria Kahn, Molly D.Smithgall, Karl Erik Hellström, Ingegerd Hellström, Omar K. Haffar, Department of Virology, Bristol Myers Squibb Pharmaceutical Research Institute, Seattle, Wa 98121

Antibodies such as 110.3,4,5,and 6 which bind the third hypervariable (V3) domain of the LAI isolate inhibit syncytia formation and neutralize cell-free virus, yet do not inhibit soluble gp 120 binding to its major receptor CD4. In fact, gp 120 bound to CD4 assumes novel configurations where exposure of the V3 domain to antibodies such as 110.3-6 is conformationally enhanced. Taken together, these data suggest that after gp 120 engages the CD4 receptor, the V3 domain facilitates cell to cell fusion and viral entry, perhaps by directly engaging or indirectly modulating an obscure cellular component which participates in the fusion or entry event, or by indirectly facilitating the fusogenic potential of gp 41.

We set out to generate molecular mimetics of the V3 domain to use as probes for potential cellular ligands of V3 by producing ten anti-idiotypic monoclonal antibodies (anti-ids) against the neutralizing monoclonal antibodies 110.3 and 110.4 (Ab 1). The anti-ids were selected for their ability to block the cognate Ab 1 binding to the V3 peptide (peptide 308-328) of the LAI isolate. Nine anti-ids bind 110.3, 110.4, and 110.5, while one, anti-id 2 binds only 110.4, and that interaction is mediated through the heavy chain. Anti-id2 recognizes a 55-65 Kd protein in CEM cells, PHA blasts, some lymphoblastoid cells, several fibroblast lines and recognizes a mouse homolog in a melanoma cell line as demonstrated by radioimmunoprecipitation (RIP) assays and Western blots. The interaction is specific since it can be blocked by preincubating anti-id 2 with 110.4 prior to either assay. By employing a differential extraction method which relies on the protein remaining insoluble in 1% NP40, 0.5 % DOC, and being subsequently solubilized by the same buffer plus 0.1 % SDS, we have purified the protein in sufficient quantity for N-terminal amino acid sequence analysis which is in progress. Further, we have prepared polyclonal rabbit sera against the cellular protein and experiments using these various reagents to assess the role of the cellular protein in the life cycle of HIV will be discussed.

Q 257 THE EFFECTS OF CEREBRAL HIV-1 gp120 EXPRESSION IN TRANSGENIC MICE, Toggas, S.M., Rockenstein, E.M., and Mucke, L., Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, CA 92037.

HIV-1 infection is often associated with neurologic impairments that can culminate in dementia and paralysis, as well as with neuropathologic changes such as activation of astrocytes (reactive astrocytosis), white matter pallor, microglial alterations and neuronal loss. The pathogenesis of these abnormalities is unclear. Because the HIV-1 envelope glycoprotein gp120 appears to have detrimental effects on neurons *in vitro* (reviewed in TINS [1992]15:75) it is interesting to assess the pathogenetic potential of this protein *in vivo*. The HIV-1 env gene was modified to encode gp120 (without gp41) and fused with regulatory sequences derived from i) the murine glial fibrillary acidic protein (GFAP) gene for astroglial expression and ii) the rat neuron specific enolase (NSE) gene for neuronal expression. The resulting fusion genes were microinjected separately into fertilized murine egg cells. Eleven transgenic (tg) founder mice were obtained for GFAP-gp120 and 5 tg founders for NSE-gp120. Cerebral gp120 mRNA expression was detectable on standard Northern blots in 3/5 NSE-gp120 tg lines and in 6/6 GFAP-gp120 tg lines tested so far. Astroglial expression of gp120 induced a marked increase in endogenous GFAP mRNA levels, widespread reactive astrocytosis and activation of microglia in several tg lines. The prominence of these changes correlated positively with the level of gp120 expression. We conclude that cerebral gp120 expression is sufficient to induce changes that resemble some of those seen in HIV-1 infected human brains. The effects of gp120 on neurons are currently being characterized by morphometric, behavioral and electrophysiologic analyses.

Q 258 CHARACTERIZATION OF HIV-1 INFECTION IN A HUMAN CORTICAL NEURONAL CELL LINE

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HCN-1A is a human cerebral cortical neuronal cell line which can be grown in culture as adherent monolayers of slowly proliferating cells. The cell monolayers were infected with HIV-1 *in vitro* by exposure to various isolates of HIV-1 and were monitored for p24 antigen in the cell-free supernatants. No cytopathic effects were observed on these cells; the cells were passaged and proliferated until termination of the cultures (as long as 5 weeks) while continuing to express low levels of p24 antigen in the supenatants. Virus-positive cells were detected by indirect immunofluorescence using characterized serum from an individual with AIDS as well as with a gp120 monoclonal antibody. Infection of HCN-1A was confirmed by polymerase chain reaction (PCR) analyses of both RNA and nuclear DNA and by *de novo* synthesis of viral proteins as shown by metabolic labeling and immunoprecipitation. Cell-free supernatants from infected HCN-1A cultures contained virus which could be passed to a permissive human T-cell line (A3.01). The mechanisms of viral binding and entry into HCN-1A cells are speculative at this time; no CD4 message was detected by PCR, and flow cytometric analyses revealed no detectable surface CD4, indicating a non-CD4-mediated infection process. The cells did express the membrane glycolipids, galactosyl ceramide and sulfatide, which may serve as gp120 receptors on cells of neuronal origin. HIV-1 infection of HCN-1A cells may provide an *in vitro* model for investigating mechanisms involved in central nervous system neuronal dysfunction associated with AIDS.

Q 259 ELEVATION OF cAMP LEVELS IN LYMPHOCYTES AND IMPAIRED FUNCTION IN HIV SEROPOSITIVE SUBJECTS, H. Wang, P. Nishanian, B. Hofmann, T. Nguyen, JL. Fahey, CIRID, UCLA School of Medicine, Los Angeles, CA 90024-1747

HIV infection results in the depletion of CD4 T cell number and also in the suppressed function of remaining lymphocytes. The current study was designed to examine the possible biochemical basis for this functional impairment. cAMP signal transduction pathway plays an inhibitory role in regulation of lymphocyte activation. To determine if cAMP is elevated in HIV infection, we measured intracellular levels of cAMP in lymphocytes of 14 HIV seropositive and 9 HIV seronegative homosexual men by the scintillation proximity assay system. cAMP levels in HIV seropositive subjects (7.8 +/- 4.7 pMol/10^6 cells) were significantly higher than in seronegative samples (5.0 +/- 2.6) ($p<0.05$). Proliferative response of PBMC to PHA, PWM and Candida albicans were shown to be reduced in HIV seropositive individuals in comparison with controls (mean cpm/$50x10^3$cells: 74,200 vs 91,000; 13,600 vs 20,000; 5,500 vs 10,000 respectively). ddAdo, an inhibitor of adenylate cyclase which is necessary for the generation of cAMP, can enhance proliferative response to Candida albicans in HIV seropositive subjects with CD4 cell number >500. A positive correlation ($r=0.53$) between proliferative response to PHA and CD4/CD8 ratio in 21 seropositive subjects was also observed ($p<0.05$). Our data indicate that both CD4 reduction and cAMP change contribute to impaired proliferation of PBMC in HIV seropositive individuals.

Q 260 BINDING AND INTERNALIZATION OF HIV-1 MEDIATED BY A NEW MEMBRANE-ASSOCIATED C- TYPE LECTIN. Andrew J. Watson and Ben M. Curtis. Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA, 98121.

We have recently described the identification, cloning and sequencing of a new cell surface receptor for HIV gp120 (B. M. Curtis, S. Scharnowske and A. J. Watson. PNAS 89 8356, 1992). This protein was identified through screening of a placental cDNA library and contains 404 amino acids with a calculated Mr of 45775. It is organised into three domains ; an N-terminal cytoplasmic and hydrophobic region, a set of 7 complete and one incomplete tandem repeats and a C-terminal domain with homology to C-type (Ca-dependent) lectins. Binding inhibition studies using saccharides indicate a specifity for mannose and fucose residues. Cells transfected with this placental mannose-binding protein (PMBP) bind gp120 with nanomolar affinity (Kd=1.8 nM+/-0.2 (n=4)). Following incubation of HIV with Hela cells transfected with PMBP, EM studies indicate that the virions are internalized and are packed into vesicles distributed throught out the cytoplasm. While no overt infection of the transfected Hela cells was detected, the outcome of HIV binding to the placental manose binding protein will most likely be strongly influenced by the type of cell expressing it. To investigate this relationship cells of lymphoid, epithelial and astroglial origin which either lack, or express CD4, are being transfected. Results from these experiments will determine more generally if the placental manose binding protein can function alone as a receptor facilitating HIV infection or whether it may have a role as an accessory molecule in those cells where CD4 expression alone is insufficient for HIV infection. The progress in these studies will be presented.

Q 261 A FELINE MODEL OF PEDIATRIC HIV NEUROPATHY

David W. Wheeler[1], Thomas W. Mitchell[1], Andrea R. Mihajlov[1], Margaret C. Barr[2], Niels C. Pedersen[3], L. Ray Whalen[1]. [1]Dept. Anatomy & Neurobiology, Colorado State University, CO, 80523. [2]Cornell Feline Health Center, NYSCVM, Cornell University, Ithaca, NY 14853. [3]Dept. of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616.

It has been estimated that 1.5 million Americans are infected with the human immunodeficiency virus, HIV. Of these people, two to three percent of the cases involve the pediatric age group. The disease in children is generally associated with progressive neurologic dysfunction and opportunistic infections. The majority of patients develop clinical manifestations of HIV infection before the age of two years.

It has been shown that HIV infects the nervous system and induces decreased mental capacity, depression, abnormal sensation, and/or muscle weakness. This combination of symptoms is called the AIDS dementia complex (ADC). The method by which HIV infects the brain and subsequently induces dysfunction remains a mystery. An animal model of ADC is necessary to expedite research into this problem.

Feline immunodeficiency virus (FIV) is a lentivirus of cats. FIV is closely related to HIV and is also associated with immunosuppression and nervous system dysfunction. This project was designed to explore the feasibility of using FIV infected cats as an animal model of the effect of retroviruses on the growth and development of the nervous system. In our previous studies we have developed non-invasive electrophysiologic testing procedures as quantitative predictors of nervous system involvement in retroviral infections. To determine the developmental consequences of lentiviral infection of the nervous system from birth to maturity, these techniques were used to sequentially monitor for *in vivo* effects of neurotropic FIVs in perinatally infected kittens.

We have demonstrated remarkable changes in lumbar evoked spinal cord potentials in 3 of 7 clinically normal cats, greater than eighteen months of age, that were infected <24 hours after birth. The electrophysiologic changes seen in these cats are consistent with fiber loss in the dorsal (posterior) columns of the spinal cord. No histopathology has been performed on these cats, however previous clinical cases of FIV infection have shown profound loss of myelin in afferent fiber tracts of the central nervous system. Currently, twelve additional FIV-infected kittens of various ages are being monitored in our prospective study of nervous system development in the presence of retroviral infection.

Q 262 HIV-1 INFECTION OF RODENT CELL LINES THROUGH A MUTANT MURINE CD4 RECEPTOR (L3T4), Kenneth J. Wieder, Jay Boltax, Irene Wieder, Terry B. Strom and Pamela Chatis, Department of Medicine, Divisions of Immunology and Infectious Diseases, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

Murine cells are resistant to HIV-1 infection because they lack receptors for the virus which result in HIV-1 binding and internalization. Murine cell transfectants expressing human CD4 bind HIV-1 but, inexplicably, do not become infected because the virus does not enter the cells. We tested a hypothesis that a murine CD4 receptor (L3T4) minimally altered to preserve potentially important L3T4 epitopes, but sufficient for binding the HIV-1 envelope glycoprotein, would permit viral entry into murine cells. These mutant L3T4 constructs were cloned and expressed in a variety of human and murine cell lines to evaluate their effectiveness as a receptor for the envelope glycoprotein of HIV-1 and their ability to facilitate viral internalization. Certain mutant L3T4 receptors bound rgp120 as determined by indirect immunofluorescence and ^{125}I-gp120 binding studies. Human HeLa cells expressing the mutant L3T4 receptors could be infected through this mutant L3T4 receptor. A variety of rodent cell lines expressing these mutant L3T4 receptors were also infected with the virus as determined by PCR analysis of cellular RNA and DNA, but viral replication was substantially lower than that found in human cells. Low level HIV-1 replication was observed as evidenced by the presence of spliced HIV-1 RNA transcripts; the presence of viral structural proteins, however, was lacking. These results suggest that HIV-1 can enter murine cells through a murine CD4 receptor, and may be useful in certain studies as receptors for HIV-1 in mice bearing mutant L3T4 receptors.

Q 263 MUTATIONAL ANALYSIS OF HIV-1 EXTERIOR ENVELOPE GLYCOPROTEIN gp120 FUNCTIONAL REGIONS, Richard Wyatt, Markus Thali, Marshal Posner, David Ho, Shermaine Tilley, Abraham Pinter, James Robinson and Joseph Sodroski, Division of Human Retrovirlogy, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115

HIV-1 is tropic for CD4-positive human cells by virtue of a high affinity interaction between the HIV-1 exterior envelope glycoprotein, gp120, and CD4, which acts as the viral receptor. Previous mutational analysis has identified discontinous residues of gp120 critical for CD4 binding. These residues are located within conserved regions of gp120, particularly C2, C3 and C4. HIV-1 infected individuals produce conformationally-dependent neutralizing antibodies whose epitopes partially or completely overlap the conserved regions involved in CD4 binding. The anti-CD4 binding antibodies can interfere with gp120-CD4 interaction, although the mechanism of neutralization is still unknown. Another important target for viral neutralization is the third variable (V3) loop of gp120. Antibodies directed against the V3 loop neutralize HIV-1 by interfering with post-receptor binding events necessary for virus fusion and entry. We have utilized both anti-CD4 binding antibodies and anti-V3 loop antibodies to examine the intramolecular relationships revealed by various mutant gp120 glycoproteins. Wild-type HXBc2 gp120 glycoproteins demonstrated decreased recognition by several anti-CD4 binding monoclonal antibodies as well as by a monospecific anti-C4 peptide antisera when compared to gp120 glycoproteins deleted of the V3 loop (Δ3). Point mutation within the base of the V3 loop (arg 298) or the C4 region (trp 427) increased the binding of the anti-V3 loop antibody 9284, as well as the binding of some anti-CD4 binding antibodies. A replication transcomplementation assay revealed the 298 R/G mutant was more sensitive to neutralization by soluble CD4, 9284 and some anti-CD4 binding antibodies. Taken together, these data indicate a structural proximity of the V3 loop and the CD4 binding site. We have also examined the structural and functional consequences of deleting the V1, V2 and V3 major variable loops of gp120. Envelope glycoproteins deleted of V1 and V2 (ΔV1/2), V3 or V1, V2 and V3 (ΔV1/2/3) were properly processed from the gp160 precursor glycoprotein, associated with the transmembrane glycoprotein gp41 on the cell-surface and maintained a native structure necessary for soluble CD4 binding and recognition by the conformationally-dependent anti-CD4 binding antibodies. All of these mutant glycoproteins were both fusion and replication defective. Further characterization of the gp120 deletion mutant glycoproteins will be presented.

Q 264 ILLICIT DRUGS AND ALTERATION OF CATALASE ACTIVITY AMONG HIV-1 (+) ASYMPTOMATIC PATIENTS. Sumio Yano, Gabriel Martinez, Maria Colon, Alma Santiago, Carmen Lefranc, Nayra Rodriguez and Yasuhiro Yamamura, Ponce School of Medicine AIDS Research Program & Dept. Biochem. & Microbiol. & Damas Hospital Dept. Medicine, Ponce PR 00732.

Illicit drugs used among i.v. drug users are believed to act as a negative co-factor that prompts the progression of the disease after HIV infection with undetermined mechanism. The progression of the disease is directly associated with a steady decline of $CD4^+$ T cells which is, in part, caused by HIV provirus activation which involves reactive oxygen intermediates (ROI). Therefore, we hypothesized that a putative decrease of antioxidants in drug addicts (DA) population may facilitate activation of HIV provirus, thus causing HIV-1 infection to progress faster than non-DA populations. To test this hypothesis, we measured catalase (CAT) activity and glutathione content of peripheral blood cells among the four groups: namely, healthy donors (HD), HIV (-) DA, HIV (+) non-DA, and HIV(+) DA. The majority of DA were cocaine and heroin users for 1-5 years. The specific activities of CAT (unit/10^6 cells) of both mononuclear cells and granulocytes were significantly lower in HIV(-) DA than HD and the decrease was correlated with the period of addiction history, while that in RBC varied significantly among the four groups. HIV (+) non-DA showed the highest activity, followed by HIV(+) DA and both DA and HD were the lowest. The total GSH level in whole blood was significantly lower in both HIV (+) non-DA and HIV(+) DA but not in HIV(-)DA compared with HD. It was suggested that a low CAT activity in the lymphocytes of DA, may lead to an activation of HIV provirus via increased hydrogen peroxide accumulation within HIV infected T lymphocytes and/or monocytes. Drug abuse may also trigger a cascade of changes in cytokine production, thus directly affecting host immune competence. (The study was supported in part by the RCMI grant G12RR03050, and also by RO1DA06037 & R29DA05510)

Regulation, Ancillary Proteins, Late Events, Transmission, Perinatal Transmission, Cytokine Regulators

Q 300 CD28 LIGATION PER SE INDUCES REPLICATION OF HIV IN NATURALLY INFECTED LYMPHOCYTES

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Induction of HIV replication in infected PBL requires cellular activation. Antigen stimulation via the CD3/TCR complex or antigen mimicry by immobilized anti-CD3 antibodies (mAb) provide signals for activation and proliferation of resting T lymphocytes. The ligation of CD28, a signal transducing receptor with a natural ligand on activated B cells, provides a co-stimulating signal for IL2 production and T-cell proliferation as well as a co-activation of the transfected HIV1-LTR in Jurkat cells. The aim of this study was to investigate the ability of the CD28 ligation to activate HIV replication in naturally infected CD4+ PBL from 29 patients. We used the anti-CD28 Mab (a gift of D. Olive, France) in combination with immobilized anti-CD3 Mab or alone. Virus was isolated from 16/29 patients. For 6/16 patients, HIV was isolated only when anti-CD28 was added in combination with anti-CD3 or in 5 of totally 13 tested cases in the presence of CD28 alone. In contrast to the potent T cell proliferation observed in all cases after CD3 stimulation, no T cell proliferation was induced at day 3 by 3H-thymidin incorporation with CD28 alone. Altogether, our results show that: 1/ the ligation of CD28 is capable of activation per se HIV replication from naturally infected lymphocytes; 2/ the CD3 cross-linking is less efficient although inducing a potent T cell proliferation. Therefore non-mitogenic CD4+ T cell activation signals, such as those provided by the ligation of CD28 to its natural ligand on antigen presenting cells, are sufficient to induce transcription and replication of HIV.

Q 301 **HIV-1 ACCESSORY GENE FUNCTION IN MACROPHAGE INFECTION** John W. Balliet, Dennis L. Kolson, Neal Nathanson, A. Srinivasan and Ronald Collman. University of Pennsylvania Medical Center and the Wistar Institute, Philadelphia, PA, 19104, USA

The essential functions of HIV-1 *vpr*, *vpu* and *nef* genes are not well understood despite extensive studies in lymphoid cells. We tested the functions of these genes, along with the *vif* gene, in infection of macrophages utilizing a biologically active molecularly cloned macrophage-tropic HIV-1 primary isolate, HIV-1/89.6.

DNA sequence showed that HIV-1/89.6 contains intact open reading frames for *vif*, *vpr*, *vpu*, and *nef*. By site-directed mutagenesis we introduced into each of these genes mutations which were predicted to generate truncated proteins, inserting termination codons within the first several amino acids. The mutations were subcloned, singly and in combination, back into the proviral clone. Mutant viruses were then tested for ability to infect and replicate in primary and transformed cells.

Wild-type HIV-1/89.6 replicated in primary monocyte-derived macrophages (MDM), peripheral blood lymphocytes (PBL), and the hybrid CEMX174 transformed cell line. Virus deficient in *vif* replicated poorly or not at all in each of the three cell types, while *nef*-deficient virus showed a profile similar to wild-type. Both *vpr* and *vpu*-deficient virus replicated in PBL and CEMX174 cells, but were markedly reduced in MDM. Viruses which carried multiple gene truncations posessed the phenotype of the most restrictive mutation.

Using a biologically active molecular clone of a native macrophage-tropic HIV-1 strain, we have shown that both *vpr* and *vpu* are essential for productive macrophage infection. *Vif* is necessary for efficient infection both of lymphoid cells and macrophages. *Nef*, however, is not necessary for macrophage infection. Since macrophages may be central in pathogenesis *in vivo*, these studies suggest that the conserved nature of *vpr* and *vpu* among primary HIV-1 isolates may result from their roles in macrophage infection.

Q 302 **ANALYSIS OF HIV-1 NEF INHIBITION OF NF-KB AND AP-1 ACTIVATION.** Juan Bandres, Thomas Niederman, and Lee Ratner, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

SIV and HIV Nef proteins function as transcriptional inhibitors. We have studied the effects of HIV-1 Nef on the DNA binding activity of transcriptional factors implicated in HIV-1 regulation. **Methods:** Stably transfected lymphoid (Jurkat, HPB-ALL) and monocytoid (U937) cells and transiently transfected COS cells were used for preparation of nuclear extracts, and analysis of binding activity with DNA oligonucleotides corresponding to the NF-kB, AP-1, SP-1, USF, URS, and NFAT sequences present in the HIV-1 LTR. **Results:** HIV-1 Nef inhibited the induction of NF-kB and AP-1 DNA binding activity in all cell types examined, whereas the binding of SP-1, USF, URS, and NFAT were not affected. The induction of NF-kB by PMA and PHA was suppressed 4-6-fold in Nef expressing cells compared to control cells transfected with vector alone, a frameshifted form of nef, or antisense nef. Additionally, Nef inhibits the induction of HIV-1- and IL-2-directed gene expression, and the effect on HIV-1 transcription depends on an intact NF-kB binding site. PHA-induced AP-1 DNA binding activity was inhibited 6-8-fold in Nef-expressing cells compared to the control cells. Moreover, Nef expressing cells were transiently transfected with a plasmid in which an intragenic HIV-1 pol AP-1 DNA recognition sequence was cloned downstream of the CAT gene. Mitogen-mediated transcriptional activation of the CAT gene in this construct was inhibited in Nef expressing cells but not in control cells.

Conclusions: Defective recruitment of NF-kB and AP-1 may underlie Nef's negative transcriptional effects on the HIV-1 and IL2 promoters. Further evidence suggests that Nef inhibits NF-kB induction by interfering with a signal emanating from the T-cell receptor complex. By inhibiting virus replication directly, and/or by blocking T-cell activation, Nef may provide a reservoir of persistently infected cells which may ultimately contribute to HIV-1 clinical latency, HIV-1 mediated CD4 T-cell depletion, and AIDS.

Q 303 FUNCTIONAL CHARACTERIZATION OF A T CELL LINE EXPRESSING A CHIMERIC CD8-NEF FUSION PROTEIN, A.Baur, S.Garber, E.T.Sawai and B.M.Peterlin. Howard Hughs Medical Institute, Department of Immunology, University of California, San Francisco, Parnassus Av. San Francisco, CA, 94143-0724.

The *nef* gene was shown to be important for maintenance of a high viral load in the host and the development of AIDS. So far, however, functional studies on Nef in vitro did not reveal a mechanism explaining these in vivo findings. Since Nef is myristilated and therefore a membrane associated protein, it was speculated that it interferes with the signalling pathways in T cells. In order to locate Nef at the membrane and establish a high expression of the protein, we expressed a CD8-Nef fusion protein in Jurkat cells. The chimeric protein was constructed by fusing an intact *nef* gene (SF2 strain) to the extracellular and transmembrane domains of the CD8 molecule. High expression of stable cell lines was achieved by FACS sorting for CD8. Subsequently, cell lines with different levels of membrane bound Nef were analyzed for T cell activation pathways, Ca-flux, T cell antigen expression and transactivation of the HIV-1 LTR. Our studies reveal that Nef interferes with signalling through the T cell antigen receptor and in particular with early steps of the tyrosine kinase pathway. Specific effects on the tyrosine kinase pathway and activation of nuclear DNA binding proteins were analyzed in greater detail.

Q 304 HUMAN IMMUNODEFICIENCY TYPE-1 VIRUS ENVELOPE GENE DIVERSITY IN MOTHER-NEWBORNS PAIRS : SEQUENCE ANALYSIS OF THE V3 LOOP REGION L.BRIANT[1], M. GUYADER[1], C. CHAPPEY[2], J. IZOPET[1], A. BERREBI[3], J. TRICOIRE[4] AND J. PUEL[1];[1]Laboratoire de virologie, CHU Purpan, Toulouse; [2] ICGM, Paris; [3] Service de gynécologie-obstétrique, hopital Lagrave, Toulouse; [4] Service de pediatrie, CHU Purpan, Toulouse.

In order to elucidate mechanisms of materno-foetal HIV-1 transmission we have analysed sequence variations in the hypervariable V3 loop region of the envelope gene from four HIV-1 infected mother-infant pairs. For each mother at least three blood samples were collected at each trimestre of pregnancy, and two samples were collected from the children at delivery and during the first year of life. DNA was extracted from PBMCs and approximately 300bp spanning within the V3 domain of the envelope gene were amplified by nested PCR. Amplified fragments were cloned in M13 vectors and at least 15 clones were sequenced. Nucleotide sequences were translated in amino-acid sequences and sequence diversity was estimated. Data obtained from 2 mother-infant pairs have already been analysed. Results show some differences. In one case, the child was HIV positive by PCR at birth. In this case the infant's isolates observed at birth were highly conserved : only one molecular variant was identified. This variant is identical to a minor variant observed in the mother at early stage of pregnancy. This data seems to support the transmission of a single maternal variant. After one month of life wide sequence diversity was observed in this child suggesting a very rapid variation rate of HIV in young children. In a second pair, the virus was not detected in the child at birth. At one month several variants were identified, none of them was identical to any maternal variant. In this case transmission could have involved one maternal variant or more. In both cases analysis of N-linked glycosylation sites in the region of the V3 loop showed no variation and cystein residues were conserved. We are currently analysing the two other pairs. We expect that this analysis will allow us to better understand molecular basis of HIV-1 vertical transmission.

Q 305 DEVELOPMENT OF AN HL-60-BASED MODEL SYSTEM FOR CELL-TO-CELL HIV-1 TRANSMISSION.

Salvatore T. Butera, Beverly D. Roberts, and Thomas M. Folks. Retrovirus Diseases Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, 30333.

Because HL-60 promyelocytes are largely recalcitrant to infection by cell-free HIV-1, we have used these cells to develop a model system for the study of HIV-1 transmission by cell-to-cell mechanisms. Normal HL-60 promyelocytes were mixed at a >10:1 ratio with HL-60-derived HIV-1 donor cells which had themselves previously undergone acute infection by cell-to-cell transmission. During the ensuing 14 day period, HIV-1 transmission occurred from the donor population to >75% of the normal HL-60 recipient cells. Cell-to-cell HIV-1 transmission in the cultures was monitored by HL-60 cell-surface CD4 down-modulation, direct anti-HIV-1 immunofluorescence, and culture supernatant reverse transcriptase activity. Similar results were obtained with acutely infected or TNF-α-activated chronically infected donor cells of either HL-60 or A3.01 derivation. As expected, the addition of cell-free HIV-1 to normal HL-60 cultures resulted in <0.1% infected cells by day 14 and cell-to-cell HIV-1 transmission in the mixed cell cultures was inhibitable by AZT and sCD4. Although the majority of HL-60 subclones (n=26) were found to be susceptible to cell-to-cell HIV-1 transmission, one subclone was found to be resistant to this mechanism of viral spread although remaining CD4 positive. Interestingly, the addition of monoclonal antibodies directed against a number of surface adhesion molecules dramatically enhanced HIV-1 transmission in the mixed HL-60 cell cultures so that peak infectivity occurred prior to day 7. Furthermore, these antibody treatments did not permit cell-to-cell HIV-1 transmission to the single resistant subclone.

Due to the relative purity of the HL-60 cells from infection by cell-free HIV-1, the brief 14-day culture period, and the convenient infection analysis by CD4 down-modulation, this HL-60 cell mixing model is ideal for the continued study of cell-to-cell HIV-1 transmission and factors that influence this important aspect of HIV-1 pathogenesis.

Q 306 DEFECTIVE HIV-1 DNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM INFECTED CHILDREN.

Nickolas Chelyapov, Alec Wittek, Philip Brunell, Victor Israele. Pediatric Infectious Diseases, Ahmanson Pediatric Center, Cedars-Sinai Research Institute, Cedars-Sinai Medical Center, UCLA School of Medicine.

Human immunodeficiency virus (HIV) like other retroviruses is known to mutate at high rates due to errors introduced by reverse transcriptase. A large proportion of proviral genetic material in infected cells in vitro and in infected humans have been found to be defective. The functional role of these genomes in HIV pathogenesis is yet unanswered. We studied the natural history of 156 HIV-1 infected children (131 perinatally acquired and 25 infected via contaminated blood transfusion), by quantitative end-point dilution culture and PCR. For PCR amplification, DNA was isolated directly from uncultured patients' peripheral blood mononuclear cells (PBMC). Oligonucleotide primers directing amplification of a 1150 bp LTR-gag fragment (positions 515-1665) were used for the first round of amplification. Of the 156 samples tested, 15 (10%) showed additional lower molecular weight DNA bands (200-600 bp) detected by hybridization to probes located on the right and left sides of the amplified segment. No additional lower molecular weight bands could be detected in the same samples when a hybridization probe complementary to the middle portion of the 1150 bp DNA fragment was used. In order to confirm the specificity of the amplified lower molecular weight bands, nested PCR was performed with a sense primer positioned 100 bp downstream and the same antisense primer. A 100 bp shift in the molecular weight of all of the amplified fragments was noticed when using the right-sided probe, and no lower molecular weight bands could be detected after reprobing with the probe complementary to the middle part of the fragment. These data strongly suggest the presence of defective HIV-1 DNA in PBMC in some HIV-1 infected children. Further studies correlating the presence of defective genomes with clinical manifestations of disease will help to clarify the functional impact of the observed deletions on viral pathogenesis.

Q 307 THE NEF ORF OF HIV NL4-3 HAS A POSITIVE EFFECT ON VIRAL REPLICATION RATE IN CELL LINES.

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The function of *nef* protein in the viral replicative cycle of HIV is not clear. There is controversy whether it has a negative influence, no influence or a positive influence on the replication of HIV *in vitro*. The influence of changes in the *nef* ORF on viral growth in cell lines was investigated. A mutant predicted to encode a 29 aa *nef* truncated protein (Δnef) displayed a hundred fold decrease in virus yield compared to wild type. This positive effect of NL4-3 *nef* ORF was apparent in a wide range of multiplicities of infection (0.01-1 $TCID_{50}$/cell), and different cell lines: CEM, MT-2, MT-4, JURKAT, U-937 and THP-1. Additional mutations were constructed. Stop mutations were introduced in the *nef* reading frame (double stop). The growth rate of this HIV mutant was similar to Δnef. Point mutations were introduced into the myristoylation signal, changing the gly-gly into ala-ala. Replication of virus derived from this construct was intermediate between wild-type NL4-3 and either Δnef or double stop. Using a high multiplicity of infection (3 $TCID_{50}$/cell) and adding leu3a (antibody to CD-4) 2h after infection to prevent secondary infection there was a ten fold decrease in viral replication with Δnef virus as measured by intra-cellular or secreted p24 antigen. These studies document a significant positive effect of *nef* on HIV replication *in vitro* consistent with the published *in vivo* observations with SIV (Kestler et al).

Q 308 **HIV-1 Rev Function is Dependent Upon Continued mRNA Synthesis.** Alan Cochrane and Sandra Iacampo, Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada H3A-2B4

The synthesis of HIV structural proteins is dependent upon expression of the virus-encoded Rev protein. In the absence of Rev, mRNAs coding for the structural proteins are either entrapped in the nucleus or, if transported to the cytoplasm, are inefficiently utilized by the translational apparatus of the cell . In order to further elucidate the mechanism by which Rev induces structural protein synthesis, the effect of inhibiting mRNA synthesis on the function of Rev was examined. To facilitate these studies, cell lines were developed which accurately reflect the affect of Rev on its target mRNAs' metabolism. The system used resulted in activation of Rev being independent of either transcription or translation over the short term. It was observed that, in this system, induction of the target gene expression was largely attributable to the appearance of the target mRNA in the cytoplasm of the cell consistent with previous observations on the mechanism of Rev function.

Using the cell lines established, it was found that Rev function was extremely sensitive to inhibitors of mRNA synthesis, including actinomycin D and DRB (5,6-dichlorobenzimidazole riboside). Addition of these compounds completely inhibited Rev action. Inhibition was observed despite the presence of significant quantities of the target mRNA throughout the course of drug treatment. Furthermore, inhibition of Rev function by DRB was observed both prior to activation of Rev and late in the response, indicating that mRNA synthesis is required throughout the response to Rev activation. In addition, it was found that, in contrast to impairing the response, prolonged treatment with DRB improved both the rate and extent of the induction following DRB removal. Together, the data outline the dependence of Rev function on continued mRNA synthesis and suggest the possibility that Rev may function through interaction with nascent target transcripts.

Q 309 IDENTIFICATION OF IL-4 AND OTHER CYTOKINE RNA IN HIV INFECTED HUMAN BRAIN IN PROXIMITY TO MULTINUCLEATED GIANT CELLS

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The pattern of cytokine expression in cerebral tissue of 4 patients with AIDS and 3 controls without neurologic disease were studied by *in situ* hybridization-histochemistry. Tissues were taken at post-mortem from the frontal, parietal and occipital cortex and subjacent white matter and processed for routine histology and *in situ* hybridization for HIV-RNA and mRNA of interleukins 1,2,3,4,5 and 6, TNF-α, interferon gamma and GM-CSF, using oligonucleotide probes or riboprobes. The sense transcripts were used as negative controls. Other controls included pre-treatment with RNAs and the use of unrelated probes. The cell types expressing HIV and cytokine mRNA were identified by immunocytochemistry using monoclonal antibodies to human endothelium (QB-END-10), CD4 lymphocytes (OKT4), all human lymphocytes (LCA), and astrocytes (GFAP). Perivascular mononuclear infiltrates and granulomata containing multinucleated giant cells were present in all HIV infected patients. None had cerebral opportunistic infections. There was an approximate correlation between the local concentrations of HIV and cytokine RNA. In all 4 patients the heirarchy of abundance of cytokine RNA was similar: TNF-α and IL-4 +++; IL-1/IL-6 ++; IL-2/IL-3 +; GM-CSF/IFN-gamma+ (IL-5 was not detected). IL-4 RNA was detected most commonly in the nuclei of lymphocytes in the perivascular mononuclear infiltrate surrounding giant cells and also in microglial cells and the occasional astrocyte. IL-2 had a similar distribution. IL-6 was expressed in microglial cells, macrophages and endothelial cells. TNF-α was strongly expressed in many lymphocytes, astrocytes, macrophages, giant cells and endothelial cells, GM-CSF was detected in perivascular lymphocytes and endothelial cells, and interferon gamma was only expressed in occasional perivascular lymphocytes. The location of these cytokines in the perivascular mononuclear infiltrates adjacent to multinucleated giant cells was of particular interest. We have recently demonstrated that interleukin 4 promotes the formation of cell clusters and multinucleated giant cells from HIV infected monocytes *in vitro*. Its localization in lymphocytes adjacent to multinucleated giant cells suggests a similar function *in vivo*. The abundant expression of TNF-α and, to a lesser extent interleukin 6, supports previous studies using immunoperoxidase identification of these cytokines in brain. The exact role of these cytokines in the pathogenesis of HIV encephalopathy remains uncertain.

Q 310 LOSS OF GROWTH FACTOR STIMULATED SIGNAL TRANSDUCTION IN NIH-3T3 CELLS EXPRESSING THE HIV-1 *nef* GENE, Swapan K. De and Jon W. Marsh, Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892.

The human immunodeficiency virus type 1 (HIV-1) nef is a myristylated 27KD protein that has been localized to the cytoplasmic surface of the cellular membrane. It is attributed to have suppressive effects on LTR-based expression and T cell activation. Additionally, SIV nef has been shown to possess an essential *in vivo* function in the development of immunodeficiency. To define the biochemical activity of HIV-1 nef in a signal transduction pathway, we have transduced murine NIH-3T3 cells with a retroviral *nef* expression vector. In *nef*-expressing cells, but not in control retroviral transduction lines, the proliferative response to bombesin and platelet derived growth factor (PDGF) was eliminated. However, the mitogenic activity of fetal calf sera was maintained in both cell lines. Analysis of an early signal pathway metabolite, inositol tri-phosphate (IP_3), following bombesin and PDGF treatment to previously serum-starved cells, revealed that both control and *nef*-transformed cells displayed similar kinetics of this metabolite. In both cases, a four-fold increase in IP_3 levels was observed 2.5 min following the exposure to the peptide growth factors. IP_3-mediated changes in the cytosolic free Ca^{2+} ($[Ca^{2+}]i$) were then studied at the single cell level through digital imaging fluorescence microscopy of fura-2-loaded control and *nef*-transformed cells. Upon stimulation with bombesin, control cells displayed a 3.5 fold increase of $[Ca^{2+}]i$ over the basal level, while the $[Ca^{2+}]i$ response in *nef*-expressing NIH-3T3 cells was lacking or highly diminished. These results suggest a specific, definable biochemical activity for the HIV-1 nef protein in the context of a well characterized cellular activation pathway.

Q 311 QUANTITATIVE PCR AS A MEASURE OF VIRAL BURDEN IN INFECTED MOTHERS AND THEIR CHILDREN: CORRELATION WITH TRANSMISSION AND DISEASE STATUS. Ruth Dickover[1], Maryanne Dillon[1], Audra Deveikis[2], Margaret Keller[3], Susan Plaeger-Marshall[1] and Yvonne Bryson[1]. [1]Department of Pediatrics, UCLA School of Medicine, Los Angeles, Ca 90024, [2]Department of Pediatrics, Long Beach Memorial Medical Center, Los Angeles, Ca 90806 and [3]Department of Pediatrics, Harbor-UCLA Medical Center, Los Angeles, Ca 90501.

Vertical HIV transmission accounts for the majority of new pediatric HIV infections. Potential factors influencing maternal transmission and disease progression in infants include viral burden. We measured HIV DNA levels in peripheral blood cells (PBMC) from 30 infected mothers and their children using quantitative PCR and correlated the results with transmission and disease status. Ficoll-hypaque separated PBMC were boiled in H_2O, treated with RNase and proteinase K and phenol/chloroform extracted. PCR was performed with primer pair SK38,39 and 1 ug of PBMC DNA with serial dilutions of pBH10 as HIV DNA standards run in parallel. After 30 cycles aliquots were dot blotted onto nitrocellulose, hybridized to ^{32}P labeled SK19 and scintillation counted. Patient samples were compared to a standard curve to quantitate the HIV DNA copy number. We evaluated 76 children at risk for HIV infection and found a negative PCR in all P0 (n=45). The mean HIV DNA copy number in 16 P2 children was significantly higher than in 27 P1 children (393$\pm$ 25 SEM vs. 86.4$\pm$ 15 SEM; $p<.0001$). The mean HIV DNA copy number was also significantly higher in 8 transmitting mothers compared to 22 non-transmitting mothers (281$\pm$ 55 SEM vs 50$\pm$ 9 SEM; $p=.0007$). These data suggest that quantitative PCR is a useful measure of viral burden and that increased DNA copy number is associated with a more severe clinical status in children and an increased risk of transmission in HIV infected mothers.

Q 312 PROTEIN-PROTEIN INTERACTIONS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 GAG AND CAPSID PROTEINS. Lorna S. Ehrlich, Beth E. Agresta, Gabriele Zybarth and Carol Carter, Dept. of Microbiology, S.U.N.Y. Stony Brook Stony Brook, N.Y. 11794.
The capsid protein (CA, p24) and the precursor, Gag, of human immunodeficiency virus type 1 expressed in E. coli and purified to >90% homogeneity are being used to examine interactions involved in the formation of immature and mature capsid structures. These studies aim to identify viral domains that can be targeted for drug development. The proteins were detected in dimeric and oligomeric forms as indicated by molecular size measurements using gel filtration chromatography and nondenaturing gel electrophoresis. Regions of protein-protein interaction and regions that affect the formation or stability of interacting surfaces in these proteins have been examined using a combination of biochemical and genetic approaches. Limited tryptic digestion converted the mature (CA) and immature (Gag) proteins to the same CA peptides, suggesting that similar regions of the CA domain are exposed in both precursor and product. A region of the CA domain that was highly resistant to trypsin in the precursor was also resistant in the mature CA protein, suggesting that the region was structured or buried in both proteins. These studies may aid in identification of regions on the surface of the proteins at early and late stages of maturation that are potentially involved in capsid interactions.

Q 313 CORRELATION BETWEEN PRESENCE OF HIV-1 IN EJACULATE, VIRAL LOAD, AND CD4 COUNT: A MACS REPORT. Homayoon Farzadegan, Roger Pomerantz, Joseph Margolick, John Palenicek, Omar Bagasra, T. Seshamma and Alfred Saah, Infectious Diseases Program, Department of Epidemiology, Johns Hopkins University, Baltimore, MD 21205 and Infectious Disease Division, Thomas Jefferson University Hospital, Philadelphia, PA 19107.
Sexual transmission of HIV-1 plays an important role in the AIDS epidemic. Ejaculate is the body fluid most frequently exchanged during sexual intercourse. We studied ejaculates from 32 homosexual men at different stages of immune suppression to determine the correlation between detection of HIV-1 in PBMC and sperm ejaculate, viral load in PBMC, and immune status. In situ PCR was used to detect the HIV-1 DNA in ejaculate. Quantitative DNA PCR, immune complex dissociation and flow cytometric analysis of T-cells in peripheral blood were employed. The CD4 counts ranged between 72 to 1632 cells/μl. All viral tests were tested blindly. All tests were negative for 6 seronegative controls. HIV-1 DNA was detected in 30% of ejaculates of seropositive gay men by in situ PCR. Among 10 homosexual men with more than 800 CD4 cells/μl, only 2 (20%) showed detectable HIV-1 DNA in sperm (3/100 to 4/1000 sperms). Four of 10 men with fewer than 200 CD4 cells/μl showed detectable HIV-1 DNA in their sperm (15/100 to 11/1000 sperms). This suggests that the presence of HIV-1 in ejaculate increases with immune suppression. The odds ratio for detection of HIV-1 in ejaculates of gay men with < 200 CD4 count was 2.6.

Q 314 KINETICS OF HIV-1 INFECTION IN HUMAN PLACENTAL TROPHOBLAST, Fatemeh Fazely, Gordon C. Douglas, Grete N. Fry, Twanda Thirkill, Jinjie Hu, Hendrik Hakim, Sonia Schmerl, Myra B. Jennings and Barry F. King, Departments of Cell Biology & Human Anatomy and Medical Pathology, School of Medicine, University of California at Davis, Davis, CA 95616.
In utero transplacental transmission of HIV is a major factor in the acquisition of fetal HIV infections. Thus, understanding how HIV gains access to the placenta, and particularly the placental syncytiotrophoblast (ST), may help devise strategies to prevent transmission. We previously demonstrated that ST cells derived from term human placentas could be readily infected when cocultured with HIV-infected lymphocytic cells. No infection was detectable when ST cells were cocultured with free HIV particles. In the present study we have used immunofluorescence microscopy, electron microscopy and *in situ* hybridization to further characterize the infection process in highly purified ST cultures. It was found that ST cells did not become infected when separated from the HIV-infected Molt-4 Clone 8 cells by a virus-permeable membrane. This indicates that contact of infected lymphocytic cells with ST cells is necessary for virus transmission. In order to determine the time course of infection, infected Molt cells were incubated with ST cells for times ranging from 15 min to 48 h. No virus particles or antigen were found associated with ST cells before 4 h of coculture. Immunofluorescence microscopy revealed that some ST colonies were positive for p24 antigen at 4 h. The number of positive colonies increased with time. Viral particles were found associated with the ST cell surface at 12 h, and budding virions were observed at 24 h. These results demonstrate that cell-mediated infection of ST proceeds rapidly compared to the infection process seen in other systems using a cell-free virus inoculum. This work was supported by Training Grant AI07398 and Research Grants AI32307 and HD11658.

Q 315 MUTATIONAL ANALYSIS OF THE VPU PROTEIN OF HIV-1, Jacques Friborg, Azim Ladha, Xiao Jian Yao and Eric A. Cohen, Laboratoire de rétrovirologie humaine, Département de microbiologie et immunologie, Faculté de médecine, Université de Montréal, CP 6128 station A, Montréal, Canada, H3C 3J7.

The product of the *vpu* gene is a membrane associated phosphoprotein unique to the human immunodeficiency virus type 1 (HIV-1). Functional studies have shown that *vpu* increases virus particles released from infected CD4+ cell lines and reduces the accumulation of cell associated viral proteins. The *vpu* facilitated export of virus capsid from infected cells is independent of the expression of the env glycoprotein and CD4 receptor. Moreover, as monitored by syncytia formation and single cell killing, the cytopathic effect of *vpu*+ virus in CD4+ cell lines is delayed as compared to *vpu*- virus infection.

The precise mechanism(s) by which *vpu* function has not been clearly defined. It was previously shown that the *vpu* protein is phosphorylated at seryl residues by a casein kinase II (CK-II) related protein. The studies described here investigate the importance of post-translation phosphorylation for the biological activity of *vpu* during the course of HIV-1 infection.

In the current experiments, *vpu* mutants at serine residues in positions 52 (ser^{52}) or/and 56 (ser^{56}), highly conserved amongst HIV-1 isolates and describes as phosphoacceptor sites for CK-II, were generated by site-specific mutagenesis using a PCR based protocol. The site of *vpu* phosphorylation was identified as ser^{52}. Functional analysis revealed that the rate of syncytia formation and single cell killing in CD4+ cells infected with virus encoding *vpu* mutant at ser^{52} and ser^{56} is increased and is as comparable to that observed in *vpu*- virus infection. Interestingly, the *vpu* facilitated export function is not altered by the mutation on the serine residues. Together these data suggest that the mutation at $ser^{52/56}$ may have revealed one of the active domain responsible for the delayed cytopathic effect of *vpu*+ virus which seems to be independent of *vpu* facilitated export function.

Q 316 FUNCTIONAL SIMILARITIES BETWEEN HIV AND SIV NEF: SIV NEF INDUCES DOWNREGULATION OF CD4 CELL SURFACE LEVELS. J. Victor Garcia, Amy L. B. Frazier, Barbara Larson, and K. Slobod. Dept. of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, 38101.

We previously have shown that HIV Nef from a laboratory isolate (SF2) induces downregulation of CD4 from the cell surface (Nature **350**: 508, 1991). To relate this observation to AIDS pathogenesis, we tested the ability of SIV Nef from the mac239^{open} isolate to induce CD4 downregulation. We chose this Nef isolate because it has been shown to be necessary for pathogenesis and disease progression *in vivo*. We first obtained the Nef gene by PCR and cloned it into the retroviral vector pLXSN. We then established high titer ($1X10^6$ CFU/ml) amphotropic retrovirus producer lines (PA317\LSnefSN). The ability of LSnefSN to transduce Nef was tested on NIH 3T3 cells. Analysis of eight independent clones of NIH 3T3 cells transduced with LSnefSN showed similar expression levels of SIV Nef as determined by Western blot. These results suggest that SIV Nef has no toxic effect on these cells. Using LSnefSN we also established populations of human T- and B-cells expressing SIV Nef. In the two cell lines tested, SIV Nef expression correlated with a significant decrease in human CD4 cell surface levels. Untransduced cells or cells transduced with a control vector show normal CD4 cell surface levels. These results indicate that the lower CD4 cell surface levels are due to Nef expression and not to transduction with a retrovirus vector. Comparison of the sequences of SIV and HIV Nef at the amino acid level show significant differences. In spite of these differences both proteins induce downregulation of cell surface CD4. These results and the fact that SIV Nef is important *in vivo* for pathogenesis suggest that this conserved function of Nef might play a role in virus persistence and disease progression *in vivo*.

Q 317 MOLECULAR INTERACTIONS AT THE HIV-1 LONG TERMINAL REPEATS IN LATENTLY INFECTED AND ACTIVATED CELLS EXAMINED BY IN VIVO FOOTPRINTING

Mauro Giacca*, Francesca Demarchi*, Pierlanfranco D'Agaro**, Fabrizio d'Adda di Fagagna*, Giuseppe Marzio*, Maria Ines Gutierrez*, Daniela Pedacchia*, Arturo Falaschi*; *International Centre for Genetic Engineering and Biotechnology,Trieste (Italy); **Institute of Hygiene, University of Trieste, Trieste (Italy)

The mechanisms underlying transcriptional activation of HIV-1 are of particular interest, since the progression of the disease usually correlates with an augmented viral expression [1]. A large number of cis-acting transcriptional regulatory elements has been identified in the LTR by in vitro binding studies. We have analyzed the actual relevance of these sites in living infected cells by in vivo dimethylsulfate footprinting using ligation-mediated PCR. By this method, we have demonstrated that in a chronically infected T cell line (H9/HIV-$1_{HTLVIIIB}$) major footprints appear over the TATA box, the three Sp1 sites, the two repeats of the enhancer, and, in the negative regulatory element, over the USF/MLTF and NFAT-1 binding sites [2]. The USF/MLTF site behaves as a downregulator of transcription [3]. Furthermore, two purine-rich sites sharing a common sequence, located between nucleotides from -260 to -275 and from -204 to -216, respectively, appear to be involved in protein-DNA interactions in vivo. The upstream site was mutated and found to behave as a positive regulator of transcription.

The same technique was used to investigate protein-DNA interactions in the monocytic U1 cell line, in which viral production is very low in basal conditions and is strongly inducible by TPA treatment. The footprinting pattern of the LTR in U1 cells is different from H9 cells, probably reflecting diversity between cell types; a marked protection over the Sp1 and enhancer sites is evident also in basal conditions. Stimulation by TPA results in a gradual enhancement of the protection of the enhancer sites within 24 hours, suggesting a progressive recruitment of new factors over the enhancer, or, alternatively, an increase in the number of cells with a transcriptionally competent LTR.

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2. Demarchi, F. et al.1992. J. Virol. 66, 2514-2518.
3. Giacca, M. et al.1992. Virology 186, 133-147.

Q 318 COCULTURED PRIMARY HUMAN ENDOTHELIAL CELLS AUGMENT HUMAN IMMUNODEFICIENCY VIRUS PRODUCTION IN PERSISTENTLY INFECTED PROMONOCYTIC CELLS AND IN ACUTELY INFECTED PRIMARY MONOCYTE DERIVED MACROPHAGES. Patrick N. Gilles, Janet Lathey, and Stephen A. Spector. University of California, San Diego, La Jolla, CA 92093

This research examined the interaction between monocyte derived cells and endothelial cells on HIV-1 infection. Endothelial cells obtained from umbilical veins (EC) were cocultured with either monocyte-derived-macrophages (MDM) or U1 cells (a persistently infected promonocytic cell line). Over a 3-6 day incubation period, HIV production, as assessed by extracellular p24 antigen, was 4-fold greater in U1/EC co-cultures (n=18, mean$\pm$SEM: 29.0$\pm$3.5ng/ml) than isolated U1 cells (n=18, 7.3$\pm$1.3ng/ml) cultured in parallel. Similarly, over a 3-6 day period following acute infection with the monotropic Ba-L strain, p24 antigen production was 3.5-fold greater in MDMs co-cultured with endothelial cells (n=10,2.2$\pm$0.6ng/ml) than in MDMs (n=10, 0.63$\pm$0.1ng/ml) infected and cultured in parallel. The disparate increase in p24 antigen produced from U1 cells (+21.7ng/ml) and infected MDMs (+1.6 ng/ml)co-cultured with EC cells as compared to that produced from Ba-L infected ECs (less than 0.2 ng/ml) suggest that direct infection of ECs does not account for the increased production of HIV in co-cultures. Rather, these observations suggest that endothelial cells either directly stimulate HIV expression in monocyte-derived cells by cell-cell contact or indirectly augment HIV production through the release of soluble cytokines. Although the role for cytokines in the endothelial cell stimulatory effect is speculative, both transforming growth factor-beta (TGFβ) and interferon-gamma (IFNγ) were found to suppress by 50-80% the EC enhanced production of HIV in U1/EC and MDM/EC co-cultures. These data suggest that HIV-1 infection of monocyte-derived cells co-cultured with endothelial cells results in an upregulation of virus production which can be partially abrogated by the cytokines TGFβ and IFNγ.

Q 319 TRANSMISSION OF HIV BY INTRACERVICAL INOCULATION IN FEMALE CHIMPANZEES, Marc Girard[1], Laurence Rimsky[2], James Mahoney[3], Françoise Barré-Sinoussi[1], Kent Weinhold[2], Elizabeth Muchmore[3], and Patricia Fultz[4]. 1. Pasteur Institute, Paris 75015, France, 2. Duke University Medical Center, Durham, NC 27710, 3. LEMSIP, NYU Medical Center, Tuxedo, NY 10987, and 4. University of Alabama, Birmingham, AL 35294.

Adult female chimpanzees were infected at the estrogenic (pre-ovulatory) peak of their cycles with cell-associated HIV-1 LAI via atraumatic inoculation of the endocervical mucosa. Cryopreserved PBMC from an HIV-1 infected chimpanzee with a titer of 30 infectious units per 10^6 PBMC were used as the inoculum. The desired number of viable PBMC was deposited through the os cervix into the endocervical canal of the animal using a syringe mounted with a catheter tubing and guided by colposcopy to ensure no bleeding was present or induced. Two female chimpanzees were inoculated by this procedure with either 30 or 300 HIV-infected cells (10^6 or 10^7 viable chimpanzee PBMC, respectively). The female that received the higher dose became seropositive and was positive for virus isolation from PBMC for at least 4 months. The female that received the lower dose remained seronegative and was only transiently positive for virus isolation from PBMC (at 4-6 weeks). However, she was consistently positive for HIV-I DNA in PBMC by nested-set PCR assay for env sequences. One additional female chimpanzee was exposed to 100 infected cells ($3x10^6$ PBMC) using the same protocol. She failed to seroconvert, and virus could not be recovered from her PBMC. A male chimpanzee infected in parallel with 20 infected cells ($7x10^5$ PBMC) from the same inoculum, but using the intravenous route, seroconverted readily and was positive for virus isolation from PBMC starting at week 4. The female that had received the lowest dose (30 infected cells) has been super-infected with 300 infected cells by the endocervical route. Follow-up studies are in progress. These results suggest that persistent HIV-1 infection can be achieved in the chimpanzee model by cervical inoculation with approximately 10- to 20-fold more infected cells than are required intravenously. Exposure by this route to lower doses of HIV-infected PBMC can induce persistent infection, but only transient viremia, and no detectable antibody response.

Q 320REAREANGEMENT OF THE CONSERVED ZINC FINGERS OF THE HIV-1 NUCLEOCAPSID PROTEIN Robert J. Gorelick, D. J. Chabot, A. R. Rein, L. E. Henderson and L. O. Arthur, National Cancer Institute-Frederick Cancer Research and Development Center, PRI/DynCorp, AIDS Vaccine Program, Frederick, Maryland 21702-1201

HIV-1 contains two copies of the zinc finger (Zn^{++}-finger) sequence **CYS-X_2-CYS-X_4-HIS-X_4-CYS** with differing amino acid residues at the "X" positions. In the NY5/LAV, pNL4-3 clone (**pNC1/2**), these sequences are **CFNCGKEGHIAKNC** and **CWKCGKEGHQMKDC** for the conserved arrays in the first and second positions, respectively. Three mutants were constructed whereby the positions of these arrays in the HIV-1 nucleocapsid protein were reversed (**pNC2/1**), or the first (**pNC1/1**) and second (**pNC2/2**) conserved sequences were duplicated. When compared to **pNC1/2** virus, the mutants contained similar levels of Gag, Pol, Env and Tat. Env proteins are functional since transfected mutant proviral clones produce syncytia in a CD4-positive HeLa cell line. The genomic RNA content is reduced to less than 10% of wild-type levels for the **pNC2/1** and **pNC2/2** mutants. The **pNC1/1** mutant contains 70% of wild-type levels of genomic RNA. No infectious virus could be detected in either **pNC2/1** or the **pNC2/2** mutants, which are at least 10^4-fold less infectious than a comparable amount of wild-type virus. The **pNC1/1** mutant appears to be very poorly infectious, but reverts to a wild-type phenotype after a 2-4 week lag period. These results strongly suggest that the two NC Zn^{++}-fingers of HIV-1 are not functionally equivalent, and that the first Zn^{++}-finger in the Gag precursor plays a more prominent role in RNA selection and packaging. The data also indicate that both Zn^{++}-fingers in the mature NC protein play as yet unknown roles in viral assembly or in the early stages of the viral infection process.

Q 321 DETECTION OF MULTIPLY-SPLICED mRNA'S AND REGULATORY PROTEINS DURING HIV INFECTION.

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HIV-1 replication depends on several highly conserved non-structural genes. Study of the kinetics of expression of these regulatory genes may give further insight in their function for viral replication and may give clues for intervention strategies.

Current methods to study HIV-regulatory gene expression (Northern blot, RT-PCR) do not allow for quantitation of individual mRNA species, encoding for these genes. For this purpose we have developed a quantitative RNase protection assay, which identifies the splice acceptor sites used. Radiolabeled anti-sense RNA was transcribed from isolate-specific cDNA clones spanning splice acceptor sites of the regulatory genes. After hybridization with viral mRNA and RNase A/T1 digestion, protected fragments were separated on gel and detected by autoradiography.

To further extend these studies, the presence of the corresponding proteins was determined using a "North-Western" blot assay. In this assay regulatory proteins were separated by SDS-PAGE, immobilized on nitrocellulose and visualized after binding of their radiolabeled target RNA.

Results indicate that HIV-1 gene expression is highly dependent on the cellular environment and that regulatory genes are involved in differences observed.

Application of these techniques provides complementary results to those obtained with Northern blot and RT-PCR analysis and identifies the cascade of events leading to HIV production.

Q 322 MUTAGENESIS OF THE HIV-1 *REV* SPLICE ACCEPTOR REGION: UNEXPECTED GENERATION OF DOMINANT DEFECTIVE VIRAL GENOMES.

John Guatelli, Nanette Riggs, and Douglas Richman. Departments of Medicine and Pathology, University of California, San Diego and the Veterans Affairs Medical Center, La Jolla, CA 92093-0679.

The nucleotide sequence 5' of the *rev* initiator codon contains two AG dinucleotides that are used as splice acceptors to generate two closely related exons named 4A and 4B. Mutations were introduced into the 4A and 4B splice acceptors by altering the AG dinucleotides required for splicing. These mutations were designed to impact minimally the overlapping *tat* reading frame. After inserting these splice acceptor mutations into the pNL43 clone of HIV-1, the phenotype of these mutant viral genomes was assessed by transfection into the T lymphoblastoid cell line CEM.

HIV-1 genomes carrying mutated 4A, 4B, or 4A plus 4B splice acceptors were defective. However, none of these mutants were complemented by co-transfection with *rev* expression vectors. The genome carrying the 4A mutation was complemented by a small amount of wild-type pNL43. However, the genomes carrying the 4B or 4A plus 4B mutations inhibited p24 production when co-transfected with the wild-type pNL43. The 4B mutant genome was inhibitory in a dose dependent fashion. The mechanism by which these HIV-1 genomes are dominantly defective is under investigation.

Q 323 EXPRESSION OF GP41 SEQUENCES AT THE CELL SURFACE IS NOT SUFFICIENT FOR PACKAGING OF THE HIV-1 ENVELOPE GLYCOPROTEIN INTO PSEUDOVIRIONS,

Omar Haffar, Emily Platt, Bruce Travis, Sridhar Pennathur and Shiu-Lok Hu, Department of Virology, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Assembly of HIV-1 virus takes place at the plasma membrane of infected T cells. Based on evidence derived from other retroviral systems, it has been postulated that assembly of the HIV virion involves direct interaction between the gag matrix protein (p17) and the env transmembrane protein (gp41). To explore the role of the env glycoprotein in virus assembly, we evaluated the ability of gp41 to be incorporated into pseudovirions. A truncated env gene comprised of sequences encoding gp41 together with 45 amino acids from the C terminus of gp120, fused to the signal sequence of gp160, was generated and introduced into a recombinant vaccinia virus vED1. Upon infection of BSC-40 cells, vED1 encoded a 48kDa protein that was transported to the cell surface. The ED1 protein was specifically immunoprecipitated with a variety of antibodies specific for various epitopes in gp41 and the C terminus of gp120. ED1 was also proteolytically processed to generate a mature gp41, albeit very inefficiently. Although the ED1 protein contained the complete gp41 sequence and was expressed at the cell surface in oligomeric form, it failed to be packaged into budded particles when coexpressed with the HIV gag protein. Interestingly, ED1 associated with the full length gp160 to form mixed oligomers at the cell surface and inhibited the incorporation of gp160 into pseudovirions. This dominant negative phenotype of ED1 appears to be due to an altered intracellular transport pathway. Our results show that expression of gp41 sequences at the plasma membrane is not sufficient for incorporation into pseudovirions. The implications of these results on the mechanism of virus assembly will be discussed.

Q 324 AN IN VITRO MODEL OF ATTACHMENT OF CRYPTOSPORIDIUM PARVUM SPOROZOITES TO MDCK CELLS Davidson H. Hamer, Honorine Ward, Saul Tzipori, Miercio E. A. Pereira and Gerald T. Keusch, Department of Geographic Medicine and Infectious Diseases, New England Medical Center, Box 41, 750 Washington St., Boston, MA 02111

The molecular pathogenesis of *C. parvum* infection, a chronic diarrheal disease in AIDS patients for which no effective therapy is known, remains poorly understood. We have developed an *in vitro* cell culture system in order to study the initial step in infection, the attachment of sporozoites to host cells. MDCK cells are grown to confluence in 16 well multichamber slides and fixed with paraformaldehyde in order to prevent cell invasion. Freshly excysted, Percoll purified sporozoites are then added to each well and incubated at 37° C. Unbound sporozoites are removed by washing and then the slides are fixed in methanol and air dried. Parasites adherent to the MDCK monolayer are detected by indirect immunofluorescence using a polyclonal, anti-sporozoite murine antibody and a FITC-conjugated goat anti-mouse antibody.

Adherence was time dependent, was maximal after 60 minutes at 37°C, and was reduced three-fold by lowering the temperature to 4°C. Dose-response studies showed a saturation of binding of sporozoites at a parasite to cell ratio of 50:1. Attachment was enhanced by manganese and calcium whereas concentrations of magnesium ranging from 0.1 to 100 mM had no effect. The effect of calcium was blocked by the addition of EDTA. Combining calcium and manganese had an additive effect on attachment. The effects of pH, cytoskeletal inhibitors, glycoconjugates, and a series of monoclonal antibodies reactive with different sporozoite structures on attachment are currently being evaluated.

Q 325 DISSOCIATION OF THE HIV-1 gp120-gp41 COMPLEX IS NOT INTEGRAL TO SYNCYTIA FORMATION (FUSION), Timothy K. Hart, Yung-Kang Fu and Peter J. Bugelski, Dept. of Toxicology, SmithKline Beecham Pharm., King of Prussia, PA.

Membrane fusion mediated by the activation of HIV-1 gp41 envelope glycoprotein is initiated by binding of gp120 to CD4. Recombinant soluble CD4 (sCD4) induces shedding of gp120 form gp41 prematurely, thereby blocking the fusion potential. The mechanism of receptor-mediated activation of the HIV-1 fusion complex and the biological significance of gp120 shedding are poorly understood. BJAB cells (TF228.1.16) stably expressing HIV-1 envelope glycoproteins were used to study shedding and fusion events resulting from the interaction of (s)CD4 and gp120-gp41. Both sCD4-induced shedding of gp120 and fusion with $CD4^+$-SupT1 cells were sensitive to pH and temperature. sCD4-induced shedding of gp120 was optimal at pH 4.5-5.5, but did not occur at pH 8.5. Fusion was permissive at pH 8.5 (optimal at pH 7.5) but was non permissive at pH 4.5-5.5. At neutral pH, sCD4-induced shedding of gp120 occurred at 22, 37, or 40° C, but not at 16 or 4° C. Syncytia formation (cell-cell fusion) occurred at 37 or 40° C but not at 4, 16, or 22° C. Incubation of cocultures of TF228.1.16 and SupT1 at 4, 16, or 22° C before shifting to 37° C, resulted in similar, increased, or decreased numbers of syncytia compared to the control 37° C incubations, respectively. It is hypothesized that a transiently activated CD4-gp120-gp41 complex is stablized at 16° C which readily decays upon shifting the cocultures to 22° C. The physicochemical dissection of shedding and fusion implies that dissociation of gp120 from gp41 is not integral in the fusion (syncytia) cascade. Inactivation of the HIV-1 fusion complex by cellular CD4 in the above experiments may procede in a process analogous to sCD4-induced shedding of gp120 but the number of interactions and time frame of the interaction would not be significant enough to affect the virulence of the HIV-1.

Q 326 **A PHYLOGENETIC ANALYSIS OF POSSIBLE HIV TRANSMISSION THROUGH INVASIVE DENTAL PROCEDURES,** **[1]Martin D. Hill, [2]Ronald W. DeBry, [2]Lawrence G. Abele, [3]Stanley H. Weiss, [4]Maria Bouzas, [4]Eric Lorenzo, [4]Folke Graebnitz, and [4]Lionel Resnick. [1]Dept. of Pediatrics, University of Miami School of Medicine, Miami, FL 33101. [2]College of Arts and Sciences, Florida State University, Tallahassee, FL 32306. [3]Division of Infectious Disease Epidemiology, UMDNJ - NJ Medical School, Newark, NJ 07107. [4]Retrovirology Research Laboratories, Mount Sinai Medical Center, Miami Beach, FL 33140.**

Five patients were reported to have been infected with HIV through invasive dental procedures. To more critically examine this conclusion a series of phylogenetic analyses were performed using newly derived HIV envelope nucleotide sequences from these patients and thirty community controls. Maximum parsimony found that a monophyletic clade was defined by only two nucleotides and each of these occurred in at least one local control. Using a bootstrap analysis the dental group clustered only 56% of the time. The range of pairwise nucleotide distances between sequences from the dental group and the dentist (1.6-8.8%) overlapped with the range of distances between sequences from the community controls and the dentist (3.9-13.5%). We determined that the signature sequence analysis of amino acids is not phylogenetically informative: we found three controls with six of the signature amino acids. Within these patients, the amino acids appeared in clusters, i.e., non-independently. We conclude that the high variability and low number of nucleotides in the V3 region render it inappropriate for establishing HIV relatedness and no conclusion of epidemiology can be made in this case.

Q 327 CELL-SURFACE EXPRESSION AS WELL AS INTRACELLULAR PROTEIN LEVELS OF THE CD4 ANTIGEN ARE DOWNMODULATED BY THE HIV-1 NEF PROTEIN, Susanne Hiller and Sundararajan Venkatesan, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

The function of the HIV-1 Nef protein in the lifecycle of the virus has remained elusive. One proposed effect is the reduction of CD4 protein expression on the cell surface. To study the mechanisms of downregulation of cell-surface expression of CD4 by the Nef-protein, HeLa cells were transiently co-transfected with expression plasmids for CD4 and Nef or a non-functional Nef mutant. The cells were stained by indirect immunofluorescence with antibodies against CD4. The number of CD4 expressing cells was reduced approximately 10-fold in the samples co-transfected with a Nef expression plasmid as compared to the control. Evaluation of CD4 expression by binding of an iodinated antibody to the cells confirmed this downmodulating effect of Nef. Immunoblotting of extracts from the transfectants with anti-CD4 antibodies showed that the decrease in surface CD4 was accompanied by a modest decrease in total cellular CD4 protein. The specificity of this effect was tested by co-transfecting Nef with CD8, a CD4 mutant retained in the ER, or a chimera containing the extracellular domain of CD8 and intracellular domain of CD4. Neither protein was affected by Nef in cell-surface expression or total protein levels. This indicates that the Nef-dependent regulation is specific for CD4 and does not require the intracellular CD4 domain. In pulse/chase labelling experiments with transiently transfected cells we could not detect any difference in the half-life of CD4 in the presence or absence of Nef, indicating that Nef does not enhance degradation of CD4. To localize Nef-domains important for the effects on CD4 expression, we analyzed different Nef mutants in co-transfection experiments. A mutant in the N-terminal sequence required for myristoylation of Nef was as competent in CD4 downregulation as the wild-type protein. Another mutant that had the internal methionine and potential initiation codon changed, did not have any effect on the CD4 expression. These data suggest, that the effects on CD4 may be mediated by the less abundant, non-myristoylated smaller Nef protein .

Q 328 THE VIF- PHENOTYPE IS A CELL-SPECIFIC BLOCK TO INFECTION FOLLOWING VIRUS ENTRY AND EARLY REVERSE TRANSCRIPTION

Gabor Illei and William James, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, U.K.

Vif has been shown to reduce the infectivity of cell free HIV on certain cell lines. Our aim was to demonstrate the stage of viral life cycle at which Vif has its effect. We compared the wild type (wt) and Vif- viruses on two HIV-susceptible lymphocytic cell lines, H9 and C8166. C8166 was equally susceptible to infection by the wt and Vif- viruses, but H9 cells were refractory to infection by Vif-.

To check if the block in infectivity was before or at the beginning of reverse transcription (RT) we tested the cells at different times after infection by PCR using primers amplifying the "strong stop" cDNA. In H9 cells this earliest product of RT became detectable later than in C8166 cells. There was, however, no difference between the wt and Vif- viruses. We showed that the block produced by the lack of Vif is after the entry of the virus and the beginning of reverse transcription.

We will report a PCR-based method to test the integration of the two viruses. We also report the effects of the Vif- genotype on infection of primary lymphocytes and monocytes in the background of proviral clones with lymphocyte or monocyte tropism.

Q 329 PND ANTIBODY REACTIVITY TO VERTICALLY TRANSMITTED QUASISPECIES IN HIV-1 INFECTED MOTHERS.

Marianne Jansson*, Britta Wahren**, Gabriella Scarlatti***, Eva Halapi*, Albert Jan**, Hans Wigzell* & Paolo Rossi*. *Dept of Immunology and ***Dept of Virology, Karolinska Institute, Stockholm Sweden, **Dept of Virology, Natl Bacteriological Lab, Stockholm, Sweden.

Objective: To investigate the antibody responses to HIV-1 principal neutralizing determinants (PND) of different virus variants found in HIV-1 infected mothers of which some are found to be vertically transmitted to their children.

Methods: From HIV-1 proviral DNA and viral RNA V3 sequences obtained by cloned and direct sequencing of HIV-1 from 10 infected mothers and their infected children, peptides were synthesized 15 amino acids (aa) long corresponding to HIV-1-MN aa 308-322. Seroreactivity in the mothers to the different quasispecies specific peptides was tested in ELISA.

Results: In eight out of ten mothers tested for seroreactivity to peptides representing the vertically transmitted virus, weak reactivity, titres below 300, was found. Among mothers where V3 apex sequences were available from both DNA and RNA , three were found to transmit a virus homologous in the PND sequence to the RNA based sequence. These three sera also showed a lower reactivity to the peptides representing the RNA and the vertically transmitted virus then to the peptides based on the DNA sequence variants.

Conclusion: The results imply that the PND of the vertically transmitted virus variant is, in a high proportion of cases, not well recognized by the maternal humoral immune system. The explanation for this could either be a general low recognition of this epitope in the transmitting mothers or an immune escape.

Q 330 THE HIV GAG PROTEIN REGULATES ASSEMBLY AND PACKAGING OF VPX, John C. Kappes[1,2], Joan A. Conway[1], Xiaoyun Wu[1], Departments of Medicine[1] and Microbiology[2], University of Alabama at Birmingham, University Station, Birmingham, Alabama, 35294. Viral protein X (*vpx*) is an HIV-2/SIV accessory protein that enhances viral infectivity and replication in natural target cells. It is a constituent of the mature cell-free virus particle and is packaged in molar amounts equivalent to that of gag proteins. Recent studies conducted in our laboratory demonstrated that the subcellular localization and incorporation of vpx into virions requires additional virus type-specific components. In order to assess the role of gag in regulating assembly and packaging of vpx with virions, we used a recombinant vaccinia virus, containing the bacteriophage T7 RNA polymerase gene under control of a vaccinia virus promoter (VV-T7), to facilitate high levels of gag protein expression and subsequent core-particle formation and extracellular release. Chimeric pGEM expression plasmids were constructed placing the coding regions of HIV-2 gag, gag-pol and vpx as well as those of HIV-1 gag and gag-pol under control of the T7 promoter. For viral protein expression, HeLa cells were infected with recombinant vaccinia virus (VV-T7) and one hour later transfected with gag, gag-pol and vpx pGEM recombinants. 32-40 hours after transfection, cells and culture supernatants were processed for Western blot analysis. Using monoclonal antibodies as probes, HIV-1 and HIV-2 gag polyprotein precursors were detected in cell lysates. Assembly and release of core particles was confirmed by thin-section electron microscopy and purification from supernatants by ultracentrifugation through linear gradients of sucrose. Analysis of HIV-1 and HIV-2 cores, purified from culture supernatants of HeLa cells transfected with gag-pol chimeras, revealed processing (cleavage) of the gag polyprotein precursor. Purified HIV-2 core particles, derived from cultures cotransfected with either pGEM-gag or pGEM-gag-pol in combination with pGEM-vpx, contained large amounts of vpx protein. Using identical assay conditions, vpx did not package with HIV-1 core particles and immunofluorescence analysis revealed that in transfected cells failed to target to the cell surface membrane. By radioimmunoprecipitation, however, vpx was detected with purified HIV-1 core particles, but only in minor amounts compared with gag. To test whether encapsidation of mRNA transcribed from pGEM-gag affected vpx packaging the HIV-2 pGEM-gag expression plasmid was reconstructed terminating transcription immediately 3' of gag and eliminating the putative RNA packaging signals located in the 5'-noncoding region of unspliced mRNAs. Purified core particles derived from cotransfected HeLa cells contained vpx protein in amounts equivalent to those found with pGEM-gag derived cores. These results demonstrate that the HIV-2 gag polyprotein precursor is sufficient to direct subcellular localization and virion incorporation of vpx. An understanding of the mechanisms of vpx packaging are likely to be important for development of novel strategies for antiretroviral therapy.

Q 331 THE MOLECULAR BASIS OF NEF FUNCTION: THE POSITIVE EFFECT OF A "NEGATIVE FACTOR", Harry W. Kestler, Janelle Salkowitz, and Daniel Premkumar, Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195

The *nef* gene of the pathogenic molecular clone SIVmac239 was shown to be required for viral pathogenicity and for maintenance of high virus loads in rhesus monkeys (Kestler et al., *Cell* 65: 651-662). Deletion of 182 base pairs in the *nef* gene produced a virus that was unable to sustain high loads observed in rhesus monkeys infected with SIVmac239/*nef*-open which encodes a full length *nef* gene. Virus load was determined by four different methods: limiting dilution coculture, quantitative PCR, plasma antigenicity, and immunohistochemistry. An intense selection pressure on *nef* was observed by innoculating animals with molecular cloned virus that contained a stop codon in *nef*. One hundred percent of virus recovered from nef-stop infected animals contained open *nef* genes. This reversion, shown not to occur in vitro, was seen in vivo as early as two weeks after inoculation. This indicates that NEF is performing some crucial function early in the in vivo life cycle of the virus. We have chosen to focus on early events in the course of infection.

Activation of T-cells is required for the virus to initiate a productive infection. *Nef* is expressed at a high level early in the course of an in vitro infection and is required very early in an in vivo infection. Therefore, we suspect that this "early" gene may play a role in the steps leading to the activation of T-cells. We have tested the ability of NEF to modulate T-cell activation. The *nef* genes of SIVmac239/nef-open and SIVmac239/*nef*-deletion were subcloned into murine retroviral gene transfer vectors. These subclones were transfected into a packaging cell line and infectious particles were produced. The particles were used to infect resting and stimulated lymphocytes.

Supernatents containing the *nef* gene in the sense orientation were capable of inducing the proliferation of lymphocytes while supernatents containing either the vector or the antisense *nef* gene were unable to awaken lymphocytes from the resting state. Thus, *nef* appears to play a role in stimulating lymphocyte proliferation and may be a required cofactor for a productive in vivo infection. In vitro viral replication could occur independent of *nef* since the growth of the host cells is generally pre-established in such experiments. *Nef* may increase virus loads and enhance pathogenicity in an indirect fashion.

Q 332 INHIBITION OF HIV INTEGRATION IN MT-2 CELLS ARRESTED IN G_1 (RESTING) PHASE OF THE CELL CYCLE. R. Kiernan and D. McPhee. National Centre for HIV Virology Research, AIDS Cellular Biology Unit, Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria, Australia.

HIV -1 requires activated target cells for a productive infection in vitro. G_1 (resting phase) arrested HTLV-1 transformed MT-2 cells were used to investigate the mechanism of cell cycle dependence of HIV replication. Cells were arrested in G_1 using 5mM hydroxyurea, shown by FACS analysis of DNA content and >90% reduction of ^{3}H-TTP uptake. Production of cell-free infectious virus, measured by plaque assay and reverse transcriptase activity, was almost completely inhibited in G_1 arrested cells compared with actively dividing cells which released progeny 24 h post-infection. However, HIV proteins were detected by immuno-fluorescence and Western blot. G_1 arrested cells were not fusogenic although surface expression of both CD4 and gp120/gp41 were detectable. PCR analysis of HIV DNA showed that full-length reverse transcripts were detectable in both cytoplasmic and nuclear compartments, and circularization of DNA also occurred. Analysis of integration showed that this step was delayed in G_1 arrested cells compared with dividing cells. These data show that the replication cycle of HIV was inhibited in G_1 arrested cells at the level of integration. This implies that unintegrated HIV DNA may be transcriptionally active.

Q 333 CONSEQUENCES OF HIV-1 SUPERINFECTION OF CHRONICALLY INFECTED CELLS, Jerome H. Kim[1], Maryanne T. Vahey[1], Robert J. McLinden[3], Joseph D. Mosca[3], Donald S. Burke[2], and Robert R. Redfield[1]. [1]Department of Retroviral Research, [2]Division of Retrovirology, Walter Reed Army Institute of Research, and the [3]Henry M. Jackson Foundation, Rockville, Maryland 20850.

Acute infection of T-cell lines by HIV-1, *in vitro*, is associated with the downregulation of the CD4 receptor and resistance to further viral infection, the phenomenon of viral interference. The ACH2 cell line serves as a model for chronic HIV-1 infection in that it possesses a single integrated copy of the HIV-1 strain LAI, is essentially CD4 negative, and can be induced to make virus by a variety of stimuli. We devised a polymerase chain reaction strategy that permits reliable and quantitative discrimination between HIV_{LAI} and HIV_{RF}. We demonstrate that ACH2 cells can be superinfected by HIV_{RF} at a frequency of 50-300 HIV_{RF} per 10^4 HIV_{LAI} and that the frequency of superinfection increases with time. Superinfection of a HIV_{IIIB}-chronically infected H9 line was similarly accomplished. Filtered supernatant from an ACH2/HIV_{RF} superinfection transmits both HIV_{LAI} and HIV_{RF}, suggesting productive infection. Antibody against CD4 blocks superinfection, implying superinfection occurs through a gp120/CD4 interaction. Reverse transcription of ACH2 mRNA from 14 and 28 days post-infection allowed a similar PCR strategy to be used to analyze full-length RF- and LAI-specific transcripts. These data suggested that superinfection of ACH2 with HIV_{RF} results in an increase in the expression of both HIV_{RF} and HIV_{LAI} mRNA. From day 14 to day 28 post-infection there is an apparent increase in the relative expression of HIV_{RF} compared with HIV_{LAI}. Interestingly, by day 28, when only 0.5% of the ACH2 cells contained superinfecting HIV_{RF}, roughly 50% of the HIV-specific full-length mRNA was HIV_{RF} in origin. Sequencing of PCR-derived LTR fragments from A3.01 cells acutely infected with HIV_{RF} or ACH2 cells shows differences in the critical U3-R region. Functional differences in CAT activity are also observed. Establishment of an ACH2 cell line infected with both host HIV_{LAI} and superinfecting HIV_{IIIB} allowed analysis of the rate of induction of full-length HIV transcripts. Transcription from the superinfecting strain was more rapidly induced by PMA. Taken together, these data establish the feasibility of superinfection and offer insight into the effect of superinfection on the expression of host and superinfecting strain mRNA and raise the possiblity that HIV-based retroviral vectors might be used to target HIV-infected cells and to modify viral expression within those cells.

Q 334 *VPU* AFFECTS THE ABILITY OF HIV-1 TO REPLICATE AND FORM SYNCYTIA IN CELLS EXPRESSING HIGH LEVELS OF CD4, Nathaniel R. Landau[1,2], Marc E. Lenburg[1] and William Paxton[1]. [1]Aaron Diamond AIDS Research Center, and [2]Dept. of Pathology, NYU School of Medicine, New York.

Recently, two apparently unrelated effects on viral replication have been ascribed to the *vpu* protein of HIV-I. In one report, *vpu* appeared to increase the amount of virions secreted by infected or transfected cells[1]. In the other, *vpu* appeared to overcome a block to gp160 processing imposed by CD4[2]. *To evaluate the possibility that a single protein could perform both functions* and to examine their possible biological significance, we have constructed HIV-1 proviruses that differ only by their ability to encode *vpu*. Our results show that *vpu* increases the efficiency of particle release as observed both in pulse-chase labeling experiments and in measurements of the amount of gag^{p24} in cells and supernatants. Cells producing vpu^- HIV-1 contained four-fold more gag^{p24} than those producing vpu^+ virus, while supernatant levels showed the opposite result (6-8 fold increase for vpu^+). In further experiments we cotransfected cells with HIV-1 proviral DNA together with a CD4 expression vector. We found that in these cells gp160 was processed efficiently in the presence of CD4 in the vpu^+ but not the vpu^- virus. *Vpu* did not affect the rate of processing of any of the other viral components, but did *decrease the amount of newly synthesized* gag^{p24} *in the cells. These results* support the viewpoint that *vpu* has a role in capsid export and in gp160 processing in $CD4^+$ infected cells.

To further determine the biological significance of these findings, we developed a tissue culture system to monitor the effect of *vpu* on HIV-1 replication, secretion of virions and syncytia formation. Our results show that when CD4 is absent from HIV-1-producing cells, *vpu* has little effect on their ability to form syncytia with $CD4^+$ cells. In the presence of increasing amounts of CD4, the cells producing vpu^- virus showed a four-fold decrease in the numbers of syncytia formed, as might be expected, due to blocking of gp160 processing. In contrast, the vpu^+ virus-producing cells showed a 2-3-fold increase in the number of syncytia formed, due in part to viral spread. Because we observed both the block to processing in the absence of *vpu* and the block to syncytia formation was only at high levels of CD4, we speculate that *vpu* may have evolved to enable HIV-1 to replicate efficiently in cells expressing a high ratio of CD4 to gp160. This ability may contribute to the increased pathogenicity of HIV-1 as compared to HIV-2 which lacks this open reading frame.

1. Yao et al., J. Virol 66, 5119-5125.
2. Willey et al. J. Virol. 66, 226-234.

Q 335 TRANSCRIPTIONAL ACTIVATION BY HIV-1 TAT IN A CELL FREE SYSTEM: MECHANISM OF ACTION AND THE ROLE OF CELLULAR PROTEINS. Michael F. Laspia Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The HIV-1 transactivator protein Tat acts to increase greatly the expression of genes linked to the long terminal repeat (LTR) of the virus. It does so by binding to a structured RNA element, known as TAR RNA, that is present in the 5' untranslated region of all HIV mRNAs. TAR RNA forms a secondary structure consisting of a base paired stem with a trinucleotide bulge and an apical loop. Studies *in vivo* in cell culture model systems suggest that Tat is a unique activator that both increases transcriptional initiation and improves the efficiency of elongation. To obtain a detailed knowledge of the mechanism of transactivation and the role that cellular proteins might play, we have utilized a cell free approach. Purified, bacterially-expressed Tat specifically stimulated HIV-1 transcription 10-20 fold in a nuclear extract and was dependent on TAR. Interestingly, high levels of Tat inhibited transcription, which may represent "squelching" due to sequestering of a factor required for basal transcription. The addition of wild type, but not mutant, synthetic TAR RNA to *in vitro* transcription reactions inhibited transactivation proving that TAR functions as an RNA element. Competition was abolished by mutations in the bulge required for Tat binding as well as by mutations in the apical loop not required for Tat binding suggesting that transactivation requires the cooperative binding of a cellular loop binding protein.

In vitro, transactivation was due an increase in the efficiency of transcriptional elongation. In addition, low basal levels of HIV-directed transcription appeared to be caused by repression since the detergent sarkosyl increased the efficiency of transcriptional elongation in the absence of Tat. A kinetic analysis of HIV transcription was consistent with ongoing transcription resulting in the activation of a general inhibitor of transcriptional elongation. We postulate that Tat may function as a gene-specific antirepressor of a general eukaryotic inhibitor of transcriptional elongation.

Q 336 *IN VIVO* FINE MAPPING OF THE TAR BINDING DOMAIN OF HIV-1 TAT.

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HIV-I LTR transactivation require Tat to bind to TAR. The N-terminal region (amino acids 1-48) was shown to be the activation domain in vivo. Once fused to other RNA binding proteins such as R-17 coat protein or REV, the N-terminal region can activate HIV-I LTR if the TAR is also replaced by coat protein operator or RRE correspondly. Although in vitro studies showed that the arginine rich region of Tat (amino acids 49-58) was required and sufficient to bind to TAR in vitro, the TAR binding domain was never been defined in vivo. In this study, we constructed series of Tat-Rev hybrid proteins to investigate whether the arginine rich region was and independent TAR binding domain which could bind to TAR without the actiation domain. Our results indicated that the activation domain is absolutely required for Tat to bind to TAR. We also furthur demonstrated that the arginine rich region and N-terminal transactivation domain have to be kept closely associated for optimal interaction between Tat and TAR.

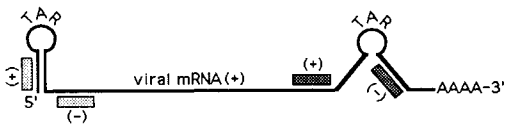
Q 337 ALTERATION OF CATALASE ACTIVITY AMONG HIV-1 (+) ASYMPTOMATIC PATIENTS. Gabriel Martinez, Sumio Yano, Maria Colon, Alma Santiago, Carmen Lefranc, Nayra Rodriguez and Yasuhiro Yamamura, Ponce School of Medicine AIDS Research Program & Dept. Biochem. & Microbiol. & Damas Hospital Dept. Medicine, Ponce PR 00732.

A level of plasma TNFα is distinctly high among patients with ARC or AIDS while that of glutathione is already significantly low among asymptomatic patients (ASP). Hydrogen peroxide as well as TNFα were shown to activate NF-kB leading to the activation of HIV-1 provirus transcription. Since exogenous TNFα decreases catalase (CAT) activity in mice liver, we hypothesized that HIV(+) patients might also have decreased levels of CAT activity relating to the progression of the disease. The specific activity of CAT (units/10^6 cells) in both lymphocytes/monocytes and granulocytes fractions showed significantly lower in ASP than HIV(-) while that in red blood cells (RBC) was significantly higher in ASP than HIV(-). RBC count was inversely correlated with the specific activity of CAT but reticulocyte analysis by flow cytometry revealed no difference between the two groups. Furthermore, the increase of CAT activity in RBC was accompanied with an increased intensity of immuno-reactive CAT and also with the appearance of more acidic isozyme bands. However, the molecular weight of immunoreactive CAT from both groups was very similar. These results suggest that the changes of CAT activity in peripheral blood cells is one of biochemical events occuring after HIV infection and also that the decrease of CAT activity in lymphocytes is probably linked with a change of lymphocyte functions, which are associated with the progression of the disease. It is speculated that the decrease of CAT activity may facilitate the activation of NF-kB by increasing the amount of hydrogen peroxide available for forming reactive oxygen intermediate. The intermediate then will be the direct activator of NF-kB. (The study was supported in part by the RCMI grant G12RR03050, and also by RO1DA06037 & R29DA05510 from NIDA)

Q 338 AMPLIFICATION AND SEQUENCING OF THE 5' AND 3' TAR ELEMENTS IN VIRAL RNA FROM HIV_{BaL} INFECTED MACROPHAGES. Hajime Matsuzaki, Douglas D. Richman, and Richard S. Kornbluth, Dept. of Biology, Depts. of Medicine and Pathology, Univ. of Calif. San Diego, and the V.A. Medical Center, La Jolla, CA 92093-0679

The TAR stem-loop element is located at both the 5' and 3' ends of all viral mRNA. Recent experiments in *Xenopus* oocytes have shown that TAR undergoes a Tat dependent adenosine to inosine modification at position 27 [Sharmeen et al., PNAS 88:8096-8100, 1991]. Assuming that the *Xenopus* oocyte model accurately reflects the Tat/TAR interaction during the course of HIV infection, one would expect to find this A to I modification in viral mRNAs isolated from infected human cells.

We have recently developed a method for highly specific reverse transcription of selective RNAs into corresponding cDNAs. The high specificity is acheived by using very long anti-sense primers and the thermostable rTth DNA Polymerase. A linear amplification of the targeted RNA during reverse transcription, followed by PCR amplification of the cDNA, allows the detection of an RNA of interest from as little as 10^4 cells in culture. We reverse transcribed total RNA from HIV_{BaL} infected macrophages using anti-sense primers targeting the 5' and 3' TAR elements; and then PCR amplified the cDNAs using sense primers that, together with the anti-sense primers, bracket the TAR elements (see diagram below). Sequencing the amplification product that brackets the 5' TAR provides the RNA sequence from position 20 to 60, while sequencing the product that brackets the 3' TAR gives the sequence from position 1 to 28. We did not detect the expected A to I modification at position 27, nor at any other residue in the TAR element. A similar analysis of RNA isolated from HIV_{LAI} infected CEM cells, gave the same result.



Q 339 FREQUENCY OF HIV-1 GAG- AND ENVELOPE-SPECIFIC CYTOTOXIC LYMPHOCYTE PRECURSORS IN HIV-INFECTED CHILDREN. Elizabeth J. McFarland, Luckey D, Conway B, Young RK, Ko D, Rosandich ME, Kuritzkes DR. Divisions of Pediatric and Adult Infectious Disease, The Children's Hospital and University of Colorado Health Sciences Center, Denver CO 80262 and Department of Microbiology and Immunology, University of Ottawa, Ottawa, CANADA

Adults infected with human immunodeficiency virus type 1 (HIV-1) develop a vigorous HIV-specific cytotoxic T-lymphocyte (CTL) response which is readily demonstrated in the peripheral blood mononuclear cells (PBMC) of these individuals without stimulation *in vitro*. By contrast, the presence of activated HIV-specific CTL in the PBMC of children with vertically-acquired HIV-infection has been less clearly defined. We assessed CTL activity of unstimulated PBMC from eight vertically-infected infants and children. Autologous EBV-transformed B-lymphoblastoid cell lines infected with vaccinia recombinants that expressed HIV envelope (ENV), GAG, and reverse transcriptase (RT) served as targets in a ^{51}chromium-release assay. Low levels of ENV-specific cytotoxicity were observed in PBMC from four children; natural killer (NK) cell depletion eliminated this activity in one child tested. No specific cytotoxicity against GAG or RT was observed. We also examined HIV-specific cytotoxicity in the PBMC of seven uninfected infants born to HIV-infected mothers. Absence of HIV-infection was verified by repeatedly negative HIV-1 culture and PCR. These infants likewise had no CTL activity against GAG or RT. Transient ENV-specific cytotoxicity was observed in three infants. HIV-infected adults are reported to have high frequencies of HIV-specific precursor CTL (pCTL) detected after *in vitro* stimulation. To determine the frequency of HIV-specific pCTL in vertically infected children, PBMC were seeded at limiting dilution and stimulated with a CD3-specific monoclonal antibody, IL-2, and irradiated autologous PBMC feeder cells. Among HIV-infected children at various disease stages, HIV GAG- and ENV-specific cytotoxic precursors were identified at frequencies ranging from 1/400-1/73,000 and 1/1300-1/62,000 PBMC respectively. Frequencies of HIV-specific cytotoxic precursors in two uninfected children were $\leq$1/93,000 for GAG and $\leq$1/58,000 for ENV. Our results suggest that despite the absence of circulating activated HIV-specific CTL, some infected children have cytotoxic precursor frequencies similar to those reported for infected adults. Analyses of pCTL frequency using cell populations depleted of either CD8 lymphocytes or NK cells prior to seeding at limiting dilution are currently underway in order to delineate further the effector cell phenotype.

Q 340 NEGATIVE-STRAND TRANSCRIPTS ARE PRODUCED IN HIV-1 INFECTED CELLS AND PATIENTS BY A NOVEL PROMOTER DOWNREGULATED BY TAT. Nelson L. Michael, Philip K. Ehrenberg*, Lisa d'Arcy*, Maryanne T. Vahey, Joseph D. Mosca*, Jay Rappaport§, and Robert R. Redfield. Department of Retroviral Research, Walter Reed Army Institute of Research and *Henry M. Jackson Foundation, 13 Taft Court, Suite 200, Rockville, Maryland 20850. §Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20852

Current understanding of human immunodeficiency virus type-1 (HIV-1) transcription is based on unidirectional expression of transcripts with positive-strand polarity from the 5' long terminal repeat (LTR). We have identified HIV-1 transcripts of negative strand polarity in acutely and persistently infected cell lines as well as in 117 mononuclear cell samples from 15 seropositive patients. We constructed a directional cDNA library from A3.01 cells acutely infected with HIV_{IIIB}. Screening of 3×10^5 plaques resulted in 60 HIV-specific clones of which 3 were identified by DNA sequence analysis as negative-stranded polarity. The 5' ends of these negative strand cDNA clones map to the R region of the 3' LTR. One of these cDNAs encodes a highly conserved 189 amino acid open reading frame that maps antiparallel to the envelope gene. This cDNA acquires a poly-A tail of over 80 residues by use of novel poly-A signal box located within the coding sequences of the gene.

We demonstrate, through the use of a reporter construct, that a novel negative strand promoter (NSP) functions within the 3' LTR and is downregulated by wild-type but not mutant Tat expression. Deletion mapping analysis of the NSP reveals that U3 region sequences are an absolute requirement for NSP function and that Tat responsiveness is dependent upon an intact TAR element. These data extend the coding capacity of HIV-1 and suggest a role for antisense regulation of the viral life cycle.

Q 341 **AN INTERFERON-INDUCIBLE GENE FAMILY INHIBITING HIV-1 EXPRESSION**

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We have identified an IFN-inducible cellular protein that binds to the HIV-1 RNA, within the region of the Rev responsive element (RRE). RRE is the binding site of the Rev protein of HIV-1, which is essential for appropriate nucleocytoplasmic transport and utilization of viral mRNAs and for virus production. The IFN-inducible cellular protein was identified by screening a human monocyte cDNA expression library with an RRE RNA probe. One clone which specifically bound to the RRE expressed a 14.5 kD protein. DNA sequencing showed that the gene encoding this protein belongs to a family of IFN-inducible genes. In transient transfection studies, this protein inhibits the Rev-dependent expression of the HIV-1 structural proteins, possibly through binding to the RRE and interfering with Rev function. Down-regulation by this protein is a posttranscriptional event, since the expression of Rev-independent genes is not affected.

In vitro binding studies after expression of the protein in bacteria showed that it binds to RRE RNA. Another member of the same gene family was shown to inhibit HIV-1 expression, although less efficiently, while a third member did not inhibit expression.

These studies demonstrate that a family of IFN-inducible genes of previously unknown function encodes RNA binding proteins which affect viral expression. We propose that one mechanism of IFN inhibition of HIV-1 production may be the interference with the Rev-RRE posttranscriptional regulatory pathway of the virus. These studies contribute to the elucidation of the mechanisms that mediate the antiviral effects of IFNs and may lead to the further understanding of the IFN effects on HIV-1 and to the development of novel therapeutic strategies for HIV-1 infection.

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Q 342 THE NATURAL HISTORY AND RELEVANCE OF ANTIBODY TO PRINCIPAL NEUTRALIZING DOMAIN IN PEDIATRIC HIV, SI Pelton, A Breña, B Potts, H Cabral, and ER Cooper Department of Pediatrics, Boston City Hospital and Boston University School of Medicine and Boston University School of Public Health, Boston, MA and Repligen Laboratories, Cambridge, MA

We have established an ELISA assay which employs a 24 amino acid peptide synthesized to match the V_3 loop sequence of GP120. The results of this V_3 ELISA against a peptide synthesized to represent the tip of the V_3 loop of HIV_{MN} has been compared with VAC-ENV assay which measures syncytia reduction and utilizes recombinant vaccinia virus expressing HIV glycoprotein 160(MN). The V_3 ELISA has been found to correlate well with this functional VAC-ENV assay.

We have evaluated HIV infected children followed longitudinally at Boston City Hospital by employing V_3 ELISA against the MN peptide. Of 20 children tested before 12 months of age, only 3 demonstrated significant reactivity to MN ($\geq$ 1:50). However, after one year of age, three patterns have been observed: children who never developed measurable antibody (to MN), children who have developed antibody to MN (usually by 12 to 24 months of age) and who have stable levels over a long period of time, and children who developed antibody but during follow-up have demonstrated decaying levels. We have begun to correlate results of this V_3 ELISA with clinical outcome, immune function, and P_{24} levels. Initial results have identified a correlation between the presence of P_{24} antigen and absence of antibody to V_3 (MN) and an association between low CD_4 and absent antibody. We are completing further data analysis to evaluate association between antibody to the PND as measured in our assay and clinical outcomes.

Q 343 CELL LINEAGE-SPECIFIC PROCESSING OF HIV-1 NEF: MULTIPLE NEF PROTEIN SPECIES ARE PRODUCED IN MONOCYTE/MACROPHAGES, Victoria R. Polonis, Jeanne M. Novak, Nelson L. Michael, *Suzanne Gartner, and Robert R. Redfield, Dept. of Retroviral Research, WRAIR, and *The Henry M. Jackson Foundation, 13 Taft CT, Rockville, MD 20850

The role of the HIV nef gene products in the regulation of virus replication in specific cell lineages has not been defined. The biogenesis of nef proteins was investigated in infected T cells and monocyte/macrophages to address potential functional differences of nef in distinct cellular targets. While one predominant species of nef is produced in T cell cultures, multiple forms of this gene product were detected in cells of monocytic lineage. In the chronically infected U-1 promonocytic cell line and in HIV-1 (Ba-L) infected 10-14 day old human macrophages, two predominant nef proteins of 26 and 28 kDa were detected, as well as a less abundant doublet of approximately 18-20 kDa molecular weight. In the presence of cerulenin, an inhibitor of fatty acid synthesis, a non-myristylated nef precursor was identified in both cell lineages. Molecular sequencing and immunoprecipitaion of nef proteins using specific antisera demonstrated a significant amino acid heterogeneity at the C-terminus of the nef protein in cells of the monocytic lineage. The differential post-translational processing of the nef gene product in T cells and monocyte-macrophages suggests potential differences in the function(s) of this protein in specific host cell lineages.

Q 344 PURIFICATION AND PARTIAL BIOCHEMICAL CHARACTERIZATION OF HIV-1-NEF EXPRESSED ON HUMAN AA2 B-CELLS, G. Pulido-Cejudo[1] J. Campione-Piccardo[1], C.A. Izaguirre[1] and J. Victor Garcia[2] [1]Laboratory Centre for Disease Control, Ottawa Canada K1A 0L2 and [2]Department of Virology & Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101-0318

The role of Nef in HIV pathogenesis appears to be related to a downregulation of membrane-bound CD4. The molecular mechanism by which this process is achieved remains unsettled. To determine some biochemical properties of Nef, a B-cell line (AA2) retrovirally transfected with SF2 nef (Nature 350, 508, 1991) was used to purify this protein. Fractions enriched in Nef were identified using a monoclonal anti-HIV-1 3' ORF antibody in both dot blot and Western blot analysis. AA2 cells were homogenized in 50mM phosphate buffer pH 7.0 (1mM DTT, 2mM $MgCl_2$, 1mM PMSF). The homogenate was spun at 17,600 X g for 30 min. and the supernatant applied onto a P11 column equilibrated and eluted with phosphate buffer. This eluent was concentrated and applied to a Partisil 10μm SAX column. Nef was eluted with a linear gradient (0-1M NaCl; 50mM phosphate buffer pH 7.0) at 1 ml/min (~1500 psi). Fractions were pooled and applied to a Bio-Sil Sec 250 column equilibrated with phosphate buffer pH 6.8, 100 mM KCl, 0.05% NaN_3. Samples were eluted at 1 ml/min (~700 psi). Based on the retention time after gel filtration Nef positive samples showed a MW of 60,000. In contrast, SDS PAGE performed on this fraction showed a single band at approximately 29 KDa both after silver staining and Western blot analysis. The isoelectric point of the Nef positive fraction was 5.5-6.0. Collectively, these data suggest that Nef in B-cells is namely expressed as a dimer with monomeric subunits of approximately 29KDa. Studies pertaining to the physiological implication and cellular factors controlling the expression of Nef dimer will be discussed.

Q 345 A STUDY OF PREVALENCE OF INFECTIOUS HIV IN SALIVA OF IVDU'S IN NEW YORK CITY, *+M. Nasar Qureshi, *Zhongda Qiu, *Charles E. Barr, *Department of Dental Medicine and +Department of Pathology and Laboratory Medicine, Beth Israel Medical Center, New York, New York 10003.

The risk of transmission of HIV through saliva is generally considered to be very low. However, isolation of infectious HIV from saliva has been reported from 40% to <1% of samples tested. Variations in methodology of processing saliva specimen may account for this discrepancy. Recent concern for transmission of HIV during dental procedures and case reports of seroconversion possibly through oral sex warrant additional studies to assess the presence of free infectious virus in saliva. Another important factor in the transmission of HIV through saliva maybe the extent of oral pathology increasing the probability of presence of infectious viral particles in saliva and increased risk of transmission. Intravenous drug users (IVDU's) are generally known to have poor oral hygiene and increased incidence of oral pathology in comparison of other high risk groups for HIV. We are studying IVDU's who present at the Beth Israel Medical Center in New York for the presence of infectious virus in saliva. The results will be correlated with the presence of virus in plasma, PBMC and the presence and extent of oral pathology and stage of disease in seropositive individuals. To control for variations in culture methods and assess the component of saliva which may harbor the virus, we use either cell free concentrate (100,000g) of whole saliva, or whole saliva pretreated with antibiotic, for coculture with PHA treated PBMC from seronegative donors. We plan to assess 400 HIV seropositive and seronegative individuals over a period of 2 years and preliminary results from approximately first 75 individuals will be presented.

Q 346 CHARACTERIZATION OF REVERSION EVENTS FOLLOWING MUTATION OF THE HIV LTR REGULATORY SEQUENCES, Arnold B. Rabson and Wen-Tse Tseng, CABM and UMDNJ-RWJMS, Piscataway, NJ 08854.

Introduction of mutations into the Sp1 binding sites in the HIV LTR results in generation of viruses that replicate in some T cell lines (such as MT4 cells) but not in others (ex. A3.01 CEM cells). Previous studies had suggested that the presence of NF-κB activity in T cells allowed the replication of these mutant viruses, suggesting that NF-κB could functionally replace Sp1 in supporting viral replication (Parrott, C. et al. J. Virol. 65:1414, 1991.) Reversion mutations can be detected that allow Sp1 deleted viruses to replicate in formerly non-permissive T cell lines (Ross, E.K. et al, J. Virol. 65: 4350, 1991). In this study, we have systematically analyzed the genotype and phenotype of second site reversion mutations that allow replication in non-permissive A3.01 cells, of a virus containing point mutations in the two 5' Sp1 sites. Such reversion mutations were detected in 8 of 30 separate infections. Direct PCR DNA sequencing of proviral DNA from these infections allowed identification of four classes of reversion mutations, which were subsequently characterized as to their replication properties and their protein binding characteristics. These classes were: (1) a single base change in the 5' mutated Sp1 site restoring a functional Sp1 binding site (4/8); (2) a two base change in the 5' Sp1 site conferring sequence identity to, and DNA binding characteristics of, an NF-κB half-site, identical to that seen in the 5' NF-κB LTR site (1/8); (3) deletion of the 3' NF-κB binding site, bringing the 5' site into direct proximity to the mutated Sp1 sites (2/8) and (4) a single base pair point mutation mapping between the NF-κB and Sp1 sites. These results support the concept of complex interactions between the LTR NF-κB and Sp1 binding sites and the possibility of functional distinctions between the two NF-κB sites.

Q 347 CONSTRUCTION AND CHARACTERIZATION OF A REPLICATION-DEFECTIVE HERPES SIMPLE VIRUS TYPE 1 VECTOR EXPRESSING THE HIV-1 *tat* GENE, Siyamak Rasty and Joseph C. Glorioso, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

We are curently constructing replication-defective herpes simplex virus type 1 (HSV-1) vectors, through mutagenesis of particular genes essential to the lytic cycle of HSV-1, that establish latent infections in the rat CNS. A functional *loxP* recombination site from bacteriophage P1 has been inserted into the thymidine kinase gene of a replication-defective HSV-1 derivative, d120 (DeLuca *et al.*, J. Virol, 56: 558), which contains a 4.1-kb deletion in both copies of the HSV-1 ICP4 gene. Insertion of the *loxP* sequence into the vector's genome has allowed for rapid incorporation of shuttle plasmids carrying the *loxP* site and individual promoter-gene cassettes into the vector. In addition, since the vector is unable to encode ICP4, an essential immediate-early HSV-1 regulatory protein, it cannot express its early and late genes in non-complementing cells and thus cannot replicate. A shuttle plasmid, containing the HIV-1 *tat* cDNA driven by the SV40 early promoter-enhancer, was recombined into the *loxP* site of the vector by an *in vitro*, cell-free recombination reaction using the cre protein of bacteriophage P1, thus creating the *tat* expression vector d120::SVTat. Transfection of a stable, ICP4-transformed Vero (African green monkey kidney) cell line by a pLTR-CAT plasmid, followed by infection with the d120::SVTat vector, resulted in high level CAT activity in the cells, indicative of *tat* expression from the vector. Moreover, a similar but lower CAT activity was demonstrated when normal Vero cells (not expressing ICP4) were used for transfection/infection. These results suggest that functional *tat*, capable of *trans*-activating the HIV-1 LTR, is expressed from the vector, and that it is expressed in the absence of HSV-1 DNA replication. We are currently studying the kinetics of *tat* mRNA and protein expression from the vector in tissue culture, and are also examining the capacity of the vector to express *tat in vivo*. Expression of *tat* in the brains of mice that have been stereotactically injected with the vector is being investigated by *in situ* hybridization, RNA PCR, and immunofluorescence techniques. One outcome of this work will be to investigate the contribution of HIV-1 tat protein to AIDS-related neuropathology, and to use this and other HSV-1 vectors expressing separate HIV-1 gene products (e.g., gp120, gp45) to develop a small animal model of HIV-1 related CNS disease for testing of antiviral therapies.

Q 348 **STRUCTURE-FUNCTION RELATIONSHIPS OF THE HIV-1 ENVELOPE V3 LOOP TROPISM DETERMINANT.** Lee Ratner, Alejandro Carrillo, Christoph Ebenbichler, and Ruili Gu, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

The V3 loop of the HIV-1 gp120 envelope glycoprotein is an important determinant of macrophage-tropism. Studies with recombinant full-length HIV clones showed that substitution of a macrophage-tropic V3 loop into a T cell line tropic isolate abolished its ability to replicate in T cell lines. Although a T cell line-tropic V3 loop is insufficient to confer T cell line tropism when inserted in a macrophage-tropic backbone, a 200 aa region encompassing the V3 loop and CD4-binding domain is able to confer T cell line tropism. Attempts to refine the minimal T cell line tropism determinant resulted in clones incapable of infection of all cell types examined. Similar results were seen with different T cell line-tropic isolates inserted into two different macrophage-tropic backbones, suggesting that non-functionality is a general phenomenon caused by incompatibility of chimeric env domains. Mutational analysis at 3 positions within the V3 loop also failed to identify a single point mutation capable of conferring or abolishish T cell line tropism.

The gp160 envelope precursor proteins from macrophage-tropic or T cell line-tropic strains of HIV-1 were expressed in recombinant vaccinia virus infected cell lines or primary lymphocytes or macrophages. No significant differences in the kinetics of synthesis of gp160, processing, glycosylation, or gp120 release were noted. However, shed gp120 was partially cleaved within the V3 loop. The gp120s from macrophage-tropic isolates exhibited lower rates of cleavage than those from T cell line-tropic strains in all cell types examined. Nine different purified envelope proteins were also prepared in a Drosophila expression system. Six monoclonal antibodies against the V3 loop reacted preferentially with T cell line-tropic gp120s, and one monoclonal antibody reacted preferentially with macrophage-tropic gp120s. In addition, T cell line-tropic gp120 envelope proteins were 1000-fold more susceptible to V3 loop proteolytic cleavage by mast cell tryptase than macrophge-tropic gp120 envelope proteins. **These findings suggest that there are two distinct conformations for the V3 loops of T cell line-tropic and macrophage-tropic gp120 envelope proteins.**

Q 349 HUMAN CORD BLOOD CELL RECONSTITUTION AND HIV-1 INFECTION OF NEONATAL SCID MICE. Barbara Reinhardt, Bruce E. Torbett, Richard J. Gulizia, Mary J. Narciso, Donald E. Mosier, Stephen A. Spector. University of California, San Diego and Scripps Research Institute, La Jolla, CA 92093.

To develop a small animal model for pediatric HIV infection, neonatal SCID mice were reconstituted i.p. with human cord blood mononuclear cells to yield hu-CBL-nSCID mice and challenged 2 wks. later with HIV-1 isolates. Lymphocyte subsets were determined by FACS analysis. Viral infection was detected by co-culture with PHA-stimulated human PBLs, plasma p24 antigen determination and PCR. Hu-CBL-nSCID mice contained readily detectable human CD3 T-lymphocytes and CD20 B-cells, and produced readily detectable human IgM and IgG (including all subclasses). CD4/CD8 ratios > 1 were maintained in peritoneal cavity, spleen, lymph nodes, and peripheral blood in uninfected animals for at least 8 wks. Two wks. following infection with HIV-1, virus could be recovered by co-culture of spleen, lymph node, peritoneal cavity, liver and plasma from hu-CBL-nSCID mice. Proviral DNA was detectable by PCR in peripheral blood of 41% of the animals infected with HIV-1 IIIB and 65% of the animals infected with HIV-1 SF2. HIV-1 p24 antigenemia was present in 81% of the HIV-1 IIIB infected chimeras (mean $\pm$ SE: 60 $\pm$ 11 pg/ml), and 65% of the HIV-1 SF2 infected animals displayed p24 antigen levels of 202 $\pm$ 63 pg/ml. Infection with HIV-1 IIIB lead to a rapid decline in circulating CD4 cells and a relative and absolute increase in circulating CD8 cells within 2 wks. Hu-CBL-nSCID mice infected with HIV-1 SF2 continued to show CD4/CD8 ratios > 1 after 2 wks., with progressive loss of CD4 cells over time. In summary, hu-CBL-nSCID mice demonstrated many of the characteristics of infants including high CD4 lymphocytes and high CD4/CD8 ratios, and, like HIV-infected infants, hu-CBL-nSCID mice showed rapid CD4 depletion with initial CD8 expansion following HIV-1 infection. The model allows for monitoring of HIV-1 infection through blood samples without sacrificing the animals. Hu-CBL-nSCID mice should prove useful for evaluating the pathogenicity of HIV-1 strains for infants, differences in viral inoculum necessary to establish infection, and strategies for treating and preventing HIV-1 infection of newborns.

Q 350 NAIVE CORD BLOOD MONONUCLEAR CELLS (CBMC) ARE PREFERENTIALLY INFECTED BY MONOCYTE/MACROPHAGE TROPIC HIV-1 ISOLATES. Peter P. Reinhardt, Barbara Reinhardt and Stephen A. Spector. University of California, San Diego, La Jolla, CA 92093.

Maternal-infant transmission of HIV-1 is an increasing problem worldwide. Because of the differences in maturity and state of activation between CBMC and adult peripheral blood mononuclear cells (PBMC), we compared the susceptibility to HIV-1 infection of these two cell populations. Non-stimulated CBMC and PBMC were incubated within 4 hrs. after collection with LAV, a well characterized, lymphotropic, syncytia inducing HIV-1 isolate, Ba-L, a well characterized monocyte-derived-macrophage (MDM) adapted HIV-1 isolate, and two clinical virus isolates of previously defined tropism for MDM and T-lymphocytes. All experiments were carried out with equal multiplicity of infection. Of 11 non-stimulated CBMC samples, 7 were permissive for LAV, whereas all 11 could be infected by Ba-L. CBMC infection with Ba-L consistently displayed 10-20 times higher virus replication than the lymphotropic isolate. The opposite was observed in non-stimulated PBMC; while Ba-L infected 3 of 6 samples, all were susceptible to LAV, which consistently replicated to higher levels than Ba-L in PBMC. Similar results were seen after infection with clinical isolates. 4 of 6 non-stimulated CBMC samples could be infected with the MDM tropic isolate, and only 1 of 6 samples was susceptible to the T-cell tropic, syncytia inducing clinical isolate. In contrast, none of the 3 non-stimulated PBMC samples was infectable with the MDM tropic isolate and 2 of 3 samples showed viral replication after incubation with the lymphotropic strain. FACS analysis of CBMC samples showed that more than 90% of T-cells belonged to the naive, CD45RA expressing subset, whereas only 20-30% of CD45RA expressing T-cells were found in PBMC. Furthermore, the activation markers CD25 and CD69 were expressed at lower levels on the surface of CBMC. The percentage of monocytes did not differ between CBMC and PBMC. Stimulation with PHA enhanced susceptibility of CBMC to lymphotropic HIV-1 strains. These data indicate that naive CBMC are more susceptible and permissive to MDM tropic HIV-1 isolates than to lymphotropic, syncytia inducing isolates. Cytotropism analysis of mother to infant transmitted HIV-1 isolates will show if there is, in fact, a preferential transmission of MDM tropic HIV-1 isolates.

Q 351 COMPARISON OF HIV env SEQUENCE VARIATION FROM UNCULTURED LYMPH NODE AND SERIAL BLOOD SAMPLES. Merlin L. Robb, Jill Ruderman, Nelson L. Michael, Ted Cieslak and Robert R. Redfield. Walter Reed Army Institute of Research, 13 Taft Ct., Rockville, MD. 20850.

We sought to characterize the relationship between peripheral blood lymphocytes (PBL) and lymph node (LN) in terms of the amount and type of HIV quasispecies which are present in these two compartments. A perinatally infected child was evaluated using quantitative PCR and sequence analysis of uncultured PBL at 20, 31, 37, 44 and 47 months of age. Lymph nodes were excised simultaneous with phlebotomy at 44 months to exclude a lymphoproliferative disorder. Viral burden was determined for each sample using quantitative RNA and DNA PCR. A 793 base pair segment of env encompassing V1,V2,C2 and V3 was amplified using nested PCR prior to molecular cloning and automated sequencing. Over the course of 27 months, the patient's CD4 count declined from 2173/mm^3 to 306/mm^3 and he clinically progressed from mild P2A symptoms to P2AF with severe failure to thrive (FTT). Proviral burden and RNA expression increased abruptly from 21 DNA copies/1000 CD4 and <1 copy genomic RNA/1000 CD4 to values of 420 and 1393 respectively following the development of severe FTT , progressive lymph-adenopathy and massive heptosplenomegaly. 99 clones have been analyzed representing 12-23 clones per sample. Clonal diversity within a sample based upon DNA sequence pair distances was 6.6% (3.8-11.1%). Predicted protein alignment identified 5 clades which corresponded roughly to sample collection dates. The LN sample and the last PBL sample demonstrate the greatest diversity. The PBL sample collected with the LN is largely confined to a clade which contains the majority of LN sequences and subsequent PBL sequences. These data suggest that V1-V3 portion of env has steadily changed over time and that the LN and late stage PBL sample embrace a greater representation of these changes than early stage PBL samples in this patient. In addition, the PBL sample collected with LN represented the majority of available LN clones. The range of diversity observed in the LN and most recent PBL specimen may reflect changes in immunological control of the virus over time or may represent changes in the number and type of infected cells analyzed in these samples (e.g. T cell vs. monocyte lineage). Further investigation addressing the contributions of host cell type, the relationship of observed changes to immune responses and the relationship of plasma and expressed RNA to provirus will contribute to understanding pathogenesis of HIV.

Q 352 HIV-1 GENES IN THE ESTABLISHMENT OF CHRONIC VIRUS PRODUCERS, Harriet L. Robinson[1,2] and Farah Mustafa[2], Department of Pathology[1] and Department of Molecular Genetics and Microbiology[2], University of Massachusetts Medical Center, Worcester, MA 01655.

Two molecularly cloned viruses, NL4-3 and HXB-2, have been used to study the role of HIV-1 genes in the establishment of chronic virus producers. Both NL4-3 and HXB-2 are cytopathic viruses that undergo a lytic phase of infection in T-cell lines. In H9 cells, the lytic phase is followed by the outgrowth of surviving populations of cells. In NL4-3 infections, only a small proportion of the surviving H9 cells are chronic virus producers. By contrast, in HXB-2 infections, the vast majority of H9 survivors are virus producing. NL4-3 encodes all known HIV-1 proteins, whereas HXB-2 is defective for three auxiliary genes: *vpr, vpu,* and *nef*. Tests of mutant NL4-3 genomes containing all possible combinations of defective *vpr, vpu,* and *nef* genes revealed that both *vpr* and *nef* limit the ability of NL4-3 to establish chronic virus producers. Tests of a series of recombinants between NL4-3 and HXB-2 revealed that 5' internal sequences of HXB-2 as well as fragments containing defective auxiliary genes were required for the efficient establishment of chronic virus producers. These results indicate that multiple regions of the HIV-1 genome determine the ability to establish chronic virus producers.

Q 353 **IDENTIFICATION OF cFOS-RESPONSIVE ELEMENTS DOWNSTREAM OF TAR IN THE LONG TERMINAL REPEAT OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1,** **Kenneth A. Roebuck, Department of Immunology and Microbiology, Rush-Presbyterian-St. Luke's Medical Center, 1753 W. Congress Parkway, Chicago, Illinois 60612.** Activation of human immunodeficiency virus type-1 (HIV-1) requires the binding of host cell transcription factors to cis-elements in the proviral long terminal repeat (LTR). My laboratory is interested in identifying the cellular transcription factors and cognate cis-regulatory elements that constitute important activation signals in the HIV-1 LTR. In particular, we are investigating the functional role of immediate-early gene products, such as the transcription factors Jun and Fos, in the activation of the HIV-1 proviral genome. The present study identifies cFos-responsive sequence motifs in the U5 transcribed noncoding leader sequences downstream of the viral transactivator responsive (TAR) element. These DNA sequence motifs are the most downstream regulatory elements described thus far in the HIV-1 LTR and they exhibit sequence similarity to functional AP-1 and CREB/ATF binding sites also known as TRE (for TPA-responsive element) and CRE (for cyclic AMP-responsive element), respectively. Functional studies, using human colon epithelial cell lines, demonstrate that the downstream TRE/CRE-like elements are transactivated by expression of the cfos protooncogene and can transmit phorbol ester and tumor necrosis factor α activation signals to the viral LTR. Moreover, the cFos-responsive elements mediate HIV-1 LTR transcription independent of Tat and the NFκB-binding enhancer element. Nuclear extracts of colon epithelial cells form distinct gel mobility shift complexes with the cFos-responsive elements. These complexes comigrate with a gel shift complex formed on a classical CRE oligonucleotide but migrate differently from complexes formed on a classical TRE oligonucleotide. Moreover, these gel shift complexes are competed by CRE and ATF-binding oligonucleotides, but not by TRE or TRE-like oligonucleotides. An anti-cFos monoclonal antibody (18C3) blocked or inhibited the formation of the comigrating gel shift complexes on the CRE and cFos-responsive elements, respectively. These data indicate that the HIV-1 LTR contains previously unrecognized functional DNA cis-regulatory elements downstream of TAR in the transcribed noncoding 5' leader sequence and suggests that early response genes such as cFos play a role in the activation of HIV-1 gene expression.

Q 354 HIV-SPECIFIC CYTOTOXIC T-CELL ACTIVITY IN AN HIV-EXPOSED BUT UNINFECTED INFANT.

Sarah Rowland-Jones, Douglas Nixon, Marian Aldhous, Frances Gotch, Nicholas Hallam, J. Simon Kroll, Richard Tedder, Karin Froebel & Andrew McMichael, Molecular Immunology Group, Institute of Molecular Medicine, Oxford, OX3 9DU, UK.

The immune response to HIV is characterized by vigorous HIV-specific cytotoxic T-lymphocyte (CTL) activity. We have observed a transient HIV-specific CTL response in a baby born to HIV-positive parents, when all standard markers of infection were negative. Cord blood was HIV-seropositive but showed no HIV-specific CTL activity. At five months the baby was healthy, HIV seropositive, but had no other evidence of HIV infection on PCR analysis, p24-antigen testing or viral culture. However, lymphocyte cultures stimulated with autologous PHA-activated-lymphoblasts, showed HIV-gag-specific CTL activity, directed against two HLA-B8-restricted gag peptides (p24-13 and p17-3), previously recognised by CTL from the father who also has B8. This was accompanied by an increase in the proportion of CD8+ lymphocytes expressing CD45RO, usually seen in infected children. At fourteen months, the child was completely healthy and all markers of infection were negative.These findings suggest that HIV-specific CTL may be a marker for recently exposed but immune individuals.

Q 355 **REGULATION OF COMPLEMENT MEDIATED LYSIS OF HIV-1,** Mohammed Saifuddin, Dan Takefman, Chandana Patki, Mahmood Ghassemi and Gregory T. Spear, Department of Immunology/Microbiology, Rush Medical College, Chicago, IL 60612

Regulation of antibody mediated complement lysis of HIV was determined *in vitro*. Complement control proteins, decay accelerating factor (DAF,CD55) and membrane inhibitor of reactive lysis (MIRL,CD59) expressed on human cells inhibit formation of the complement cascade by accelerating dissociation of C3 convertases (C4b2a,C3bBb) and by blocking C8 binding to C5b67, respectively. HIV was grown in mutant cells which did not express DAF and MIRL on their surface and in wild type cells, and tested for its complement mediated lysis in the presence of complement and anti-HIV antibody. The level of complement mediated lysis of HIV was quantitatively determined by the amount of reverse transcriptase released from HIV particles. HIV grown in mutant cells was more sensitive to lysis by complement as compared to HIV grown in the wild type cells, which suggests that DAF and MIRL cellular proteins expressed on the virion regulate the complement mediated lysis of HIV. Further, the expression of complement control proteins on the surface of HIV was assessed by incubation of virus with specific antibodies to host cell proteins along with complement. Both anti-DAF and anti-MIRL caused lysis of several strains of HIV-1 produced in H9 cells in the presence of complement indicating that DAF and MIRL were expressed on the surface of HIV. These results suggest that HIV is resistant to complement mediated lysis due to virion-surface, host cell-derived complement control proteins.

Q 356 EFFECTS OF ONCOPROTEINS ON TAT-DEPENDENT TRANS-ACTIVATION FROM THE HIV-I LTR

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The pathogenesis of AIDS is characterized by an extended period of latency which typically spans several years. Many stimuli are reported to activate signal transduction pathways ultimately leading to activation of the latent provirus. These activators include cytokines, mitogens, hormones, chemicals such as phorbol esters and viral proteins such as the Adenovirus E1A gene product. We are attempting to decipher the pathways that mark the transition from latency to productive infection. We are utilizing the plasmid pU3-III-CAT encoding the HIV-1 LTR fused to a CAT reporter to transiently transfect lymphocytes and monocytes. CAT activity is increased several-fold when the cells are cotransfected with pCV-1, a plasmid expressing the TAT gene product. A 10-25 fold increase is typical. The cells are also cotransfected with plasmids expressing different oncoproteins each having a well-characterized role in intracellular signal transduction. These include tyrosine kinases, serine-threonine kinases and the Ras family of monomeric G-proteins. The effects of these proteins on TAT-dependent trans-activation from the HIV LTR is being monitored. Preliminary data shows that a dominant negative Ha-Ras mutant represses TAT-dependent trans-activation in monocytes. Our progress on these experiments will be reported.

Q 357 BIOCHEMICAL CHARACTERIZATION OF T-CELL LINES EXPRESSING CHIMERIC CD8-NEF FUSION PROTEINS

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The Nef gene product of HIV is a myristylated phosphoprotein of about 29 Kd. Genetic studies have shown that expression of the Nef gene from specific HIV-1 isolates results in a differential ability to suppress HIV replication. Although effects of Nef at the level of HIV transcriptional activation have been observed, the exact mechanism of its action is not clearly understood.

In order to establish a system where Nef expression can be monitored, we have generated T-cell lines that constitutively express HIV-1_{SF2} Nef in the form of a chimeric fusion protein. CD8-Nef chimeras were constructed by fusing an intact Nef gene to the extracellular and transmembrane domains of the CD8 molecule.

These cell lines were used to facilitate biochemical and functional studies. The 49 Kd. CD8-Nef fusion protein is stably expressed and can be immunoprecipitated by either a CD8-specific monoclonal antibody or a polyclonal anti-Nef serum. The half-life of the fusion protein is similar to that of native Nef found in virally infected cells. Phosphorylation analyses indicate that in unstimulated cells, Nef is weakly phosphorylated. In contrast, Nef is hyperphosphorylated in cells stimulated with phorbol esters. Preliminary results indicate that a kinase coimmunoprecipitates with Nef. We are currently characterizing this kinase activity and determining its relationship to Nef function.

Q 358 MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF HIV-1 FROM 10 MOTHER-CHILD PAIRS;

Gabriella Scarlatti*, Thomas Leitner#, Eva Halapi**, Paolo Rossi**, Jan Albert## and Eva Maria Fenyö*; *Departments of Virology and **Immunology, Karolinska Institute; ##Department of Virology, National Bacteriological Laboratory, and # Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.

It has been recently recognized that mother-to-child transmission of HIV-1 frequently occurs during delivery. However, little is known about the pathway of the virus from the mother to the child. The identification of specific molecular or biological features of the transmitted virus would be essential for a better understanding of the transmission process and thereby give some highlights for prevention trials.

We have sequenced the V3 region from 10 seropositive mothers at delivery and compared those to the sequences obtained from the corresponding child (age: 0 to 4 months). Proviral DNA derived from peripheral blood mononuclear cells (PBMC) directly as well as from cultured PBMC, and from viral RNA extracted from the serum was amplified by nested PCR with primers specific for the V3 region of gp120 env gene. The PCR product was then used for direct solid-phase DNA sequencing. Pairwise comparisons were performed to calculated inter- and intrapatient distances. Furthermore, the replicative capacity of the isolates from the same mother-child pairs were tested in a variety of cell lines of T-lymphoid and monocytoid origin (Jurkat, Jurkat tat, CEM, U937 clone2 and MT-2).

The amino acid sequence of the V3 region from each of the ten children was homogeneous. In contrast the mothers showed a varying degree of heterogeneity. Comparison of the children's sequences with those of the corresponding mothers showed that 4 children presented a sequence similar to the maternal derived DNA sequence and the other 4 to the RNA derived sequence. Three mothers harboured mixed virus populations with and without the N-glycosylation site in position 300 (according to HIV-1 MN), however, the virus variant with the mutation was only transmitted to 1 child. All 7 mothers with a slow/low replicating virus transmitted a slow/low virus to their children, instead mothers with rapid/high virus transmitted in 2 out of 3 cases a slow/low type.

In conclusion, mothers in contrast to their infants harbour a homogeneous virus population. The results indicate that selection of an HIV-1 variant occurs either at transmission or during initial replication in the child. No characteristic molecular features of the transmitted virus could be identified. Moreover, viruses with rapid/high replicating capacity do not have a selective advantage during transmission.

Q 359 COLLABORATIVE STUDY OF HIV QUANTITATION IN SEMEN,

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Knowledge of the biological factors that influence sexual transmission of HIV including changes in HIV infectivity in genital secretions is critical to prevention efforts and vaccine development. Currently, assays of HIV from genital tract secretions are problematic because of the limited volume of specimens, presence of toxic and inhibitory factors, and variable stability of HIV. The prevalence of HIV in genital secretions may also be affected by clinical and biological variables such as the frequency of sample collection and processing of specimens, disease stage, inflammation, presence of HIV antibodies, and therapeutic intervention.

The objective of this study is to optimize methods to detect and quantitate HIV in semen. Eight laboratories with established interest and expertise are participating in a collaborative study utilizing co-culture with PBMCs, RNA and DNA PCR to compare sensitivity and specificity of HIV detection in human blood plasma vs seminal plasma.

Standardized frozen aliquots of cell-free HIV-1 JR-CSF and PBMC infected with HIV-1 JR-CSF, aliquots of pooled frozen seminal plasma and blood plasma from seronegative men were provided to participating laboratories by the NIH AIDS Research and Reference Reagent Program. The results of this collaborative study will be presented.

Q 360 HIV-1 TAT STIMULATES LFA-1/ICAM-1 DEPENDENT AGGREGATION, Sandra C. Smole, J. Wade Harper, JoAnn Trial, Arline Laughter and Roger D. Rossen, Departments of Microbiology & Immunology, Medicine, and Biochemistry, Baylor College of Medicine and the VA Medical Center, Houston, TX 77030

Infection of monocytoid cells with HIV-1 increases cell surface expression of the β_2 integrins, induces homotypic aggregation in the presence of PMA, and stimulates LFA-1/ICAM-1 dependent adhesion to activated endothelial cells. To investigate whether these phenotypic changes require infection or simply stimulation with a viral protein product, U1 cells and uninfected U937 cells were treated with purified recombinant HIV-1 tat protein. Tat alone had no effect in concentrations up to 4 μM, while costimulation with 1nM PMA and 1-4 μM HIV-1 tat resulted in significant homotypic aggregation of both cell lines. Treatment with low dose PMA (1 nM), tat buffer, "mock tat", or trypsinized tat (all +/- 1 nM PMA) did not induce homotypic aggregation. Reaggregation after resuspension could be blocked by incubating tat/PMA-treated cells (U937 or U1) with mAb to CD18 and CD54, although flow cytometric analysis revealed no change in cell surface expression of CD18, CD11a, or ICAM-1. Furthermore, a transactivation -defective mutant tat protein (dipeptide Glu-Phe insertion between Pro-18 and Lys-19) was able to induce a similar aggregation phenotype. We found that less tat was required to transactivate CAT expression in cells transfected with HIV-1 LTR-CAT constructs than was required for homotypic aggregation. In order to differentiate between tat that might signal at the cell surface and that which localizes to the nucleus, we compared the phenotype of cells treated with recombinant wild type tat protein versus those transduced with a tat retrovirus with or without PMA. Our studies show that comparable levels of CAT activity may be induced by exogenously added tat or transduction with a tat producing retrovirus. However, only exogenous tat plus PMA mediated homotypic aggregation. These findings suggested that tat can alter the activation of a constitutive host cell receptor/ligand pair by means of an initial 'outside-in' signalling. The ability of tat to induce homotypic aggregation defines an additional function for tat which is independent of its nuclear, transactivation function.

Q 361 HIV-1 PHENOTYPE IN PERINATALLY INFECTED CHILDREN. L. Terry Spencer, Jr., Wayne M. Dankner and Stephen A. Spector. University of California, San Diego, La Jolla, CA 92093.

In adults infected with HIV-1, phenotypic properties of viral isolates including replication rate and syncytia-induction (SI) capacity, have been associated with progression of disease. No data exist for children concerning clinical manifestations of HIV-1 infection and viral phenotypes; however, the often rapid progression of HIV-1 infection in infants makes the question of phenotypic variants associated with clinical disease particularly important. In these studies, peripheral blood isolates obtained within 7 months of birth (mean 2.8 ± 2.0 mos., median 2.8 mos) from 11 perinatally-infected infants were examined for SI capacity, by their ability to replicate in MT-2 cells. All 11 isolates were found to be non-syncytia-inducing (NSI) in MT-2 cells. In addition, 1 isolate obtained from a HIV-1 culture-positive cord blood sample did not induce syncytia. The infectivity of all isolates examined was confirmed by infection of peripheral blood mononuclear cells obtained from HIV-1 seronegative donors. In contrast to observations made in adult patients, the NSI phenotype did not correlate with asymptomatic HIV-1 infection. 7 (64%) of 11 patients developed *Pneumocystis carinii* pneumonia within 9 months (median 3.5 mos) of birth. Of the 10 patients followed for at least 12 months, all were classified as P-2 (symptomatic infection), and 3 died. Examination of sequential HIV-1 isolates obtained every 4-6 months from 8 of the patients (range: 6-39 months) revealed the development of MT-2 tropism and SI capacity in one isolate from a 35 month old patient. These data suggest that children infected perinatally with HIV-1 may undergo rapid disease progression without the appearance of SI HIV-1 isolates, and that the NSI isolates are frequently associated with symptomatic disease. The observation of NSI isolates in early infant samples and a single cord blood sample may reflect maternal characteristics, *in utero* selection, and/or characteristics of the fetal and neonatal immune system. It may also have implications for the mechanism of maternal-fetal transmission of HIV-1.

Q 362 **THE IMPORTANCE OF NEF IN THE INDUCTION OF HIV-1 REPLICATION FROM PRIMARY, QUIESCENT CD4 LYMPHOCYTES.** Celsa A. Spina, T. Jesse Kwoh, Michal Y. Chowers and Douglas D. Richman. UCSD School of Medicine, VA Medical Center and Baxter Diagnostics, San Diego, CA 92093.

HIV-1 can enter quiescent CD4 lymphocytes, establish a latent nonproductive infection, and subsequently be induced into productive replication through T cell activation and proliferation. Of the several regulatory genes which are unique to HIV and related primate lentiviruses, *nef* is proposed to have a role in viral latency due to proported negative effects on control of viral transcription. However, *in vitro* studies to address this hypothesis have produced contradictory data, while the *in vivo* model of SIV_{mac} infection demonstrates an essential, positive effect of *nef* on the development of disease pathogenesis.

During prior studies using acute *in vitro* infection of primary, quiescent CD4 lymphocytes with the Lai (LAV_{Bru}) strain of HIV-1, we observed the late appearance and accumulation of *nef* RNA transcripts in the absence of cell stimulation and productive virus replication. To investigate the potential impact of *nef* on HIV infection of primary T cells, virus clones with open and mutated *nef* reading frames were compared for replication competence. The *ORF* of the NL4-3 clone was used to make 2 isogenic *nef* mutants: a 185 bp deletion within the unique reading frame and a double stop within the initiation sequence. Even at a high multiplicity of infection, the *nef* mutated clones were severely handicapped (100-fold) in their ability to replicate following mitogen induction from quiescent T cells. Replication defects in the mutated clones were less apparent with infection of prestimulated, proliferating T cells. These results show a positive contribution of the *nef* gene to HIV life cycle, and indicate that *nef* may function in the biological setting of primary CD4 cells to facilitate viral transition from a latent state to a fully productive replicative cycle.

Q 363 PRODUCTION OF HIV FROM CERVICAL EPITHELIAL CELLS:IMPLICATION FOR SEXUAL TRANSMISSION Xin Tan and David M. Phillips, The Population Council, 1230 York Avenue, New York, NY 10021.

The cervical derived ME180 epithelial cells used in this study are characterized by numerous desmosomes and epidermal filaments. Although these human epithelial cells can be infected by HIV, immunocytochemistry, immunoblotting, and the inability of antibody to CD4 to significantly block infection suggests that they are CD4 negative. Electron microscopy revealed that infection was initiated by direct contact with H9 lymphocytes chronically infected with HIV strain MN. Following adherence to the epithelium the H9 cells shed numerous virions into a narrow space between the lymphocyte and the epithelial monolayer. Virions subsequently entered the ME180 cells via large vesicles. A few days after infection cytopathic effect and syncytia formation were observed, and some cultures subsequently died off. In other cultures, however, we rescued stable lines of productively infected cervical epithelial cells. PCR Southern blot hybridization revealed integrated HIV-1 genome, and the virus produced by the epithelial cells was normal as accessed by western blot analysis and the virus produced by the epithelium was infectious in human T cell lines. One of these cell lines produced an amount of virus equivalent to that of high secreting HIV infected lymphocytic cells as measured by p24 ELISA. In the electron microscope numerous virions were seen budding from the basal, lateral and apical surface of the epithelium. In addition, we saw a novel mechanism of virus production involving virus secretion into vesicles which subsequently released virus by fusing with the plasmalemma. These observations suggest that the cervical epithelium could be both a site for infection and a reservoir for HIV.

Q 364 SEQUENCE COMPARISON OF SEQUENTIAL ISOLATES OF HIV DERIVED FROM AN INFECTED MOTHER / INFANT PAIR, Nancy A. Wade and Lorraine Flaherty, Wadsworth Center for Laboratories and Research of the New York State Department of Health Albany, New York, 12208 and Department of Pediatrics , Albany Medical College, Albany , New York 12208

Factors involved in the transmission of HIV from mother to infant may include abnormalities in maternal immune status, phenotype and genotype of the virus, and intercurrent maternal infections. The level of maternally derived neutralizing antibody to the V3 loop of the HIV envelope may also affect transmission. We describe the sequence comparison from sequential isolates derived from DNA from an HIV infected mother and infant over an eighteen month period. A 982 bp fragment of DNA was amplified by the polymerase chain reaction in a nested procedure. The V3 loop and CD4 region were sequenced by the dideoxy chain termination method. Amino acid sequence comparisons were analyzed. In addition, maternal and infant CD4 counts and clinical course over this eighteen month period were corrrelated with amino acid sequence changes.

Q 365 VPU MEDIATES HIV-1 PARTICLES RELEASE. Xiao Jian Yao, Françoise Boisvert and Eric A. Cohen. Laboratoire de rétrovirologie humaine, Département de Microbiologie et Immunologie, Faculté de Médecine, Université de Montréal, CP 6128, Station A, Montréal, Canada, H3C 3J7.

The vpu protein is a phosphorylated integral membrane protein encoded by HIV-1 but not other closely related human and simian retroviruses. Previous studies have shown that expression of vpu decreases the rate of syncytium formation in infected CD4+ human T cells; ii) increases greatly the rate of release of virus particles from infected cell. This effect does not require expression of the envelope glycoproteins and CD4. Vpu has also been shown to affect the maturation of env glycoproteins by altering the intracellular association between the nascent envelope glycoprotein and CD4.

To study the mechanism by which vpu mediates the release of virus particles we have investigated the localization of the vpu protein in infected MT4 cells by indirect immunofluorescence using a specific rabbit anti-vpu polyclonal antibody. Our results indicate that while vpu accumulates mainly in the Golgi apparatus it can also be localized as very discrete spots in the cytoplasm. Association of vpu with the plasma membrane was not detected. Our immunolocalization experiments demonstrate that intracellularly vpu colocalized partially with the env glycoproteins but does not colocalized with the gag precursor. Cycloheximide treatment of infected cells followed by immunolocalization of vpu indicate that the possible pathway of vpu is from ER to Golgi to vesicles of the endosome. Vpu may affect the release of virus particles by altering properties of the plasma membrane where budding is occurring.

Pathogenic Mechanisms, Apoptosis, Superantigens, Antibodies, Antibody Responses, Animal Models

Q 400 HIV-2 INFECTION OF BABOONS: AN ANIMAL MODEL FOR PERSISTENCE AND PATHOGENESIS, Susan W. Barnett*, Krishna Murthy**, and Jay A. Levy*, *Cancer Research Institute, University of California, San Francisco, CA 94143-0128, **Southwest Foundation for Biomedical Research, San Antonio, TX 78227

Early studies in our laboratory indicated that the cytopathic HIV-2_{UC2} (UC2) strain from West Africa was able to persistently infect 1/1 baboon *in vivo* (Papio papio). For more than four years following challenge, virus can be consistently recovered from the PBMC of this animal and high anti-HIV-2 antibody titers are observed. In addition, this UC2-infected baboon is now exhibiting a decline in its total CD4+ lymphocytes. We have since extended these studies to include seven additional animals. Four of these were inoculated intravenously with approximately 5000 human $TCID_{50}$ of the UC2 strain following pre-selection of the animals for a high susceptibility of their PBMC to *in vitro* infection with this virus. Two of these animals demonstrated positive viral cultures from week 2 through at least week 48, and three of the four exhibited substantial anti-HIV-2 antibody titers during this period. Clinically, one animal presented with skin lesions, rash, and lymphadenopathy at four weeks. Two other HIV-2 strains, UC12 and UC14, have been identified, which like UC2, are able to replicate efficiently and consistently in baboon PBMC *in vitro*. The UC14 strain has been recently inoculated into three additional baboons. The results of these infections will be reported.

Q 401 ANTIBODIES TO CLASS I MHC INHIBIT SIVsmmPBj 1.9-INDUCED PROLIFERATION BUT NOT INFECTION IN PIG-TAIL MACAQUE LYMPHOCYTES

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Previously, we have shown that Simian immunodeficiency virus, SIVsmmPBj 1.9, induced proliferation in human peripheral blood mononuclear cells (PBMC). In the present study we show that PBj 1.9 induced proliferative response in the PBMC from the pigtail macaque monkeys. This proliferative response was profoundly inhibited by monoclonal antibodies against human class I MHC, class II MHC and CD4, and partially inhibited by antibodies against CD18 and CD11a. The crossreactivity of these anti-human antibodies with PBMC from pigtail macaques was confirmed in binding assays followed by flow cytometry. Although antibody to class I molecules inhibited the PBj-induced proliferation, it did not inhibit viral infection as shown by p27 analysis. By contrast, the other antibodies inhibited virus-induced proliferation as well as virus infection. Based on these result, we discuss the probable ligand molecules involved in proliferative response of PBMC from macaque to SIVsmmPBj 1.9 and the relationship with virus infection.

Q 402 DOES LYMPHOCYTE FUNCTIONAL IMPAIRMENT CORRELATE WITH OTHER HIV-1 INDUCED IMMUNOLOGICAL CHANGES? Hong Z. Bass, Susan Plaeger-Marshall, Parunag Nishanian, Daisy Wang, Roger Detels, William Cumberland, Margaret Kemeny and John L. Fahey, CIRID at UCLA, UCLA School of Medicine and Public Health, Los Angeles, CA. 90024-1747

Human immunodeficiency virus type-1 (HIV-1) induce substantial impairment of immune function which can be evaluated by reduced lymphocyte proliferative response to antigen and mitogen stimulation. This impairment is a good indicator of the relative hazard of disease progression and is only partly dependent on reduction in CD4 cell number. However, testing for proliferative function has not been compared to other major immune changes in HIV-1 infection. The present study is undertaken to determine if immune functional defects are paralleled by other major alterations caused by HIV-1. These include immune system activation, indicated by increased serum levels of soluble products which are good predictors of disease prognosis or changes in lymphoid phenotypic markers detected by flow cytometry. Our study was conducted in 118 HIV-1 seropositive subjects. Changes in lymphocyte function have been determined by proliferative response to PHA and PWM mitogens. Other significant indicators of disease activity that have been assessed included (a) three serum immune activation markers (neopterin, β2 microglobulin and soluble IL-2 receptor; (b) four phenotypic markers that may reflect activation of the lymphocytes (CD38, HLA-DR, CD71 and CD25); and (c) serum p24 levels. Calculations of correlation coefficients revealed that the lymphocyte functional impairment reflected by decreased proliferative response in vitro is largely independent of other immunological changes induced by HIV-1 infection. Based on these results, we suggest that proliferative capacity should be an additional measurement, separate from other parameters, for evaluation of HIV-1 disease status and therapy.

Q 403 REGULATION OF BCL-2 EXPRESSION IN T CELLS, H. Elizabeth Broome and Catherine M. Dargan, Department of Pathology, University of California, San Diego 92093

Forced expression of the bcl-2 protein in transgenic mice thymocytes and post-thymic T cells inhibits the induction of apoptosis in these cells by various stimuli or by withdrawal from growth factors. There is recent evidence that T cells from patients infected with the HIV I virus are more susceptible to the induction of apoptosis after various stimuli including anti-CD3 and pokeweed mitogen. Therefore, we decided to further investigate the role of bcl-2 levels in the susceptiblity of T cells to apoptosis. Murine splenic T cells have relatively high levels of bcl-2 compared to thymocytes, and about 50% remain viable in culture for over 4 days without activation. However, over the 4 days, there is a gradual decline in cell numbers by apoptosis without any change in the overall bcl-2 levels as determined by immunoblotting. Concanavalin A (Con A) activated splenic T cells undergo IL2 dependent proliferation. Our data show that there is a correlation between the dose of IL2 supplied to Con A activated T cells and the level of bcl-2. Also, our data show that there is a correlation between the levels of bcl-2 in activated post thymic T cells and their susceptibility to apoptosis. Therefore, the IL2 concentration dependent level of bcl-2 expression in T cells probably affects their susceptibility to apoptosis. Freshly isolated peripheral blood T cells from HIV-I infected individuals have the same overall levels of bcl-2 as controls by immunoblotting. Currently, we are investigating the levels of bcl-2 in human peripheral blood by flow cytometry to determine the heterogeneity of expression among T cells, both freshly isolated and activated in culture.

Q 404 A TRANSGENIC MODEL OF HIV GENE EXPRESSION: DEVELOPMENTAL REGULATION Jeanine M. Buzy, Lynn M. Lindstrom, Janice E. Clements, Dept. of Neuroscience and Comparative Medicine, Johns Hopkins School of Medicine, Baltimore, MD 21205

HIV infection of the central nervous system (CNS) in children frequently results in encephalopathy causing developmental delays including a deterioration of motor skills and intellectual abilities. HIV antigens, DNA and RNA have been detected in prenatal and postnatal CNS tissue establishing the brain as a major target organ of HIV in children. We have previously described a model of HIV gene expression in the CNS. Transgenic mice were generated using the LTRs of two CNS-HIV isolates from the frontal lobe and cerebrospinal fluid of a single patient (JR-FL and JR-CSF, respectively). These LTRs were linked to the reporter gene for B-gal. Expression of B-gal mRNA was quantitated by RNAse protection and the localization of protein by X-gal staining. The CNS derived HIV-LTRs controlled expression in neurons of the brain of adult transgenic mice (Science, in press).

In this study we examined the expression controlled by CNS derived HIV LTRs in the developing CNS. Strikingly different levels and patterns of expression of B-gal were observed compared to adults. In the developing nervous system B-gal expression was present in the telencephalon and future brainstem in the HIV-LTR-FL lines as early as embryonic day 13. Endothelial cells in the skin and the developing lens demonstrated strong expression in the HIV-LTR-CSF lines. Strikingly, within the dorsal root ganglion, X-gal was observed in both LTR transgenic lines in utero and postnatally, in contrast to the adult spinal cord and dorsal root where no X-gal was detected. On postnatal day 1 both transgenic lines contained strong X-gal staining in subcortical regions including the pontine nuclei, the brainstem and cerebellum. X-gal staining was found in cells of both neuronal and glial origins suggesting that both cell types contain cellular factors that activate expression of the LTRs. The dramatically different levels and localization of gene expression from the two HIV CNS-LTRs in the embryonic and newborn mouse compared to the adult suggests that cells in the developing nervous system support higher levels of gene expression than the adult.

Q 405 **HIV-1 VIRUS PRODUCTION AND APOPTOSIS IN CD4$^+$ T CELLS DURING AN IMMUNE RESPONSE TO VIRUS PULSED DENDRITIC CELLS.** Paul Cameron, Melissa Pope, Stuart Gezelter, Jeanne Barker, Ralph Steinman. Laboratory of Cellular Physiology and Immunology, Rockefeller University 1230 York Ave, New York, NY 10021.

We have previously shown that HIV-1 pulsed but uninfected dendritic cells are able to efficiently carry virus to CD4$^+$ T cells during antigen specific or mitogen induced clustering. The ability of allogeneic dendritic cells to cluster CD4$^+$ T cells was used to study the mechanism of loss of antigen specific CD4$^+$ T cells. Dendritic cell enriched fractions or sorted dendritic cells were prepared from blood mononuclear cells by depletion of T cells and FcR$^+$ cells and floating on metrizmide gradients. DCs pulsed with HIV-1 or with heat inactivated virus efficiently cluster resting allogeneic CD4$^+$ T-cells. By reverse transcriptase assay on the culture supernatants, virus production is evident as early as 3 days in the separated clusters but little if any production occurs in the non cluster T cells. After 5 days an increased proportion of non-viable cells is seen in the cluster fractions of cultures with live virus but not heat inactivated virus or unpulsed controls. Acridine orange / ethidium bromide staining shows chromatin changes of apoptosis in the viable and non viable cells. Combined Immunofluorescence for HIV-1 p24 antigen and in situ nick translation to detect cells undergoing apotosis shows a correlation between the apoptosis and productive infection. cells. This relationship can be seen at the single cell level. The dendritic cells that appear as strongly class II positive cells with characteristic morphology within clusters show no cytoplasmic p24 staining but accumulate viral antigen. Electron microscopy shows abundant viral particles associated with dendritic cells but viral budding occurs only on the T blasts.

Loss of antigen specific T cells in this model occurs by mechanisms including apoptosis of productively infected CD4$^+$ T cells. Dendritic cells accumulation of virus and transmission to the clustering T cells facilitates this process.

Q 406 ANTIBODIES AGAINST CD4 PEPTIDES BLOCK BINDING OF GP120 TO NATIVE CD4

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Effectiveness of immunity to HIV that is based on disruption of binding of gp120 to CD4 by means of Ab raised against gp120 may be limited by antigenic variability of the gp120 protein. Thus we are investigating an alternative approach to a therapeutic HIV vaccine, based on disruption of this critical molecular interaction by means of Ab versus CD4. We have selected three peptide sequences within the V1 region of CD4, based on their proposed functions as deduced from published studies on crystallography and functional mapping. After synthesis, these peptides were encapsulated in liposomes and used in immunization of rabbits. Without exception, every rabbit responded to its respective immunogen, producing high titer antisera reactive against peptide. All antisera also cross-reacted strongly with a (recombinant) soluble CD4 fragment. Preliminary results of ELISA-type experiments with a subset of these antisera demonstrate inhibition of binding of gp120, presumably as a result of Ab binding to CD4. We plan to extend these studies to include cell binding experiments using CEM cells as a source of native CD4.

Q 407 EFFECT OF ANTIBODY VALENCY AND SIZE ON INTERACTION WITH CELL-SURFACE EXPRESSED VIRIONS AND VIRAL NEUTRALIZATION

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Antibody fragments [F(ab) and $F(ab')_2$] generated from the human monoclonal antibody, F105, reactive with the CD4 binding site of HIV-1/gp120 were compared to intact F105 for cell surface binding and viral neutralization. Antibody binding to cell surface expressed antigen was measured by flow cytometry using equivalent concentrations of antibody and goat anti-human kappa chain secondary antibody. Identical binding curves were obtained with the IIIB isolate for F105 IgG (bivalent) as well as F(ab) (univalent) and $F(ab')_2$ (bivalent) fragments. In contrast, despite identical binding curves on RF infected cells for F105 IgG and $F(ab')_2$ antibody, F(ab) antibody fragments failed to bind to this isolate. Equimolar concentrations of F105 IgG and fragments were tested for viral neutralization of cell free virus in an MT-2 assay. While identical binding curves for both F(ab) and $F(ab')_2$ antibody fragments and intact F105 was seen with IIIB infected cells, neutralization equivalent to F105 IgG was obtained only with $F(ab')_2$. At all concentrations tested, viral neutralization by F(ab) fragment was four-fold lower than either F105 IgG or $F(ab')_2$. Binding and neutralization results similar to IIIB were obtained using the MN isolate. F105 IgG and $F(ab')_2$ were comparable in neutralization of the RF isolate which requires high concentrations of F105 to achieve modest neutralization. F(ab) had no neutralization activity on the RF isolate. Thus, the interaction of antibody with viral antigen is a complex phenomenon. For some viral isolates, e.g. RF, despite excellent reactivity with cell-surface expressed antigen, only modest neutralization of cell free virus occurs. From these results it appears that bivalent interaction may be important in binding and neutralization of virus and that this interaction is more dependent on possible changes in oligomeric gp120 conformation on intact virions and cell surface than on affinity. Antibody size appears to be less a factor than valency. Further characterization of this interaction may have important implications in the design and use of immunotherapeutics and vaccines. Supported by NIAID AI26926 and AI07387.

Q 408 A $CD8^+CD5^-$ SUB-POPULATION IS EXPANDED IN PERIPHERAL BLOOD OF AIDS PATIENTS

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A small T cell sub-population showing the CD8+CD5- phenotype accounts for 14.6±7.1% of circulating T cells in seronegative subjects; we observed an increase in this subset (27.4±13.6, $p<0.01$) in seropositive patients with limited disease progression (stages WR1-WR3), and a much more evident rise in patients with advanced disease (stages WR4-WR6; 37.6±14.8, $p<0.001$). To better understand the meaning of this alteration, we characterized the phenotypic and functional properties of this T cell subset in both seronegative and seropositive individuals. Compared to their CD5+ counterpart, CD8+CD5- cells are exclusively TCR alfa/beta+, and show preferential expression of NK cell function-associated markers (CD16, CD56, CD57); activation markers (CD25, CD71, HLA-DR) are not evident on these cells. Compared to the unfractionated CD8+ population, CD8+CD5- cells are less able to respond in vitro to allogeneic stimulation; CD8+CD5- cells are capable of mediating cytotoxic activity after primary MLR, but cytotoxicity levels against unrelated targets are comparable to those exerted against the primary stimulator. The Vbeta usage of these cells is now under study. As BMT patients undergoing GVHD is the only condition in which such an expansion of CD8+CD5- cells has been described, our observations deserve further study, in view of the possibility that GVHD mechanisms are at work in AIDS pathogenesis.

Q 409 ANTIBODIES AGAINST THE THIRD VARIABLE LOOP OF GP120 BLOCK THE BINDING OF GP120 TO GALACTOSYL-CERAMIDE

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Galactosyl-Ceramide (GalCer) is expressed in a variety of cell types including, oligodendrocytes, schwann cells, and epithelial cells of the colon. Recent work has indicated that gp120 specifically binds GalCer and antibodies against GalCer inhibit HIV-1 infection of the GalCer expressing cell lines, SK-N-MC and U373 (both derived from the brain), as well as HT29 cells derived from the colon. These findings suggest that GalCer may have a critical role in facilitating the infection of these cells which all lack the CD4 receptor. Thus, it is important to better understand the interactions between gp120 and GalCer.

The objective of this study was to specify the gp120 epitope(s) that bind GalCer. To address this question a panel of site-specific anti-gp120 antibodies were pre-incubated with gp120 (gift of Raymond Sweet, SmithKline Beecham) and screened for the ability to block the binding of gp120 to GalCer immobilized on HPTLC plates.

We tested four monoclonal antibodies against the V3 loop and found that all blocked gp120/GalCer binding. In contrast, anti-gp120 antibodies directed against the amino-terminus, first variable loop, third disulfide loop, and carboxyl-terminus failed to inhibit gp120/GalCer binding. Only 2 of 11 non-V3 antibodies interfered with gp120/GalCer binding: 110-1(carboxyl-terminus); and F105 (CD4 binding domain) both of which blocked to intermediate levels. We subsequently found that 110-1, but not anti-V3 antibodies bind gp120 already bound to GalCer. This indicates that the 110-1 binding domain is distinct from the GalCer binding site. The ability of 110-1 to block gp120/GalCer binding when pre-incubated with gp120 may be due to other factors such as steric hindrance.

Consistent with the results obtained in these gp120/GalCer binding experiments, we found that the anti-V3 antibody, 0.5β neutralized the GalCer-dependent infection of HT29 cells by HIV-1(IIIB), while 110-1 failed to interfere with infection.

Taken collectively, these results suggest that binding of gp120 to GalCer involves the V3 loop and possibly other components of the CD4 binding domain that may interact with the V3 region of gp120.

Q 410 PROBLEMS IN ESTIMATING THE DISTRIBUTION OF CD4+, NEOPTERIN, AND OTHER FACTORS AT THE TIME OF AIDS_DEFINING DIAGNOSES,

Robert J. Currier, William G. Cumberland and Jeremy M. G. Taylor, Department of Biostatistics, University of California at Los Angeles School of Public Health, Los Angeles, CA 90024

A fundamental unknown is the distribution of any of the markers, such as CD4+ level, at the time of diagnosis with an AIDS-defining illness. One should rather say distributions, since it is clear that, for example CD4+ levels of people diagnosed with Kaposi's sarcoma are significantly higher than those of people diagnosed with Pneumocystis carinii pneumonia.

A naive analysis based on only people who have been diagnosed leads to a biased estimate of these distributions; it fails to consider those people who have not yet been diagnosed. In traditional survival analysis, these are "censored" observations. In the standard situation, Kaplan-Meier estimates of the survivor function take into account both the "failures" and the "censored observations".

Our approach differs in two important ways from this traditional setting. First, we replace time in the survival model by the level of some marker, like CD4+ level. We use the fact that CD4+ level has an underlying downward trend, around which there are random fluctuations. We use estimates of the trend to provide a scale. Second, we use bivariate or multivariate generalizations of the standard Kaplan-Meier estimates to give estimates of the joint survivor function. From this estimated joint distribution, it is possible to derive conditional distributions of one factor, given levels of other factors. A possible use of these conditional distributions would be to provide an interpretative adjustment to CD4+ levels based on values of other markers.

Q 411 INTERNATIONAL COLLABORATIVE STUDY TO COMPARE NEUTRALIZING AND IMMUNOCHEMICAL ASSAYS FOR MONOCLONAL ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS,

M. Patricia D'Souza and Gregory Milman, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

In a second Antibody Serological Project (ASP) study sponsored by the National Institutes of Health and the World Health Organization, 21 laboratories characterized a coded panel of monoclonal antibodies (MAbs) to HIV-1 envelope protein. The panel included seven human MAbs, seven murine MAbs, recombinant soluble CD4-immunoglobulin hybrid molecules (CD4-IgG), normal human immunoglobulin and normal murine immunoglobulin. The MAbs were evaluated by immunochemical (radioimmune precipitation, immunoblot, enzyme linked immunosorbent assay, radioimmune assay, peptide mapping) and neutralization assays.

The ASP MAbs were classified into three groups based on the epitopes they recognized in gp160: (1) anti-V3 MAbs that were effective at low MAb concentrations but neutralized only the homologous viral isolate, (2) an anti-gp41 MAb that recognized a conserved linear sequence in gp41 and was broadly neutralizing, and (3) anti-CD4 binding region MAbs that were broadly neutralizing at high MAb concentrations. The anti-CD4 binding region MAbs were 50-100-fold less efficient at neutralization when compared to CD4-IgG. The collaborative study demonstrated that laboratories using diverse neutralization assays varying considerably in sensitivity could agree on the relative neutralization characteristics of a diverse group of MAbs.

Q 412 SIV-ASSOCIATED INTESTINAL ABNORMALITIES AND CELLULAR TARGETS OF SIV IN THE INTESTINAL TRACT OF RHESUS MACAQUES,

Satya Dandekar, Carla Heise, Peter Vogel and Andrew Lackner, Dept of Internal Medicine and California Primate Research Center, University of California, Davis, CA., 95616.

Intestinal dysfunction is frequently observed in HIV and SIV infection. To investigate the pathogenesis of SIV-associated intestinal disease, 24 rhesus macaques were followed prospectively for 24 wks. Animals were inoculated with either a biological isolate (SIVmac), a pathogenic (SIVmac-239) or non-pathogenic clone (SIVmac-1A11). At baseline, 2, 8, 13 and 24 wks, D-xylose absorption was assessed and intestinal biopsies were taken to analyze changes in clinical and functional parameters. Two animals from each group were killed at these timepoints and intestinal tissues were collected for combined in situ hybridization and immunohistochemistry to determine viral distribution and cellular targets of SIV. By 2 wks, D-xylose malabsorption was observed in 6/24 animals with the most severe changes noted in the group infected with the biological isolate. All animals had SIV infection of CD3+ T lymphocytes in gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes by 2 wks. The distribution of SIVmac and SIVmac-239 was primarily perifollicular, in contrast to infection of follicular centers by SIVmac-1A11. By 8 wks, one animal infected with SIVmac-239 appeared to develop a macrophage tropic variant, with SIV-infected macrophages distributed throughout the GI tract. In addition to SIV-infected cells in the mesenteric lymph node, free viral particles were detected in the interstitium of the germinal centers. Malabsorption and decreased digestive enzyme activity were observed. At necropsy, a proliferative enteritis of the small intestine was diagnosed, however no opportunists were identified. The distribution of SIVmac was unchanged at 8 wks whereas the infection was significantly decreased in the SIVmac-1A11 group. By 24 weeks, SIVmac-1A11 was virtually absent and SIVmac-239 infection was significantly reduced, however the biological isolate was widespread. One animal developed a typical SIV-associated enteropathy with severe diarrhea and wasting. The majority of SIV+ cells were CD3+ T lymphocytes localized in GALT, lamina propria and villus epithelium. These results indicate that SIV infection of T lymphocytes and macrophages within the intestinal tract is an early occurance and allows persistence and dissemination of the viral infection. In addition, intestinal SIV infection is associated with epithelial cell dysfunction as determined by clinical malabsorption and decreased digestive enzyme activities. The effect on epithelial cells may be due to cytokines disrupting the integrity of the epithelium or interfering with epithelial cell differentiation.

Q 413 THE COMBINED ASSESSMENT OF CELLULAR APOPTOSIS, MITOCHONDRIAL FUNCTION AND PROLIFERATIVE RESPONSE TO PWM HAS PREDICTIVE VALUE IN HIV/SIV INFECTION,

Ana M. del Llano, Julio A. Lavergne, University of Puerto Rico, Medical Sciences and Humacao Campuses.

Routine monitoring of HIV/SIV infection includes the assessment of clinical parameters of disease progression, such as persistent lymphadenopathy, diarrhea or weight loss. However, these clinical signs usually become evident only after significant immunologic impairment has already occurred. The need for the development of early markers of disease progression, which correlate with the later appearance of viral antigenemia and depletion of blood CD4 lymphocytes is therefore evident. This work describes a new approach to the prognostic evaluation of HIV/SIV infection, utilizing a three-parametric functional assessment of peripheral blood mononuclear cells. This tri-functional in vitro evaluation allows for the classification of infected individuals in convenient stages, according to the number of in vitro parameters affected. The classifying parameters used are: the mitochondrial activity of freshly isolated blood mononuclear cells (BMNC), measured by an MTT-reduction assay; the detection of apoptosis in 72 hour cultures of BMNC, assessed by propidium iodide staining and dual-parametric flow cytometric analysis; and the proliferative response, measured by flow cytometry, of BMNC to pokeweed mitogen (PWM), an established correlate of immune function. Results obtained indicate that the BMNC from HIV/SIV-infected subjects may present one or more of the following in vitro characteristics: 1) an impairment in their mitochondrial dehydrogenase activity; 2) development of various degrees of apoptosis when cultured for 72 hours and then examined for their nuclear granularity and DNA content (A_0 zone) by flow cytometry and 3) a poor proliferative response to the mitogen PWM. Thus, the combination of observed parametric results among individuals has allowed us to develop a versatile staging system with prognostic value in HIV/SIV infection. Based on these observations, patients or infected macaques have been classified in four stages: Stage 0, which includes uninfected controls and all other individuals with unaffected parameters; Stage 1, with subjects having only one parameter affected; and Stages 2 and 3, which include subjects presenting two or all three impaired parameters, respectively. The patient or animal progression through stages 0 to 3 may take place even before CD4 cell depletion occurs, indicating its early prognostic value in HIV/SIV disease. Supported by NIH-RCMI RR-03051.

Q 414 HUMAN MONOCLONAL ANTIBODIES AGAINST HIV

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Human monoclonal antibodies (HMabs) against HIV antigens were obtained after immortalisation with Epstein-Barr virus or fusion with a murine myeloma of B lymphocytes from HIV seropositive individuals or after primary or secondary *in vitro* stimulation of spleen cells and peripheral blood lymphocytes from seropositive or seronegative subjects. Antigens used for *in vitro* stimulation were conserved "octopus"-conjugated synthetic peptides of gp41 or gp120 V3 loop of MN. A total of 12 HMabs will be presented with their biological and immunological characteristics. Two types of neutralizing HMabs were obtained, some directed against 583-599 epitope presented the capacity to inhibit syncitia formation between H9 infected with different HIV strains and SUPT1 target cells and some others (against 642-665 and V3 loop) neutralized cell-free virus infection of MT4 cells, PBLs and CEM-SS cells with different HIV strains. Activation of complement by these HMabs will be presented as well as their enhancing effect on HIV1 infection. The cooperative neutralization of HIV1 by some of these HMabs were studied and the difterent levels of synergy will be presented. The synergistic neutralization of HIV by a combination of HMabs may be particularly effective in passive immunotherapy against the virus.

Q 415 EARLY AND PROGRESSIVE HELPER T CELL DYSFUNCTION IN FELINE LEUKEMIA VIRUS INDUCED IMMUNODEFICIENCY,

Lauri J. Diehl and Edward A. Hoover, Department of Pathology, Colorado State University, Ft. Collins, CO 80523.

Cats infected with the highly pathogenic feline leukemia virus isolate FeLV-FAIDS develop an immunodeficiency syndrome characterized by progressive loss of CD4+ T cells and eventual pan-lymphocyte depletion. Prior to the decline in circulating CD4+ cells, infected cats are unable to mount primary antibody responses to T-dependent antigens. We investigated whether altered ability of helper T cells to produce cytokines necessary for B cell growth and differentiation might be a primary event in the pathogenesis of FeLV-FAIDS infection. We found that as early as 9 weeks after infection, lymphocytes from cats with normal CD4+ cell numbers produced significantly lower levels of B cell stimulatory cytokines. This deficit became progressively more severe with time. By contrast, similar levels of IL-2 are produced by lymphocytes from control and infected cats. Also, resting B cells isolated from infected cats did not differ from controls in ability to undergo activation and differentiation to antibody secreting cells. The results suggest that a selective and progressive defect in Th function occurs prior to CD4+ T cell depletion early in the course of FeLV-FAIDS induced immunodeficiency.

Q 416 HIGH LEVELS OF MONOCYTE INFECTION CORRELATE WITH FELINE IMMUNODEFICIENCY VIRUS PATHOGENICITY,

Steven W. Dow, Lauri J. Diehl, Lynne L. O'Neil, Edward A. Hoover, Department of Pathology, Colorado State University, Fort Collins, CO, 80523.

Cats infected with pathogenic strains of feline immunodeficiency virus (FIV) typically develop lymphadenopathy, weight loss, severe CD4+ lymphocyte depletion, and opportunistic infections. We have isolated several new FIV isolates that rapidly induce immunodeficiency in SPF cats and reach high titer peripheral blood mononuclear cell - associated and cell-free (plasma) viremia (Hoover EA; manuscript in preparation). To identify the major FIV target cells in circulating PBMC, we utilized immunocytochemical techniques to detect FIV antigen expressing cells in separated populations of lymphocytes and monocytes at various times post culture. We have found unexpectedly high levels of monocyte infection, with up to 30% of this cell population expressing large amounts of FIV antigens after several days in culture. FIV p26 antigen assay and PCR for proviral DNA also confirmed the high level of monocyte infection. By contrast, fewer than 1 in 10^4 to 1 in 10^5 lymphocytes expressed FIV antigens after mitogen activation and several days in culture. Infected monocytes were detected in blood of 24 cats infected with 6 pathogenic FIV strains. Moreover, the level of monocyte infection correlated with the severity of CD4 depletion and clinical disease. By contrast, FIV-positive monocytes could not be detected in 6 cats infected with avirulent FIV strains. We conclude that infected monocytes represented a major FIV reservoir in infected cats and that the level of monocyte infection is associated with FIV disease pathogenesis.

Q 417 **APOPTOSIS IN HUMAN HIV-1 INFECTED THYMOCYTES**

, Athena Economides, Deborah J. Anisman, Ingrid Schmid, Jerome A. Zack, Esther F. Hays, and Christel H. Uittenbogaart. UCLA School of Medicine, Los Angeles, CA 90024

To determine if programmed cell death (apoptosis) plays a role in HIV infection of immature human T cells, we studied human thymocytes that were infected with the clinical HIV-1 isolate JR-CSF, and were cultured in serum free medium alone or in the presence of cytokines interleukin 2 (IL-2) in combination with IL-4. These cytokines cause synergistic HIV production by thymocytes as measured by p24 levels. Apoptosis was measured by DNA staining using 7-amino-actinomycin-D (7-AAD) and hypotonic propidium iodide (PI) and flow cytometry at different times during in-vitro culture. In addition, surface staining with CD8 and CD4 was performed on viable cells. Thymocytes normally undergo apoptosis, but our results on non-infected thymocytes showed that that there is great variability as to when apoptosis is most pronounced during culture. We found that HIV-infected thymocytes show an increase in apoptosis as compared to non-infected cells cultured in parallel. Therefore, in-vitro exposure to the virus and/or HIV proteins may play an important role in changing the conditions for apoptosis, by intensifying this normally occuring process. We are currently examining which cytokines induce and which inhibit programmed cell death in human thymocytes.

Q 418 SIVmac ENVELOPE GENE DIVERSITY AND DISEASE PROGRESSION, Edmonson P.F.[1], Murphey-Corb M.[2], and Mullins J.I.[1,3]. [1]Committee on Virology, Harvard Medical School, Boston, MA 02115, [2]Tulane Regional Primate Center, Covington, LA 70433, [3]Dept. of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402,

The molecularly cloned virus SIVmac251 clone BK28 (BK28) is minimally pathogenic in rhesus macaques, causing death in only 1 of 12 macaques by 2 years post-inoculation (p.i.). However, upon in vivo passage the BK28 virus has become more virulent. The virus isolate SIVmacF965 (F965) was obtained from bone marrow of a BK28 infected macaque that died 17 months p.i. Inoculation with F965 has led to death by opportunistic infection in 18/20 macaques by 2 years p.i. In an effort to understand what genetic changes are responsible for transforming a minimally pathogenic virus into an acute pathogen, sequence analysis of the proviruses present within macaques infected with either the minimally pathogenic molecularly cloned virus BK28, or the acutely pathogenic F965 isolate was undertaken. Proviral DNA was examined at early and late times p.i., and during asymptomatic and disease states. Comparison of over 150 kb of gp110 envelope sequences from 140 independently PCR amplified viruses has yielded the following observations.

Virus sequences obtained after in vivo passage contain a small number of consistent changes relative to the molecular clone BK28. Notably, viruses are found which carry a group of 3-5 additional sites for potential N-linked glycosylation in all of the animals examined. Selection for viruses carrying these specific glycosylation sites suggests that the sites are important for virus growth in vivo. Mutations affecting sites for potential N-linked glycosylation are found throughout the 1.1kb region examined, whereas mutations not directly involved in N-linked glycosylation are concentrated within the V1 and V4 regions.

A replication competent chimeric virus representative of the consensus genotype from the F965 infected macaques was constructed and used to infect 5 macaques. The chimera consists of the 5' half of the BK28 genome, and the 3' half of a genome amplified directly from the thymus of a macaque that died 5.5 months after infection with the F965 isolate. Early results indicate that the chimera displays in vivo growth properties more characteristic of the acutely pathogenic F965 isolate than of BK28, as demonstrated by the rapid induction of antigenemia.

Q 419 IDENTIFICATION OF PROTEOLYTIC PROCESSING SITES WITHIN THE Gag AND Pol POLYPROTEINS OF FELINE IMMUNODEFICIENCY VIRUS, J.H. Elder, M. Schnölzer, C.S. Hasselkus-Light, M. Henson, D. A. Lerner, T.R. Phillips, P.C. Wagaman and S.B.H. Kent; The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA. 92037

N-terminal amino acid sequencing, ion spray mass spectrometry, and cleavage of synthetic peptide substrates was employed to identify the N- and C-termini of the mature Gag and Pol proteins of FIV. The Gag polyprotein encodes matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. The Gag-Pol polyprotein encodes, in addition to the above proteins, aspartate protease (PR), reverse transcriptase (RT), deoxyuridine triphosphatase (DU), and integrase (IN). Secondary cleavage of RT at Trp^{595}/Tyr^{596} of Pol yields a truncated form lacking the C-terminal RNAse H domain. Observed versus expected molecular weights of the viral proteins were in agreement, with three exceptions. 1) The molecular weight of MA was 14,735 daltons, compared to a predicted mass of 14,649, based on a single cleavage at Tyr^{135}/Pro^{136} of Gag. The observed molecular weight is consistent with myristolation of MA, which was confirmed by metabolic labeling of FIV MA with ^{3}H-myristic acid. 2) The N-terminus of the NC protein is generated via cleavage at Gln^{366}/Val^{367} of Gag, which predicts a mass of 25,523 for CA and 9,101 for the major form of NC. The observed mass of CA was 24,569, consistent with loss of nine C-terminal amino acids by a second cleavage of CA at Leu^{357}/Leu^{358}. Synthetic FIV protease accurately cleaved synthetic peptide substrates containing this site. 3) The actual mass of NC was approximately 2 Kd smaller than the mass predicted by synthesis to the stop codon at the end of the Gag (7,118 actual, versus 9,101 predicted mass). Experiments are in progress to characterize additional cleavage(s) in NC.

Q 420 ESTABLISHMENT OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I (HTLV-I) T-CELL LYMPHOMAS IN SCID MICE, Gerold Feuer, Jerome A. Zack, William J. Harrington, Jr*, Ramon Valderamaφ, Joseph D. Rosenblatt, William Wachsmanψ, Stephen M. Bairdω, Irvin S.Y. Chenδφ, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA; *Center for Blood Diseases, Jackson Memorial Medical Center, 1475 N.W. 12th Avenue, University of Miami, Miami, FL; φ30 E. 60th Street, New York NY; ψHematology/Oncology V-III-E, UCSD School of Medicine, 3350 La Jolla Drive, San Diego, CA; ωLaboratory Service 113, Department of Veterans' Affairs, Medical Center, 3350 La Jolla Village Drive, San Diego, CA; δDepartments of Microbiology & Immunology and Medicine, UCLA School of Medicine, Los Angeles, CA.

Factors leading to tumor progression following human T-cell leukemia virus (HTLV) infection remain largely unknown. Studies of the mechanisms important in tumorigenesis have been hampered by the lack of a suitable animal model. To explore the possibility of developing a mouse/human chimera model to study HTLV type I (HTLV-I), severe combined immunodeficient (SCID) mice were injected intraperitoneally with peripheral blood lymphocytes (PBL) from patients diagnosed with adult T-cell leukemia (ATL), HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) or from asymptomatic HTLV-I-seropositive patients. Many of these mice became persistently infected with HTLV. Lymphoblastic lymphomas of human origin developed in animals injected with PBL from two ATL patients. The tumor cells resembled ATL cells in that they had monoclonal or oligoclonal integrations of the HTLV-I provirus and predominantly expressed CD4 and CD25 cell surface markers. Of note, cell lines derived by HTLV immortalization of T-cells *in vitro* did not persist or form tumors following introduction into SCID mice. This system represents the first small animal model to study progression of HTLV-I tumorigenesis *in vivo*.

Q 421 *Abstract Withdrawn*

Q 422 ALTERATIONS IN RNA SPLICING PATTERNS THAT CORRELATE TO DISEASE PROGRESSION IN HIV-1 INFECTIONS, Manohar Furtado and Steven Wolinsky, Infectious Diseases Section, Department of Medicine, Northwestern University, Chicago IL 60611 An increase in the levels of unspliced mRNA relative to spliced mRNA is characteristic of HIV-1 infections of cells in culture and is also observed when chronically infected cells are induced by cytokines. We evaluated changes in proviral DNA content and in the levels of different HIV-1 transcripts in sequentially obtained patient PBMC. Three patients who exhibited a rapid decline in their CD4+ cell counts to levels below 200/mm^3 within 30 months after seroconversion and three patients whose CD4+ cell counts remained stable and over 400/mm^3 for over 36 months after seroconversion were studied.

Following reverse transcription, oligonucleotide primers designed to specifically amplify multiply spliced, singly spliced, and unspliced RNA were used to amplify transcripts. 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 exon specific probes and quantified.

Our results indicate a very rapid increase in the ratio of unspliced to spliced RNA in the rapid progressors and moderate to no increase in the slow progressors. Transcripts encoding vif and vpr were detected only in the rapid progressors. RNA splicing profiles appear to be better correlates of disease progression than proviral DNA content probably because they reflect active viral replication.

Q 423 INFECTION OF PIG-TAILED MACAQUES WITH HIV-1 Suzanne Gartner,Yiling Liu, Mark Lewis, Victoria Polonis*, Yvonne Rosenberg, Charles Brown, Philip Zack* and Gerald Eddy Henry M. Jackson Foundation Research Laboratory; Rockville, MD *Div. of Retrovirology, WRAIR; Washington, D.C.

Towards developing a macaque model for HIV-1 infection, we evaluated the susceptibility/permissivity of peripheral blood mononuclear cells from three species of macaques to HIV-1 in vitro. Peripheral blood-derived T lymphocytes and monocyte/macrophages from pig-tailed macaques were considerably more permissive for HIV-1 than comparable cells from cynomolgus and rhesus macaques. Optimal infectivity was dependent upon the virus strain and multiplicity of infection used, and in the case of the T lymphocytes, their proliferative status. The kinetics of HIV-1 infection in pig-tailed macaque T lymphocytes was comparable to that observed for normal human T cells, but peak expression was only 1/10-1/100th that of human cells. Semi-quantitative PCR analyses indicated that the low virus expression observed was a consequence of limited permissivity rather than limited susceptibility of the cells. Although electron microscope examination revealed the presence of morphologically normal HIV-1 particles in the cultures, the infectiousness of these particles was frequently limited, as determined by infectivity experiments using human and macaque target cells.

Four pig-tailed macaques were inoculated with cultured HIV-1-infected autologous cells harvested at peak virus expression. HIV-1 was isolated from peripheral blood mononuclear cells from 3 of the 4 animals at various times during the first 10 weeks post inoculation (PI), and from biopsied lymph node tissue from one animal at week 6 and another at week 10. Additionally, HIV-1 genome was detected in some uncultured specimens from which infectious virus was not recoverable. During the first 12 weeks PI, no HIV-1-specific antibody was detected by Western blot, but in some cases, was detected by radioimmunoprecipitation. In vivo passage experiments are ongoing.

Q 424 NOVEL HUMAN LENTIVIRUS *pol* SEQUENCES ISOLATED FROM HIV-NEGATIVE AIDS CASES, Irwin H. Gelman*, Stephen M. Morse‡, and Jeffrey Laurence†, *Dept. of Microbiology, Mount Sinai Medical Center, New York, NY 10029, ‡The Rockefeller University, New York, NY 10021-6399, †Laboratory for AIDS Virus Research, Cornell Medical College, New York, NY 10021.

Co-cultures of PBMC from two AIDS patients (*Lancet*, 340:273-274, 1992) were positive for reverse transcriptase (RT) activity yet negative for HIV-1 or -2 infection by ELISA, western blot, p24 assay, and PCR using standard *gag*, *pol*, or *tat* primers. The RT activity could be banded on sucrose gradients at similar densities to HIV-1 in parallel gradients. RT activity could be induced in PHA-treated PBMC and in human macrophage/U937 hybridomas by cell-free transmission, resulting in the production of syncytia. Budding forms and mature, extracellular lentivirus-like particles were detected by electron microscopy in RT-positive PBMC co-cultures. PBMC co-culture DNA and cDNA synthesized from virion RNA purified from density gradients were amplified by PCR with degenerate primer sets complementary to *pol* sequences shared by all retroviruses (VLPQG/YMDD) or by lentiviruses alone (*AIDS Res Human Retrovir.*, 8:1981-1989, 1992). Analysis of cloned PCR products revealed sequences which were 74-93% similar at the amino acid level to HIV-1 *pol*. Almost all nucleotide changes encoded amino acid substitutions, often non-conservative. Primers specific for these novel sequences facilitated the amplification of appropriately-sized products by RT-PCR from PBMC co-culture RNA, which when cloned and sequenced, contained the collection of non-synonomous mutations derived by the lentivirus *pol* primer sets. Larger provirus and cDNA clones will be isolated using the novel *pol* sequences as probes. Data will be presented on efforts to develop a cell culture system for this virus. These results demonstrate the association of novel or highly variant strains of HIV, not detectable by standard means, with AIDS.

Q 425 A MOLECULAR CLONE OF HIV-1 TROPIC AND CYTOPATHIC FOR HUMAN AND CHIMPANZEE LYMPHOCYTES. Sajal K. Ghosh, Patricia N. Fultz, Michael S. Saag, Paul M. Sharp, Beatrice H. Hahn and George M. Shaw. Department of Medicine, University of Alabama at Birmingham, AL 35294.

Previous studies of HIV-1 replication in chimpanzee lymphocytes have been limited to a small number of virus isolates which generally replicated poorly and without cytopathic effect. Here, we describe an HIV-1 provirus (SG3), cloned as a single proviral unit, which replicated more efficiently in chimpanzee than in human lymphocytes, resulting in syncytium formation and cell death. This provirus also replicated efficiently and with extraordinary cytopathic effect in immortalized human T-cell lines. The SG3 genome was completely sequenced and found to contain all genes typical of HIV-1 with the exception of *vpu*. Phylogenetically, SG3 is representative of North American/European strains of HIV-1 and shows no greater similarity to SIV_{CPZ} in its envelope sequence than to other HIV-1 strains. In order to identify the molecular determinant(s) for chimpanzee lymphocyte tropism of this virus, we constructed chimeric viruses between SG3 and a prototype HIV-1 virus YU-2 (which does not grow in chimpanzee lymphocytes) by reciprocal exchange of the envelope gene and subgenomic portions of it. Preliminary studies indicate that the preferential replication of SG3 in chimpanzee lymphocytes is determined by the envelope gene and detailed studies mapping this determinant will be presented. Pilot studies also indicate that SG3 can infect and replicate in chimpanzees and SCID-hu mice *in vivo* following intravenous or intrathymic inoculation. The SG3 proviral clone, because of its replication efficiency in human and chimpanzee cells and its complete molecular description, represents a new virologic reagent for the coordinated molecular analysis of HIV-1 replication and pathogenesis in relevant animal model systems.

Q 426 TISSUE-SPECIFIC EXPRESSION OF HUMAN CD4 IN TRANSGENIC ANIMALS AS MODELS FOR HIV-1 INFECTION,
Frances P. Gillespie, Linda Doros, James A. Vitale, Catherine Blackwell, Joe Gosselin, Benjamin W. Snyder and Samuel Wadsworth, TSI Corporation, Innovation Drive, Worcester MA 01605.

The search for a vaccine or cure for AIDS has been hampered by the lack of suitable animal models. It is our intention to develop a small animal model through transgenic technology by expressing the human CD4 gene in certain species. CD4 is a glycoprotein found on the surface of lymphocytes which serves as the receptor for the HIV-1 virus. Recent evidence from several laboratories has demonstrated that rabbits and rabbit T-cell lines can be infected with HIV-1 under specific experimental conditions; and that certain T-cell lines become more susceptible to HIV-1 when human CD4 is expressed on the cell surface. We have cloned the gene for human CD4 and its accompanying regulatory sequences, and assembled these components into a transgene. This construct was initially tested in cultured cells and transgenic mice and found to direct high-level expression of human CD4 in those target cells and tissues which normally express murine CD4. Based on this evidence, we are developing transgenic rabbits expressing human CD4 in a tissue-specific manner.

Q 427 HIV ENHANCEMENT BY ANTI-CD4 MONOCLONAL ANTIBODIES

Marisela P. Gomez, James E. K. Hildreth, Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV infects cells by binding via its envelope glycoprotein, gp120, followed by fusion with the cell membrane. Infection with HIV can also occur by fusion of infected cells with uninfected CD4+ cells (syncytium formation). T cells, monocytes, and other CD4-bearing cells that are normally present in the host have been reported to be susceptible to infection by HIV. Antibodies against CD4 inhibit syncytia formation and viral entry into CD4+ cells. Preliminary experiments in our laboratory have shown that inhibition of HIV infection by murine monoclonal anti-CD4 antibody does not occur at all concentrations of antibody, and that an antibody-dependent enhancement of HIV infection occurs at 0.2 to 1.0 ug/ml of antibody. Antibody enhancement can be seen as a 10 to 50 fold increase in the viral p24 core antigen over that of infected cells without treatment with CD4 antibody. In addition, a decrease in syncytia formation and an increase in cell viability is also observed within this range of antibody concentrations. Studies in which AZT is added to the cultures at fixed time intervals, suggest that this increase in viral production occurs following reverse transcription of viral RNA. These studies could have important implications in light of the potential use anti-CD4 antibodies and soluble CD4 in the treatment of HIV infection. In addition, these studies may have implications with respect to the role of auto-anti-CD4 antibodies in disease progression.

Q 428 ENHANCED HIV-1 EXPRESSION CORRELATES WITH DEVELOPMENT OF AIDS: A MULTICENTER AIDS COHORT STUDY,
Phalguni Gupta, Lawrence Kingsley, John Armstrong and Charles Rinaldo, Department of Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, PA 15261

The development of AIDS may be significantly related to the level of HIV-1 replication. We have used quantitative cell culture and quantitative RNA PCR to measure viral expression in peripheral blood mononuclear cells (PBMC) obtained cross sectionally and longitudinally from HIV-1 seropositive homosexual men enrolled in the Pittsburgh portion of the Multicenter AIDS Cohort Study. Quantitative DNA PCR was also used to measure the level of proviral DNA in these cells. Results indicate that the number of virus-producing $CD4^+$ lymphocytes increased in association with the decrease in the absolute number of $CD4^+$ T cells. However, there was no correlation between the level of virus-producing $CD4^+$ cells and the duration of infection. Furthermore, the expression of HIV-1 gag RNA increased as the disease progressed and $CD4^+$ cell numbers declined in subjects who developed AIDS. Subjects who remained asymptomatic, however, exhibited a very low level of HIV-1 RNA expression during the entire period of follow up (38-60 months). In contrast to expression of viral RNA, the level of proviral DNA did not change significantly as the disease progressed. However, the levels of proviral DNA were consistently higher in AIDS patients than in men who remained asymptomatic. In most subjects, the levels of HIV-1 gag DNA were comparable to that of LTR-gag DNA, indicating that most of the subjects harbored HIV-1 DNA as a full length genome. These results support the role of HIV-1 RNA expression in the development of AIDS.

Q 429 HIV ENHANCING ANTIBODIES,
Carl V. Hanson, Terri Wrin, Lynette Sawyer, Leta Crawford, Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, CA 94704

Assays for the effect of HIV antibodies on HIV infectivity measure the net sum of antibody-dependent neutralization and antibody-dependent enhancement. Because human leukocytes express both F_c receptors and several types of complement receptors, we attempted to better mimic <u>in vivo</u> conditions by including high levels of active human complement in <u>in vitro</u> assays. Using MT-2 indicator cells (which express a high level of complement receptor CRII), strong antibody-mediated enhancement was observed using human immune serum or purified IgG, but to only a limited extent with purified $F(ab')_2$ fragments. HIV grown in PBMC is enhanced to a much higher extent than is HIV grown in H9 cells. With some samples, low-titered complement-mediated enhancement occurs in the absence of any net neutralization, and in others the enhancing titer greatly exceeds the neutralizing titer. Such enhancement is not seen, however, when PBMCs are used as the indicator cell. Cases of complement-independent antibody enhancement of HIV-1 have also been observed, and HIV-1 antibody can enhance the expression of HIV-2 in the absence of complement. While HIV enhancing antibody remains a concern in vaccine design, the possible artifactual nature of <u>in vitro</u> enhancement is suggested partly by the observation that there is much less enhancement with 50% complement than at the artificially low level of 10% complement.

Q 430 PROTEASE INHIBITORS REVERSE TcR MEDIATED PROGRAMMED CELL DEATH IN A $CD4^+$ MURINE T CELL HYBRIDOMA AND RESTORE IN VITRO T CELL RESPONSES IN SOME HIV+ INDIVIDUALS, Pierre Henkart, Mario Clerici, Apurva Sarin, and Gene M. Shearer, Experimental Immunology Br, NCI, Bethesda,MD 20892

As shown by others, the murine CD4+ T cell hybridoma 2B4 undergoes programmed cell death (PCD) and secretes IL-2 when stimulated by antigen or immobilized anti-TcR MAb. We find that at non-toxic concentrations, the cysteine protease inhibitors E-64, leupeptin, or Ac-Leu-Leu-nor-Met-H (ALLM), block the death process (measured after 12-20 hours by trypan blue or ^{51}Cr) down to unstimulated control levels, whereas the secretion of IL-2 is enhanced. Thus these inhibitors block at a later, death-selective step rather than an early step in TcR-mediated signal transduction. However, they enhance rather than block corticosteroid-induced PCD in 2B4. Because the PCD model for HIV pathogenesis predicts that the decline in immune function and $CD4^+$ cell number is a result of a TcR induced death similar to 2B4, we examined the in vitro responses of lymphocytes from asymptomatic HIV^+ individuals to a recall antigen, to allogeneic cells, and to PHA in the presence and absence of E64 and ALLM. Both IL-2 secretion and ^3H thymidine incorporation responses were measured. Of 32 HIV^+ individuals whose functional responses were deficient to one or more of the stimuli used, 9 had one or more of their responses improved by E-64 and 10 had one or more of their responses improved by ALLM. In some cases undetectable responses were restored to control levels by the inhibitors. The inhibitors were not stimulatory alone, nor did they generally enhance the responses of normal controls. We have also carried out preliminary studies of PCD of these cells in response to a mixture of pokeweed mitogen and Staphylococcal enterotoxin B after 48 hours in culture. A significant percentage of the HIV+ individuals who responded to this TcR signal with a PCD response showed reversal by the protease inhibitors. These results support the PCD model for HIV-induced immune dysfunction and pathogenesis, and suggest the possibility of therapy by cysteine protease inhibitors.

Q 431 ROLE OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS AND SYMPATHETIC NERVOUS SYSTEM (SNS) IN VIRAL PATHOGENESIS AND IMMUNITY Gerlinda Hermann and John F. Sheridan, Depts. Med. Micro. and Immunology and Oral Biology, Ohio State Univ., Columbus, OH 43210

Development of the immune response to antigenic challenges may elicit host defensive reactions that could result in excessive tissue damage if allowed to progress unchecked. Immunoregulatory mechanisms, such as elevated levels of corticosteroid hormones in response to infection, may have evolved to limit these defensive immune responses. The degree to which an immune response may develop before being checked may be genetically determined (Mason, 1991). Immunoregulatory mechanisms may be exacerbated during psychological or physiological stress. Thus, genetic variation in the response to stress may play a critical role in the susceptibility of an individual to autoimmune and inflammatory diseases. Physiological responses to stressors are mediated via the HPA axis and the SNS. These two systems are able to modulate the activity of various immune effector cells directly as well as alter the general physiology of the individual. The purpose of these studies was to investigate the role of glucocorticoids (GC) and catecholamines (CA) in viral pathogenesis. Our studies demonstrated that both C57BL/6 and DBA/2 mice elevate GC in response to infection with influenza A/PR8. This increase in GC levels was amplified when infected animals were subjected to daily restraint stress (RST). Elevated GC levels were associated with a reduction in the cell density of draining lymph nodes, reduced inflammation and pathosis of lungs when compared with food-water deprived (FWD)/infected animals. Although RST/infected DBA mice demonstrated an enhanced survival rate compared to their FWD/infected cohorts, this was not solely attributable to differences in their GC levels since tissue CA levels were elevated in the RST/infected DBA group relative to the FWD/infected group. Thus, the coordinated elevation of GC and CA might be responsible for the increased survival ratios seen in the RST/infected DBA/2 mice. Subsequent studies indicate that GC may be responsible for minimizing pulmonary inflammation and mononuclear cellular infiltration while the adrenergic innervation of lymphoid tissue may play a role in activation of virus-specific immune effector cells.

Q 432 ISOLATION AND TRANSMISSION OF HIGH EXPRESSION PATHOGENIC FELINE IMMUNODEFICIENCY VIRUS ISOLATES, Edward A. Hoover[1], Steven W. Dow[1], Matthew J. Dreitz[1], Stephen A. Gardner[2], and James I. Mullins[3]. [1]Colorado State University, Fort Collins, CO, 80523, [2]Albany Veterinary Clinic, Albany, CA, 94707, [3]Stanford University School of Medicine.

Feline immunodeficiency viruses isolated and studied to date have proven to be of low pathogenicity. To determine whether more highly expressed and rapidly pathogenic FIVs exist in nature, to isolate and characterize such viruses, and to determine whether donor disease state correlated with immunopathogenicity, we isolated unpassaged FIVs from 44 cats: 24 cats with various symptoms of immunodeficiency and 20 cats which were asymptomatic. In a selected subset of 7 virus isolates we assessed in vitro phenotypic features and in vivo pathogenesis in age-matched groups of 4 specific pathogen-free (SPF) cats per virus. These studies have produced the following results thus far: (1) FIVs have been readily recovered from both blood mononuclear cells (PBMC) and plasma of animals with symptoms of immunodeficiency. (2) FIVs isolated from cats with immunodeficiency have proven to be highly replicative, cytopathic, and strictly lymphotropic (i.e., restricted to PBMC vs. cell lines). (3) In vivo infection of SPF cats with 7 FIV field isolates has produced high and sustained levels of virus expression, significant decrements in CD4+ lymphocytes, and early clinical signs of immunodeficiency (lymphadenopathy, weight loss, diarrhea, dermatitis, and gingivitis). In these studies high virus burdens ranging from 10,000 to 100,000 FIV-infected cells per million PBMCs have been demonstrated. We conclude, therefore, that infection with high expression FIV isolates produces early and progressive T cell subset alterations, immunologic impairment, and a constellation of clinical symptoms associated with other immunodeficiency-inducing lentiviruses.

Q 433 EARLY POST -TRANSMISSION DIVERSIFICATION OF SIV DOES NOT CORRELATE WITH DETECTABLE ANTIBODY, Noreen A. Hynes, Simoy Goldstein, W. Randall Elkins, and Vanessa M. Hirsch, LID/NIAID/NIH, Rockville, MD 20852

The earliest post-transmission events in human immunodeficiency virus (HIV) infection remain poorly defined. Since immediate post-transmission samples from HIV infected persons are rarely available, the SIV infection of macaque model provides a surrogate for the study of HIV pathogenesis and may provide important insights into these early events. In this study of genetic variability (from the time virus was first detectable through seroconversion) no correlation between virus diversification and detectable antibody was found. Specifically, sequential samples (from 1 to 56 days post inoculation) were collected from the peripheral blood mononuclear cells (PBMC), macrophages, plasma, cerebral spinal fluid, and lymph nodes of 4 female juvenile pig-tailed macaques intravenously inoculated with PBMC-associated virus from a rhesus macaque terminally ill with AIDS. Single-stranded DNA conformational polymorphism (SSCP) of PCR-amplified products was used to estimate and follow genetic variation from sequentially collected specimens. This technique allows detection of single nucleotide changes based on differential migration of single stranded DNA under non-denaturing conditions. The 200 base-pair region of the most variable domain of the SIV envelope (V-1) was analyzed by SSCP followed by sequence analysis of cloned PCR products. SSCP detected genetically distinct species from different samples. Two distinct 7-day patterns emerged among the different specimens collected from individual animals: (1) minimal variability and (2) extensive variability. Furthermore, 2 distinct tissue-specific temporal variability patterns, minimal and extensive, emerged over the 56 day observation period. Two animals were found to have V-1 region SSCP patterns and nucleotide sequences from 7-day post inoculation PBMC DNA which were virtually identical to the dominant clone of the inoculum. The SSCP patterns for all other examined specimens for these 2 animals at 7 days and over the 56-day observation period revealed minimal variation. Extensive variation at 7 days and over time was seen in the remaining 2 animals. One animal was noted to have the earliest and most vigorous immune response (as measured by ELISA and Western blot assays) whereas the other animal had no detectable antibody during the study period. These results suggest that factors other than early measured antibody may be important in virus diversification within the host.

Q 434 HUMAN CYTOMEGALOVIRUS INFECTION IN THE SCID-hu MOUSE, Hideto Kaneshima, Mark Bonyhadi, Suzan Salimi, Joseph M. Mc Cune and *Edward S. Mocarski, New Enterprise Research Division, SyStemix, Inc., Palo Alto, CA 94303 and *Department of Microbiology, Stanford University School of Medicine, Stanford, CA 94305

Animal models of human cytomegalovirus (HCMV) infection have not been available to study pathogenesis or to evaluate antiviral drugs. We have shown that severe combined immunodeficient (SCID) mice implanted with human thymus and liver (Thy/Liv, J. M. Mc Cune et al., *Science*, 241:1632, 1988 and R. Namikawa et al., *J. Exp. Med.*, 172:1055, 1990) can serve as an animal model for infection and pathogenesis with HIV (R. Namikawa et al., *Science*, 242:1654, 1988 and M. Bonyhadi et al., abstract in this issue). When Thy/Liv SCID-hu mice were infected with HCMV, clinical HCMV strain Toledo into, consistent and high-level viral replication was detected by plaque forming assay with cultured fibroblasts. Other human tissue implants, including lung, colon, skin and bone marrow, were also found to support viral replication but at lower level and for a shorter duration of time. The majority of virus-infected cells in the thymus were found to be keratin-positive thymic epithelial cells, localized in the medulla. The treatment of infected animals with ganciclovir by either oral or intraperitoneal administration reduced viral replication in a dose-response manner. This animal model opens the way for a range of investigations not previously possible with HCMV, alone or in co-infections with other viruses including HIV.

Q 435 *Abstract Withdrawn*

Q 436 DEBRIS FROM APOPTOTIC, HIV-1-INFECTED T CELLS INFECTS MACROPHAGES IN A MANNER THAT IS DISTINCT FROM A MACROPHAGE-TROPIC VIRUS.
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CEM T cells infected with the lymphotropic strain HIV-LAI (LAI) undergo apoptotic cell death (J. Clin. Invest. 87:1710, 1991). This process generates cellular debris containing degraded cellular DNA but intact HIV DNA. Scavenging macrophages avidly take up this debris and proceed to release infectious virions. In contrast, lymphotropic virions of LAI (prepared from supernatants taken from chronically infected, non-apoptosing CEM cells) contain little or no full length viral DNA by PCR and are essentially unable to infect macrophages. For comparison, the macrophage-tropic strain HIV-BaL (BaL) serves as a positive control for virion-mediated infection of macrophages.

Whereas BaL spread through macrophage cultures to generate multinucleated giant cells and an exponentially increasing amount of p24 core antigen over time, LAI-containing debris did not cause giant cell formation and produced only modest amounts of linearly increasing p24. AZT (10 μM) completely blocked BaL infection in macrophages but only partly reduced p24 release (30-80%) from macrophages infected with debris containing LAI DNA. Soluble CD4 (100 μg/ml) markedly reduced BaL infection but had much less effect on infection by debris and sometimes even enhanced p24 production. Rabbit polyclonal antibody produced against uninfected CEM cells had little effect on BaL infection but completely prevented infection by HIV-infected CEM debris. Pretreatment of macrophages with TNF-α had no effect on BaL replication but markedly enhanced the production of p24 in debris infected cultures (5-20X). These data suggest a different pathway for infection of macrophages by apoptotic debris derived from HIV-1-infected cells. If similar debris were formed *in vivo* (either by HIV-induced cytotoxicity or by cytotoxic immune cells), therapeutic interventions other than inhibitors of reverse transcriptase may be necessary to prevent infection of macrophages that scavenge the remains of dead infected cells.

Q 437 EXPRESSION AND PURIFICATION OF RECOMBINANT HTLV-II ENVELOPE PROTEINS

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We have generated recombinant vaccinia viruses which express human T-cell leukemia virus II (HTLV-II) envelope glycoproteins gp61 and gp46. Infection by these recombinant vaccinia viruses resulted in expression of significant levels of HTLV-II envelope proteins, as determined by RIPA and Western blot assays. Infection of human cells, or mouse cells containing only human chromosome 17, with full-length envelope recombinant viruses, results in cell fusion. HTLV II envelope mediated cell fusion can be blocked by sera from HTLV II infected individuals. Our data suggest that HTLV-II Env is sufficient to mediate cell fusion during HTLV-II infection of receptor bearing cells. Our work is also consistent with the previous mapping of the gene encoding cellular receptor for HTLV-I and HTLV-II to human chromosome 17. In contrast, infection of cells with viruses, which express the external Env gp46 only, does not result in cell fusion but produces high levels of this protein. HTLV-II external glycoproteins expressed by vaccinia vectors are not secreted in contrast to external proteins of other retroviruses e.g. HIV gp120. Instead, gp46-II was found both within cells and on the cell surface, which made its purification difficult. A novel recombinant vaccinia virus expressing gp46-II with the 9 AA epitope, YPYVDVPDYA of the influenza virus hemagglutinin added to its C-terminus has been engineered. An immunoaffinity column using MAB 12CA5, which recognizes the added epitope, has been used to purify the gp46-II-YPYVDVPDYA to homogeneity. Furthermore, MABs against this purified gp46-II have been generated by immortalizing antibody-producing cells using MuLV(abl-myc) virus. We are currently characterizing these MABs, and one of MABs, SB1, has been used successfully to purify the recombinant full-length gp61-II. We are currently investigating the biological properties of these purified glycoproteins as well.

Q 438 HIV INFECTION OF MESENCHYMAL AND EPITHELIAL CELLS DERIVED FROM THE GASTRO-INTESTINAL TRACT. Mary Pat Moyer[1], Ricardo Torres[1], Alejandro A. Cantu[1], L. Sheri Anderson[1], Marianna P. Cagle[2], and Hal B. Jenson[2], Departments of Surgery[1] and Pediatrics[2], The University of Texas Health Science Center at San Antonio, TX 78284

Primary cultures of human submucosal mesenchymal (SM) and epithelial cells from the gastrointestinal (GI) tract were infected with the human immunodeficiency virus (HIV). Kaposi's Sarcoma (KS)-like changes in growth and morphology were displayed by HIV-infected SM cells. In cytokine studies, more Interleukin 6 (IL-6) was released from HIV-infected than control cultures at early times (3-6 days post-infection) even though virus replication did not occur. The quantity of released tumor necrosis factor (TNF) was unchanged. In long-term studies (weeks to months), replication of persistent or latent virus and shifts in specific markers of angioblast/endothelial cells were observed. Angiogenesis studies of culture supernatants with mice and chick embryos were positive. Similar effects on cell growth and cytokine induction were found when the HIV tat gene in either the pGEMtat or pSVtat72 vector was transfected into the SM cells, but production of IL-6 was more dramatic than with the virus alone. This in vitro model demonstrates cytokine induction of a KS phenotype, differentiation of endothelial and mesenchymal cells, and angiogenesis. Epithelial cells from various regions of the GI tract could be infected with HIV, but specific cell-associated phenotypic changes were not evident. Differences were seen in virus strains with regard to their ability to replicate and to establish a persistent infection in a subset of the GI epithelial cells. These studies suggest that different cells of the GI tract vary in their response to HIV and that pathogenesis may result from limited virus replication, persistence of virus, or cytokine release. Support by NIH-R01-HL48497 and DK40625 are acknowledged.

Q 439 A MONOCLONAL ANTIBODY (1F7) SPECIFIC FOR HUMAN ANTI HIV Ig INDUCES APOPTOSIS AND INHIBITS CTL ACTIVITY IN CD8$^+$ LYMPHOCYTES FROM HIV-1 INFECTED INDIVIDUALS. Sybille Müller, Heli Collins, Peter Brams, Haitao Wang, Oliver Dorigo, Michael McGrath**, Michael Grant*, Ken Rosenthal* and Heinz Köhler, San Diego Regional Cancer Center, San Diego, CA 92121, the Molecular Virology and Immunology Program, McMaster University, Hamilton, Ontario, Canada* and AIDS Program, University of California, San Francisco, CA 94110.

A murine monoclonal IgM antibody, 1F7, recognizes an idiotope on anti-HIV antibodies against different HIV antigens. A common idiotope on anti-HIV antibodies with different specificity favors anti-idiotypic regulation of the antibody response against HIV. Since T cells are also subject to anti-idiotypic regulation, we analyzed T cells from HIV$^+$ persons for evidence of anti-idiotypic regulation via the idiotope recognized by 1F7. We found that 1F7, but not isotype control antibodies induced apoptosis after a minimum of 72 hours of culture in PBMC cultures from 13/17 HIV$^+$ individuals and 0/5 HIV$^-$ controls. Flow cytometric analysis and lymphocyte subset depletion experiments demonstrated that the cells undergoing apoptosis were CD8$^+$. Apoptosis was determined by flow cytometric measurement of fragmented DNA in propidium iodide stained cells. Apoptotic cells appear in the A0 region with a DNA below that of the G0/G1 cells. Addition of 1F7 at the start of cell culture reduced T cell-mediated cytotoxicity in IL-2 stimulated PBMC cultures from HIV-infected individuals, as measured by anti-CD3 mediated lysis of P815 cells. Incubation of CTL derived from HIV$^+$ individuals with 1F7 immediately before ^{51}Cr release assays also reduced lysis of specific targets. These experiments suggest 1F7 binds a subset of CD8$^+$ lymphocytes selectively expanded in HIV infection via molecule(s) linked to cell-mediated cytotoxicity and apoptosis. By selectively binding to subsets of B and T lymphocytes expanded in HIV infection, 1F7 might (favorably) alter the immune response in HIV infection.

Q 440 CLONING OF A HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 STRAIN MN [HIV-1(MN)] CAPABLE OF EFFICIENT REPLICATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC), Stephen M. Nigida, Jr., Michelle R. Shoemaker, and Larry O. Arthur, Viral Diseases and Immunity Section, AIDS Vaccine Development Program, PRI/DynCorp, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

Evaluation of the efficacy of experimental vaccines against HIV-1 infection in chimpanzees requires a quantified, infectious virus stock. No in vitro correlate of this property has been identified, although efficient replication in human (Hu) and chimpanzee (Ch) PBMCs has been suggested. Laboratory stocks of HIV-1(MN) which replicated efficiently in H9 cells in vitro demonstrated poor replicative capacity in HuPBMCs when compared to that of HIV-1(B) and HIV-1(RF). Replication of HIV-1(MN) was maintained in HuPBMCs by the semi-weekly addition of fresh, PHA-stimulated, noninfected HuPBMCs. Reverse transcriptase (RT) activity first reached positive levels in culture supernatant fluids at 70 - 78 days postinoculation (PI). Cell-free virus obtained at 396 days' culture efficiently infected and replicated in both H9 cells and HuPBMCs with elevated RT activity first detected at 11 and 7 days PI, respectively, and a titer of $\geq 10^9$ $TCID_{50}$/ml in both cell types. Cell-free virus obtained at 442 days' culture was used to infect H9 cells. After 24 hours' incubation, these infected cells were cloned in 96-well plates containing gamma-irradiated monolayer cell cultures. Forty-four (44) cloned cell lines were established (1.9% efficiency). Thirty-seven (37) of these cloned cell lines (84.1%) expressed HIV-1 RT activity and *gag* product; cell-free supernatant fluids from some clones efficiently and rapidly infected H9 cells and human and chimpanzee PBMCs. The envelope regions of five (5) of these HIV-1(MN)-containing cell lines are currently being sequenced. These preparations will be used for the production of infectious virus stocks and for the inoculation of chimpanzees.

Q 441 HIV-1 REPLICATION CAN BE INCREASED IN BLOOD FROM SEROPOSITIVE PATIENTS FOLLOWING INFLUENZA IMMUNIZATION, William A. O'Brien, Stanislava Ovcak, Ali Namazie, Hamidreza Kalhor, Si-Hua Mao and Jerome A. Zack, Department of Medicine, West Los Angeles VA Medical Center and UCLA School of Medicine, Los Angeles, CA

Symptoms of AIDS typically occur many years following acute infection. Factors which influence the duration of this clinical latency remain poorly defined, however, in vitro activation of HIV-infected blood CD4+ lymphocytes and macrophages by mitogens or cytokines markedly increases virus production. To determine if cell activation by influenza immunization affects virus replication in blood cells of HIV infected patients, we utilized sensitive and quantitative culture and RNA PCR methods to measure blood infectious titres and HIV specific expression, respectively. Blood samples were obtained on 3 occasions from 16 patients followed in the VA Immunodeficiency Clinic just prior to influenza immunization, and again, 3 to 5 times after immunization. Significant increases in virus replication (> fivefold increase in plasma or blood cell infectious virus titre, and/or > than tenfold increase in viral RNA) were seen in 9 of 16 patients. No changes were seen in 5 HIV+ patients who did not receive influenza immunization but had blood samples obtained at similar intervals. The peak increase in virus replication was seen at 1 to 2 weeks after immunization, and levels fell to approach baseline values after several months. Studies to examine long-term clinical correlation are in progress. These data demonstrate that immune activation with influenza antigens may also stimulate virus replication. Results of these studies have broad implications for health care policies regarding immunization in general in HIV positive patients, and for immune-based therapies.

Q 442 IMMUNISATION WITH VIRUS ANTIGENS HAD NO EFFECT ON THE INDUCTION AND OUTCOME OF MAIDS.

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We used the murine immunodeficiency disease (MAIDS) model which is in many ways comparable to human AIDS for monitoring the effect of immunization with specific virus antigens on the induction and outcome of disease. The LPBM5 MuLV virus mixture containing the Duplan, helper and MCF (1) was inactivated by alkaline hydrolysis and administrated subcutaneously with complete Freund adjuvent to C5BL/6 mice 5,2 and 1 weeks before infection with LPBM5 MuLV stock. At the end of immunization period the presence of antibody to viral proteins in the serum was demonstrated by immunoperoxidase staining of LPBM5-infected cells, although the level of neutralizing antibodies and the extent of cellular immunity were not determined. The phenotypical changes in mouse blood lymphocytes (lymphonemia, increase in B cells and decrease in T cells subsets, the latter mainly due to the fall in CD8 + cells) were comparable in all experimental groups. All animals exhibited lymphadenopathy (by gross observation at 6-8 weeks after infection independently of previous treatment. The great diversity of survival time was observed in the mice within each experimental group with no significant difference between immunized and control animals. Recent work (2) has shown that successfull immunization against virally induced immune deficiency in cat and monkey models depends on the induction of an efficient cellular immunity and the humoral response induced in this experiment was probably insufficient to fend off disease.

1. Buller RM, et al. J Virol 1987, 61:383 - 2. Gardner MB. Antiviral Res 1991, 15:267.

Q 443 EPITOPE MAPPING AND FUNCTIONAL CHARACTERIZATION OF NEUTRALIZING MONOCLONAL ANTIBODIES DIRECTED AGAINST SITES WITHIN THE V2 DOMAIN OF HIV-1 gp120. Abraham Pinter, S. Kayman, Z. Wei, W. Honnen, H. Chen, J. McKeating, C. Shotten, S. Warrier and S. Tilley. Laboratory of Retroviral Biology, Public Health Research Institute, 455 First Ave., New York, New York 10016.

The hypervariable V1/V2 domain of HIV-1 gp120 is a highly conformational and heavily glycosylated region. Mutational studies have established that these sequences are required for function of gp120, and monoclonal antibodies (MAbs) binding to this region possess neutralizing activities. In order to facilitate characterization of epitopes in these domains that were either conformational or glycosylation-dependent, a fusion vector based on the murine leukemia virus *env* gene was used to express the native HXB2 V1/V2 domain. Biochemical and immunological analyses demonstrated that the resulting construct expressed the correctly folded and glycosylated V1/V2 domains. The fusion product was reactive with a number of MAbs, including several potent neutralizing antibodies. Three classes of neutralization targets in the V2 domain were detected by these studies. One group of antibodies reacted with linear peptides, and thus were seeing nonconformational epitopes that were not dependent on glycosylation. A second group of antibodies did not detect linear peptides and reacted only with native molecules possessing intact disulfide bonds and N-linked glycans. These epitopes were both conformational and glycosylation-dependent. At least one potent neutralizing antibody was affected by deglycosylation but not by disulfide reduction, indicating that it was seeing a glycosylation-dependent nonconformational epitope. More precise mapping of these epitopes will be reported. Similar V1/V2 fusion proteins were prepared from a number of additional HIV-1 strains, including MN, SF2 and JR-CSF. An analysis of the reactivity of these constructs with the anti-V2 MAbs and with panels of human sera will be presented. Finally, studies relating to the mechanism of neutralization by MAbs directed against several V2 epitopes will be discussed.

Q 444 IMMUNOLOGICAL AND VIROLOGICAL EVENTS IN LYMPH NODES AND SPLEENS OF SIV-INFECTED MACAQUES AND PROGRESSION TO AIDS.Yvonne J. Rosenberg*, Philip M. Zack#, Jack Greenhouse*, Enrique C. Leon*, Sue F. Papermaster*, Brian D. White*, Gerald A. Eddy*, and Mark G. Lewis*. Henry M.Jackson Foundation Research Laboratory, Rockville, MD. WRAIR#, Rockville, MD.

Lymphocytes in the blood constitute only 1-2% of the total pool. Studies in 15 monkeys infected with SIV-PBj-14 or 6 immunized monkeys challenged with the E11S isolate have both indicated that despite the well characterized CD4+ cell loss from the circulation during the chronic phases of infection, the CD4+ pool in LN remains within the normal range (30-50% CD4+ cells, 1.2 - 2.9 4/8 ratios). Only when blood 4/8 ratios fall below a value of around .5, are declines in LN CD4% and CD4/CD8 ratios observed. Several major changes in LN occur concominantly (i) the appearance of SIV antigen staining on follicular dendritic cells within germinal centres by immunohistochemical techniques (ii) a loss of PHA responsiveness (iii) a marked phenotypic change in the LN CD8+ pool from predominantly CD45RAhi to an activated CD45RAlo population (iv) the generation of potent non-restricted cytolytic CD8+ cells and (v) migration of these cells into GC with loss of FDC morphology. In macaques given a highly virulent SIV-251 isolate, these changes in LN often occur shortly after infection with no latent phase. By PCR analysis, immunological collapse of LN is not associated with high rates of viral replication and appears over a wide range of total proviral DNA levels. A comparison of such levels following infection with different isolates indicates that SIV-251 results in >one log higher levels of proviral DNA than E11S or PBj-14 and probably accounts for the more rapid disease progression but does not predict AIDS. Overall, the best correlate with LN collapse, advanced disease and death appears to be the levels of unintergrated virus present in lymphoid tiissue.These findings suggest that the CD4+ cell loss from the circulation throughout SIV and HIV infection is minimal relative to the total body pool and it is the immunological collapse of the LN which results in the disease susceptibility characteristic of AIDS. In addition, whilst anti-viral responses may be protective, many of the underlying mechanisms leading to the collapse in LN appear to be autoimmune in nature.

Q 445 EVALUATION OF HIV-1-ASSOCIATED CLASS II PROTEIN IN THE INDUCTION OF ANERGY AND APOPTOSIS, J. L. Rossio, J. W. Bess, Jr., P. Cresswell, D. D. Taub, L. E. Henderson and L. O. Arthur, AIDS Vaccine Program, Program Resources, Inc./DynCorp, NCI-Frederick Cancer Research & Development Center, Frederick, MD 21702; Yale University, New Haven CT; Laboratory of Molecular Immunoregulation, NCI-FCRDC, Frederick, MD

It is known that engagement of superantigen with MHC Class II-coded structures on antigen-presenting cells and the T cell receptor can lead to apoptosis. We have shown that HIV-1 produced in human cells carries significant amounts of host MHC Class II gene products such as HLA-DR (Science, In Press). To determine the functionality of this viral HLA-DR in inducing apoptosis, we have propagated HIV-1 in the cell line 174xCEM.T1, a T x B human somatic cell hybrid known to exhibit cell surface HLA-DR, as well as in 174xCEM.T2, a genetically modified clone lacking Class II coding sequences in MHC, and thus not expressing cell surface HLA-DR. The absence of HLA-DR on the 174xCEM.T2-produced virus was demonstrated by western blot as well as by the ability to precipitate intact 174xCEM.T1 virus, but not 174xCEM.T2 virus with antibodies to HLA-DR. The two HIV-1 preparations can be used to present the superantigen staphylococcal enterotoxin A (SEA) to the antigen-specific murine cell line AE7, a T1 helper cell clone responsive to pigeon cytochrome C. Since HIV-1 will not infect murine cells, only cell surface interactions are involved. The virus-treated AE7 cells are challenged with antigen and examined for both apoptosis and anergy. This system will allow the determination of the role of viral Class II structures in HIV pathogenesis.

Q 446 A HEXAPEPTIDE EPITOPE LIBRARY USED TO IDENTIFY THE LIGAND OF A HIV-1 NEUTRALIZING HUMAN MONOCLONAL FAB MOLECULE.
Astrid Samuelsson[2], Jorma Hinkula[3], Francesca Chiodi[2], Mats A. A. Persson[1]. [1]Department of Medicine, Karolinska Institute, [2]Department of Virology, Karolinska Institute, S-105 21 Stockholm, Sweden, [3]Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden

A human monoclonal Fab molecule has been obtained, using the combinatorial immunoglobulin library approach, from a rgp160 immunized HIV-1 infected patient. The Fab molecule shows reactivity to gp120 and neutralizes different HIV-1 strains in vitro. To identify the ligand of the monoclonal Fab, we used a filamentous phage epitope library (a gift from Dr G. P. Smith) where random hexapeptides are displayed as a fusion complex with phage coat protein III. The library consists of 2 x 10^8 clones representing about 70% of all possible hexapeptides. After multiple rounds of screening, eleven individual clones from the library were isolated and sequenced. Nine clones contained the common amino acid sequence motif P W X W L. Two of the sequences, P W E W L D and P W E W L W, were represented by two and three clones respectively. Interestingly, different codons were used for the leucine residue, emphasizing a strong preference for the mentioned motif. Two other sequences were completely unrelated. Although HIV-1 gp120 does not contain a linear peptide sequence with the identified motif, the enrichment of common sequences indicates that we have identified the ligand of the monoclonal Fab molecule.

Q 447 **T CELL RECEPTOR V_β12 GENE USAGE IN HIV-1 SERONEGATIVE AND SEROPOSITIVE PATIENTS SUPPORTS THE HYPOTHESIS THAT HIV-1 ENCODES A SUPERANTIGEN,**
Premlata Shankar and Judy Lieberman, Division of Hematology-Oncology, New England Medical Center, Boston, MA 02111

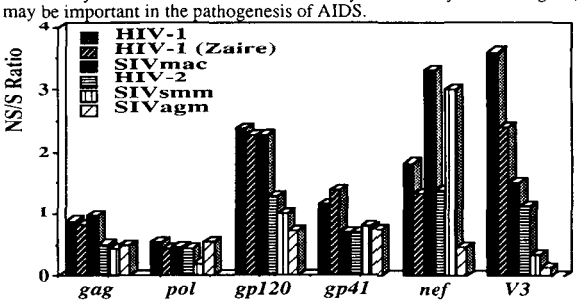
PBL from HIV-1 seronegative subjects were tested for proliferation against autologous HIV-infected PHA lymphoblasts. In 3 of 4 seronegative subjects a small amount of proliferation (stimulation index 2-5) was observed in the response to HIV-infected, but not mock infected, blasts. In one subject studied in more detail, both CD4 and CD8 T cells proliferated in response to HIV-1 infected autologous cells. Analysis of T cell receptor V_β gene usage by RNA PCR in these HIV-1 stimulated cells compared with mock stimulated cells showed an increase in the V_β12-expressing population. These results are consistent with the hypothesis that HIV-1 encodes a superantigen especially if interpreted in light of the results of Laurence et. al. that HIV-1 preferentially proliferates in CD4 T cells expressing V_β12. When we examined V gene usage by RNA PCR in mitogen-stimulated T cell lines from HIV-1 infected subjects, we could not detect V_β12 message in 4/5 patients, although it was readily detectable in normal controls. The 4 patients without detectable V_β12 gene usage had not had AIDS-defining opportunistic infections (2 CDC group II, 1 CDC group III and 1 with Kaposi's sarcoma) whereas the patient who had detectable V_β12-expressing T cells had AIDS opportunistic infections and a CD4 count of only 60/mm^3. The significance of this difference in V_β12 usage with clinical disease stage is unclear.

Q 448 **MOLECULAR CHARACTERIZATION OF 8:14 CHROMOSOMAL BREAKPOINTS IN AIDS-RELATED B-CELL LYMPHOMA**
Chien-Kou Shieh and Suraiya Rasheed, Laboratory of Viral Oncology and AIDS Research, USC School of Medicine, Los Angeles, CA 90032

We have previously reported establishment of a B-cell lymphoma cell line from a patient with AIDS. The B-cells do not produce HIV, and the cellular DNA does not contain an integrated HIV genome. However, the c-myc gene is translocated from 8 to 14 chromosome and is expressed at a high level in these cells. Analysis of the chromosomal DNA indicated that the c-myc translocation breakpoint is located 5' to the first intron of this gene. This breakpoint appears to be distinct from those reported for other B-cell tumors with similar 8:14 translocations. To understand the mechanism/s by which c-myc is activated in these cells we have isolated clones containing various regions of this gene, including the chromosomal breakpoints. Analyses of DNA nucleotide sequences and characterization at these breakpoints may lead to identification of regions that may be critical for the expression of c-myc in B-cells of patients with AIDS.

Q 449 RATES OF AMINO ACID CHANGES IN THE ENVELOPE PROTEIN CORRELATE WITH PATHOGENICITY OF PRIMATE LENTIVIRUSES,
Eugene G. Shpaer and James I. Mullins, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402

A spectrum of pathogenicity has been observed for primate lentiviruses in their natural hosts. For example, human immunodeficiency virus type 1 (HIV-1) is a potent etiologic agent for AIDS in man, whereas there is no evidence to date which indicates that simian immunodeficiency virus from African Green monkeys (SIV_{AGM}) causes immunodeficiency in AGM. We measured the relative rates of amino acid change, as the ratio of the number of nonsynonymous to synonymous (silent) nucleotide substitutions (NS/S), for six primate lentiviruses evolving in their respective hosts. These rates for the external envelope glycoprotein (gp120) and *gag* coding sequences are 2-3 times higher for pathogenic HIV-1 and SIV_{mac} (macaque) than for minimally pathogenic SIV_{AGM} and SIV_{smm} (sooty mangabey), and intermediate for HIV-2. We speculate that the increased rates of non-synonymous changes in *gp120* and *gag* coding sequences are due to viral escape from immune surveillance, and indicate higher immunogenicity of these proteins in their hosts. Based on these results and available experimental data, we conclude that there is a positive correlation between lentiviral pathogenicity and immunogenicity of the Env and Gag proteins in a given host. This hypothesis is consistent with recent data suggesting that immune system activation or autoimmunity induced by viral antigens, may be important in the pathogenesis of AIDS.



Q 450 **FIV *env* GENE DIVERSITY IN CATS,** Donald L. Sodora, Barbara Kitchell, Steven W. Dow#, Edward A. Hoover#, and James I. Mullins. Dept. Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402; #Dept. of Pathology, Colorado State University, Fort Collins, CO.

Feline immunodeficiency virus (FIV) is a feline lentivirus associated with an AIDS like illness in cats. The utility of FIV as a model for HIV disease progression and vaccination is in part dependent on the methods by which the two viruses establish a persistant infection in their respective host. For example, during HIV infection a rapid mutation rate of the *env* gene may allow the virus to escape immune surveillance. We have therefore set forth to determine if the FIV *env* gene varies to a similar extent. These experiments utilize FIV infected cat samples obtained from the U.S. and Canada. Genomic DNA was extracted from PBMC and subjected to nested-PCR to amplify the entire *env* gene. Alignment of 9 full length *env* genes revealed five variable regions within the SU and TM coding regions. There were fewer in-frame insertion/deletions observed in the FIV *env* genes than has been previously described for HIV. These gaps were almost exclusively located in the third variable region (Vc). Further diversity studies focused on a 300 bp fragment including Vc. To date, the 7 new and 8 known isolates examined can be divided into three homology groups (A,B,C), the B group subdivides into B1 and B2 subgroups. Diversity within a group ranged from 2.4 to 11.0%, between the B subgroups 10.3 to 17.1%, and between the groups 19.2 to 26.2% . These ranges are similar to what has been observed between the 5 HIV-1 subtypes defined by Myers and McCutchen. Lastly, diversity of viruses within infected cats was examined and found to be as high as 2% within the 300 bp region studied. This value appears lower than the intrapatient diversity of HIV which can be as high as 6% over the entire *env* gene. Therefore both similarities and differences in *env* gene diversity of FIV and HIV have been observed at this time.

Q 451 **Vβ-SPECIFIC VARIATIONS IN THE T CELL RECEPTOR REPERTOIRE OF HIV-INFECTED SUBJECTS SUGGEST THE PRESENCE OF AN HIV-ASSOCIATED SUPERANTIGEN.** H. Soudeyns*, G. P. Pantaleo+, T. Boghossian*, C. Ciurli*, N. Rebai*, H. C. Lane+, A. S. Fauci+, and R.-P. Sékaly*. *Laboratoire d'Immunologie, IRCM, Montréal, Canada, H2W 1R7. +Laboratory of Immunoregulation, NIAID, Bethesda, MA 20892.

Viral superantigens (v-sAg) have been shown in mice to induce anergy and deletion of T cells bearing specific T cell receptor Vβ subsets, these perturbations being mainly restricted to T cells expressing the CD4 cell surface molecule. In accordance with this model, a putative HIV-1-associated v-sAg could conceivably contribute to the pathogenesis of HIV-1 infection and AIDS. In order to reveal the presence of such an HIV-associated molecule, four different study protocols were designed which relied on the fact that the similarity of the expressed Vβ repertoire of a given pair of individuals is proportional to the relative likeness of their respective HLA-D/Dr background. 1- Flow cytometric analysis with panels of Vβ-specific monoclonal Abs, and a quantitative PCR technique that allows simultaneous typing of the expression of 24 known Vβ families were used to compare the Vβ repertoire of a cohort of HIV-infected mothers with that of their HIV-infected and -uninfected children. 2- Sequential Vβ typing was performed on single individuals progressing through HIV disease and AIDS. 3- The Vβ repertoire found in the lymph nodes of HIV-infected subjects was contrasted with that found in peripheral blood samples from the same individuals. 4- The respective Vβ repertoires found in purified CD4+ and CD8+ T cell subpopulations from HIV-discordant monozygotic twins were compared. Results from these various approaches have revealed that significant perturbations of the TCR Vβ repertoire were taking place in HIV-infected subjects, and that these alterations were restricted to T cells expressing particular Vβs. Moreover, analysis of purified T cell subpopulations has shown that the perturbations were taking place predominantly in the CD4+ T cell compartment. These results are consistent with the presence of an HIV-associated v-sAg in HIV-1 infection. This molecule might play a role in the development and pathogenesis of AIDS.

Q 452 ROLE OF dsRNA-DEPENDENT 2-5A SYNTHETASE/ RNASE L AND p68 KINASE PATHWAYS IN THE PATHOGENESIS OF HIV-1 INFECTION, Robert J. Suhadolnik[1], David R. Strayer[2], William A. Carter[2], Heinz C. Schröder[3] and Werner E. G. Müller[3], [1]Temple University School of Medicine, Philadelphia, PA 19140; [2]Hahnemann University, Philadelphia, PA 19102; [3]Universität Mainz, Mainz, Germany

HIV-1 causes immune cell depletion and a subsequent loss of immune function. To better understand the pathogenesis of HIV-1, we have examined key components of dsRNA-dependent 2-5A synthetase/RNase L and p68 kinase antiviral pathways in PBMC extracts from HIV-1 infected individuals. Compared to normals, latent 2-5A synthetase is elevated; however, little or no intracellular bioactive 2-5A ($p=.05$) and significantly reduced levels of 2-5A-dependent RNase L ($p<.05$) were detected. HIV-1 also down regulates the p68 kinase. RNase L can be photolabeled by an azido 2-5A photoprobe in PBMC extracts from healthy individuals, but not in PBMC extracts from HIV-1 infected individuals. These findings are consistent with our earlier report of a correlation between 2-5A metabolism and the growth cycle of HIV-1 (Schröder et al., J. Biol. Chem. 264, 5669, 1989). A new strategy has been developed which yields a selective antiviral effect of 2-5A against HIV-1 infection, namely the intracellular immunization approach using 2-5A synthetase cDNA linked to HIV-1 TAR. HIV-1 trans-activated 2-5A synthetase selectively inhibits HIV-1 replication in vitro. Therefore, drug modalities capable of regulating the endogenous 2-5A synthetase/RNase L and/or p68 kinase pathways such as dsRNAs or 2-5A derivatives may be important adjuncts to current approaches to anti-HIV-1 chemotherapy (e.g., nucleoside analogs).

Q 453 LACK OF CORRELATION BETWEEN ANTIBODY BINDING TITERS TO HIV-INFECTED CELLS AND ADCC IN HIV-POSITIVE SERA.

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The presence of ADCC mediating antibodies in HIV-positive (HIV$^+$) sera is currently determined by use of ^{51}Cr radiolabelled, HIV infected cells in an assay with peripheral blood mononuclear cells (PBMCs). Antibodies in the HIV patients sera recognize epitopes on infected cells; in turn the antibodies are recognized by the Fc receptors of PBMCs (e.g. NK cells, monocytes). The PBMCs then lyse the HIV infected cells. To date, ADCC lysis has been predominately shown to be due to IgG antibodies. We sought to use flow cytometry (values recorded as MCF: mean channel fluorescence) in measuring the IgG antibody binding to infected cells to answer the question of whether antibody-binding/MCF would correlate with observed lysis values from ADCC assays. Using FITC (fluorescein isothiocyanate) labelled secondary antibodies to IgG, 41 HIV$^+$ sera samples were studied. Also ^{51}Cr ADCC assays were done on these 41 sera samples, quantifying the ADCC mediating abilities of the antibodies. The first antibody binding study was done using anti-human IgG; no correlation between MCF values and ADCC lysis was found. Next, antibodies against specific isotypes of IgG were used. The MCF values for anti-human IgG2 & IgG3 were not significantly above background and thus showed no correlation to ADCC. Anti-human IgG1 also showed no correlation. However 10 of the strongest and 10 of the weakest sera samples, as measured in the ^{51}Cr ADCC assay, appeared to show an inverse correlation between MCF values and percent lysis from the ^{51}Cr ADCC assay.

Q 454 ADCC IN HIV-2/SIVmac INFECTED INDIVIDUALS AND/OR IMMUNIZED MONKEYS.

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

Characterization of a broadly reacting immune response and identification of specific target epitopes seems essential for an efficient AIDS vaccine. The humoral response is able to mediate several functions such as antibody-dependent cellular cytotoxicity, ADCC. The progression of disease in HIV-1 infected patients may be faster than in HIV-2 infected individuals. We have shown high frequencies of ADCC in HIV-2 sera from Guinea-Bissau against three HIV-2 strains as well as SIVmac and the HIV-2 sera have a broader ADCC reactivity than has been shown for HIV-1. This suggest that HIV-2 may harbor regions that can evoke a more effective immune response. To futher estimate the variability of ADCC target regions, we evaluated the antigenic similarities between the virus strains. Thus the HIV-2/SIVmac isolates seem to be more related to each other than the HIV-1 isolates and by using peptides from diffrent regions of the envelope glycoprotein, we suggest that the V3 region and the C-terminal end are involved in ADCC. Moreover, we have shown HIV-2/SIVmac specific ADCC in sera from immunized and infected monkeys. About 90 % of these monkeys develope strain-specific ADCC with high titers and both autolougus and heterolougus ADCC developes rapidly after immunization/infection.

Q 455 A COMPLEMENTARY-DETERMINING REGION (CDR) SYNTHETIC PEPTIDE ACTS AS A MINI ANTIBODY AND NEUTRALIZES HIV-1 IN VITRO, Britta Wahren, Michael Levi, Matti Sällberg, Ulla Rudén, Dorothee Herlyn*, Haruhiko Maruyama*, Hans Wigzell# and James Marks**, Department of Virology, National Bcteriological Laboratory, S-105 21 Stockholm, Sweden; *The Wistar Institute, Philadelphia, PA 19104-4268; #Department of Immunology, Karolinska Institute, S-104 01 Stockholm, Sweden; **Cambridge Centre for Protein Engineering, Cambridge CB2 2QH, UK, Present address: Department of Anaesthesia, San Francisco General Hospital, University of California, CA 94110.

A complementarity-determining region (CDR) of the mouse monoclonal antibody (Mab) F58 was constructed with specificity to a neutralization-inducing region of HIV-1, human immunodeficiency virus type 1. The Mab has its major reactivity to the amino acid sequence I--GPGRA in the V3 envelope region. All CDRs including several framework amino acids were synthesized from the sequence deduced by cloning and sequencing Mab F58 heavy (H) and light (L) variable chain domains. Peptides derived from the third heavy chain domain (CDR-H3) alone or in combination with the other CDR sequences competed with F58 Mab for the V3 region. The CDR-H3 peptide was chemically modified by cyclization and then inhibited HIV-1 replication as well as syncytium formation by infected cells. Both the homologous IIIB viral strain to which the F58 Mab was induced and the heterologous SF2 strain were inhibited. This synthetic peptide had unexpectedly potent antiviral activity and may be a potential tool for treatment of HIV-infected persons.

Q 456 HIV-1 MUTANTS THAT ESCAPED NEUTRALIZATION BY MONOCLONAL ANTIBODIES DIRECTED AGAINST CONFORMATIONAL DETERMINANTS ON gp120. Hironori Yoshiyama, Yaoxing Huang, John P. Moore, Mandal Singh, James E. Robinson, Michael Fung, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, 455 First Avenue, New York, NY 10016; University of Connecticut Health Center; Tanox Biosystems, Inc.

The NL4-3 molecular clone of HIV-1 is susceptible to neutralization by 21h, a HMab directed against the CD4-binding site on gp120. Two variant viruses that are refractory to 21h neutralization have been generated by in vitro passages. Interestingly, these two escape mutants show enhanced replicative and syncytia-forming capacity. Molecular characterizations reveal that two mutations in the V3 domain account for the escape from 21h, suggesting an interaction between V3 and CD4-binding regions. Starting with a biologic clone of HIV-1RF, three escape mutants have also been generated to G3-4, a MMab directed against a conformational epitope in the V2 loop. Further studies on these viruses should provide important information on this recently defined, novel neutralization site on gp120.

Q 457 BIOLOGICAL AND ANTIGENIC PROPERTIES OF SEQUENTIAL REISOLATES FROM MACAQUES EXPERIMENTALLY INFECTED WITH HIV-2.

Y-j Zhang, P. Putkonen, J. Albert, E.M. Fenyö, Department of Virology, Karolinska Institute, Department of Virology and Immunology, SBL, Stockholm, Sweden.

Sequential reisolates and serum samples obtained during a two-year period from six cynomolgus macaques (*Macaca fascicularis*) experimentally infected with HIV-2/SBL6669 were studied for autologous neutralization assay and for in vitro biological properties. All macaques seroconverted shortly after infection but remained healthy with only transient initial drop of $CD4^+$ cell counts.

The sequential reisolates from all macaques could replicate in HUT-78 and U937-2 cell lines, similarly to the inoculum virus. Four of the macaques produced neutralizing antibodies against the inoculum virus and sequential reisolates. Once neutralizing antibodies were procuced, sera could neutralize sequential reisolates obtained at both early and late time after infection indicateing that at least some neutralizing epitopes of HIV-2 remain stable in the course of infection. Only one reisolate (of fifteen tested) showed resistance to neutralization by autologous sera taken simultaneously with virus isolation. The results show that antigenic and biological variability of sequential reisolates from experimentally HIV-2 infected monkeys is relatively limited, unlike the pathogenic SIVsm infection.

Cellular Immune Responses, Cytokine Expression, Variation, Drug-Induced Mutations, Vaccines

Q 500 UGANDAN HIV-1 V3 LOOP SEQUENCES CLOSELY RELATED TO THE U.S./EUROPEAN CONSENSUS

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Objective: The HIV-1 V3 loop is an important determinant for virus neutralization and cell tropism. In this study we have compared V3 sequences from 22 Ugandan individuals with sequences from U.S./European and other African sequences.

Methods: Blood samples were collected in 1990 from 22 Ugandan individuals with symptoms ranging from asymptomatic infection to AIDS. The V3 domain was directly amplified from uncultured lymphocytes by PCR and cloned. Multiple clones from each sample were sequenced. Peptides corresponding to the 15 most central amino acids of each individual were synthesized and used in peptide ELISA.

Results: Twenty of the 22 Ugandan V3 loop sequences could be divided into two distinct groups, which both were more closely related to the U.S./European consensus than to earlier Ugandan sequences. One group differed from the U.S./European consensus by five amino acids only. The sequence data were supported by peptide serology. The degree of cross-neutralization between the two groups is now being investigated.

Conclusion: The demonstration of these two previously unrecognized Ugandan V3 loop genotypes, which are closely related to the U.S./European consensus has implications for the understanding of the evolution of HIV-1 and for the future design of a vaccine for use in Africa.

Q 501 EXAMINATION OF HIV-1 PROVIRAL SEQUENCE DIVERSITY USING DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) REVEALS DOMINANT FORMS WITHIN PATIENT SAMPLES.

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The human Immunodeficiency virus type 1 (HIV-1) is known to evolve rapidly and to exhibit a high degree of DNA sequence diversity, both within and between isolates from infected patients. Very extensive variation where all HIV-1 genomes are different, has been suggested from results obtained by DNA sequencing of subcloned PCR products. In order to obtain an overall view of the HIV-1 sequence diversity in patient samples, denaturing gradient gel electrophoresis (DGGE) combined with direct automated DNA sequence analysis, of PCR-amplified DNA-fragments from the envelope and nef genes of HIV-1 have been performed. DNA fragments suitable for DGGE-analysis were obtained using PCR-primers with 40-bp GC-clamps and using theoretical calculations of the melting behaviour of the DNA fragments. The theoretical calculations were performed using specialised computer software which had been compiled for use on UNIX. Using these fragments sequence differences can be detected in more than 1 kb of HIV-1 sequence.

Nine patients were examined for variation in two variable env regions, and the results showed that a majority of the patients had a pattern where only one major form was present. The minor forms only constituted a small fraction of the total sample. In the remaining patients 2-4 major forms could be detected for each region. In general, the diversity in the two env fragments was greater for patients in later stages of infection. In the nef gene 6/8 patients showed relatively high diversity with 3-5 major forms present.

Using DGGE the pattern of different forms in the two env fragments was examined for a patient sample before and after cultivation in vitro. It was found that a minor form in the patient sample became the dominant form in culture, while the dominant form in the patient sample could no longer be detected.

It was shown that the separated forms can be excised from the gel and their DNA sequence determined using direct automated sequencing. Using this procedure, single base substitutions causing separation by DGGE were characterized.

This indicates that HIV-1 forms which have greater clinical significance can be isolated and sequenced, and possibly correlated to events occuring during the HIV-1 infection.

Q 502 SUBSTITUTION OF PBMC-DERIVED gp120 CODING SEQUENCES INTO AN INFECTIOUS MOLECULAR CLONE OF HIV-1.

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Introduction: To date, studies on the relationship between structure and function of HIV-1 gp120 have only been possible on virus strains selected for growth in culture. We have developed a cassette vector system based on the infectious molecular clone HXB2, HXB2-MCS, which allows the generation of homogeneous virus stocks containing specific full-length gp120 sequences from an infected patient, which have been PCR-amplified directly from PBMC proviral genomes.

Methods: Full-length gp120 sequences are amplified from PBMC DNA with nested primers using the thermostable polymerase *Pfu*. Internal primers carry restriction sites which allow cloning into a *BstII* site created at 5875 and an *XbaI* site created at 7624 in the HXB2R sequence. These newly created sites do not affect the coding potential of HXB2. The cloned gp120 sequences can be shown to contain an intact reading frame by *in vitro* translation before insertion into HXB2-MCS and transfection into C8166 cells.

Results: 1) We have tested the viability of HXB2-MCS and shown it to have virtually identical growth properties to the parent clone.
2) We found that *Taq* polymerase incorporated errors in one third of clones examined. Using *Pfu* polymerase we have found, to date, no PCR-induced errors. 3) We have obtained 12 gp120 clones derived from single proviral molecules, representing the major gp120 forms found over a 7-year period of infection in an asymptomatic hemophiliac, as defined by sequence variants in the V3 and V4/V5 hypervariable regions. 4) Virus from one of these clones has already been obtained and we are producing virus stocks from the remaining 11 for subsequent analysis of gp120-associated properties, including neutralisation by autologous sera, cell tropism, replicative capacity and conformation.

Q 503 ASSEMBLY OF DEFECTIVE INTERFERING HIV-1 PARTICLES USING A DEFECTIVE HIV-1 HELPER VIRUS DNA

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We have developed six generations of defective interfering (DI) HIV-1 proviral DNAs which interfered with HIV-1 replication by two independent mechanisms: 1. the expression of a chimeric CD4/Env receptor protein which forms intracellular complexes with Env and 2. a multitarget-ribozyme which cleaved HIV-1 Env RNA at up to nine conserved sites. Both mechanisms inhibited syncytia formation and drastically decreased the total amount of p24 antigen release. Expression of the DI genome was dependent on the regulatory proteins of HIV-1 but it did not inhibit HIV-1 gene expression, suggesting that viral progeny were less infectious and unable to spread to susceptible cells.

Towards the use of these DI particles as potential antivirals against HIV-1, we have developed a defective helper virus DNA (HDPACK1) by making specific deletions in the infectious DNA, pNL4-3. HDPACK1 DNA did not contain the previously identified packaging signal (39 bp). It contained a 2506 bp deletion in Env, and Nef as well as 3' LTR were removed and replaced by SV40 poly(A) signal. This construct encoded all structural and regulatory proteins of HIV-1 except Nef and Env. HDPACK1 was able to transactivate a CAT gene that was placed under control of the HIV-1 LTR promoter. Enhanced production of p24 antigen was observed when transfected into HeLa-TAT cells,
(7 ng/ml, approx. 7×10^7 virus particles/ml). Most of the p24 antigen (90%) could be pelleted by high speed centrifugation through 10% sucrose and virus particles were further purified by isopycnic banding on sucrose gradients. Packaging of prototype HIV-1 DI (HD4) specific poly(A) RNA was observed in released virus particles that were generated by cotransfecting HeLa-TAT cells with HDPACK1 and HD4 DNAs. Characteristic HIV-1 like viruses budding from cell membranes were observed by electron microscopy.

Q 504 IMPORTANCE OF CD8+ CELLS' ABILITY TO SUPPRESS HIV-1 REPLICATION IN PROLONGING THE ABILITY OF INFECTED INDIVIDUALS TO REMAIN ASYMPTOMATIC, Edward Barker, Mitchell B. Fadem and Jay A. Levy. Cancer Research Institute, University of California School of Medicine, San Francisco, CA, 94143

Several effector cell functions were evaluated to determine which cell types may be important for the healthy state of individuals who remained asymptomatic for > 8 years following HIV. infection (long term survivors). Several aspects of effector cell functions of long term survivors (LTS) with CD4 counts greater than 400 cells/ul were evaluated and compared to HIV seronegative individuals. We evaluated natural killer cell (NK) lytic activity, monocyte/macrophage effector cell function as measured by the production of superoxide anion in resting or activated cells, and the ability of $CD8^+$ cells to suppress HIV-1 replication (in a nonlytic fashion) in $CD4^+$ cells infected with the virus. No changes were seen with monocyte/macrophage effector cell function. NK cell lytic activity was depressed compared to levels measured in HIV seronegative individuals. The only elevated response was the ability of $CD8^+$ cells to suppress HIV-1 replication in $CD4^+$ cells. In LTS, 1 CD8+ cell was able to control viral replication in 8 $CD4^+$ cells. In addition, we showed that individuals who have $CD4^+$ counts <350 cells/ul (some symptomatic) required at least 3 $CD8^+$ cell to control virus replication in 1 $CD4^+$ cell. Preliminary data indicate that lysis of target cells possessing HIV antigens by CTL from these long term survivors did not correlate with the HIV suppressing activity of CD8+ cells. Thus the ability of $CD8^+$ cells to suppress HIV replication may play a role in prolonging the asymptomatic state in HIV infected individuals.

Q 505 AMINO ACIDS DETERMINING ENZYME AND VIRUS SENSITIVITY TO HIV-1 PROTEASE INHIBITORS. Edward D. Blair, A. Joseph Wood, Carolyn Blance, Satty Bains, Graham Darby, Andrew Wilderspin*, Richard Sugrue*. Department of Molecular Sciences, Wellcome Research Laboratories, Beckenham BR3 3BS, and *Birkbeck College, London WC1E 7HX, UK.

Alterations in the sensitivity of HIV protease to inhibitors of the enzyme, and concomitant virus resistance, are of considerable importance as several such inhibitors are currently in clinical development. We have used three approaches to assess the nature and relevance of changes of enzyme and virus sensitivity to two prototype inhibitors, A-75925[1] and Ro-XI[2]. Firstly, we have compared the relative sensitivities of HIV-1, HIV-2 and SIV protease's to these inhibitors, and attempted to relate differences to variation in the amino acid sequences of these enzymes. Secondly, based on such comparisons, we have expressed and purified 16 single amino acid mutants of HIV-1 protease (courtesy of Dr R Swanstrom[3]) as polyhistidine fusion proteins, and we are now correlating sensitivity to specific amino acid changes. Thirdly, we have looked at the development of resistance by passing HIV-1 (HXB2) in the presence of the protease inhibitors and have found that the pattern of resistance varies for the different inhibitors.

Sequence changes in the protease gene upon emergence of resistance will be correlated with *in vitro* enzyme sensitivities. The ultimate aim of such studies is to enable the prediction of mutations likely to emerge with particular classes of inhibitor(e.g., C_2, mimetics) and their implications for virus growth.

[1]Kempf et al, *J. Med. Chem.I.* **33** 2687 (1990).
[2]Roberts et al, *Science***248** 358 (1990).
[3]Loeb et al, *Nature***340** 397 (1989).

Q 506 ANALYSES OF THE TCR V_β REPERTOIRE IN CD4+ and CD8+ LYMPHOCYTE POPULATIONS OF HIV-1 INFECTED SUBJECTS, Deborah M. Boldt-Houle, Thomas Zavoral, Xiao-Li Huang, Charles R. Rinaldo, Jr., and *Garth D. Ehrlich, Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261

Previous reports of TCR V_β clonal deletions of V_βs 14-20 among HIV-1 infected individuals with CD4+ counts <200, generated the hypothesis that HIV-1 depletion of CD4+ cells was achieved through a superantigen mechanism. Our cross sectional analysis of HIV-1 infected subjects, with CD4+ counts < 200, enrolled in the Multi-center AIDS Cohort Study do not support these findings, but instead suggest a random loss of clones probably due to overall low T-cell numbers. Subsequent longitudinal studies of the V_β repertoire, conducted with stored frozen cells collected over an interval of 5-6 years from controls and HIV-1 seroconvertors that did not experience disease progression, revealed consistent results over time for a given individual. Mitogen stimulation of these samples did not result in qualitative changes in the repertoire. In contrast, the variation in TCR repertoires between persons was quite pronounced in both seronegative and seropositive groups. These findings of subject-specific perturbations argue that to determine the effects of HIV-1 infection it is necessary to perform longitudinal studies applying early times points as controls for later time points.

To assess whether the TCR repertoire varies between an individual's CD4+ or CD8+ cells, phenotypic panning was performed. The CD4+ and CD8+ cell populations of the seronegative individuals and nonprogressors mimicked results obtained with PBMCs. In contrast, qualitative differences were seen between the separated CD4+ and CD8+ cells obtained from HIV-1 positive patients with CD4+ counts < 200. Together these data (equivalent TCR repertoires for CD4+ and CD8+ cells in normals; and qualitative differences in phenotypically separated cells from AIDS patients) are not supportive of a superantigen model of CD4+ depletion which would be independent of HIV-1 infection and would, therefore, affect CD4+ and CD8+ cells in a like manner.

Q 507 THE EFFECTS OF SIMIAN IMMUNODEFICIENCY VIRUS ON ALVEOLAR MACROPHAGE-MEDIATED PHAGOCYTOSIS AND KILLING OF *CRYPTOCOCCUS NEOFORMANS*. S.J. Brodie[1], D. Pauley[1], M. O'Connell[1], D.G. Walsh[1], P.K. Sehgal[2], and D.J. Ringler[1]. Divisions of Pathology[1] and Medicine[2], Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts, 01772.

Phagocytosis and killing of encapsulated and acapsular forms of *Cryptococcus neoformans* by alveolar macrophages (AM) from rhesus macaques infected with cytopathologically-distinct molecular clones or uncloned isolates of simian immunodeficiency virus (SIV) was studied. The AM were collected by bronchoalveolar lavage from animals (n = 21) in different clinical stages of AIDS. Additionally, AM from normal retrovirus-free animals (n = 6) were infected *in vitro* with cloned or uncloned SIV. The AM from SIV-infected macaques without clinical signs (lymphadenopathy, chronic diarrhea, dementia, and/or weight loss) or laboratory evidence (reduced CD4 lymphocytes, SIV p24 antigenemia, and/or relative decrease in antiviral antibody titer) of immunodeficiency were functionally normal. However, AM from chronically infected animals with clinical signs and/or laboratory evidence of AIDS demonstrated a significant ($P < .05$; *t*-test) decrease in killing of acapsular *C. neoformans*. Infection of AM *in vitro* with macrophagetropic strains of SIV resulted in increased cytopathogenicity (no. of syncytia and no. of nuclei per syncytia) and increased numbers of cells containing SIV capsid antigen (p27), but did not alter the percent killing of acapsular *C. neoformans*. Encapsulated forms of *C. neoformans* were not readily phagocytized, regardless of virus strain used or mode of infection, and there was no evidence of extracellular killing. These results suggest that recurrent and opportunistic respiratory infections associated with SIV infection may result from a defect in AM function and are observed only in animals with overt AIDS. However, based on *in vitro* studies, direct viral infection of AM does not appear to be the principle mechanism associated with a defect in killing of protozoal targets.

Q 508 HIV-1 REVERSE TRANSCRIPTASE RESIDUES THAT INFLUENCE SENSITIVITY TO NONNUCLEOSIDE INHIBITORS, Vera W. Byrnes, Vinod V. Sardana, Jon H. Condra, William A Schleif, Christine L. Schneider, Julie A. Waterbury, Jill A. Wolfgang, Donald J. Graham, Leah Gotlib, Abner J. Schlabach, Bohdan S. Wolanski, William J. Long, Audrey Rhodes, Donna L. Titus, Elizabeth Roth, Olga M. Blahy and Emilio A. Emini, Merck Research Laboratories, West Point, PA 19486

An essential step in the replicative cycle of the human immunodeficiency virus type 1 (HIV-1) is the synthesis of a DNA copy of the viral RNA, which is catalyzed by the virally encoded reverse transcriptase (RT). Therefore, the development of RT inhibitors has been the focus of many anti-HIV therapeutic research programs. To date, the only approved anti-HIV-1 therapies are the nucleoside analogs AZT, ddI and ddC. Prolonged treatment with either AZT or ddI results in the emergence of resistant variants. The RT residues that mediate sensitivity to these nucleoside analogs have been described. Recently, a class of several structurally diverse nonnucleoside inhibitors, including the pyridinone L-697,661, has been described. Virus variants resistant to this compound have been derived both in cell culture and in HIV-1 infected individuals enrolled in clinical studies. Substitutions at RT residues 103, 181 and 188 have been identified as important for mediating cross-resistance to L-697,661 and other nonnucleoside inhibitors.

Given the possibility of developing therapeutic combinations of either nonnucleoside and nucleoside inhibitors or nonnucleoside inhibitors with non-ovelapping profiles of resistance, we attempted to identify additional RT residues that mediate sensitivity to L-697,661. A series of recombinant RT genes and viruses was constructed which contained single substitutions at RT residues 98, 100, 101, 106, 108 and 179. All of these bacterially expressed mutant RT's exhibited low level resistance ($\leq$15 fold) to L-697-661, but unique resistance profiles were seen for other nonnucleoside inhibitors. Combinations of these mutations within the same RT resulted in greater-than-additive resistance. Furthermore, to address the question of functional interactions of RT residues that mediate sensitivity to the nucleoside analogs and those which mediate sensitivity to the nonnucleoside inhibitors, we are examining resistance patterns of RT enzymes and viruses that contain substitutions engendering resistance to both classes of inhibitors. Analyses such as these will aid in the selection of inhibitors to be used in combination anti-HIV-1 therapy.

Q 509 ENZYMOLOGIC STUDIES OF HIV-1 AZT AND DDI RESISTANCE. A.M. Caliendo, J.J. Eron, Y-K. Chow, K.M. DeVore, J. Kaplan, and R.T. D'Aquila. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129.

We have noted in earlier work that the addition of AZT resistance mutations in the HIV-1 RT gene augmented the degree of in vitro virus didanosine (ddI) resistance conferred by the ddI-selected RT Leu^{74}-> Val substitutuion. A non-radioactive heteropolymeric primer-template RNA-dependent DNA polymerase activity assay (RT-DETECT, Dupont) was used. We studied recombinant-expressed RTs with AZT resistance mutations, ddI resistance mutations as well as RTs with both AZT and ddI resistance mutations. Virions were also PEG-precipitated from PBMC culture supernatants of clinical isolates with the codon 74 mutation and tested for ddATP resistant RT activity.

Enzymologic studies of recombinant-expressed RT showed a 2.5 fold increase in the 50% inhibitory concentration (IC_{50}) for ddATP in RTs with the Leu^{74} -> Val substitution compared with wild type RT. In addition, RTs with Leu^{74} -> Val plus Thr^{215} -> Tyr and Lys^{219} -> Gln substitution showed a 5 fold increase in IC_{50} when compared to wild type RT. These enzymatic differences correlated with differences in ddI IC_{50} seen in cell culture studies. Slight differences in the IC_{50} for AZT-TP were seen for RT containing the AZT resistance substitutions Thr^{215} -> Tyr and Lys^{219} -> Gln compared with wild type RT. The addition of the Val^{74} mutation to Thr^{215} -> Tyr and Lys ^{219} -> Gln did not change the AZT-TP IC_{50}. Virus AZT susceptibilities did not parallel these RT enzyme findings. The virion-associated RT activity of clinical isolates containing the 74 mutation also showed an increase in ddATP IC_{50} when compared with wild type virion-associated RT.

These enzymologic results are consistent with different mechanisms of resistance for ddI and AZT. Studies of other functions of RT such as processivity or mispair extension may provide insight into the mechanism of resistance of AZT. Also, the polymerase activity assay used in these studies may allow more rapid monitoring of ddI resistance than the current virus susceptibility testing assays.

Q 510 IMMUNOLOGICAL PROPERTIES OF SIV NEF PROTEIN EXPRESSED IN A SALMONELLA LIVE-VACCINE STRAIN, Margherita E. Cattozzo, Marianne Hovi and Bruce Stocker, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305

Nef , a regulatory protein of human and simian immunodeficiency viruses (HIV and SIV), is associated with the inner face of the membrane of infected cells and is produced at an early stage of the viral infection. A vaccine strategy based on the use of this protein might allow elimination of infected cells before the release of new viral particles could occur. For this purpose we have constructed an aroA$^-$ Salmonella live-vaccine strain expressing the SIV *nef* gene in the bacterial body as a fusion protein. The expression vector used is named pGEX-1 in which the entire *nef* gene is linked to the carboxy terminus of the glutathione transferase gene. The sequence coding for Nef protein was obtained by PCR amplification from a clone containing the 3' half of the entire genome of PBj14: this SIV variant, isolated from pig-tailed macaques, is extremely virulent and may provide a rapid assay system for testing a new potential vaccine.

Humoral and cellular responses to the nef protein expressed in such a system, were tested *in vivo*. Groups of 5 BALB/c mice were inoculated i.p. on days 0, 13, 21, 29 and 35 with 10^6 colony forming units of the Salmonella live vaccine carrying the recombinant plasmid pGEX-1 with the *nef* gene. The pooled sera collected were titrated by ELISA with purified SIV Nef protein as test antigen: after the III and IV booster dose significant anti-Nef activity was evident.

To investigate cellular immune response, animals were injected with one dose (10^6 bacteria) i.p. with the live recombinant Salmonella vaccine and the T cell proliferative response to the fusion protein was measured *in vitro* 1 week and 1 month after the injection. We found that the stimulation index, that is the ratio between the values obtained from each sample (mean of triplicates) and the background value, was 2.5 and 3.8 after 1 week or 1 month respectively using the fusion Nef protein, as the stimulating antigen, at a concentration of 10 μg/ml.

Q 511 $CD8^+$ T-LYMPHOCYTES FROM ASYMPTOMATIC HIV-1 INFECTED INDIVIDUALS SUPPRESS TAT-MEDIATED HIV-1 LTR TRANSCRIPTION, Chin-Ho Chen[1], Kent J. Weinhold[1], John A. Bartlett[2], Michael J. MacDougall[1] and Michael L. Greenberg[1], [1]Departments of Surgery, and [2]Department of Medicine, Duke University Medical Center, Durham, NC 27710

HIV-1 infection evokes a vigorous anti-viral response that may participate in resolving the initial peak of plasma viremia and maintenance of the asymptomatic state. $CD8^+$ T-lymphocytes of HIV-1^+ individuals play a critical role in the cellular anti-HIV response. Specific killing of HIV infected cells by cytotoxic T-lymphocytes as well as nonlytic suppression of HIV-1 replication are two major anti-HIV activities mediated by $CD8^+$ T-lymphocytes. In agreement with previous reports, we have observed a potent suppressive effect on HIV-1 production from autologous $CD4^+$ T-lymphocytes by $CD8^+$ T-lymphocytes from asymptomatic HIV-1 infected individuals. To elucidate the mechanism(s) of the nonlytic suppressive anti-viral activity, we tested the effect of $CD8^+$ T-lymphocytes on the transcriptional activity of the HIV-1 promoter (HIV-LTR). $CD8^+$ T-lymphocytes from HIV-1 infected asymptomatic individuals consistently suppressed tat-mediated HIV-LTR transcription activity in autologous $CD4^+$ lymphocytes. In contrast to the suppressive effect seen with $CD8^+$ lymphocytes from HIV-1^+ individuals, $CD8^+$ lymphocytes from HIV-1^- individuals had no effect on tat-mediated transcription activity. $CD8^+$ lymphocytes from either HIV-1^+ or HIV-1^- individuals failed to alter HIV-LTR (i.e. basal) transcription activity. In a minority of samples, culture supernatants from $CD8^+$ T-lymphocytes from HIV-1^+ individuals exerted an inhibitory effect on tat-mediated HIV-LTR transcription. Preliminary experiments suggest that the suppressive effect of $CD8^+$ T-lymphocytes on tat-mediated HIV-LTR transcription may be $CD4^+$ cell specific, as $CD8^+$ T-lymphocytes from HIV-1^+ individuals did not exert a significant effect on tat-mediated HIV-LTR transcription in autologous EBV transformed B lymphocytes or in Jurkat cells (a T lymphoblastoid cell line). In conclusion, $CD8^+$ lymphocytes from asymptomatic HIV-1^+ individuals manifest virus suppressive activity at the level of virus transcription. This suppressive effect of $CD8^+$ T-lymphocytes on tat activity could play a role in the regulation of HIV-1 replication during the asymptomatic period of HIV-1 infection.

Q 512 A PHASE I DOSE-RANGING TRIAL OF HGP-30 CANDIDATE VACCINE IN HIV SERO-NEGATIVE ADULTS, Rebecca Coleman[1], Daniel Stites[1], Nelson Murcar[1], Prem Sarin[2], A. Goldstein[2], Peter Heseltine[3], Vincent Simon[4] and James Kahn[1]
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A Phase I study was undertaken to examine the safety and relative immunogenicity of 3 doses of HGP-30 candidate vaccine in HIV sero-negative adults. Twenty-one subjects were enrolled as follows: 10 μg/kg (6 subjects), 25 μg/kg (6), 50 μg/kg (6) and 100 μg/kg (3). Subjects received doses intramuscularly on Day 0, and in Weeks 4 and 10. Subjects were followed at monthly intervals for one year. A uniform fourth dose (25 μg/kg) is being administered at least 12 months after the third immunization. All subjects tolerated their doses well to date, reporting tolerable local pain at the injection site. Clinical evaluations have detected no toxicity at any dose in association with the injections. Lymphocyte phenotype analysis to date demonstrates no significant changes from baseline. Lymphoproliferative responses to date suggest a significant response to the carrier molecule, KLH, with a much smaller, though detectable, response to HGP-30. Preliminary analysis of antibody development in 16 subjects reveals antibody to KLH in all 16, antibody to HGP-30 in 11 and antibody to p17 in 5. One subject had immunoblot evidence of HIV sero-conversion at week 22. Further studies are underway evaluating this event. Full lymphoproliferative response, lymphocyte phenotyping and antibody development data will be presented.

Q 513 **A RAPID SCREEN FOR DRUG-RESISTANT HIV PROTEASE MUTANTS.** Jon H. Condra, Emilio A. Emini, Leah Gotlib and Donald J. Graham. Department of Virus and Cell Biology, Merck Research Laboratories, West Point, PA 19486.

The aspartic protease of Human Immunodeficiency Virus is responsible for cleavage of the viral polyprotein into mature viral proteins. The protease has been shown to be essential for viral infectivity, and is thus an attractive target for antiviral intervention. Numerous studies of HIV reverse transcriptase inhibitors have indicated that drug-resistant viral variants rapidly appear in response to antiviral therapy, both in cell culture and in clinical settings. The potential appearance of mutants resistant to HIV protease inhibitors, therefore, is of great concern in the development of effective antiviral agents.

Baum *et al.* (PNAS **87**:10023-10027 (1990)) have described an *in vitro* system for measuring the activity of HIV protease when expressed in *E. coli*. In this system, the protease is coexpressed with a modified ß-galactosidase that is cleaved and inactivated by the protease. In the absence of protease, colonies appear blue in the presence of Xgal. When protease is active, the ß-galactosidase is cleaved, leading to white bacterial colonies.

We have modified this system to permit the rapid identification, by colony color, of protease mutants resistant to a variety of antiviral inhibitors. Screening a randomly mutagenized library of HIV protease genes expressed in *E. coli* has permitted the identification of mutants resistant to protease inhibitors *in vitro*.

This screen is useful to predict the appearance of drug-resistant viral variants in advance of clinical trials, thereby accelerating the design of inhibitors active against these mutants.

Q 514 CONVERSION OF 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) TO ITS TOXIC METABOLITE,3'AMINO-3'-DEOXYTHYMIDINE (AMT) IS MEDIATED BY CYTOCHROME P450 AND NADPH-CYTOCHROME C REDUCTASE IN LIVER MICROSOMES. E. M. Cretton, L. Placidi, and J-P Sommadossi. Dept. of Pharmacology, Univ. of Alabama at Birmingham, Birmingham, AL.

In the present study, the metabolism of AZT to its toxic catabolite AMT, was characterized in liver microsomes, using AZT as substrate. Microsomes were exposed to 1 mM AZT, 6mM NADPH and incubated under various conditions. AMT formation was enhanced in the presence of FAD and/or FMN, under anaerobic conditions and is inducible by phenobarbital. Pre-incubation with various P450 inhibitors such as chloramphenicol, n-octylamine, SKF 525A and alpha-naphthoflavone decreased AZT reduction; exposure to carbon monoxide completely inhibited AMT formation. However, when FAD was added to the incubation media following carbon monoxide, no inhibition was observed. In addition, pre-incubation with polyclonal antibodies to rat NADPH-cytochrome C reductase and a cytochrome P450 (IIB1) resulted in 50% and 80 % inhibition of AMT formation, respectively. When microsomes from different human liver specimens were assayed, wide variations in AMT formation, as much as 5 to 6 fold, were observed. A correlation ($r = 0.85$) between AMT formation and total P450 was observed. These data demonstrate that AZT reduction is mediated by both cytochrome P450 and NADPH-cytochrome P450 reductase. The large variations in AMT formation observed in human liver specimens suggest that large variations in the pharmacokinetics and formation of this metabolite may be observed in vivo thereby influencing the AZT pharmacodynamic properties. The potential drug-drug interactions between AZT and drugs which interact with P450 may potentially affect the toxicity and/or activity of AZT and should be carefully evaluated.

Q 515 PHENOTYPIC AND IMMUNOLOGIC CHARACTERIZATION OF LYMPHOCYTES IN HIV-ASSOCIATED CD8+ LYMPHOCYTOSIS, Tyler J. Curiel, Lisa Starke, Nancy Madinger, Xing-Quan Zhang and Elizabeth Connick, Box B-168 Infectious Disease Division, University of Colorado Health Sciences Center, Denver, CO, 80262.

CD8+ lymphocytosis is an unusual syndrome of unknown etiology first described in HIV negative individuals. In HIV infected individuals, the syndrome may be asociated with organ infiltration with CD8+ cells, and has been associated with HLA DR5. Little is known regarding the immune status and ultimate clinical course of these patients. We have established a prospective study of patients who are HIV infected and who have more than 1700 CD8+ cells/ml in peripheral blood. Nine patients are currently under investigation. All are males with median CD8+ counts of 2322/ml (range 1785-6432) and median CD4+ counts of 561/ml (range 153-602). HIV-specific cytotoxic T cell killing was detected in the peripheral blood mononuclear cells (PBMC) of 2/3 patients. High background lysis interfered with interpretation of this assay in the third. Normal blastogenesis of PBMC was detected in response to PHA in 2/2, to tetanus in 0/2, to Herpes simplex in 1/2 and in a mixed lymphocyte reaction in 0/2. One subject (with 6432 CD8+ cells/ml) appeared to have a clonal population of circulating CD8+ cells by analysis of T cell receptor rearrangements. HTLV-I culture was negative in 0/2. HLA DR5 was present in 2/7. Karyotypic analysis was normal in 2/2, and is pending in 2 more. FACS analysis of PBMC was done using CD3/CD4, CD3/CD8, CD4/CD8, CD8/CD25, CD8/CD38, CD8/S6F1, CD45RA/CD8, DR/CD8 and NKH.1/CD8. FACS data as well as analysis of larger numbers of patients will be presented at the meeting. Study of these patients may afford insights into the role of CD8+ cells in the pathogenesis of HIV disease.

Q 516 Heteroduplex Analysis of HIV-1 Envelope DNA Sequence Variation. Eric L. Delwart, Francine McCutchan*, Haynes Sheppard#, Jaap Goudsmit@, Helga Rübsamen-Waigman§ and James I. Mullins. Dept of Microbiology and Immunology, Stanford University Medical Center, Stanford CA; *Henry M. Jackson Foundation, Rockville MD; #California Dept. Health Services, Berkeley CA; @Human Retrovirus Lab, University of Amsterdam, Holland; §Georg-Speyer-Haus Institute, Frankfurt, Germany.

Nucleotide gaps and mismatches in DNA heteroduplexes affect electrophoretic mobility in polyacrylamide gels. DNA heteroduplex mobility decreases with increasing levels of sequence divergence between the annealed strands. This observation led to the development of a heteroduplex gel shift assay (HGSA) for the rapid analysis of human immunodeficiency virus (HIV-1) sequence variation through direct analysis of the DNA products from nested polymerase chain reactions (PCR). Using HGSA we found that the level of sequence variation varies greatly between epidemiologically unlinked individuals in different geographic regions. HIV-1 from Nothern Thailand and from the Bombay region of India, sites of recent HIV-1 introduction, shows low levels of HIV-1 env variation, comparable in some cases to what is observed within a single individual. The levels of DNA sequence divergence was larger in the US and Europe and largest on the African continent. Phylogenetic analysis of heteroduplex fragment mobility data revealed clustering of sequences resulting in a single US/Western European subtype. Highly related viral sequences could be identified on different continents.

HGSA was also used to monitor the generation of env sequence complexity following primary infection. All pre-seroconversion quasispecies examined appeared highly homogeneous and variation developed at variable rates in different individuals, with rapid progressors to AIDS not developing high levels of quasispecies complexity.

Q 517 *IN VITRO* CHARACTERIZATION OF BISHETEROARYLPIPERAZINE-RESISTANT RECOMBINANT HIV-1 RT

T.J. Dueweke, S.M. Poppe, S.M. Swaney, and W.G. Tarpley, Upjohn Laboratories, Kalamazoo, MI 49001

Several nonnucleoside inhibitors of HIV-1 RT have been described, including the dipyridodiazepinone nevirapine, the pyridinones (i.e. L-697,661), TIBO compounds, and the bisheteroarylpiperazines (BHAPs). These drugs all compete for binding to RT and are thus thought to share a common mode of action. HIV-1 resistant to L-697,661 or nevirapine emerges rapidly *in vitro* and is primarily caused by RT substitutions at amino acids 103 or 181 that also confer cross resistance to other nonnucleoside RT inhibitors. To characterize resistance development to the BHAPs, resistant HIV-1 has been derived in cell culture and characterized by nucleotide sequencing (see S.M. Poppe *et al.* abstract). Recombinant RTs with amino acid substitutions thought to confer resistance to the BHAPs were created by site-directed mutagenesis and characterized enzymatically. Biochemical analysis of these mutant RTs and other nonnucleoside-resistant RT mutants (i.e. K103N and Y181C) will be presented.

Q 518 CYTOKINE DYSREGULATION IN CD4 AND CD8 T CELLS IN HIV INFECTION, **Fan J, Bass H, Guan WM, Fahey JL. Center For Interdisciplinary Research in Immunology and Disease, and Multicenter AIDS Cohort Study, University of California, Los Angeles, CA 90024**

Both immune deficiency and activation occur in the immune system in HIV infection. Because cyokines have a central role in the performance and regulation of the immune system, studies were undertaken to evaluate the gene expression of several key cytokines comparing specific mRNA in the lymphocytes of HIV seronegative and seropositive individuals. Furthermore, because of the selective binding of HIV on CD4 T cells and the striking differences in CD4 and CD8 T cell numerical changes, purified CD4 and CD8 T cells from HIV seropositive homosexual men were compared with two reference populations (10 persons in each group) and evaluated for cytokine mRNA. The mRNA levels for IL-2, INF-gamma, IL-10, and TNF-alpha were measured by quantitaive RT PCR. We found that the profiles of cytokine mRNA differ for CD4 and CD8 T cells. Major changes were seen in CD4 T cells on a per cell basis, the median mRNA levels of IFN-gamma, IL10, and TNF-alpha are increased approximatelt 3 times in HIV seropositive subjects ($p < 0.05$). IL-2 showed lesser increases when compared to HIV seronegative homosexual men. CD8 cells from HIV seropositive men, in contrast, had IL-2 and IFN-gamma cytokine mRNA levels that were decreased when compared to HIV seronegative subjects. Thus expression of many cytokine genes in circulating CD8 cells is reduced during HIV infection. Thus, the activation of CD8 T cells (as indicated by increased cell number and phenotypic marker changes) presumably is response to increased cytokine production in other (non-CD8) cells of the body. Increased cytokine gene expression in CD4 T-cell, on the other hand, indicates where substantial amounts of cytokine are produced. (The per cell production of IFN-gamma is especially high). This substantial activation of CD4 T cells may represent the effects of soluble HIV products rather than direct infection. The altered balance of cytokines can contribute to immune malfunction and impared effector function.

Q 519 HIV-1 INDUCES INTERFERON α IN BLOOD DENDRITIC CELLS, John Ferbas and Charles Rinaldo, Departments of Infectious Diseases and Microbiology and Pathology, University of Pittsburgh, Pittsburgh, PA 15261

The ability of the host to produce IFNα in vitro has been proposed to be a correlate of HIV-1 disease progression. The cellular origin of IFNα, however, has not been adequately established and precludes focused investigations on the role of IFNα-producing cells during HIV-1 infection. We determined the relative capacities of cell subsets from all major PBMC lineages to produce IFNα in response to HIV-1 (IIIb, RF and BaL), Sendai virus and HSV-1. Initial experiments indicated that IFNα was detectable within 6 hours of stimulation and reached maximal titers at 18-24 hours. The IFN was characterized as subtype α with type-specific antibodies and was acid stable. Negative selection and enrichment experiments indicated that HLA DR^+ null cells produced the majority of the IFNα. We designed, therefore, a flow cytometric sorting protocol using a cocktail of monoclonal antibodies to purify HLA DR^+ null PBMC, i.e., HLA DR^+, $CD3^-$ (T), $CD19^-$ (B), $CD16^-$, $CD56^-$ (NK), $CD14^-$ (monocyte). These cells, which comprised 0.5±0.1% of unfractionated PBMC, were enriched to >95% purity. The cells were morphologically identified as blood dendritic cells by scanning electron microscopy. The purified dendritic cells produced 50-, 60-, 5- and 15-fold more IFNα compared to purified monocytes in response to HIV IIIb, HIV BaL, Sendai virus and HSV-1, respectively. These viruses did not induce IFNα in $CD3^+$ T cells or $CD56^+$ NK cells. B cells produced a minimal amount of IFNα to Sendai virus only. In addition, psoralen/U.V. inactivated HIV IIIb, and viable or inactivated HIV IIIb infected CEM cells, induced IFNα production in dendritic cells, suggesting that this was independent of the state of the viral inducer. We conclude that the production of IFNα constitutes a previously unrecognized, major function of blood dendritic cells. The ability to prepare >95% pure dendritic cells with facilitate future experiments designed to address the mechanisms of IFNα production in vitro, as well as other aspects of dendritic cell interactions with HIV-1.

Q 520 **TH1 and TH2 CD4 Subset Responses in HIV-1 Infected Individuals at Various Stages of Disease**

Guido Ferrari[1,3], Mark V. Packard[2], John A. Bartlett[2] and Kent J. Weinhold[3]. [1]Laboratorio di Virologia, Ospedale S. Raffaele, Milan, Italy, Departments of [2]Medicine and [3]Surgery, Duke University Medical Center, Durham, NC 27710

HIV-1 infection initiates a cascade of immunologic dysfunction which continues to escalate throughout disease progression. To date, the early impairment of immune function has not been examined in the context of functional CD4 subsets. To examine the functional integrity of TH1 and TH2 subsets of CD4 cells, PBMC from HIV-1 infected patients at various stages of disease were co-cultivated with a panel of antigens consisting of Candida albicans extract (CASTA), psoralen-inactivated HIV-1, recombinant gp160, and a synthetic peptide analogue (DP6) of a p24 determinant. PBMC cultures from non-infected individuals served as controls. After 7 days, cell-free culture supernatants were collected and analyzed for the presence of the TH1 derived cytokines IL-2 and IFN-γ as well as the TH2 associated cytokines IL-4 and IL-6. Additionally, *in vitro* cultures were examined for the presence of memory and activated lymphocytes by flow cytometry. The two cytokines which proved to be most revealing were IFN-γ and IL-6. No spontaneous cytokine production was detected in unstimulated wells. Production of both IFN-γ and IL-6 in response to CASTA was detected for nearly all patients regardless of disease status. However, early stage patients, like the HIV-1 seronegative controls, produced higher levels of IFN-γ than IL-6. In late-stage patients this pattern was reversed. The response to HIV-1 related antigens was sharply skewed in favor of TH2 associated cytokine production. The appearance of the cellular activation markers CD25, CD38, HLA-DR, as well as CD45RO generally correlated with a high level of antigen-induced IFN-γ and IL-6 production although cytokine production was found in some cultures where no significant cellular activation was observed. In conclusion, these studies suggest that a TH2-related CD4 cellular response is favored in HIV-1 infected individuals. Whether this is due to immunologic dysregulation or to preferential loss of TH1 cellular subsets remains to be elucidated.

Q 521 HIV SPECIFIC CYTOTOXICITY AND CD8 CELL SUB-POPULATIONS IN HIV INFECTED CHILDREN Karin S Froebel, Marian C Aldhous, Karen C Watret, Jacqueline Q Y Mok and A Graham Bird. HIV Immunology Unit, University Dept of Medicine, Edinburgh, UK.

The cytotoxic T cell (CTL) response, mediated by CD8 cells, is thought to be an important part of the immune response against HIV. A number of antigens have been described on CD8 cells which are associated with cytotoxicity. In an earlier study we showed that HIV infected children have an abnormally raised proportion of CD45R0+CD8 cells (1). In this study we have asked whether raised levels of CD8 subpopulations in peripheral blood lymphocytes correlate with HIV-CTL activity. CTL activity against HIV 'gag', 'tat', 'pol' and 'env' proteins was measured after in vitro culture of pbl, in 8 HIV infected children, together with the proportion of CD8 pbl which are CD45R0, DR or S6F1 positive. The results show that all of the infected children have raised levels of CD45R0+, DR+ and S6F1+ CD8 cells, but that only 5 of the 8 children have HIV specific CTLs. The CD8 subset antigens in pbl therefore do not define functional CTL cells. However, analysis of the subpopulations after in vitro culture suggests that those cultures in which the CD8 subpopulations expand contain HIV specific CTLs.

1. Froebel et al AIDS (1991) **5** 97-99.

Q 522 ACTIVATED PHENOTYPE OF CIRCULATING HIV-SPECIFIC $CD8^+$ CYTOTOXIC T CELLS, Janis V. Giorgi, Hong-Nerng Ho and Ronald T. Mitsuyasu, UCLA School of Medicine, Los Angeles, CA 90024

CD38 is an activation antigen (Ag) with multilineage distribution and the MHC class II molecule HLA-DR (DR) is also an activation Ag when expressed on T cells. The expression of these two molecules, which have markedly elevated levels of expression on $CD8^+$ cells of HIV-infected people, was investigated on circulating HIV-specific $CD8^+$ CTL. Purified $CD8^+$ lymphocytes from twenty-two HIV-seropositive participants in the UCLA Multicenter AIDS Cohort Study were screened for CTL activity against autologous EBV-immortalized lymphoblast targets infected with vaccinia vectors that carried HIV_{IIIB} *gag*, *pol*, and *env* genes. Sixty-seven percent (14/21), 64% (14/22), and 9% (2/22), respectively of the subjects had HIV-specific $CD8^+$ CTL activity against gag, pol, and env proteins. $CD8^+$ cells from eleven of the subjects who had high CTL activity were then FACS separated using three-color immunofluorescence sorting. Circulating DR^-CD38^- $CD8^+$ cells had little activity. Highly purified DR^+CD38^+ $CD8^+$ cells had higher HIV-specific CTL activity than other $CD8^+$ cells. DR^+CD38^- or DR^-CD38^+ $CD8^+$ cells also mediated significant activity, but only about half as much on a per cell basis as DR^+CD38^+ $CD8^+$ cells. This is the first report that the CD38 molecule is expressed in vivo on Ag-specific $CD8^+$ CTL, and confirms previous reports that DR is expressed on these cells. Both asymptomatic HIV-seropositive subjects (144 ● 132/mm^3) and AIDS patients (253 ± 178/mm^3) had markedly elevated levels of DR^+CD38^+ $CD8^+$ cells compared with the levels in HIV-seronegative controls (7 ± 3/mm^3). These results indicate that $CD8^+$ T cells with the phenotype of CTL are present throughout the course of HIV disease, but the relative activity of these $CD8^+$ CTL declines as disease progresses.

Q 523 **SELECTION AND CHARACTERIZATION OF MUTANTS OF FELINE IMMUNODEFICIENCY VIRUS RESISTANT TO THE COMBINATION OF 3'-AZIDO-3'-DEOXYTHYMIDINE AND 2',3'-DIDEOXYINOSINE,** Judy M. Gobert, Kathryn Martin Remington, Ya Qi Zhu, and Thomas W. North, Division of Biological Sciences, The University of Montana, Missoula, MT 59812.

We selected mutants of feline immunodeficiency virus (FIV) using the combination of 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxyinosine (ddI). This is the first report of mutants arising to combination chemotherapy in an *in vitro* cell culture system. Mutants of FIV were also selected with ddI alone. A focal immunoassay developed by this lab was used to quantitate the susceptibility of the FIV mutants to various inhibitors. The AZT,ddI-selected mutant was found to be resistant to AZT and phosphonoformate (PFA), slightly resistant to ddI, and hypersensitive to 2',3'-dideoxycytidine (ddC). The ddI-selected mutant was also found to be resistant to PFA, slightly resistant to ddI and hypersensitive to ddC, but remained sensitive to AZT. These patterns of susceptibility are different than the patterns found with mutants selected with AZT alone (Remington et al, J. Virol. 65:308-312, 1991), which were resistant only to 3'-azidonucleosides. The FIV mutants reported here will be useful for studies of combination chemotherapy and mechanisms of drug-resistance. (Supported by NIAID Grant AI-28189).

Q 524 SAFETY AND IMMUNOGENICITY OF A MONOCLONAL ANTI-IDIOTYPE ANTIBODY (3C9) PLUS SAF-M ADJUVANT FOR HIV INFECTED PATIENTS. R.H. Haubrich, J.A. McCutchan, V. Caralli, S.A. Spector, C. Jacobsen, C. Kang, J. Merritt. University of California, San Diego, CA 92103; IDEC Pharmaceuticals, Mountain View and La Jolla, CA

Objective: To assess the safety and immunogenicity of 3C9, a mouse monoclonal anti-idiotypic antibody which defines a marker on human B cells that produce broadly neutralizing anti-gp120 antibodies and evokes HIV neutralizing antibody responses in monkeys.
Method: Six asymptomatic patients with CD4 > 600 were assigned to each of four groups using a double blind randomization: (1) 2.5 mg 3C9, (2) 3C9 + emulsion (polysorbate 80, poloxamer 401, squalane) (3) 3C9 + SAF-m [emulsion + temurtide (an analog of MDP)] (4) emulsion + temurtide. Patients were immunized on weeks 0, 2, 4, 6, 14, and 22.
Results: Vaccination resulted in tolerable local and systemic toxicity. Mean duration of local pain at the injection site was 46 hours and systemic symptoms (fever, myalgia, arthralgia) lasted from 16 to 27 hours. One patient required a blinded dose reduction of the adjuvant component because of fever and myalgia. Laboratory abnormalities were minimal and clinically insignificant except for two elevated CPK values (1462 and 6212) which resolved by the next visit. Eight patients had episodes of diarrhea not consistently related to immunization. CD4 lymphocyte counts and quantitative PBMC and plasma HIV cultures showed no consistent change in the cohort. A total of 10 of 18 possible subjects who received 3C9 developed an Ab3 response to immunization. The treatment groups will be broken out for analysis and presentation.
Conclusion: 3C9 is well tolerated and is immunogenic. SAF-m adjuvant appears safe in this population.

Q 525 A RETROVIRAL VECTOR ENCODING HIV-1_{IIIB}ENV INDUCES POTENT CTL AND ANTIBODY RESPONSES IN MICE AND MACAQUES, Michael J. Irwin, Lisa S. Laube, Sunil Chada, Melissa Arce, Virginia Lee, Douglas J. Jolly, and John F. Warner, Department of Immunobiology, Viagene, Inc., San Diego, CA 92121

A murine MoMLV-based recombinant retroviral vector (N_2IIIB-env) containing the envelope and rev genes from HIV-1_{IIIB} was developed as a potential HIV vaccine or immunotherapeutic for HIV infected individuals. Previous studies using syngeneic cells transduced with this retroviral vector and expressing HIV-1 envelope protein (HIV-1env) have demonstrated induction of HIV-1env-specific cytotoxic T lymphocyte (CTL) and antibody responses. In this study we have evaluated the ability of N_2IIIB-env to induce specific anti-HIV immune responses in mice and rhesus macaques after direct in vivo administration of the retroviral vector. In mice, both intramuscular (IM) and intraperitoneal (IP) administration of a single dose (10^5cfu) of the N_2IIIB-env vector have generated substantial HIVenv-specific CTL and antibody responses. In addition, rhesus macaques injected IM with 10^6 cfu of N_2IIIB-env vector particles developed substantial HIVenv-specific CTL responses. Thus retroviral vectors appear to be an effective means of inducing immune responses, particularly CTL responses.

The N_2IIIB-env retroviral vector is currently being evaluated as a potential immunotherapeutic for post-exposure HIV infected individuals as well as a vaccine for protection against HIV infection. As with any anti-HIV therapeutic, the ability to recognize divergent HIV-1 isolates is an important consideration. CTL lines generated by injection of N_2IIIB-env and propagated with HIV-1_{IIIB}env-expressing transduced fibroblasts show clinical cross coverage by their ability to lyse target cells expressing env epitopes of different HIV isolates. We have also demonstrated lysis of target cells infected with prototypic, clinical, and sequential patient HIV isolates with effectors generated by injection of syngeneic cells transduced with N_2IIIBenv. The ability to induce CTL cross reactivity against various HIV isolates could have important implications regarding vaccine and therapeutic applications.

Q 526 PHASE 1 STUDY OF AN HIV-1 GP 120 VACCINE COMBINED WITH THE NOVEL ADJUVANT EMULSION, MF59 AND THE BIOLOGIC RESPONSE MODIFIER MTP-PE IN HIV SERO-NEGATIVE ADULTS J. Kahn, D. Chernoff, F. Sinangil, J. Baenziger, N. Murcar, D. Wynne, R. Coleman, K. Steimer and C. Dekker. University of California San Francisco, San Francisco General Hospital, CA 94110, Chiron Corp, Emeryville CA.

A phase 1 randomized double-blind study was performed to determine safety and immunogenicity in HIV-seronegative adults of three injections of a vaccine composed of 25 μg of recombinant HIV gp 120 antigen combined with MF 59 emulsion containing a muramyl tripeptide (MTP-PE) in a dose escalation format. The vaccine antigen is recombinant gp 120 from the SF2 isolate of HIV-1, expressed in Chinese hamster ovary cells. The gp 120 vaccine is glycosylated and exhibits CD4 binding activity. Forty-two healthy HIV-seronegative adult men and women, with normal laboratory studies and without identifiable high-risk behavior for HIV infection were vaccinated. Vaccination occurred at day 0, 1 month and at 6 months. Each vaccine contained MF59 emulsion and each subject received MTP-PE (μg) dosing as follows: Group 1 (0); Group 2 (1); Group 3 (10); Group 4 (50); Group 5 (10) on the first immunization and (0) at month 1 and 6; Group 6 (100) MTP-PE at initial immunization and (0) on month 1 and 6. Two subjects in each group were randomized to receive placebo-antigen while 6 received gp 120. A fourth immunization was administered at month 12.

All subjects except one received all three vaccinations. Symptoms post immunization included mild muscle aches, headache, low grade fevers and injection site pain. ELISA antibodies directed to gp120 developed in the expected number of subjects. All subjects developing ELISA antibodies also developed neutralizing titers to SF-2 comparable to titers observed in naturally infected subjects. Virus neutralizing to a heterologous strain (MN) has also been observed. Durability of antibody responses has been documented 6 months following the third immunization. Lymphocyte proliferation data has been documented in most of the subjects developing gp 120 antibodies. Six months following the third immunization, these responses remained high in a majority of subjects.

Initial information suggests that this candidate HIV vaccine is well tolerated and immunogenic. The safety and immunogenicity will be presented. The role of MF59 with and without MTP-PE in eliciting humoral and cellular responses will also be discussed. Durability of responses and broadening of responses will also be presented.

Q 527 T CELL RECEPTOR GENE USAGE IN HIV-1 SPECIFIC CYTOTOXIC T LYMPHOCYTES, Spyros Kalams*, R.P. Johnson*, A. Trocha*, M.J. Dynan*, J.T. Kurnick**, B.D. Walker*, *Infectious Disease Unit, Massachusetts General Hospital, Charlestown, MA 02129, ** Dept. of Pathology, Mass. General Hospital.

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

Peripheral blood mononuclear cells (PBMC) obtained from a seropositive individual were cloned at limiting dilution in the presence of IL-2, feeder cells, and a CD3-specific monoclonal antibody or PHA as a stimulus to T cell proliferation. CTL epitopes were defined using autologous EBV lymphoblasts incubated with synthetic HIV-1 peptides. cDNA was prepared from approximately $5x10^6$ cloned T cells. PCR was then performed with 5' specific oligonucleotides from the variable regions of the 29 known Vα genes and 24 known Vβ genes in conjunction with a 3' primer from the constant region of the respective TCR α or β gene. PCR products were then sequenced directly using the dideoxy chain termination technique.

Envelope specific CTL clones have been isolated from an HIV-1 infected individual over a 29 month span. All clones are HLA-B14 restricted and recognize the same nine amino acid minimal epitope. All clones thus far analyzed have utilized Vα14 and Vβ4 T cell receptor (TCR) genes. Sequencing of these genes has revealed identical diversity and joining regions, indicating a restricted TCR usage for this epitope which is maintained over time. Another HLA-B14 restricted clone isolated from this same patient, which is specific for an epitope in the reverse transcriptase (RT) molecule, utilizes the Vα 21 and Vβ 14 genes. Studies are in progress to determine whether CTL clones specific for other HIV-1 epitopes from this individual show limited TCR gene usage.

This study demonstrates that HIV-1 specific CTL of a given epitope specificity and HLA restriction can exhibit limited T cell receptor gene usage in a given individual which is maintained over time. Analysis of clones with the same epitope specificity and HLA restriction from different individuals will aid in the understanding of the role TCR gene rearrangement plays in CTL recognition of HIV-1 epitopes.

Q 528 A SINGLE MUTATION IN HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) REVERSE TRANSCRIPTASE CONFERS RESISTANCE TO THE NUCLEOSIDE ANALOGUE 2',3'-DIDEOXY-5-FLUORO-3'-THIACYTIDINE (FTC), Sharon D. Kemp, Margaret Tisdale, Nigel R. Parry and Brendan A. Larder, Department of Molecular Sciences, The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K..

Resistant variants of human immunodeficiency virus type 1 (HIV-1) have been selected by limited passages in cell culture of wild-type or 3'-azido-3'-deoxythymidine (AZT) resistant strains with the nucleoside analogue 2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC). *In vitro* selection with 2',3'-dideoxy-3'-thiacytidine (3TC) gave rise to resistant variants at a rate similar to FTC. This rapid selection of resistant virus has not previously been seen with nucleoside analogues but is reminiscent of that seen with the non-nucleoside reverse transcriptase (NNRT) inhibitors. The combination of AZT with FTC resulted in a slower rate of resistance developing to FTC. Virus variants independently selected with either FTC or 3TC were cross resistant to both inhibitors. DNA sequence analysis of the reverse transcriptase (RT) coding region from the FTC resistant variants revealed a mutation at codon 184. An infectious molecular clone constructed with an M184 to V mutation yielded virus after transfection of T-cells which was about 1000-fold more resistant to FTC than wild-type virus. This degree of resistance was similar to that seen with the passaged virus. In order to assess the influence of this mutation in a background of AZT and NNRT inhibitor resistance mutations, a series of HIV-1 variants were created by site-directed mutagenesis. The susceptibilities of these viruses to nucleoside analogues and NNRT inhibitors was determined. This study assessing the effects of multiple drug resistance mutations may help to establish a rationale for using these drugs in the future therapy of HIV disease.

Q 529 IMMUNOGENICITY OF BACULOVIRUS DERIVED HIV-1 gp160 IN INBRED STRAINS OF MICE, Ronald C. Kennedy and Ronald Q. Warren, Center for AIDS Research at the Southwest Foundation for Biomedical Research, Department of Virology/Immunology, San Antonio, TX 78227

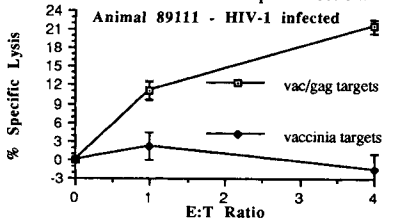
We examined the extent of antibody cross-reactivity to a panel of V3 based synthetic peptides in six inbred strains of mice following repeated immunization with a baculovirus-derived recombinant gp160 (rgp160) preparation formulated with alum. Following five injections with rgp160 all six strains developed antibodies to the homologous IIIB based V3 peptides, designated 304-321 and RP135. However, antibody cross-reactivity to the other non-homologous V3 peptides was either undetectable or limited among the strains of mice examined. No *in vitro* neutralizing activity against HIV-1 was observed in sera from any of the six inbred strains of mice that were examined. The antibody titers to gp160 and the fine specificity to linear defined gp160 epitopes indicated a relatively weak humoral immune response was induced. These results suggest that repeated immunization of mouse strains with a rgp160/alum formulation leads to non-neutralizing antibodies directed against the V3 region which remain predominantly type specific.

Q 530 HIV-1 SPECIFIC CYTOTOXIC AND PROLIFERATIVE T CELL RESPONSES IN HIV-1 INFECTED MACACA NEMESTRINA. Stephen J. Kent, L Corey, J McElrath, P Greenberg. Departments of Medicine, Laboratory Medicine and Immunology, University of Washington, Seattle, WA 98195.

INTRODUCTION: M. Nemestrina can be infected with HIV-1, and are being evaluated as a model for studying protective immunity to this virus. As part of this evaluation we examined CTL and T cell lymphoproliferative (LP) responses elicited by HIV-1 infection in M. Nemestrina.

METHODS: Fresh PBMC were obtained at multiple time points following infection with cell-free or cell-associated HIV-1. To assess LP responses, fresh PBMC were incubated with psoralen-inactivated HIV-1 for 7 days prior to measuring ^{3}H-Thymidine incorporation. For CTL assays, the PBMC were incubated for one week with vaccinia/gag infected autologous PBMC and then stimulated weekly with autologous irradiated vac/gag infected H.Papio transformed B cell lines (BLCL) and filler. Lytic activity was assessed with ^{51}Cr labeled BLCL. From selected cultures, CTL were cloned by limiting dilution with vac/gag infected BLCL.

RESULTS: LP responses to HIV-1 were detected 6 to 90 weeks post infection in 5/6 animals, with stimulation indices that ranged from 6 to 35. CTL activity from PBMC obtained 10 to 90 weeks post infection were detected following multiple sequential stimulations of bulk cultures in 2/4 animals (Fig). Limiting dilution culture permitted isolation of gag specific CTL clones, which are currently being characterized for fine specificity and restriction, from one of these animals at 10 weeks post infection.



CONCLUSION: Both LP and CTL responses to HIV-1 have been detected in HIV-1 infected M. Nemestrina. The presence of such T cell responses following viral infection suggest that it should be possible to explore the role of components of the T cell response in preventing and controlling HIV-1 infection in this animal model.

Q 531 DEVELOPMENT IN VIVO OF GENETIC VARIABILITY OF CLONED SIV AND HIV-1, Reinhard Kurth, Heike Merget, Matthias Dittmar, Stephen Norley, Michael Baier and Klaus Cichutek, Paul Ehrlich Institute, D-6070 Langen/Frankfurt, Germany

Rapid development of variability may contribute to the pathogenicity of lentiviruses and it was thus of interest to compare infection of SIV in a healthy natural host with HIV-1 infection of humans. We have investigated development of virus variants following injection of molecularly cloned SIVagm into its natural host, the African green monkey, and after accidental infection of a few hemophilia B patients with a biological clone of HIV-1.

SIV of AGM rapidly developed variants in the V1- and V2-like variable domains. Dominating major virus variants were never detected. Extensive variability evolved from a single genotype. A remarkable non-immunological selection for non-synonymous mutations was observed.

In all nine hemophiliacs acutely infected with an HIV-1 biological clone only the consensus sequence was initially demonstrable. Later, true intermediates between input and multiply mutated genotypes were found. Variability initially clustered outside of the V3 loop, whereas after more than one year p.i. V3 variability began to develop. The evolution of SIV and HIV-1 quasispecies starting from a single genotype will be illustrated.

Q 532 RESISTANCE OF DNA SYNTHESIS BY MUTANTS OF HIV-1 TO AZT-TP, ddATP, AND NEVIRAPINE DEMONSTRATED USING AN ENDOGENOUS ASSAY, Simon F. Lacey*, Katyna Borroto-Esoda#, Brendan A. Larder*, and Lawrence R. Boone#, *Department of Molecular Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS,UK, and #Department of Virology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

It is now well documented that amino acid substitutions at 5 codons in the HIV-1 reverse transcriptase (at positions 41, 67, 70, 215, and 219) are responsible for conferring resistance to AZT (zidovudine, 3'-azido-3'-deoxythymidine, Retrovir). However, to date there have been no published data demonstrating that this resistance is due to an altered interaction between mutant RT and AZT-TP (Larder et al Science 243 1731-1734,1989, Lacey et al J. Biol. Chem. 267 15789-15794, 1992).

To study this problem we have used a recently developed endogenous RT assay (Borroto-Esoda and Boone in press) to test the sensitivity of DNA synthesis by virion-associated RT on viral genomic RNA using the native tRNAlys primer. The major product is a 9.4Kb reverse transcript which corresponds to one template jump with synthesis of all but one LTR of the viral genome, and quantitation of this was used as a measure of RT activity.

We used a panel of recombinant mutant viruses in the assay with combinations of the 5 AZT-resistance mutations (41Leu, 67Asn, 70Arg, 215Tyr or Phe, 219Gln), 74Val which confers resistance to dideoxyinosine (ddI), and 181Cys which confers resistance to a range of non-nucleoside inhibitors including nevirapine (BI-RG-587). We were able to show an approximately 8-fold decrease in sensitivity to AZT-TP in the case of the most resistant virus (41/67/70/215Y/219), with lesser resistance in the case of viruses with fewer mutations. Standard exogenous RT assays employing poly(rA):oligo(dT) with the same virus preparations gave similar sensitivities to AZT-TP for both wild-type and mutant, demonstrating the importance of template and/or conformation for AZT-TP resistance. We were also able to demonstrate ddATP (the active form of ddI in cells) resistance for the mutants with the 74Val change and nevirapine resistance for mutants with the 181Cys.

Q 533 CYTOKINE EXPRESSION IN ACTIVATED B-CELLS OF PATIENTS WITH AIDS Shing-Yi Lee and Suraiya Rasheed, Ph.D. Laboratory of Viral Oncology and AIDS Research, USC School of Medicine, Los Angeles, CA 90032

Several cytokines such as Interleukin-6 (IL-6) and TNF-a, have been shown to be secreted by the activated B-cells in HIV-infected individuals. To determine the role of cytokines in the development of B-cell lymphoma in patients with AIDS, we compared the levels of IL-1a, IL-2, IL3, IL-5, IL-6, IL-7, IL-8, IL-10, TNF-a and IFNa in various established T- and B- cell lines. The gene expression was assessed in the cellular RNA by RT-PCR and the PCR product was analyzed semi-quantitatively, by normalizing the cDNA product to the B-actin gene expression. Our results indicate that IL-6 and IL-10 RNA expression are significantly enhanced in the B-cell tumors of patients with AIDS but not in the non-HIV-associated B- or T-cell tumors.

Q 534 ANALYSIS OF HETEROGENEOUS VIRAL POPULATIONS BY DIRECT GENOMIC SEQUENCING

Thomas Leitner 1,2), Eva Halapi 3), Gabriella Scarlatti 2), Paolo Rossi 3), Jan Albert 4), Eva-Maria Fenyö 2) and Mathias Uhlén 1). 1) Department of Biochemistry and Biotechnology, Royal Institute of Technology,2) Department of Virology and 3) Department of Immunology, Karolinska Institute, 4) Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden

The ability of direct PCR sequencing to detect and quantify sequence polymorphisms were investigated using samples containing mixed populations of HIV-1. A part of the genome encoding the polymorphic variable region 3 (V3-loop) of the envelope was directly sequenced to yield a consensus sequence of the virus population. The results were compared with sequences obtained by analysis of multiple clones derived from the same clinical samples. The results of five patients suggested that the direct sequencing method can be used as a rapid tool to analyse and quantify heterogeneous viral populations. Reconstitution experiments using cloned material demonstrated that it was possible to detect and quantify minor sequence variants present in as little as 10 % of the total virus population.

Q 535 IL-6 INDUCED RESISTANCE OF HIV EXPRESSING TARGET CELLS TO T CELL MEDIATED CYTOTOXICITY Min Liu[1], Otoniel Martinez-Maza[1,2], Janis V. Giorgi[3], and Susan Plaeger-Marshall[4], Departments of Microbiology and Immunology[1], Obstetrics and Gynecology[2], Medicine[3], and Pediatrics[4], UCLA School of Medicine.

MHC-restricted CD8 cytotoxic T cell (CTL) activity against target cell expressing HIV proteins can be detected in vitro in the peripheral lymphocytes of most HIV-infected adults. CTL activity is variable and tends to diminish with disease progression. CTL activity may be an important component of immune protection in HIV infection. Thus, we sought to characterize mechanisms by which CTL assay system utilizing EBV-transformed autologous B cells infected with vaccinia virus vectors expressing env or pol proteins as target cells. The cytokine IL_6 is known to induce resistance of target cells to killing in some non-MHC restricted cytotoxic assays. Further, since IL-6 production is elevated in HIV-infected individuals, and is produced by their transformed B cells, we hypothesized a role for IL-6 in CTL killing of HIV-expressing B cell targets. Preliminary experiments from six asymptomatic HIV-infected subjects showed that 1). detectable levels of IL-6 were produced in the supernatant from transformed B cells of HIV-infected but not healthy control individuals, 2). preincubation of target cells with exogenous IL-6 resulted in diminished CTL activity, 3). preincubation of target cells with antibody to IL-6 resulted in enhancement of CTL activity, 4). the enhancement of activity associated with anti-IL-6 treatment varied in the six individuals, and correlated with the levels of IL-6 detected in their B cell supernatant. These results indicate that IL-6 induces resistance to CTL killing of HIV-expressing target cells. Confirmatory experiments with additional subjects are in progress. We thank the participants of the Los Angeles Multicenter AIDS Cohort Study for their contribution to this study.

Q 536 THE NAIVE HUMAN T HELPER REPERTOIRE SPECIFIC FOR HIV REVERSE TRANSCRIPTASE

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Reverse transcriptase (RT) of HIV can be recognized by antibodies, Th and CTL detected in seropositive individuals, even though RT represents a minor component of HIV. RT specific CTL may kill HIV infected cells, but it is harder to propose a role for RT specific antibodies and Th cells. Cooperative interaction between RT specific Th cells and env specific B cells is suggested by data by Sherle and Gerhard (PNAS 1988,85,4446) in the influenza virus system. Thus we investigated the human Th repertoire specific for RT in naive individuals.

We generated T cell lines and clones from naive individuals by in vitro stimulation with RT and presenting cells plus IL2. The fine specificity of T cells was examined with a panel of synthetic peptides provided by M. Pierres, CIML, Marseille, France.

RT specific lines were obtained from 6/7 individuals. The lines display a helper phenotype (>95% CD4+, WT31+). The bulk lines respond only to one peptide in the panel, but the peptides were different among the individuals.

These data suggest that the high frequency of RT specific T cells in naive repertoire of non primed individuals may be due to cross-reactivity with other unrelated antigens, or to previous exposure to cross-reactive RT molecules of yet undefined human retroviruses.

Q 537 MUTATIONAL ANALYSES OF THE SIX PEPTIDE-BINDING POCKETS OF HLA-A2 MOLECULE IN VIRUS-SPECIFIC AND ALLO-SPECIFIC CTL RESPONSES: A SYSTEM FOR THE STUDY OF HIV PEPTIDE PRESENTATION BY CLASS I MOLECULE, Masanori Matsui and Jeffrey A. Frelinger, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

To evaluate the contribution of the six peptide-binding pockets of class I molecules in CTL recognition, we have constructed an extensive library of HLA-A2 mutants with different amino acid substitutions in each of the six pockets, using a combination of saturation mutagenesis and site-directed mutagenesis. These mutants were tested in cytotoxicity assays with influenza A (Flu) virus-specific CTL and anti-A2 allo-specific CTL. In the Flu response, 4/7 mutants with substitutions in B pocket altered recognition of virus infected cells by CTL, whereas none of these substitutions abrogated recognition for FMP synthetic peptide pulsed targets. We might interpret these results as indicating that these mutations have altered the affinity for peptide, or that biochemical pathway of naturally processed, endogenous peptide association with A2 in the endoplasmic reticulum is not identical to the binding of exogenous peptide to already folded, cell surface A2 protein. Because of the striking predominance of Leu and Met at position 2 of A2-associated self-peptides, we had expected that alteration of residues in pocket B would easily be detected by the loss of CTL recognition, whereas mutations in other pockets would be less likely to have widespread effects. Indeed, we have found mutations in pocket B have a strong impact on recognition of both Flu-specific and allo-specific CTL. Surprisingly, the majority of the mutations in the other pockets also affect CTL recognition of the A2 molecule. Recently, we have established HLA-A2 positive EBV transformed B cell lines expressing endogenous HIV1 tat or rev protein. We are now attempting to produce A2-restricted CTL specific for peptides derived from these regulatory proteins. Effects of these mutations in each pocket will be evaluated on HIV specific CTL responses.

Q 538 CTL RESPONSES IN HIV-1 UNINFECTED VOLUNTEERS PARTICIPATING IN PHASE I HIV-1 VACCINE TRIALS.

M. Juliana McElrath, Lawrence Corey, Mike Rabin, Mark Hoffman, Sara Klucking, and Philip D. Greenberg. Depts. of Medicine, Laboratory Medicine, and Immunology, Univ. of Washington, Seattle, WA 98195

A major goal in the development of a preventive HIV-1 vaccine is to stimulate cytotoxic T lymphocytes (CTL), which function to clear virus in acute infection and control virus replication in persistent infection. The analysis of the CTL responses induced by immunization is a high priority, but has been technically difficult in preventive vaccine trials. We report a sensitive method to detect vaccine-induced CTL in HIV-1 uninfected recipients of envelope subunit vaccines that circumvents the problem of inducing during culture high levels of lysis against target cells expressing vaccinia and/or EBV antigens that interfere with analysis of responses in persons immunized with vaccinia vectors, and provides a method useful for comparing CTL responses elicited by various vaccine candidates.

PBMC from vaccinees were stimulated for 2 one-week cycles with autologous macrophages previously infected with HIV-1_{Ba-L}, a monocytotropic strain. No exogenous IL-2 was required during stimulation. Bulk, CD4-, and CD8-enriched effectors were tested for lytic activity in a 4 h ^{51}Cr release assay against BLCL infected with vaccinia, recombinant vaccinia containing the *env* or *gag* gene, and BLCL transduced with the CD4 gene and productively infected with HIV-1.

Env-specific CTL were detected in 2 of 4 individuals who received recombinant vaccinia/HIV-1 envelope priming followed by rgp160 boosting (AVEG 002). The CD8-mediated, MHC-restricted CTL responses against HIV-1 infected and *env*-expressing targets were detected in one individual multiple times from 3-15 months after rgp160 boosting, at low E:T ($\lesssim$5:1). CTL responses are also being evaluated in 12 volunteers from trial AVEG 002 who have received a second course of Vac/Env, and in 9 volunteers participating in subunit protein vaccine trials (AVEG 003B and AVEG 007A).

In summary, we have developed a methodology that can be of general use to elucidate the potential efficacy of candidate HIV-1 vaccines at eliciting CTL responses to one or more HIV-1 gene products. Our findings in ongoing phase I trials will be described.

Q 539 SEQUENTIALLY DERIVED HIV-1 ISOLATES DISPLAY PROGRESSIVE HETEROGENEITY IN THE NEF GENE,

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To assess the role of naturally occurring NEF divergence, DNA was isolated from peripheral blood mononuclear cells sequentially derived from four unrelated adults over a 2.5-4.5 year period. The DNA fragments encoding NEF and the first half of the 3' LTR (nested primers included nt. 8305-9202) were amplified, cloned and sequenced. Comparisons of the various clones with HXB2 revealed a sequence divergence of 17 to 32% among the four patients. Among clones derived from the same blood sample, sequence divergence from early and late time point samples ranged from 1.4-3% to 5-16% respectively. Increasing amino acid divergence correlated with the decreasing CD4 + cell count associated with the later blood samples. In contrast to available sequences previously derived from the V3 region (McNearney et.al., PNAS 89:10247-10251, 1992), no conserved consensus sequences could be identified among the early time point isolates. A loss of amino acid residue 15 phosphorylation site was noted in several late time point isolates. Previously described B- and T-cell epitopes were well conserved throughout the full length isolates. Deletion mutants and aberrant products could be demonstrated in all patients, disproportionately represented in the late time points. Sequence divergence in NEF may contribute to disease progression in HIV-1 infection.

Q 540 NC-30 and IL-4, TH-2 Products, inhibit HIV-1 growth in Tissue Culture Differentiated Macrophages

Montaner L.J. , Herbein G., Collin M., Gordon S.; *Sir William Dunn School of Pathology, University of Oxford, England OX1 3RE*; Minty A. J. , Caput D. and Ferrara P.; *Sanofi-Elf Biorecherches, 31676 Labege, France*

It is currently thought that Th-2 products may contribute to the pathogenesis of AIDS by down-regulating Th-1 type cellular immune responses. However, we have observed inhibition by IL-4 and NC-30 (newly cloned "Th-2 cytokine") of HIV-1 ADA growth in primary macrophages, without affecting its growth in PHA stimulated, IL-2 maintained PBLs. In initial experiments, NC-30 dependent inhibition has been up to 30 fold; in the present experiment, IL-4, NC-30, INF-γ, and INF-α 2A were compared in relation to increasing multiplicity of infection (MOI) of HIV-1 ADA. As the MOI decreased, IL-4 and NC-30 reached a maximum inhibition of 5 and 24-fold respectively, in comparison to control. The two interferons also inhibited HIV-1 ADA production, INF-α 2A being 50% more effective than INF-γ at high MOI, whereas both inhibited HIV-1 ADA completely at low MOI. These experiments show that both Th-1 and Th-2 derived lymphokines are able to suppress HIV in macrophages *in vitro*, and therefore may contribute to preservation of cell mediated immune functions *in vivo*.

Q 541 TRACKING TRANSMISSION OF AN UNUSUAL HIV-1 VARIANT IN CANADA.

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Patients suspected of being infected from a single source of HIV-1 were studied to ascertain possible transmission links and the infective strain of HIV.

Samples were received as white blood cell pellets or sera. These were processed for polymerase chain reaction (PCR) amplification of the V3 region of the *env* gene in cellular DNA or viral RNA respectively using previously published primers (M.M.W.R. 40, 21, 1991). Sequencing of cloned PCR products revealed extensive homology between the patient sequences and the rare SF170 (Rwandan) strain (P.N.A.S. 85, 2815, 1988). Novel primers based on the sequenced clones greatly enhanced the efficiency of amplification, eliminating the need for nested PCR reactions.

Analysis of the cloned PCR products from a number of patients revealed that all appeared to be infected with this single unusual strain. Sequence variability within any given patient was below 2% while the rate of sequence evolution between patients remained below 2%/year and consisted mainly of the expected G→A hypermutations.

Q 542 NATURAL SELECTION OF IMMUNE BLOCKING, SLOW /LOW STRAINS OF HIV: A MATHEMATICAL MODEL.

George W. Nelson and Alan S. Perelson, Group T-10, Los Alamos National Laboratory.

Our model considers macrophage infecting strains of HIV. We suppose that HIV may be transferred from infected macrophages to CD4+ Th cells during interactions between these cells. In this way HIV may be transferred preferentially to those CD4+ Th cells which recognize macrophage presented antigen.

To test the differential ability of varying strains of HIV to avoid immune response, we incorporate a process of initial stimulation of immune response in our model. We suppose that the initial stimulating factor is CD4+ Th cell recognition of macrophage presented antigens, and that stimulation occurs when the rate of these recognition events reaches a threshold level. We consider that different strains may vary both in their ability to block CD4+ Th cell activation, and in their ability to replicate in CD4+ Th cells. Computer simulations of the model show that strains with maximal activation blocking, and minimal CD4+ Th cell replication, are able to grow to a larger population before inducing an immune response.

This model provides a possible explanation of natural selection of slow/low HIV strains, and provides an alternative explanation of the adaptive function of reported HIV factors which block CD4+ Th cell activation.

Q 543 HIV-2_{HOM}, AN HIV-2 SUBTYPE WITH LOW VIRULENCE,

Sigrid Nick[1], Carlos A. Diniz[1], Marlies Sauter[2], Hans W. Pees[3], Nikolaus Müller-Lantzsch[2], Gerhard Jahn[1], [1]Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, D-W 8520 Erlangen, Germany; [2]Abteilung Virologie, [3]Innere Medizin, Universität des Saarlandes, D-W 6650 Homburg/Saar, Germany

Infections with the human immunodeficiency virus type 2 (HIV-2) gain increasing importance in African and Asian countries. Although a few isolates have been characterized molecularly, many questions are still open. Especially the pathogenic potential of this virus group that, in addition, comprises simian isolates (SIV) remains controversial. One reason might be the high diversity. Single isolates cannot be alloted to phylogenetic subtypes according to human or simian origin. However, genetic subtypes that share biological and molecular features may exist. In 1991 a new HIV-2 variant (HIV-2_{HOM}) was isolated from a German healthy individual most likely infected in the Ivory Coast during 1970 to 1974. The virus was recovered from both, the plasma and the peripheral blood lymphocytes of the patient by using OKT-3 stimulated cord blood lymphocytes. The viruses could be further propagated on Jurkat cells and exhibited an overall broad cell tropism. Radioimmunoprecipitations revealed that biochemical and antigenic properties of HIV-2_{HOM} polypeptides were similar to those of HIV-2_{ROD}. For a more detailed molecular characterization a 557 bp env fragment and a 722 bp pol fragment were amplified by polymerase chain reaction, cloned and sequenced. A comparison of both sequences to prototypic HIV-2 and SIV isolates revealed a close relationship to HIV-2_{ST}. HIV-2_{ST} originates from a healthy Senegalese individual and is supposed to be of reduced pathogenicity.

Taking together genetic and clinical data of the two isolates, we conclude that HIV-2_{HOM} and HIV-2_{ST} form a new HIV-2 subtype with low virulence.

Q 544 INDEPENDENT DIVERGENCES IN THE CD4 BINDING SITE AND V3 LOOP ENCODED IN TWO SEROPREVALENT UGANDAN HIV-1 ISOLATES, Gary Pestano, Andrew Atkin, Yang Tao Zheng, Alfred Prince*, Jerry Guyden, Ken Harewood, and William M. O. Boto, Dept of Biology, The City College, City University of New York, NY 10031. *Lindsley F. Kimball Research Institute, NY Blood Center, 310 East 67 Street, NYC, 10021

A conformation-dependent epitope located at or near the CD4 binding site in the envelope glycoprotein gp120 has been recently shown to neutralize a broad selection of HIV-1 strains. The finding has lead to the proposition that the immunogenic CD4 recognition site may be conserved despite the marked variation in the V3 loop principal neutralizing determinant (PND) expressed in diverse strains. This hypothesis was tested in the present investigation using two seroprevalent Ugandan clinical isolates, UG06c and UG23c. A family tree analysis of the predicted amino acid sequences in the putative CD4 binding site in V4/C3 regions suggested that UG06c is more closely related to a previously reported Ugandan clone U455 than to UG23c. In contrast, the UG23c strain appeared to cluster with NDK, JY1 and OYI in another subgroup. The UG06c and UG23c isolates were less related to each other than they were to the North American prototypic strain, MN. A similar analysis of the V3 loop amino acid sequences appeared to cluster UG06c with OYI, Z321, MN and HXB2R in the same subgroup, whereas UG23c was more closely related to ELI, Z2Z6, Z6 and JY1 in another subgroup. The V3 loop and CD4 binding site have apparently diverged independently of each other in UG06c, UG23c and other HIV-1 strains. Computer-assisted secondary structure analysis was conducted to predict the potential antigenic sites in the V4/C3 and V3 regions. These data indicated that the antigenic sites in the V3 loop and CD4 binding regions are retained despite the variation in the amino acid primary sequence encoded in the two Ugandan isolates.

Q 545 LYMPHOCYTE IN VITRO PROLIFERATIVE RESPONSE TO HIV-1 p24 IS ASSOCIATED WITH A LACK OF $CD4^+$ CELL DECLINE. Oscar Pontesilli, Maurizio Carlesimo, Anna Rita Varani, Rosetta Ferrara, Giampiero D'Offizi, Fernando Aiuti. Department of Allergy and Clinical Immunology, University of Rome "La Sapienza" , Roma, Italy

To investigate whether HIV-specific T-helper lymphocytes are functional in early HIV infection, we studied peripheral blood mononuclear cells from 16 patients with asymptomatic HIV-1 infection (CDC groups II and III, $CD4^+$ lymphocyte numbers at the time of proliferation studies $>350/mm^3$ and at least 4 $CD4^+$ lymphocyte determinations in the previous 2 years) and 13 healthy HIV seronegative subjects for the *in vitro* lymphocyte proliferative response to recombinant HIV-1 p24 (American Bio-Technologies, Cambridge, MA, USA) added at the concentration of 2 μg/ml to 7 day microcultures. Proliferation was assessed by tritiated thymidine incorporation and expressed as tstimulation index (s.i.). To quantify the variation of $CD4^+$ lymphocyte counts over time, the regression line relative to the two years preceding the proliferation study was calculated by the least square method. A negative slope of the regression line was considered indicative of $CD4^+$ cell number decrease; conversely, positive slopes revealed a relative steadiness or increase of the $CD4^+$ cell number, at least during the two-year span considered. No lymphocyte proliferation in response to p24 was found in HIV seronegative healthy controls (s.i.=0.99±0.32; range: 0.47-1.64); five of the 16 HIV-1 infected asymptomatic patients studied showed a p24 specific s.i. higher than 2, which is considered a conventional cutoff for significant proliferative responses, even though the p24 specific stimulation indices of the whole group (mean 1.99; range: 0.66-8.94; Mann-Whitney U-test vs. controls $p=0.30$) were not statistically different from those of controls. No significant correlation was found between the magnitude of the proliferative response and the number or the percentage of circulating $CD4^+$ lymphocytes at the time of the study.

A significant association between the slope of the $CD4^+$ cell count regression line and the response to p24 was found: none of the 6 patients showing a decline of $CD4^+$ cell numbers (negative slope) in the previous 2 years had a significant lymphocyte proliferative response to p24, whereas, of the 10 remaining patients showing steady or increasing $CD4^+$ cell numbers (positive slope), 5 had p24 specific s.i. higher than 2 (chi-square test, $p<0.05$). According to the preliminary data reported here, we propose that lymphocyte proliferative response to p24 could represent a useful marker of non-progression of the disease, and should be therefore included in larger studies to be evaluated more precisely.

Q 546 DEVELOPMENT OF RESISTANCE TO THE BHAP NON-NUCLEOSIDE HIV-1 RT INHIBITORS

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The bisheteroarylpiperazines (BHAPs) are non-nucleoside inhibitors of the HIV-1 reverse transcriptase (RT) that potently block HIV-1 replication in cell culture. The BHAPs bind purified RT at a single site with high affinity (Kd of 50 nM). Other non-nucleoside RT inhibitors (i.e., neviripine, L-drugs, and TIBO) compete with the BHAPs for binding to RT. Data by Nunberg *et al.* (J. Virol. **65**:4887, 1991) and Richman *et al.* (Proc. Natl. Acad. Sci. **88**:11241, 1991) indicated that HIV-1 resistance to this class of inhibitor may occur rapidly *in vitro*. To study the development of HIV-1 resistant to the BHAPs, HIV-1 (JRCSF) was passaged in peripheral blood mononuclear cells in the presence of partially inhibitory concentrations of drug. At weekly intervals, HIV-1 was recovered from culture supernatants and serially passaged with increasing levels of BHAP. Within 3-5 passages, HIV-1 at least 10-fold resistant to the BHAPs was recovered. This virus retained full sensitivity to AZT. Data characterizing this resistant virus population will be presented.

Q 547 RAPID REVERSION OF 3'-AZIDO-3'-DEOXYTHYMIDINE-RESISTANT MUTANTS OF FELINE IMMUNODEFICIENCY VIRUS, Kathryn Martin Remington, Thomas W. North, and Tom R. Phillips, Division of Biological Sciences, University of Montana, Missoula, MT 59812 and Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

The nucleoside analog 3'-azido-3'-deoxythymidine (AZT) has been widely used for treatment of patients with the acquired immune deficiency syndrome (AIDS). Within the last several years, human immunodeficiency virus (HIV) that is resistant to AZT has been isolated from AIDS patients that have been receiving long-term AZT therapy. The nature of these AZT-resistant variants, especially with respect to their potential for pathogenicity, is not known. It is difficult to dissect these characteristics of the mutant HIV without an animal model. We have selected, *in vitro*, AZT-resistant mutants of feline immunodeficiency virus (FIV) that are very similar to the AZT-resistant clinical isolates of HIV. FIV is a lentivirus that produces a natural AIDS-like disease in cats. We have used these mutants of FIV to determine the stability of the AZT resistant phenotype. When AZT-resistant populations of FIV were grown in the absence of AZT, they reverted to a wild-type phenotype within three rounds of replication. To facilitate subsequent sequence analysis of the mutants, we selected and plaque-purified AZT-resistant FIV that had been derived from a genetically homogeneous molecular clone. These mutants were similar in their patterns of drug susceptibility to the AZT-resistant mutants that had been derived from the wild-type parent population. In the absence of AZT, they also reverted to wild-type levels of AZT susceptibility within three rounds of replication. The reverse transcriptase encoding region of the *pol* gene from the molecular clone parent, the plaque-purified mutant and plaque-purified revertants have been analyzed at the DNA sequence level. This information will be useful in understanding the mechanism of AZT-resistance.

This work was supported by NIAID grant AI-28189.

Q 548 AUTOLOGOUS AND ALLOGENEIC CD4 T CELL KILLING BY CD8 T CELLS FROM HIV-1 INFECTED INDIVIDUALS. Nayra Rodriguez, Waleska Alvarado & Yasuhiro Yamamura. Ponce School of Medicine AIDS Research Program, Ponce, PR 00732.

During the progression of HIV-1 associated disease, an unusual CD8 T cell subset with CD57 and/or HLA-DR appeared; the number of which increased as the disease progressed. Some such CD8 T cells obviously included ,cytotoxic T cells which could destroy HIV-1 infected CD4 T cells. Such a decline of CD4 T cells is directly associated with the immunodeficient status of the patients. The present study examined, by a new flow cytometric analysis of mitogen-induced lymphocyte proliferation, how the activated CD8 T cells from HIV-1 infected individuals (hivCD8) affected CD4 T cells from either HIV-1 infected (hivCD4) or normal seronegative individuals (nCD4). Briefly, peripheral blood mononuclear cells (MNC) were isolated by a Ficoll-Hypaque method and labelled with a cell-linker, PKH-26 (Zynaxis Cell Science; Malvern, PA). CD4 and CD8 T lymphocytes were purified by utilizing the Dynal's DYNABEADS with the DETACHaBEAD solution. Cell were cultured in RPMI-1640 medium plus 10% defined calf serum (Hyclone) and were stimulated with 3 μg/ml of purified PHA (Wellcome) for 5 days. Cells were then labeled with FITC-conjugated anti-CD4 or -CD8 monoclonal antibody and the proliferation profile of each cellular subset was analyzed by FACScan utilizing the Lysis II software. In a PHA-stimulated hivMNC culture, a large majority of CD4 T cells were dead by day-5; while its CD8 T cell subset vigorously proliferated. Removal of hivCD8 T cells from an hivMNC culture, protected hivCD4 cells; and the addition of nCD8 to the culture of isolated hivCD4, did not affect the proliferation of the latter. On the other hand, addition of hivCD8 to the culture of nCD4 resulted in massive destruction of the CD4 cells. In unstimulated hivMNC or nMNC cultures, no significant CD4 cell death was observed. Possible mechanisms by which hivCD8 T cells destroyed both nCD4 and hivCD4 T cells are discussed.

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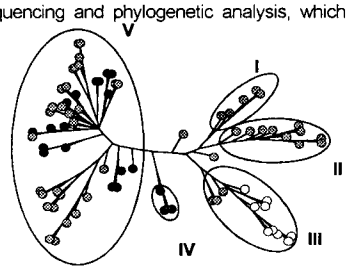
Q 549 AN IMPROVED METHOD FOR ASSESSING ANTIBODY RESPONSES TO THE PRINCIPAL NEUTRALIZING DETERMINANT (PND) OF HIV-1 gp120. Doreen Sakamoto, Jürg Baenziger, Faruk Sinangil, Diana Lee, Chris Turck* and Kathelyn Steimer. Chiron Corporation, Emeryville, CA., and *University of California, San Francisco.

It is generally accepted that an HIV-1 vaccine must be able to induce an antibody response directed to the principal neutralizing determinant (PND) in the V3 region gp120. By characterizing the V3-specific antibody responses of infected individuals within a particular population, it should be possible to design a vaccine with the highest probability of eliciting an effective V3-specific neutralizing antibody response in the target population. Usually, V3-specific antibody responses are assessed in ELISAs with peptides adsorbed directly to the wells of microtiter plates. However, the amount of each peptide bound to microtiter plates will vary depending upon the charge of the peptide. We have developed an assay that ensures that the amount of peptide coated onto the solid phase is independent of its sequence and/or length which allows for a comparison of antibody titers to different peptides. Peptides (~20 mers) were synthesized with a biotin residue separated from the amino terminus by a spacer arm. They were captured on microtiter plates by avidin. Thus, the amount of each peptide captured onto the solid phase depends only upon the amount of avidin adsorbed to the wells. The utility of this assay was demonstrated by an assessment of the V3-peptide reactivity of a panel of sera obtained from 44 HIV-1 infected patients in San Francisco. The results observed were consistent with the V3 sequences that we would expect for viruses within this cohort. 43 of these sera had very high titers of antibodies that reacted with V3 peptides from the SF2 and MN isolates which both have V3 regions closely related to the consensus sequence from North American/European isolates. However, only 21 sera from this cohort showed significant titers with the V3 peptide from the HIV-LAI isolate. Moreover, the LAI V3 titers were at least 40 fold lower than the SF2 and MN V3 titers. Only three sera reacted significantly with a V3 peptide from an African isolate, HIV-Zr6. The details of this study, along with the results of an assessment of the breadth and cross reactivity of the V3-specific antibody responses of HIV-1 seronegative human volunteers immunized with candidate gp120 subunit vaccines will be presented.

Q 550 SOLID PHASE DIRECT GENOMIC SEQUENCING AND PHYLOGENETIC ANALYSIS REVEAL FOUR MAJOR HIV-1 LINEAGES IN FINLAND, Mika Salminen, Anne Nykänen and Pauli Leinikki, Department of HIV, National Public Health Institute, Mannerheimintie 166, sf-00300, Helsinki, Finland.

As HIV-1 has revealed itself as one of the genetically most unstable RNA-viruses known, characterization and classification methods of virus strains have become increasingly important. Knowledge of circulating strains in geographic locales around the world provides important information about the prevailing types of viruses. This kind of information will most probably be of importance for possible future vaccination strategies, as well as for monitoring of direction of virus evolution. The extreme variability of the virus makes immunological classification by current methods almost impossible. Therefore, classification is based on sequence comparison methods. We have previously described a method (AIDS Res. Hum. Retrovir. 1992; 8, 1747-56), based on solid phase direct genomic sequencing and phylogenetic analysis, which allows for the rapid characterization and classification of *in vivo* patient proviruses. Using this method, a number of samples from patients in Finland were analyzed, and found to represent at least four, possibly even five, different, highly diverged major lineages (figure). Serial samples from the same patients, taken over a period of several years showed limited variation over time, indicating that the analyzed region (*gag* p7 nucleic acid binding protein) is well suited for phylogenetic analysis of major virus lineages, in contrast to the rapidly evolving hypervariable regions of the envelope. Two potential cases of patient-to-patient transmission of common source of infection were identified based on the sequence analysis.



Q 551 T-CELL PROLIFERATION IN HIV INFECTED INDIVIDUALS Rachel D. Schrier, J. Allen McCutchan, and Clayton A. Wiley Departments of Pathology and Medicine, University of California San Diego, La Jolla, California, 92093

T-cell proliferative response to HIV and opportunistic agents Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV) are being evaluated in 108 HIV infected subjects. HIV response is detected using 31 synthetic peptides representing conserved regions of gag, pol and env proteins. Responses to HCMV and HSV are assayed using inactivated virus lysates. Study participants are at various stages of disease and are assayed longitudinally. Overall response patterns represent information from 175 proliferation assays. Analysis of immune reactivity to all HIV peptides indicated that the number of peptides recognized declined with CD 4 T-cell counts, suggesting that the number of different T-cell clones responding to HIV decreases as disease progresses. However, the magnitude of the response to HIV did not decline. Testing of individual donors repeatedly indicated that the specific HIV peptides recognized can vary considerably over time. Responses to CMV and HSV followed a different pattern than did reactivity to HIV. As CD4 levels dropped below 700, the average response to HCMV and HSV increased, peaking at 450-550 CD4 cells, and then declined. An increase in reactivity to these viruses following reduction of CD4 cells is reminiscent of the herpesvirus infections (and immune responses) which follow (induced) transplantation associated immune suppression. Study participants have been HLA phenotyped to study HLA-DR associations with responses to specific peptides. Analysis of HLA DR types and overall responses to HIV peptides, HSV, and CMV indicated a slight increase (40% versus 20%) in the frequency of HLA-DR 7 in the donor population lacking response to any of the viral antigens.

Q 552 CHARACTERISTICS OF A V3 LOOP EPITOPE IN HIV-1 BY SERIAL DELETION MAPPING Stephen J. Seligman, Department of Medicine and Department of Anatomy and Cell Biology, SUNY/Health Science Center, Brooklyn, NY 11203

The determination of epitope characteristics is important for the rational design of a subunit vaccine. The recent development in this laboratory of a method of epitope mapping has made possible not only precise epitope mapping, but also the characterization of the contribution of individual amino acids to binding affinities. The method, termed serial deletion mapping, consists of competition ELISA assays in which the C-terminus is defined by a series of peptides with deletions of one amino acid at the C-terminus. The N-terminus is then determined by a similar series of peptides with N-terminal deletions. Study of a patient's serum with high titer antibody against the central portion of the V3 loop revealed that the shortest peptide with the most activity was IGPGRAF. Accordingly that peptide was considered to be the epitope. Since the apparent affinity of the antibody varied with the peptide used as coating antigen, a binding assay was developed using ^{3}H-acetylated peptide. The assay revealed a K_A of $6x10^7$/M. Serial deletion of three N-terminal amino acids from the epitope resulted in a step-wise 10,000 fold reduction in affinity. Deletion of the next N-terminal amino acid did not result in additional decrease in affinity; but that peptide, RAF, was the shortest peptide recognized. Serial deletion mapping indicates epitope characteristics not revealed by previous methods and may be especially helpful in choosing epitopes for inclusion in a subunit vaccine.

Q 553 MAPPING OF ANTIBODY RESPONSES OF HIV-1 SERO-NEGATIVE INDIVIDUALS VACCINATED WITH RECOMBINANT HIV-SF2 GP120, Faruk Sinangil, Gordon Tribbick*, Tom Mason*, James Kahn#, David Chernoff, Kathelyn Steimer. The Biocine Company, Emeryville CA 94608, *Chiron Mimotopes Pty. Ltd. Clayton, Australia & #San Francisco General Hospital, San Francisco, CA.

In two Phase 1 trials, HIV-1 seronegative human volunteers were vaccinated with three doses (0, 1, and 6 months) of either nonglycosylated, denatured gp120 (30 µg, env 2-3$_{SF2}$) or native, glycosylated gp120 (25 µg, rgp120$_{SF2}$) combined with a microfluidized oil in water emulsion adjuvant (MF59) with or without the immunomodulator MTP-PE. Both antigens were highly immunogenic in all adjuvant formulations, including the formulations without MTP-PE. However, rgp120$_{SF2}$, was much more effective in the induction of both homologous (SF2) and cross-reactive (MN) neutralizing antibodies than env 2-3$_{SF2}$. To further characterize the antibody responses induced in man by these two versions of recombinant HIV-1 gp120, we set out to map them using octapeptides with 7 amino acid overlaps spanning the entire HIV-SF2 gp120 sequence. The peptides were synthesized with a biotin residue at their N-termini, streptavidin was used to capture them on microtiter plates, and reactivity with these peptides was assessed by ELISA as described. [Geysen, H.M. *et al.*, 1987. *J. Immunol. Methods* **102**: p. 259] We characterized the responses of 12 subjects immunized with env 2-3$_{SF2}$ and 13 subjects vaccinated with rgp120$_{SF2}$. Prior to vaccination, there was no evidence of reactivity with any of the 479 gp120-SF2 octapeptides with sera from these 25 volunteers. However, two weeks after the second immunization, a clear response against the V3 region of HIV-SF2 gp120 was detected in 5 of the 13 volunteers. Following the third dose of rgp120$_{SF2}$, 12 of these 13 volunteers exhibited a predominant V3-specific antibody response and 11 had also developed a response against peptides within the V2 region of gp120. There were no other specific regions of gp120 detected with these octapeptides that were recognized by antisera from these rgp120$_{SF2}$-immunized volunteers. The responses of volunteers immunized with env 2-3$_{SF2}$ were quite different. In general, they were unfocused and directed to random epitopes along the entire length of the molecule. There was no clear evidence of immunodominance of either the V3 or V2 regions using this denatured, nonglycosylated antigen. We conclude that both the V3 and V2 regions of gp120 are immunodominant in native, but not denatured, nonglycosylated gp120. Since both the V3 and V2 regions of gp120 contain linear and conformational determinants, it is critical to utilize a native antigen in order to maximize these responses.

Q 554 CHARACTERIZATION OF VIRAL MUTANTS RESISTANT TO A C2-SYMMETRIC INHIBITOR OF HIV-1 PROTEASE. Mandal. K. Singh, Takuo Toyoshima, Martin Markowitz, Dale J. Kempf, Daniel W. Norbeck, John Erickson, and David D. Ho. The Aaron Diamond AIDS Research Center and NYU School of Medicine, New York, NY; Abbott Laboratories, Abbott Park, IL; NCI-Frederick Cancer Research Center, Frederick, MD.

HIV-1 protease exists as a homodimer, and its activity can be blocked in vitro by a number of inhibitors. One C2-symmetric compound (A-77003) that showed potent antiviral effect on HIV-1 replication in vitro is currently under investigation in phase 1 clinical trials. We studied viral resistance to this potential therapeutic agent by serially passaging HIV-1$_{NL4-3}$ in the presence of increasing concentrations of A-77003. After 19 passages, HIV-1 variants that emerged showed a 20-fold increase in the ID_{90} but no difference in the replicative capacity. PCR was then used to amplify the sequence encoding the protease gene from drug-resistant viruses followed by molecular cloning and nucleotide sequencing. Amino acid substitutions were consistently found in two distinct positions in the protease. These mutations have been subsequently introduced back into the parental virus to confirm their role in mediating the resistance to A-77003. In addition, the effect of these mutations on the protease-drug interaction has been structurally analyzed by computer modelling.

Q 555 COMPLEMENT AS AN EFFECTOR MECHANISM IN CHALLENGE RESISTANCE OF MACAQUES VACCINATED WITH HUMAN-CELL-DERIVED SIMIAN IMMUNODEFICIENCY VIRUS, Gregory T. Spear, Daniel M. Takefman, Brenda L. Sullivan, Alan L. Landay, Myra B. Jennings and James R. Carlson, Dept of Immunology/Microbiology, Rush Medical School, Chicago, IL and Dept of Pathology, School of Medicine, University of California at Davis, Davis, CA

Previous studies show immunization with uninfected human cells can protect macaques from challenge with SIV produced in human cells. Although anti-human cell antibody was induced by the immunization in those studies, the mechanism by which the antibody mediated resistance to the challenge was not determined. This study utilized both HIV and SIV systems to investigate whether complement may play a role in anti-cellular antibody-mediated protection.

While decomplemented sera from six HIV-immunized macaques had little or no neutralizing activity for HIV, the sera contained antibodies which reacted with the human cell line in which the immunogen was produced. Addition of human complement to the macaque sera substantially increased neutralizing activity against several HIV isolates. These sera were able to induce complement-mediated virolysis of HIV as well as SIV and the virolytic antibody was absorbed from the sera with uninfected cells.

Similar results were found with sera from SIV immunized macaques in that: serum antibody reacted with the human cell line in which the immunogen was produced, caused complement-mediated virolysis of HIV, and absorbtion of the serum with uninfected H9 cells removed the HIV-virolytic antibody.

Taken together, these in vitro studies indicate that complement-mediated virolysis initiated by anti-human cell antibodies can destroy either HIV or SIV. This may account for the resistance of human-cell immunized macaques to human-cell-produced SIV.

Q 556 COMBINATION VACCINE APPROACH FOR THE PREVENTION AND TREATMENT OF INFECTION BY SIV AND HIV, José V. Torres, Nancy Leung, Heather Vermazen, Michael Jarvis, David Anderson, Paul Luciw, Anna Aldovini, Richard Young and Murray B. Gardner, Department of Medical Microbiology and Immunology and Department of Medical Pathology, University of California, Davis, CA 95616 and Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

An effective vaccine for protection against retroviral infection or disease development should induce both humoral and cellular immune responses. Induction of cell-mediated immunity is very important for the elimination of virus-infected cells. In addition, neutralizing or virus-specific antibodies are necessary for the clearance of cell-free virus. An immunogen containing two major components has been designed, prepared and used for the immunization of rhesus macaques. SIVmac239 proteins were inserted into BCG using a shuttle plasmid vector. This live vector was designed to induce virus-specific cytotoxic T lymphocytes (CTL) in naive or SIV-infected monkeys. Expression of each SIV protein was tested in *E. coli* before shuttling the plasmids into BCG.

Variability of specific regions of the envelope glycoprotein of SIV and HIV is one of the major obstacles in the development of an effective vaccine. To address this problem, we have devised a new synthetic strategy for the production of constructs capable of representing the in vivo variability of individual epitopes. Following analysis of the SIV envelope glycoprotein sequences available from the various databases, constructs representing each hypervariable and antigenic region were prepared. We have demonstrated that these constructs are capable of inducing broadly crossreactive antibodies in mice. Immunogenicity of this combination vaccine either before or after infection with SIVmac239 was investigated in rhesus macaques. Virus presence in plasma, PBMC, lymph nodes and other tissues was monitored to assess the influence of this vaccine in infection and disease progression.

Q 557 SOLID PHASE DNA SEQUENCING AS A CLINICAL TOOL

Mathias Uhlén, Johan Wahlberg & Thomas Leitner
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The development of the polymerase chain reaction (PCR) has created new possibilities in clinical analysis, since virtually any target DNA can be amplified from complex samples. A potentially important application is the use of the *in vitro* amplification system for direct genomic sequencing of viral, bacterial or parasitic genomes to yield structural, functional and epidemiological data. However, large scale routine sequencing of clinical samples has been hampered by the lack of automated and reproducible methods for direct sequencing of genomic DNA. Integrated systems for sample preparation, sequencing, separation and detection are therefore required. The use of a solid phase sequencing method for direct analysis of viral drug resistance has been designed to allow for automated routine application and to yield the "consensus" sequence of the various viral genomes present in each patient. A semi-automated protocol for template preparation, sequencing reactions and electrophoresis was used and is schematically outlined in figure 1. Three separate instruments were used; (i) a thermocycler for the amplification of DNA (ii) a robotic work station for the magnetic separation and sequencing reactions and (iii) an automated sequencer for the fluorescence detection. Briefly (see figure 1), the amplified material is immobilised on the solid support using the interaction between biotin and streptavidin. A clean single-stranded template is obtained by strand-specific elution using alkali without the need for precipitation, filtration, centrifugation or other purification steps.

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Q 558 CONTRIBUTION OF ANTIBODIES DIRECTED TOWARDS LINEAR AND CONFORMATIONAL EPITOPES INDUCED BY MICROGENESYS GP160(LAI) AND GENENTECH GP120(IIIB) VACCINATION OF HIV-1 INFECTED INDIVIDUALS. Thomas C. VanCott[1], V.R. Polonis[1], F.R. Bethke[2], N.E. Jacir[2], G. Smith[3], R.R. Redfield[1] and D.L. Birx[1], [1]Walter Reed Army Institute of Research, Dept of Retroviral Research, [2]Henry M. Jackson Foundation, Rockville, MD 20850 and [3]MicroGeneSys, Inc., West Haven, CT 06516.

We are currently evaluating several different recombinant proteins (Genentech gp120(IIIB) and MicroGeneSys gp160(LAI)) as immunogens for vaccine therapy of early stage HIV-1 infected patients. The ability of these therapeutic vaccines to modulate the native host immune response to HIV-1 is being investigated with respect to their capacity to alter/boost antibody reactivity to epitopes accessible on native, conformationally intact gp120 (rgp120) versus denatured gp120 (drgp120). Our previous results on gp160(LAI) vaccinees utilizing various peptide serological assays (Western Blot Mal E fusion proteins, peptide ELISA and PEPSCAN) have demonstrated seroconversions to novel linear epitopes within gp160 as well as boosts to homologous, heterologous and autologous gp120/gp160 by Western Blot. Boosts in neutralization titers versus homologous, heterologous and autologous isolates have also been demonstrated. To further analyze the immune response with respect to linear versus conformational antibodies, we have examined serum reactivity using a variety of techniques including: 1. HIV-1(iiib, mn and viral lysates) Western Blotting, 2. peptide, rgp120, drgp120, rgp160 ELISA and BIAcore studies and 3. flow cytometric analysis of the processed oligomeric gp120/gp41 complex expressed on the surface of acutely and/or chronically infected cells. Comparisons of the antibody profiles in the separate vaccine therapy trials with respect to natural infection will be highlighted. Additionally, changes in the percentage of the spectrum of antibodies directed against particular linear regions (C1, C2, C3, V3, C4, C5) over the course of vaccine therapy are examined.

Q 559 VARIABILITY IN THE REVERSE TRANSCRIPTASE GENE, STUDIED BY DIRECT DNA SEQUENCING.

Johan Wahlberg[1], Jan Albert[2] & Mathias Uhlén[1]
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ABSTRACT

The use of a solid phase sequencing method for direct analysis of the viral pol gene has been designed to allow for automated routine application and to yield the "consensus" sequence of the various viral genomes present in each patient. Three separate instruments were used; (i) a thermocycler for the amplification of DNA (ii) a robotic work station for the magnetic separation and sequencing reactions and (iii) an automated sequencer for the fluorescence detection.

One important clinical application for direct genomic sequencing is the analysis of resistance in human immunodeficiency virus type 1 infected patients (1). AZT inhibits HIV-1 replication *in vitro* and is effective for the treatment of patients with acquired immunodeficiency syndrome (AIDS) or AIDS related complex (ARC). However, patients frequently deteriorate during the second year of therapy, a phenomenon which coincides with the occurrence of AZT-resistant viruses. AZT resistance is associated with specific amino acid substitutions in the reverse transcriptase (RT) enzyme of HIV-1.

The same method was used for a follow up study on patients with terminated AZT treatment (2). Here, the virus were reverting back to "wild trype" in 9-18 months. In this later study we analysed RNA from virus, provirus and isolates used in the IC_{50} culture assay.

Results on patient to patient transmission will also be presented, since we have used the same system on a rape case in Sweden where the virus was transmitted to the victim.

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2. Albert J. *et al.* ; Persistance of Azidothymidine-resistant human immunodeficiency virus type 1 RNA genotypes in posttreatment sera. J. Virol.(1992) **66(9)**, 5627-5630

Late Abstracts

MECHANISM OF RESISTANCE TO ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) IN CHIMPANZEES VS HUMANS. Nirmal K. Banda, William Satterfield, Roland Kurrle and Terri Helman Finkel, NJCIRM, Denver, CO,80206. Human immunodeficiency virus (HIV-1) has tropism for CD4+ T cells in humans. During HIV-1 infection, it has been widely documented that there is a profound and selective loss in the CD4+ population of T lymphocytes. We have recently reported that crosslinking of bound gp120 on CD4+ T cells followed by activation through the T cell receptor for antigen results in activation-dependent cell death by apoptosis or programmed cell death[1]. We have shown that apoptosis is not inhibitable by IL-2, although it is inhibitable by cyclosporin A and anti-CD28 Mab (9.3), agents which have been shown in other systems to inhibit apoptosis and anergy, respectively. This mechanism may be responsible for the progressive CD4+ T cell death seen in AIDS, with activation stimuli provided by the antigens and superantigens of opportunistic infections and/or by the superantigen(s) which may be encoded by HIV itself.

HIV-1 infects both humans and chimpanzees, but while human infection leads to AIDS as a result of qualitative and quantitative defects in CD4+ lymphocytes, in the chimpanzee, HIV infection does not cause AIDS-like disease. In contrast, SIVs that are closely related to HIVs do not cause disease in their natural host (African monkeys), but produce AIDS-like disease upon infection of macaque and rhesus monkeys. Our current studies involve the nonhuman primates, chimpanzee and macaque monkey, in order to determine whether apoptosis plays a pathogenetic role in AIDS in humans. Specifically, we predict that while cells from the human and macaque monkeys will undergo apoptosis in response to CD4 ligation and subsequent activation, cells from the chimpanzee will not. Our preliminary studies suggest that chimpanzee CD4+ cells do not undergo apoptosis after they are CD4-primed by gp120 and subsequently activated through their antigen receptors. In addition, proliferation assays suggest that chimpanzee CD4+ T cells also do not undergo the anergy or non-responsiveness seen in human cells in response to CD4 ligation. Thus, it is possible that chimpanzees do not get AIDS-like disease due to the absence of the cellular anergy and apoptotic cell death (the qualititative and quantitative CD4+ T cell defects) seen in human AIDS. We are currently pursuing studies of the macaque monkey to test these hypotheses. In addition, we are comparing the signal transduction mechanisms used by chimpanzee vs. human cells in an effort to understand the biochemical basis for the differential response to CD4 ligation and subsequent activation.

[1] Banda, N. K., et al. J. Exp. Med. 176: 4, 1099-1106, 1992.

EFFECT OF HIV-NEUTRALISING ANTIBODIES ON INFECTION OF TROPHOBLASTS. Aldar S. Bourinbaiar, William Borkowsky, Keith Krasinski, and *Susan Zolla-Pazner. Department of Pediatrics, Division of Infectious Diseases, and *Department of Pathology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

Although placental trophoblasts, the only fetal cells in direct contact with maternal blood, can be susceptible to HIV infection, the precise cause of the low transmission rate of virus from mother-to-fetus is not known. It is possible that maternal anti-HIV antibodies may contribute to protection of the fetus against HIV infection. This possibility has been examined in vitro using choriocarcinoma-derived BeWo line exposed to HIV (8×10^6 viral particles/ml) in the presence of serial dilutions of neutralizing monoclonal antibodies either to V3 loop (#694) or CD4-binding domain (#588). Preliminary results based on measurement of p24 release from virus-exposed cells reveal that titers of antibodies sufficient to inhibit infection of control MT-4 lymphocytes were ineffective in protecting trophoblasts.

Ab/Cells	Antibody concentration (μg/ml)					
	0	0.1	0.5	1.0	5.0	10
#588/MT-4	173	118	100	65	45	35
#694/MT-4	173	124	76	53	11	11
#588/BeWo	150	144	156	158	178	176
#694/BeWo	150	142	133	115	124	122

Amount of released p24 (pg/ml)

Further studies are in progress to confirm the preliminary data. The results derived from this in vitro model can advance our knowledge on the mechanism of viral transmission in utero and may provide a basis for designing antiviral strategies in clinics.

FUNCTIONAL AND PHENOTYPIC CHANGES WITHIN THE CD8+ T CELL SUBSET IN HIV-1 INFECTION, Jan E. Brinchmann and Torstein Egeland, Institute of Transplantation Immunology, Rikshospitalet National Hospital, 0027 Oslo 1, Norway

Staphylococcal enterotoxin (SE) superantigens, when bound to HLA class II molecules, provide a powerful activation signal via the T cell receptor/CD3 complex. We have used SE to study the functional competence of T cells in HIV-1 infection. CD4+ T cells from both HIV-1-infected individuals and their age- and HLA DR-matched healthy controls secreted IL-2 and proliferated strongly in response to SE. In contrast, CD8+ T cells from HIV-1-infected individuals proliferated poorly and secreted little or no IL-2 compared to CD8+ T cells from healthy controls. Poorly functioning CD8+ T cells from HIV-1-infected individuals at a relatively late stage of infection were found to contain a reduced proportion of cells expressing CD28, a cell surface molecule known to provide a costimulatory T cell activation signal leading to increased production of IL-2. CD28- CD8+ T cells from patients and controls expressed CD3 and $\alpha\beta$ T cell receptors, but could not be induced to proliferate or secrete IL-2 by stimulation via these surface molecules.

We have previously shown that activated CD8+ T cells from asymptomatic HIV-1-infected individuals and uninfected controls secrete a soluble HIV-1-inhibitory factor, while CD8+ T cells from patients with AIDS have little or no HIV-1-inhibitory effect (Brinchmann et al., J Immunol 144:2961,1990). The presently described changes in expression of CD28 on CD8+ T cells in advanced HIV-1 infection may provide an explanation for these observations. We are now examining the expression of CD28 on CD8+ T cells from HIV-1-infected individuals at different stages of disease development.

HIV-1 ENVELOPE ELICITED NEUTRALIZING ANTIBODY TITRES CORRELATE WITH PROTECTION AND VIRUS LOAD IN CHIMPANZEES, Claudine Bruck, Clotilde Thiriart, Luc Fabry, Myriam Francotte, Pietro Pala, Omer Van Opstal, Jeff Culp*, Martin Rosenberg*, Michel De Wilde, Peter Heidt° and Jonathan Heeney°, SmithKline Beecham Biologicals, 89, rue de l'Institut, 1330 Rixensart, Belgium, *SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406, °Institute of Applied Radiobiology and Immunology - TNO, 2288 GJ Rijswijk, The Netherlands.

In order to compare the protective effect of vaccination with 2 forms of envelope antigens and to define immunological correlates of protection against HIV infection, chimpanzees were vaccinated with either recombinant gp160 or gp120. Homologous HIV challenge was performed 3 weeks after the fourth immunisation. The results demonstrated that the animal with the highest level of serum neutralizing antibodies (gp160 immunogen) was protected against HIV infection. The protective effect correlated with the intensity of the serological immune response at day of challenge, but not with lymphoproliferative T cell responses or the nature of the immunogen (gp120 vs. gp160). Furthermore, the virus neutralizing titre at day of challenge correlated with the evolution of virus burden in the PBMC of vaccinated chimpanzees which became infected. This suggests a potential benefit of HIV vaccination in a situation where sterilizing immunity is not achieved consistently.

RETROVIRAL INFECTION OF NONDIVIDING CELLS: MOLECULAR MECHANISMS OF HIV-1 NUCLEAR TARGETING, Michael Bukrinsky[1], Sheryl Haggerty[2], Mario Stevenson[2] and David Goldfarb[3], [1]The Picower Institute for Medical Research, Manhasset, NY 11030; [2]University of Nebraska Medical Center, Omaha, NE 68198; and University of Rochester Medical Center, Rochester, NY

Integration of the retroviral genome with cellular DNA and establishment of the provirus is an essential step in retrovirus replication. Integration requires targeting of the viral preintegration complex to host cell DNA and is, therefore, dependent on transport of this complex to the nucleus of the host cell. Analysis of the HIV-1 preintegration complex transport revealed that this process is independent of cell division and requires ATP, features indicative of active transport. Analysis of the preintegration complex revealed HIV-1 matrix antigen (MA) p17 as a component. Our studies indicate that by virtue of a nuclear localization signal (NLS) at the N terminus of MA, this protein is important for nuclear import of the HIV-1 preintegration complex. Mutations within this NLS restrict nuclear import of HIV-1 DNA following virus infection of non-dividing, but not of actively proliferating cells. Therefore, HIV-1 appears to use different strategies for infection of different types of cells: one strategy similar to the one used by oncogenic retroviruses relies on nuclear membrane dissolution during mitosis, and another one common to the subgroup of lentiviruses exploits active transport for entering the nuclear compartment.

The presence of an active transport pathway for nuclear import of HIV-1 preintegration complexes explains the ability of HIV-1 to infect non-dividing cells (i.e. macrophages) and suggests the possibility for modification of current retroviral vectors to acquire the ability to infect non-dividing cells.

DEFINITION OF THE USF-I TRANSCRIPTION FACTOR BINDING MOTIF IN HIV1 LTR.
Richard Doherty, Eva Tanskanen, Melissa Churchill & Nicholas Deacon. Macfarlane Burnet Centre for Medical Research, Melbourne Australia.

To identify HIV1 LTR binding nuclear transcription factors present in cell lines transformed by strains of human papillomavirus, we prepared nuclear extracts of the cervical carcinoma cell lines HeLa (HPV 18 transformed) and SiHa (HPV 16). Nuclear proteins were incubated with ^{32}P labelled synthetic DS oligonucleotides and identified by electrophoretic mobility shift assay. Oligonucleotides were made corresponding to overlapping 35 base segments upstream from the site of initiation of transcription. HeLa nuclear extracts contained a protein which bound specifically to the oligonucleotide spanning bases -178 to -139, and the precise binding site of this protein was further defined by use of a series of related synthetic oligonucleotides. In these variants, the WT sequence was replaced in overlapping 13 base regions by nonsense sequences chosen to preserve the WT frequency of individual bases, and to avoid inadvertent introduction of known transcription factor binding motifs. Oligonucleotides were tested for their ability to compete with the labelled WT oligonucleotide for binding by proteins in the nuclear extract.
Alteration of nucleotides from -159 to -164 abolished the ability of the variant oligonucleotide to compete with WT sequence oligonucleotide for nuclear protein binding. An oligonucleotide with variant sequence at bases -165 to -177 was only weakly able to compete with the WT oligonucleotide. The HIV_{NL43} LTR sequence over bases -159 to -164, CACATG, closely resembles the consensus motif CACGTG for the cellular transcription factor USF1. The binding motif for this factor has been previously mapped by DNAse I footprinting to the region from -152 to -174 (Giacca et al, Virology (1992), 186:133-147), but our results suggest that the site to which USFI binds can be more precisely localised to bases upstream from -159.

GENETIC VARIATION BETWEEN ISOLATES OF FELINE IMMUNODEFICIENCY VIRUS AND OVER TIME IN A SINGLE CAT WITH NATURALLY ACQUIRED INFECTION.
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Feline immunodeficiency virus (FIV) is a T-lymphotrophic lentivirus that causes an AIDS-like syndrome in cats, and as such is a useful small animal model for HIV infection. To help elucidate the evolution and origin of this group of lentiviruses, proviral DNA from infected lymphocytes from four Australian isolates was amplified by PCR and the nucleotide sequence determined for two subgenomic regions in *gag* and *pol*. A comparison was made with the corresponding sequences of previously described isolates of FIV from the U.S. and Japan. This revealed a remarkable similarity between the Australian isolates and two isolates, Petaluma and PPR, from California with 95-98% nucleotide and 95-100% amino acid homologies, thereby indicating a common ancestry, presumably of European origin. By contrast, a Japanese isolate, TM2, appeared to have diverged much earlier with only 85-87% nucleotide and 91-95% amino acid sequence homology with either the Australian or Californian isolates.

To further investigate of the evolution of FIV *in vivo,* sequential viral isolates from a persistently infected cat were examined by direct sequencing following amplification by PCR of regions in *gag, pol* and *env* genes. Three isolates taken over a two year period revealed no changes to critical genes within *gag* and *pol*, such as that coding for reverse transcriptase or major capsid protein (p24). Changes were, however, detected within an *N*-terminal portion of gp120 which contained the first and second hypervariable regions. These consisted of both isolated and clustered nucleotide point mutations, some of which were predicted to give rise to amino acid substitutions. The predicted amino acid changes were not random but tended to cluster within the hypervariable regions. The rate of evolution of FIV was estimated to be 2×10^{-2} nucleotide substitutions per site per year for the *env* gene and at least 10^{-4} for *gag* and *pol* genes, values concordant with that found for HIV-1. Both nucleotide and amino acid changes in the gp120 region were found to be directional in nature, suggesting that selective pressures determine the evolutionary fate of FIV envelope gene sequences.

IMMUNOLOGICAL REACTIVITY AGAINST GAG-PROTEINS IN PATIENTS, Stefan Haist, György Stuber*, Josefine März, Anette Rohrhofer, Hans Wolf and Susanne Modrow, Institut für Medizinische Mikrobiologie und Hygiene, 8400 Regensburg, Box 100662, Germany; *Karolinska-Institut, S-10401 Stockholm, Box 60400
Due to their particle-forming properties GAG-proteins of HIV-1 may serve as a basis for recombinant vaccines. For this approach the exact immunological characterization of specific epitopes and functions localized in GAG-amino acid sequences is a prerequisite.
For examination of the humoral immune reaction in HIV-patients 41 overlapping peptides spanning the GAG-precurserprotein were used as antigens in ELISA-assays. The reactivity of GAG-specific antisera from HIV-positive individuals was tested in combination with HIV-negative sera showing GAG-specific reactivity on Western Blots in order to define the domains of unspecific reactivity. Antibody reactivities in HIV-positive sera were mainly directed to peptides derived from the carboxyterminus of p17 and to the aminoterminal part of p15. All p24-derived peptides showed slightly elevated reactions, predominant sequential epitopes recognized by the majority of sera could not be identified. Adsorption of the peptide specific reactivity by incubation with peptides and scanning of the remaining p24-specific reaction on Western Blots showed that up to 50% of the p24-reaction could be reduced. The remaining antibodies are thought to be directed to discontinous epitopes prevalent in particle forming proteins. Crossreactions of HIV-negative sera with GAG-proteins could be aligned with four protein regions.
The capacity of the peptides to MHC-I proteins was tested on mouse RMA-S cell line specific for H2-K^b, D^b, D^d and human .174/T2 line specific for HLA-A2.1 by quantitativly measuring amounts of formed complexes on the cell surface by immunfluorescence.
For further functional examination of the cellular immunity PBL from HIV-1 positive asymptomatic persons were isolated and immortalized by EBV to establish permanent B-cell lines. To use these cell lines as autologous targets with cytotoxic T-cells from respective patients continuous GAG-protein expression was achieved using eukaryotic expression vectors containing the GAG-encoding sequence under controll of the SV40 early promotor together with the rev- and RRE-elements. Transfection of the plasmide into the B-cell lines was done and optimized by electroporation. This allowed continuous production of GAG-proteins, the intracellular interaction with MHC-I proteins and transport of the complex to the surface. Those cell lines can be used for the propagation and characterisation of cytotoxic T-cells. Finemapping of the epitopes has to be done by the GAG-protein specific oligopeptides.

INTEGRIN EXPRESSION IN WOUND GRANULATION TISSUE FIBROBLASTS, Lari Häkkinen, Jyrki Heino, Leeni Koivisto and Hannu Larjava, University of Turku, Finland.

Recent studies have provided evidence that changes in cell behavior and phenotype are mediated by differential expression of integrins by the cells. Tissue regeneration during wound healing occurs through specific changes in cell function which are probably controlled by integrins. In this study, we analyzed the biosynthesis, expression, and function of integrins by wound granulation tissue fibroblasts. We used metabolic labelling of integrins, immunoprecipitations, Western blotting, and cell spreading assays. Granulation tissue fibroblasts expressed $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, αv, $\beta 1$ and $\beta 3/\beta 5$ integrin subunits. αv integrin subunit formed heterodimers with both $\beta 1$ and $\beta 3/\beta 5$ subunits. These findings indicate that these cells have multiple receptors for fibronectin, collagen, and laminin. However, wound fibroblasts expressed decreased levels of αv subunit compared with fibroblasts from normal connective tissue. They also processed the pre$\beta 1$ polypeptide in a slower rate, the t½ being 16 h and 8 h respectively. Additionally, spreading of wound fibroblasts on fibronectin but not on collagen or laminin was slower. Wound fibroblasts showed also different sensitivity to inhibition of cell spreading by RGD-containing peptide and anti-$\beta 1$ antibody. These findings suggest that expression, processing, and function of integrins in wound fibroblasts is different from normal connective tissue fibroblasts. In addition to $\alpha 3\beta 1$ and $\alpha 5\beta 1$ integrins, $\alpha v\beta 1$ and $\alpha v\beta 3$ heterodimers can serve as receptors for fibronectin. Therefore, we suggest that decreased adhesion of wound fibroblasts to fibronectin is due to decreased expression of integrin αv subunit by these cells. Supported by The Academy of Finland, Sigrid Juselius Foundation, and Hilkka Brusin Foundation.

HUMAN MONOCLONAL Fab MOLECULES GENERATED FROM BONE MARROW OF A gp160 IMMUNIZED HIV-1 SEROPOSITIVE INDIVIDUAL NEUTRALIZE THE VIRUS IN VITRO

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The combinatorial immunoglobulin library approach was used to obtain human monoclonal antibodies from bone marrow cells from an HIV-1 infected, recombinant gp160 immunized individual. A random combinatorial Fab library ($\gamma 1/\kappa$) expressed on the surface of filamentous M13 phage was prepared using the pComb3 vector. Panning with $rgp120_{LAI}$ resulted in a 250-fold enrichment of specific phages. Out of 44 clones initially selected, 20 produced detecable amounts of soluable Fab. Nine Fabs gave virus neutralization and/or syncytium formation inhibition of 50% at 1µg/ml concentration (approx. 20nM); a majority of these were effective against several HIV-1 strains. Approximate affinities, as determined by inhibition ELISA with native and recombinant gp120, were in the range of 10^8-10^9 M^{-1}. Inhibition of cell-to-cell viral spread was also shown (at 2µg/ml).

CELL CULTURE-SELECTION AND CHARACTERIZATION OF VARIANT HIV-1 WITH REDUCED SENSITIVITY TO AN INHIBITOR OF THE VIRAL PROTEASE

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HIV-1 strain GB8 was serially passaged on CEM cells in the presence of increasing concentrations of the virus proteinase inhibitor Ro31-8959. Virus with reduced sensitivity to inhibitor was obtained after 8 to 10 passages. An increase in IC_{90} of 11 to 60fold was determined between passages 8 and 14. To study the molecular basis of the reduction in sensitivity, total DNA of cells of passage 10 (320nM Ro31-8959) was isolated, the proteinase ORF of integrated virus was cloned by PCR and the sequence of individual clones was determined. Comparison of the proteinase peptide to the corresponding sequence of the wt virus revealed two mutations present in all the resistant virus proteinase, i.e. substitution of valine for glycine at position 48 and methionine for leucine at position 90. Mutation G48->V is caused by a G->T and mutation L90->M by a T->A transversion. Both mutations are localised to proteinase domains which are highly conserved among HIV-1, HIV-2 and SIV. Molecular modelling suggests that the G48->V exchange may directly affect binding of inhibitor whereas the significance of the L90->M exchange is less obvious. No resistant genotype was detected among ca.20 clones isolated from wt virus in accordance with the prolonged selection necessary to obtain partially resistant virus. The relevance of the G48->V and L90->M exchanges for reduced sensitivity to Ro31-8959 was confirmed by specific mutagenesis of the HIV-1 BRU proteinase.

MONOCLONAL ANTIBODIES DEFINE FOUR RELATED BUT DISTINCT CONFORMATIONAL NEUTRALISING EPITOPES ON SIVmac251 gp120. Kent KA[1], Powell C[1], Corcoran T[1], Jones I[2] and Stott EJ[1]. 1. National Institute for Biological Standards and Control, Potters Bar, Herts, UK. 2. Institute of Virology, Mansfield Road, Oxford, UK.

A panel of monoclonal antibodies to SIV envelope were generated from mice primed with a vaccinia recombinant expressing the envelope gene and boosted with SIVmac251-infected C8166 cells. Seven of the MAbs recognise conformational epitopes on native SIVmac251 gp120. All seven MAbs react well in RIPA and also bind to the surface of unfixed SIV-infected cells in an immunofluorescence assay but they do not react with denatured antigen in Western blot. None of the MAbs react with overlapping peptides spanning gp120. Competition studies using biotin labelled purified antibody from mouse ascites show that all seven MAbs cross-compete. The seven MAbs can be divided into 3 groups according to their ability to cross-react with SIVmac251, SIVsm3 and SIVsm7. KK5, KK57 and KK58 react with mac251 and sm7 (Group A), KK17 reacts with mac251, sm3 and sm7 (Group B) and KK44, KK56 and KK9 react only with mac251 (Group C). None of the MAbs reacted with SIVsmmB670. KK9 is unique in its ability to compete with MAbs recognising the SIV V3 epitope (aa321-340) and can therefore be assigned to a fourth group (Group D). In a CD4-gp120 binding assay, whilst all MAbs showed some inhibitory activity, only KK44 and KK56 were able to completely inhibit CD4-gp120 binding. Hence, the groups defined by cross-reactivity studies and competition analysis are being confirmed by studies of biological activity. Neutralisation escape mutants are being generated and sequenced to define the binding sites of these MAbs.

RACIAL DIFFERENCES IN SOCIAL SUPPORT AND PSYCHOSOCIAL OUTCOMES AMONG HIV INFECTED ADULTS, J. Gary Linn, Avis S. Easley, and Donna F. Crawford, Center for Health Research, Box 1306, Tennessee State University, Nashville, TN 37209.

Using data gathered on 150 HIV infected clients of care and referral centers in Nashville and Memphis, Tennessee, and Louisville, Kentucky, this study systematically examines black and white differences in social support (network size and frequency of contact), perceived stress, and psychosocial outcomes (depression, anxiety, lower self-esteem, and coherence). Analyses are done within stage of illness, HIV positive, ARC, and AIDS. Inferences are drawn with regard to appropriate counseling/medical interventions to offset racial deficits in social support and their consequences.

T_H1 / T_H2 Cytokine Profiles in HIV Infected Intestinal Mucosa. McGowan I*, Keshav S**, Radford-Smith G*, Jewell DPJ*. * Department of Gastroenterology, Radcliffe Infirmary, Oxford. ** Sir William Dunn School of Pathology, Oxford.

Background: Mucosal HIV infection can be demonstrated in 30-70% of HIV infected patients. HIV has been identified in lymphocytes and macrophages within the *lamina propria*. The demonstration of mucosal abnormalities in the absence of opportunistic infection suggests a primary enteropathogenic role for HIV. The mechanism remains unclear but may involve altered expression of cytokines by HIV infected lymphocytes and macrophages. Dysregulation of T_H1 / T_H2 cytokine production has been implicated in AIDS pathogenesis manifested by decreased cell mediated immunity and polyclonal gammopathy. This study aims to see whether such dysregulation occurs in the intestinal mucosa.

Methods: RNA was extracted using RNAzol from gut biopsies (16 duodenal, 2 rectal) obtained from 18 HIV infected (7 pathogen negative diarrhoea, 5 oesophageal candidiasis, 1 non-specific duodenitis, 1 sclerosing cholangitis, 1 CMV duodenitis, 1 stage II and 2 stage IV patients) and 13 control patients (normal endoscopy and normal duodenal histology). All patients had presented with gastrointestinal symptoms. cDNA synthesis was primed with oligo dT and PCR carried out using the following primers; IL-1β, IL-4, IL-10, IFNγ, TNFα, β actin and lysozyme. PHA/IL-2 activated lymphocyte cDNA and LPS stimulated macrophage cDNA were used as positive controls.

Results: Strong bands were seen in all samples using β actin and lysozyme primers. Expression of IFNγ mRNA was seen in 8/18 HIV positive samples and 3/13 of the control samples. TNFα was present in 15/18 HIV samples compared to 5/13 controls. IL-1β was seen in 2/7 HIV and 4/4 control samples. IL-10 was seen in 17/18 HIV and 12/12 controls. No bands were seen using the IL-4 primers apart from the positive control samples.

Conclusions: RNA extraction from small intestinal biopsies followed by RT PCR is a useful method of screening samples for cytokine mRNA. The pattern of mucosal cytokine gene expression in this study does not reflect the decreased cell mediated immunity and polyclonal gammopathy which are characteristic of late HIV infection.

CD4 DOWNREGULATION BY *nef* ALLELES ISOLATED FROM HIV-1 INFECTED INDIVIDUALS.
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Polymerase chain reaction was used to clone new isolates of the HIV-1 *nef* gene directly from peripheral blood leukocytes of HIV-1 infected individuals. A transient expression system with human CEM T cells was used to assess the effect of a large number of *nef* alleles on CD4 antigen expression on the cell surface. We show that CD4 downregulation is a frequent property of primary HIV-1 *nef* alleles. Moreover, expression of the SIV MAC239 *nef* allele also resulted in dramatic decrease in CD4 antigen expression on the cell surface. Mutations in conserved amino-acid motifs of Nef disrupted CD4 downregulation. Our observations strongly suggest that CD4 downregulation reflects a conserved function of *nef*, which is selected *in vivo* in human HIV-1 infection. Methodology described here provides quantitative assays to establish whether alterations in *nef* correlate with dynamics of disease progression in human AIDS.

SELECTION OF A SPECIFIC HIV-1 QUASISPECIES DURING IN VITRO COCULTIVATION USING DISTINCT DONOR CELLS. Alexander I. Spira and David D. Ho, The Aaron Diamond AIDS Research Center and New York University School of Medicine, New York, NY 10016.

It has been shown that during vertical transmission, the HIV-1 in the infant often consists of a homogeneous quasispecies representing only a minor form found in the mother. Similar results have been shown in horizontal transmission. To look at possible mechanisms that may be underlying these selections, we looked at the effects of cocultivation of peripheral blood mononuclear cells (PBMC) from an HIV-1 infected patient with normal PBMC from three distinct individuals. Cells were cocultivated together for 14 days, at which point their DNA was isolated and the sequence of the C2-V5 region of the gp120 protein determined. Of the 15 sequences obtained from the patient's PBMC, one (CZ-15) was highly related to 53%, 83%, and 100%, respectively, of the sequences obtained following cocultivation with the three donor PBMC. Major sequences of the patient's PBMC were only minor representatives of the sequences obtained following cocultivation. Major structural variation in the C2-V5 region of CZ-15 versus the other quasispecies was not apparent. These results suggest that the selection mechanism for a given quasispecies, at least for in vitro transmission of HIV-1 via infected PBMC, is primarily the result of a viral factor and not a host-cell factor. Studies are now underway to characterize phenotypic differences between CZ-15 and the other viral variants, and to determine potential mechanisms underlying this selection.

INHIBITION OF HIV REPLICATION IN HUMAN MONOCYTES BY DEFECTIVE HERPESVIRUS VECTOR DELIVERY OF AN INTERFERON α GENE, Jerry P. Weir and Karen L. Elkins, Henry M. Jackson Foundation, Department of Cellular Immunology, Walter Reed Army Institute of Research, Rockville, Maryland 20850.

Human monocytes and macrophages are non-dividing cells that serve as a major reservoir for human immunodeficiency virus (HIV) at all stages of infection. To investigate viral mediated gene delivery as a means of inhibiting HIV replication in human monocytes, a defective herpes simplex virus (HSV) vector was developed that expressed human interferon α (IFNα). Monocytes infected with this defective HSV vector expressed IFNα and, when challenged with HIV, showed dramatically reduced cytopathic effects and HIV replication compared to control vector treated or mock treated monocytes. Similar effects on HIV replication were observed if monocytes were first infected with HIV and then treated with the recombinant vectors. These results demonstrate that defective HSV gene delivery of IFNα directly to human monocytes can greatly decrease HIV replication, and suggests that such a vector, or possibly vector-treated cells, might deliver therapeutically important genes directly to sites of HIV infection.